Human Reproduction Update 1998, Vol. 4, No. 1 pp. 3–24

Role of nitric oxide in the biology, physiology and pathophysiology of reproduction

Marinella Rosselli[1,3], Paul J.Keller[1] and Raghvendra K.Dubey[1,2]

[1]University Hospital, Department of Obstetric and Gynaecology, Clinic for Endocrinology, 8091 Zurich, Switzerland and [2]Center for Clinical Pharmacology, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA

TABLE OF CONTENTS

Following its benchmark discovery, nitric oxide (NO) is now known to play important functional roles in a variety of physiological systems. Within the vasculature, NO induces vasodilation, inhibits platelet aggregation, prevents neutrophil/platelet adhesion to endothelial cells, inhibits smooth muscle cell proliferation and migration, regulates programmed cell death (apoptosis) and maintains endothelial cell barrier function. NO generated by neurons acts as a neurotransmitter, whereas NO generated by macrophages in response to invading microbes acts as an antimicrobial agent. Because neurons, blood vessels and cells of the immune system are integral parts of the reproductive organs, and in view of the important functional role that NO plays in those systems, it is likely that NO is an important regulator of the biology and physiology of the reproductive system. Indeed, in the past 10 years, NO has established itself as a polyvalent molecule which plays a decisive role in regulating multiple functions within the female as well as the male reproductive system. This review provides an overview of the role of NO in various reproductive organs under physiological and pathological conditions.

Key words: nitric oxide/pathophysiology/physiology/reproductive system/steroidogenesis

Nitric oxide synthesis and mechanism of action

History

In 1916, Mitchell *et al.* observed that oxides of nitrogen were produced in mammals. In 1928, Tannenbaum *et al.* confirmed that mammals produce nitrogen oxide. Five decades later, Furchgott and Zawadski (1980) provided evidence that acetylcholine-induced relaxation of vascular rings was mediated by a non-prostanoid, endothelium-dependent relaxing factor (EDRF). Then in 1985, Stuehr and Marletta reported that activated macrophages synthesize nitrite/nitrate. In 1987, Hibbs *et al.* suggested that L-arginine was the substrate for murine-derived nitrite/nitrate. Independently, Palmer *et al.* (1987) and Ignarro *et al.* (1987) provided evidence that EDRF is nitric oxide (NO). One year later, Palmer *et al.* (1988) reported that NO is synthesized from L-arginine, and Marletta *et al.* (1988) reported that macrophages generate nitrite and nitrate from L-arginine. Garthwaite *et al.* (1988) also provided evidence for the presence and synthesis of NO in brain. During the period from 1988 to 1992, research on the biology and functions of NO escalated, and then in 1992, 'NO was named 'Molecule of the Year' by D.E.Koshland, Jr., Editor for *Science*.

NO synthesis

NO is synthesized from L-arginine by the action of NO synthase (NOS), an enzyme existing in three isoforms. Brain NOS (bNOS) or neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3), also referred to as constitutive NOS (cNOS), are responsible for the continuous basal release of NO and both require calcium/calmodulin for activation (Griffith and Stuehr, 1995; Snyder, 1995).

[3]To whom correspondence should be addressed. telephone: 01-41-255-5367; fax: 01-41-255-4439; e-mail: mari@fhk.smtp.usz.ch

A third isoform is an inducible calcium-independent form (iNOS or NOS2) that is expressed only in response to inflammatory cytokines and lipopolysaccharides (LPS; reviewed in Nussler and Billiar, 1993; Morris and Billiar, 1994). The three isoforms of NOS are products of separate genes that share 50–60% amino acid homology (Nathan and Xie, 1994). However, all three isoforms are presumed to function as a homodimer during activation, sharing a carboxy-terminal reductase domain homologous to the cytochrome *P*-450 reductase and an amino-terminal oxygenase domain containing a haem prosthetic group, which are linked roughly in the middle of the protein by a calmodulin-binding domain (Dinerman *et al.*, 1993; Abu-Soud *et al.*, 1994, 1995a,b; Forstermann *et al.*, 1994; Sessa, 1994; Su *et al.*, 1995). The reductase domain of each NOS isoform contains tetrahydrobiopterin (THB) which is required for the efficient generation of NO and is necessary to maintain a stable conformation for electron transport, possibly by promoting homodimerization (Cho *et al.*, 1995; Klatt *et al.*, 1995; Tzeng *et al.*, 1995; Ghosh *et al.*, 1996). Moreover, the haem prosthetic group appears to be necessary for THB binding and enzyme dimerization (Klatt *et al.*, 1996).

Increases in intracellular calcium triggers a cascade of events leading to cNOS activation and NO synthesis. Intracellular calcium binds to calmodulin to form a calcium–calmodulin complex and regulates the binding of calmodulin to the 'latch domain', which permits electron transfer from NADPH via flavin groups within the reductase domain to a haem-containing active site, thereby facilitating the conversion of O_2 and L-arginine to NO and L-citrulline (Abu-Soud *et al.*, 1994, 1995a,b; Su *et al.*, 1995; Siddhanta *et al.*, 1996.). In contrast to cNOS, iNOS contains calmodulin tightly bound to each subunit of the enzyme, and therefore activation of iNOS does not require an increase in intracellular calcium and results in the permanent activation of the enzyme (Cho *et al.*, 1992). Indeed, calcium chelators have been shown to reduce cNOS activity significantly; however, with regard to iNOS activity, the role of calcium remains unclear, as Gellar *et al.* (1993) showed that calcium chelators significantly reduced iNOS activity, whereas Charles *et al.* (1993) reported that iNOS activity was not influenced by calcium chelators.

Apart from calcium, several other factors can regulate NOS activity. Pre- as well as post-translational and transcriptional mechanisms have been shown to regulate the expression of all forms of NOS and are beyond the scope of this review (Morris and Billiar, 1994; Kelly *et al.*, 1996). Although iNOS is cytosolic, cNOS possesses a site for myristylation, enabling it to link to the cell membrane (Busconi and Michel, 1993). Phosphorylation of cNOS by protein kinase C or A is associated with the detachment of the enzyme from the cell membrane and loss of activity (Bredt *et al.*, 1992; Michel *et al.*, 1993). Potential phosphorylation sites also exist in iNOS (Geller *et al.*, 1993); however, no functional significance has been demonstrated.

L-Arginine is the only physiological nitrogen donor for NOS-catalysed reactions; hence availability of this essential substrate could determine rates of NO generation. Arginine synthesis as well as its transport into the cells can also influence NO synthesis. Arginine is synthesized from citrulline by the actions of arginosuccinate synthetase and arginosuccinate lyase (Morris, 1992) and catabolized by arginase (Albina *et al.*, 1988). It has been shown that endogenous arginine synthesis is coupled to NO synthesis and that induction of arginosuccinate synthetase is not secondary to increased NOS activity (Morris *et al.*, 1994; Nussler *et al.*, 1994). Moreover, low concentrations of arginine caused by the release of arginase in wounds are responsible for the reduced NO synthesis (Albina *et al.*, 1988). Apart from synthesis, transport of arginine into the cells can also regulate NOS activity. In this regard, it has been shown that hepatic L-arginine transport, which is normally low, is enhanced during sepsis and after treatment with LPS (Sax *et al.*, 1988; Inoue *et al.*, 1993). Competitive inhibition of arginine uptake by L-lysine, L-ornithine (naturally occurring amino acids) and nitro-L-arginine methyl ester (L-NAME) or N^g-monomethyl-L-arginine (L-NMMA, arginine analogues) results in decreased NO synthesis (Bogle *et al.*, 1992; Inoue *et al.*, 1993). NOS activity may not always be co-regulated by L-arginine transport, as dexamethasone inhibits induction of macrophage iNOS but not arginine transport (Baydoun *et al.*, 1993). Overall, arginine synthesis and transport represents a potential target for experimental and therapeutic manipulation of cellular NO synthesis.

Because NOS activity is dependent on substrate availability and the co-factors NADPH, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), THB, the availability of these factors determines the cellular rates of NO synthesis. Maximal activation of NOS requires NADPH, FAD, FMN, THB and reduced thiol (Morris and Billiar, 1994; Davies *et al.*, 1995; Kelly *et al.*, 1996). This implies that the activities of metabolic pathways that generate or compete for these cofactors under physiological and pathological conditions could play an important role in determining rates of cellular NO production. NADPH synthesis has been shown to be co-induced with iNOS. Moreover, the activity of the NADPH-generating pentose phosphate pathway, and of the rate-limiting enzyme glucose 6-phosphate

dehydrogenase, in particular, has been shown to correlate with NO production (Corraliza *et al.*, 1993; Morris and Billiar, 1994). Additionally, glucose, which stimulates NADPH synthesis via the pentose phosphate pathway, stimulates the conversion of L-arginine to L-citrulline, possibly via NOS (Blachier *et al.*, 1991). Suppression of endogenous glutathione in endothelial cells has been shown to result in a decrease in NO synthesis, and these effects are likely due to a non-specific reduction in cellular redox capacity (Murphy *et al.*, 1991). THB, a reduced cofactor which is synthesized from guanosine triphosphate (GTP) by the action of GTP-cyclohydrolase I (GTP-cyclohydrolase; the first and rate-limiting enzyme in de-novo synthesis of THB), plays a crucial role in maintaining NOS activity (Gross and Levi, 1992; Kaufman, 1993). Stoichiometric evidence suggests that each molecule of THB allows for the generation of 18 molecules of NO without any recycling of THB (Giovanelli *et al.*, 1991), suggesting that THB has a catalytic or structural role for NOS. Indeed, THB is essential in forming the active dimeric enzyme for iNOS monomers and maintaining iNOS in an active configuration (Baek *et al.*, 1993). In pulmonary artery smooth muscle cells stimulated with cytokines or LPS, increases in guanosine triphosphate cyclohydrolase (GTP-CH) concentrations are co-induced with iNOS (Nakayama *et al.*, 1994). Similar co-induction was first observed in fibroblasts in response to interferon (IFN)-γ (Werner-Felmayer *et al.*, 1990). Increases in GTP-CH alone, and not NOS expression, has been shown to account for tumour necrosis factor (TNF), IFN-γ or LPS-induced increases in eNOS activity (Werner-Felmayer *et al.*, 1993).

NOS activity can also be inhibited by blocking flavoproteins and inhibiting haem (Nathan, 1992). Moreover, NO itself can regulate its own activity. This is clearly evident from the findings that NO synthesis is not linear after 20 min and suggests the possibility of feed-back inhibition (Assreuy *et al.*, 1993). NO donors have been shown to inhibit cNOS activity in a partially reversible manner, whereas NO scavengers prolong the linear kinetics of the enzyme (Rogers and Ignarro, 1992; Buga *et al.*, 1993). Because NO reacts with haem and non-haem iron, it has been postulated that NO inactivates the haem centre in the NOS enzyme (Assreuy *et al.*, 1993). In this regard, both cNOS and iNOS have been shown to form ferrous–nitrosyl complexes in their haem prosthetic groups (Abu-Soud *et al.*, 1995a,b; Hurshman and Marletta, 1995). Increased production of NO by cytokines has been shown to reduce haem availability and insertion into newly synthesized iNOS monomer (Albakri and Stuehr, 1996). Regulation of NO synthesis by product inhibition may have important implications under physiological and pathological circumstances, as it may increase NO synthesis in the presence of substances that decrease NO and decrease NO synthesis in situations when NO accumulates.

Because of the close homology and sharing of enzymatic properties with cytochrome *P*-450, studies have linked the oxidative component of NOS to cytochrome *P*-450 (Bredt *et al.*, 1991). In this regard, NOS has been shown to generate hydrogen peroxide, superoxide as well as NO (Heinzel *et al.*, 1992; Pou *et al.*, 1992). In L-arginine-depleted cells, superoxide generation is increased, whereas the presence of L-arginine reduces superoxide generation (Xia *et al.*, 1996). Superoxide ($\cdot O_2^-$) generated at low concentrations of L-arginine reacts with NO, resulting in increased generation of peroxynitrite ($ONOO^-$) and cell toxicity. Moreover, the generation of $\cdot O_2^-$ and $ONOO^-$ as well as cell toxicity was blocked by the specific NOS blocker *N*-nitro-L-arginine methyl ester (L-NAME; Xia *et al.*, 1996).

Different methodology has been used to identify NO in physiological media or in different biological models. The various assays available for measurement of NO were recently reviewed by Archer (1993).

NO transport and mechanism of biological action

Initially it was believed that NO reaches its intracellular targets by freely diffusing through the cell membranes. However, because of its ultra-short half-life (<30 s) and high reactivity with $\cdot O_2^-$, haem and non-haem-iron, the concept of free diffusion of NO was hard to accept. Because NO reacts with thiol (-SH) groups of proteins, it can form stable, biologically active, *S*-nitrosyl compounds (Stamler *et al.*, 1992). Indeed, NO has been shown to circulate as an *S*-nitroso adduct of albumin and possess biological activities identical to those of NO *in vitro* and *in vivo* (Keaney *et al.*, 1993). Since iron–nitrosyl compounds are associated with thiol-containing ligands, formation of dinitrosyl iron cysteine has been suggested as a second pathway for NO transport (Mulsch *et al.*, 1993).

NO is now known to mediate multiple biological effects, although initially the effects of NO were thought to be solely mediated via activation of soluble guanylate cyclase and guanosine 3',5'-cyclic monophosphate (cGMP). Generation of cGMP is known to mediate relaxation, inhibit vascular smooth muscle cell growth and prevent platelet aggregation and adherence of neutrophils to endothelial cells (Murad, 1994). However, recent studies suggest that NO can also induce its biological effects via non-cGMP-dependent pathways. Since NO is paramagnetic, it is capable of forming high-affinity-nitroso complexes with a variety of metal complexes (Lancaster *et al.*, 1992). NO binds to the haem-containing proteins, for example oxyhaemoglobin, and to

iron–sulphur-containing proteins of the tricarboxylic acid cycle enzyme *cis*-aconitase. NO can also bind to the thiol (-SH) groups of glyceraldehyde-3-phosphate dehydrogenase (GADPH), and via this mechanism NO is thought to decrease glycolytic activity associated with myocardial stunning, reperfusion injury and neurotoxicity and to inhibit mitochondrial respiration (Moncada *et al.*, 1991; reviewed by Kelly *et al.*, 1996). Other relevant mechanisms may include *S*-nitrosylation of thiols, formation of nitrotyrosines, NO binding to iron–sulphur clusters and NO binding to haem-containing proteins of the respiratory chain (McDonald and Moss, 1993; Mohr *et al.*, 1996). NO has been shown to inhibit GADPH activity by covalent linkage to NAD or NADH. NO can also act as a free radical scavenger and inactivate $\cdot O_2^-$, thereby preventing cell toxicity (Cook and Tsao, 1993). However, under appropriate conditions, reaction of NO with $\cdot O_2^-$ can also result in the generation of peroxynitrite ($ONOO^-$), a potent oxidant (Beckman *et al.*, 1990; Beckman and Crow, 1993). Peroxynitrite decomposes to form the reactive hydroxyl radical HOONO. Moreover, peroxynitrite and its metabolite are capable of inducing cytotoxicity by inducing lipid peroxidation, nitrosation of several tyrosine molecules that regulate enzyme function and signal transduction and Na^+ channel inactivation. Together, 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 nucleophil targets (Davies *et al.*, 1995; reviewed by Kelly *et al.*, 1996).

NO as a regulator of physiology and biology of the male and female reproductive systems

NO is now known to be produced by various cells in different organs, including smooth muscle cells, mesangial cells, neurons, platelets, hepatocytes, macrophages, fibroblasts and epithelial cells. NO regulates smooth muscle cell tone, platelet aggregation and adhesion, cell growth, apoptosis, neurotransmission and injury as well as infection-induced immune reactions. Because these processes are also associated with the biology, physiology and pathophysiology of various reproductive processes, it is highly likely that NO plays an important role in reproduction. Indeed, in the past decade NO has been recognized as a molecule that importantly regulates the biology and physiology of the reproductive system (Figure 1).

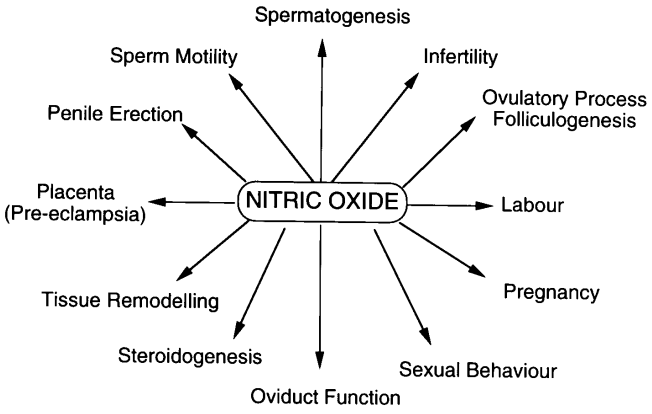

Figure 1. Various reproductive processes regulated by nitric oxide.

NO in the female reproductive system

Ovary

The role of NO in regulating ovarian function was evident from the observation that NO synthesis increases with follicular development. Moreover, the role of hormones in regulating NO synthesis during follicular development and ovulation was also obvious, as increases in NO correlated with increases in oestrogen (Rosselli *et al.*, 1994a; Figure 2). Similar changes in circulating NO concentrations with follicular development were observed in women undergoing in-vitro fertilization and treated sequentially with gonadotrophin-releasing hormone (GnRH) and human menopausal gonadotrophin and human chorionic gonadotrophin (HCG), thereby implicating other hormones [HCG, luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone] in the regulation of NO synthesis and folliculogenesis (Rosselli *et al.*, 1994a). Subsequently, Shukovski and Tsafriri (1994) demonstrated that administration of NOS inhibitors, intraperitoneally or via the ovarian bursae, inhibits ovulation in the rat, thus providing evidence that NO participates in the ovulatory processes. Although the above findings provided evidence that NO regulates ovarian function, it remained unclear whether these effects were due to NO generated in the vasculature and neurons within the ovary or were due directly to NO generated by various cells within the ovary. The role of non-vascular NO in mediating the ovulation process was evident from the observation that a reduction of blood flow to the same extent as after NO inhibition does not suppress the ovulation rate to the same extent as blocking NO synthesis in perfused rat ovary (Bonello *et al.*, 1996). An indirect role of NO in

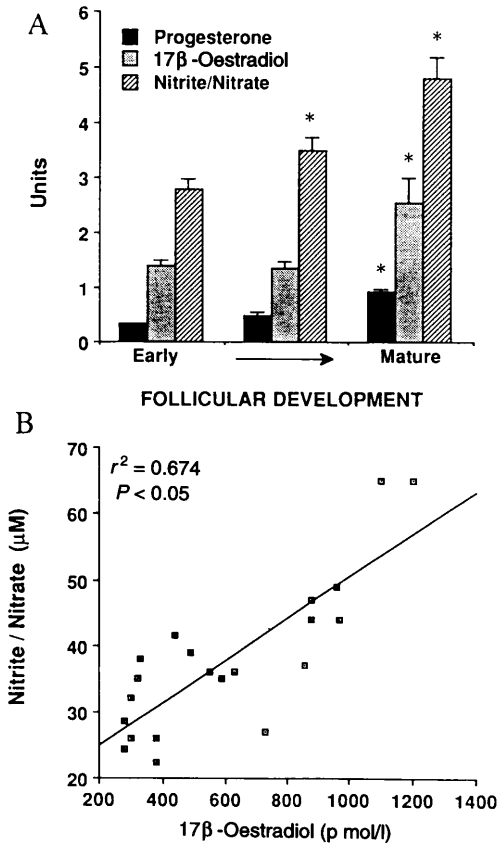

Figure 2. (**A**) Increases in serum nitrite/nitrate (stable metabolites of NO; ×100 μmol/l), 17β-oestradiol (nmol/l) and progesterone (nmol/l) concentrations with the development of follicles during spontaneous menstrual cycle in normal subjects. Data are means ± SEM; $n = 7$ subjects. (* = $P < 0.05$ versus samples collected at early follicular development). (**B**) Linear regression analysis showing the correlation between increases in serum nitrite/nitrate concentrations during follicular development phase of spontaneous menstrual cycle with 17β-oestradiol. Data represent all the samples taken from seven subjects, during follicular development. Reprinted with the permission of Rosselli *et al.* (1994a) and *Biochem. Biophys. Res. Commun.*

regulating ovarian function was also evident from the finding that interleukin (IL)-1β, which induces NO synthesis, is involved in the ovulatory process (Bonello *et al.*, 1996).

Recent findings have provided direct evidence for the role of NO generated locally by ovarian cells, as well as NO generated within the ovarian vasculature, in regulating ovulation: Van Voorhis *et al.* (1995) demonstrated that mature rat ovaries express iNOS and eNOS, but not nNOS. iNOS was localized in the granulosa cells of primary, secondary and small antral follicles in the ovary, whereas eNOS was present in the blood vessels of the ovary. Stimulation of the ovary with gonadotrophin increased expression of both eNOS and iNOS, suggesting that both isoforms participate in the ovulatory process; however, whether they play different roles could not be concluded from these studies.

Because of the lack of inhibitors to inhibit different isoforms of NOS specifically, it has been difficult to evaluate the roles of iNOS and eNOS in the ovulatory process. However, inhibition of iNOS using the specific inhibitor N^g-methyl-L-arginine and the putatively specific inhibitor aminoguanidine has been shown to inhibit ovulation in rats, in a dose-dependent manner (Shukorski and Tsafriri, 1994), suggesting that iNOS is involved in the ovulatory process. Since, inhibition of iNOS reduced ovulation by a maximum of 54%, it is feasible that eNOS also participates in this process. Immunohistochemical localization of eNOS and iNOS in various ovarian cells during follicular development demonstrated a distinct cell-specific expression pattern that was differentially regulated (Zackrisson *et al.*, 1996; Jablonkashariff and Olson, 1997). In control ovaries, eNOS was expressed in the theca cell layer, ovarian stroma and the surface of the oocytes. During follicular development, eNOS was expressed in theca cells and in mural granulosa cells. After ovulation, homogeneous eNOS staining was observed in cells of the corpus luteum. Quantitative estimates revealed that as compared to control ovaries, eNOS concentrations increased by 2.5-fold after pregnant mare's serum gonadotrophin induced follicle growth, and further by 5–7-fold in response to HCG administration. In the same study, the pattern and cell-specific expression of iNOS during follicular development was found to be different from that observed for eNOS. In immature ovaries and during follicular development, iNOS expression was found within the theca cell layer and stroma. After ovulation, iNOS was expressed in the external layers of corpus luteum, but in the degenerating corpus luteum, strong staining was observed in the non-parenchymal cells in the entire corpus luteum. Quantitative estimates of iNOS showed that, in contrast to eNOS, iNOS concentrations did not change during follicular development. However, a 6- and a 10-fold increase in iNOS was observed at the time of ovulatory HCG injection and 10 days after HCG injection respectively. The above findings provide evidence that intra-ovarian eNOS and iNOS regulate the ovulatory process simultaneously. Because theca cells, granulosa–luteal cells and cells of the corpus luteum are involved in steroidogenesis, it is feasible that NO also regulates steroid synthesis (see the section on steroidogenesis).

In most organs, iNOS is only expressed in response to an immune stimulus such as infection or trauma. The physiological relevance of the expression of iNOS in normal ovaries at all stages is unclear. It is feasible that this

expression is without any functional importance and largely due to the presence of macrophages and IL-1β in the ovary. Alternatively, since the follicles are continuously being generated and regulated by growth factors, it is feasible that iNOS-derived NO acts as a growth regulatory molecule. In this regard, it is interesting to note that NO has dual effects on cell growth. NO alone inhibits smooth muscle cell growth, however, in the presence of basic fibroblast growth factor (bFGF), NO is a potent mitogen and induces smooth muscle cell growth (Dubey *et al.*, 1997). Similarly, IL-1β-induced NO synthesis has been shown to enhance bFGF-induced growth of smooth muscle cells (Dubey *et al.*, 1997). Because IL-1β as well as bFGF are importantly involved in follicular growth (Adashi, 1989; Hurwitz *et al.*, 1992; Kaipia and Hsueh, 1997), it is feasible that NO facilitates this process. The growth-promoting effects of NO within the ovary are also evident from the findings that (i) NO donors increase, whereas NO synthesis inhibitors decrease, the receptors for epidermal growth factor (a potent growth factor) on granulosa cells (Hattori *et al.*, 1996); (ii) NO mediates gonadotrophin-stimulated regulation of the blood–follicle barrier (Powers *et al.*, 1995); (iii) NO mediates IL-1β-induced expression of glucose transporter 3 (Kol *et al.*, 1997). Because glucose increases during mid-cycle and glucose stimulates NO synthesis, it is feasible that NO and glucose facilitate follicle development in a complimentary fashion.

The importance of IL-1β-induced NOS and NO synthesis in regulating ovarian function was deduced from the observation that, in contrast to IL-1β, LPS and IFN-γ were unable to induce iNOS activity in cultured granulosa cells (Tabraue *et al.*, 1997). Moreover, IL-1β increased guanosine triphosphate cyclohydrolase (GTP-CH) activity, the rate-limiting step for de-novo biosynthesis of tetrahydrobiopterin (THB), which is an essential factor for iNOS activity. Similar to IL-1β, FSH induces GTP-CH activity and enhances the effects of IL-1β (Tabraue *et al.*, 1997). Although earlier studies with isolated ovarian dispersions suggested that IL-1β may play an important role in mediating toxic effects and cell death (Ellman *et al.*, 1993), subsequent studies provided evidence that IL-1β-induced cell toxicity was not NO mediated (Ben-Shlomo *et al.*, 1994). Moreover, NO seems also not to be an obligatory mediator of IL-1 effects on plasminogen activation and prostaglandin E generation in rat ovary (Huwitz *et al.*, 1997).

Folliculogenesis involves the participation of both growth and programmed cell death, and NO regulates both of these processes. It is feasible that the participation of NO in both these processes largely depends on interactions with other factors generated within the ovary. With regard to apoptosis, several lines of evidence suggest that NO prevents it. Sugino *et al.* (1996) studied the relationship between NO concentrations in follicular fluid and apoptosis. They demonstrated that, compared to large and medium-sized follicles, smaller follicles had a higher incidence of apoptosis, although NO (nitrite/nitrate), arginine and citrulline concentrations in these follicles did not differ. Similarly, DNA fragmentation studies revealed that IL-1β-induced anti-apoptotic effects in cultured follicles were NO-mediated (Chun *et al.*, 1995). Moreover, Anteby *et al.* (1996) noted that NO concentrations were increased in human follicular fluid, and these values were positively correlated with follicular volume and oestradiol concentrations. Together, these observation suggest that local generation of NO induces follicle development and not apoptosis. Although low concentrations of NO may prevent apoptosis, pathologically high concentrations of NO, as well as increased O_2^- generation by NOS due to lack of arginine, may promote cell death, via peroxynitrite generation.

In conclusion, the above findings provide convincing evidence that NO, both ovarian cell-derived and vascular endothelial cell-derived, plays an important role in the physiology and biology of the ovary with regard to regulation of folliculogenesis and ovulation. The clinical implications of these findings have not yet been addressed. Future studies are needed to investigate whether the ovarian dysfunction observed in certain pathological conditions is associated with decreased vascular and intra-ovarian NO synthesis and whether these defects can be corrected by NO. It may be possible to treat ovarian dysfunction related to decreased perfusion of the ovary in vaso-occlusive disorders either directly by administering NO donors or indirectly via administration of IL-1β.

Oviduct

In oviducts, enhancement of endothelin-induced contraction in the presence of L-NAME provided the first evidence for the presence as well as a physiological role of NO in regulating oviduct function (Rosselli *et al.*, 1994b). Ekerhovd *et al.* (1997) provided evidence that NO mediates contractile activity in isolated strips of human Fallopian tube. Subsequently, Bryant *et al.* (1995) as well as studies from our laboratory (Rosselli *et al.*, 1996) reported the presence of calcium-dependent as well as independent forms of NOS in rat, bovine and human oviducts (Figure 3). Immunostaining revealed the presence of eNOS in the epithelial cells of the oviduct; moreover, IL-1β stimulated NO synthesis in both human and bovine

oviducts. The distribution of calcium-dependent NOS activity was not different in the isthmus, fimbria and ampulla (Rosselli *et al.*, 1996). However, NOS activity in the oviduct was relatively low during pro-oestrus, as compared to all other stages of the oestrus cycle. Since the oviduct plays a critical functional role by being the site of fertilization and early embryonic development, it is feasible that NO may play an important role in promoting this process. Basal release of NO may promote sperm motility and protect the ovum as well as the spermatozoa against oxygen free radical-induced damage. Because NO has been shown to regulate contractility of the Fallopian tube (Rosselli *et al.*, 1994b), it may also regulate ciliary beating of the ciliated epithelial cells. NO has been shown to up-regulate the receptors for growth factors such as epidermal growth factor as well as those for anchoring proteins and integrins (Hattori *et al.*, 1996; Dubey *et al.*, 1997). It is feasible that, during early embryonic development, NO primes the embryo to develop these growth regulating and anchoring proteins on the cell surface and this eventually facilitates implantation within the uterus.

In contrast to the physiological situation, NO synthesis within the oviduct may increase under certain pathological conditions, such as infection or endometriosis, and this may evidently lead to decreased fertilization via deleterious/toxic effects on the spermatocytes as well as on the oocyte. Moreover, increased generation of NO may also influence ciliary beats and thus embryo transport, and may consequently result in abortion. With regard to the expression of NOS within the oviduct under pathological conditions, we compared the expression of eNOS and iNOS in oviducts obtained from normal cycling women, postpartum patients, patients with extrauterine pregnancies and postmenopausal women. eNOS was localized in the epithelium lining of all the oviducts from all the four groups. In contrast, iNOS expression was only observed in groups of cells present in the muscle layer of oviducts obtained from postpartum patients, whereas in oviducts obtained from patients with an extrauterine pregnancy (personal communication, Rosselli *et al.*, 1997) iNOS expression was observed all through the muscle and epithelial layers. Together, these findings suggest that NO plays an important role in the physiology as well as the pathophysiology/pathobiology of the oviduct.

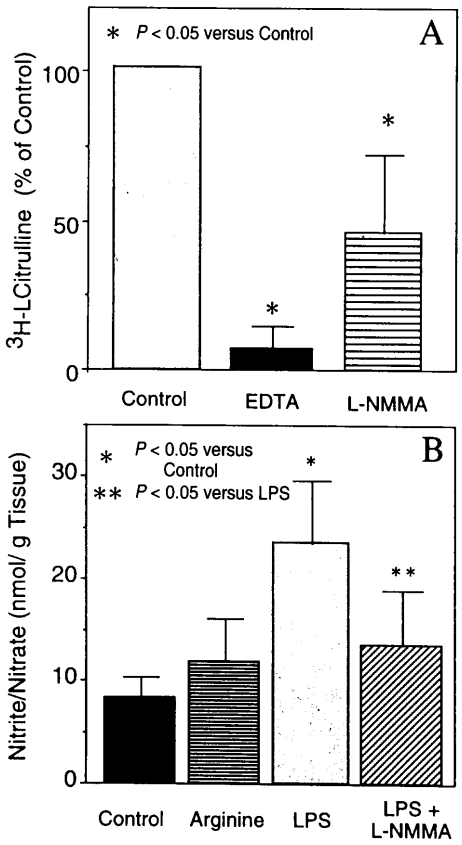

Figure 3. (**A**) Calcium-dependent nitric oxide synthase (NOS) activity measured in cytosolic extracts of ampullary segments of human oviduct. NOS activity was assayed by measuring the formation of [^{3}H]-L-citrulline from [^{3}H]-L-arginine in the presence or absence (control) of 2 mM EDTA or 1 mM N–monomethyl-L-arginine monoacetate (L-NMMA). Values are given as a percentage of control values (fmol [^{3}H]-l-citrulline formed per min per mg protein). (**B**) Inducible NOS activity in bovine oviducts. Production of NO was measured by analysing the concentrations of nitrite/nitrate in the incubation medium in which ampullary segments were incubated for 36 h in: Hanks' balanced salt solution (control), 7.3 mM L-arginine, L-arginine + 10 μg/ml lipopolysaccharide (LPS), L-arginine + LPS + 1 mM L-NMMA (LPS + L-NMMA). Each bar represents the mean ± SEM from three experiments. Reprinted with the permission of Rosselli *et al.* (1996) and *Mol. Hum. Reprod.*

Uterus

The uterus undergoes important structural alterations in pregnancy as well as during menstruation. Because NO regulates smooth muscle cell contractility, and spontaneous contraction as well as distension of the uterus during pregnancy, the role of NO in regulating the pathophysiology and biology of the uterus has gained intense attention. Uterine vasculature as well as nerve supply both express NOS activity and have been shown to modulate the contractile effects of multiple vasoactive factors (Azuma *et al.*, 1995; Dong and Yallamplalli, 1996; Jaing *et al.*, 1996; Magness *et al.*, 1996, 1997; Veille *et al.*, 1996). Apart from in the vessel wall and neurons, the presence of NOS has been demonstrated in glandular epithelium, endometrial stromal cells, myometrial smooth muscle cells and mast cells, suggesting that NO plays a local role in the control of uterine function (Telfer *et al.*,

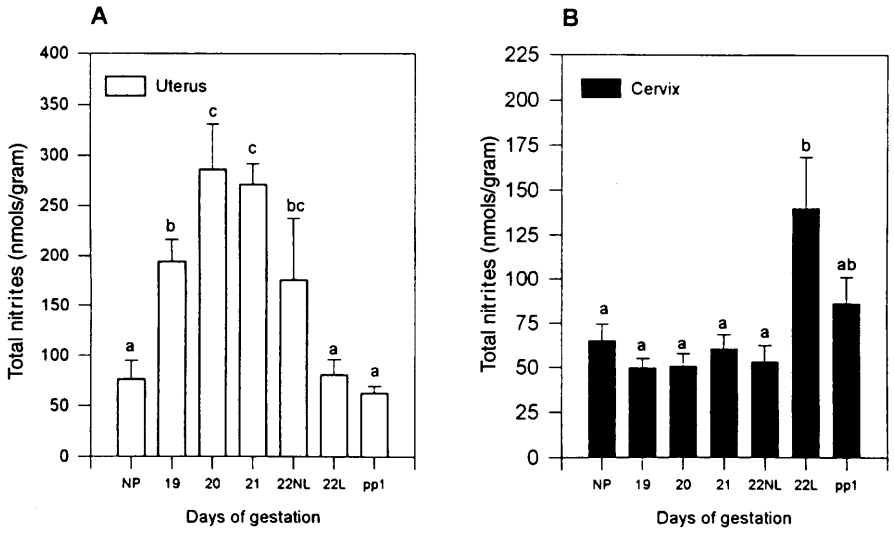

Figure 4. Nitrite production by the non-pregnant (NP) and pregnant rat uterus (**A**) and cervix (**B**) during gestation (days 19–21), day 22 morning of the delivery date (22NL), during term delivery (22L) and 1 day post-partum (pp1). Statistical analysis consisted of analysis of variance (ANOVA) followed by post-hoc tests using Fisher's least significance criteria. Means (±SEM) with at least one common superscript were not different at a value of $P < 0.05$, $n = 5$ animals in each group. Reprinted with the permission of Buhimschi *et al.* (1996) and *Hum. Reprod.*

1995). Although myometrial smooth muscle cells express eNOS (Gangula *et al.*, 1997), it is one of the rare tissues which expresses iNOS even under unstimulated conditions (Buhimschi *et al.*, 1996; Bansal *et al.*, 1997). The expression of eNOS and iNOS in non-pregnant and decidualized human endometrium was also comprehensively studied by Telfer *et al.* (1997). The physiological role of eNOS and iNOS during pregnancy and labour was studied in depth by Buhimschi *et al.* (1996), who demonstrated that both eNOS and iNOS are up-regulated during pregnancy but decreased when labour begins (Figure 4). Gestational differences in the response to NO are well established. It has been shown that, during pregnancy, the relaxing effects of NO are enhanced, compared with the uterus during term or preterm labour (Natuzzi *et al.*, 1993; Sladek *et al.*, 1993; Yallampalli *et al.*, 1994; Buhimschi *et al.*, 1995b, 1996). The above findings, together with the observations that (i) nitroglycerine relaxes the uterus to facilitate extraction of retained placenta (Peng *et al.*, 1989), (ii) nitroglycerine arrests preterm labour and prolongs gestation (Lees *et al.*, 1994), (iii) amyl nitrate decreases the magnitude of uterine contractions induced by oxytocin, but not spontaneous activity (Kumar *et al.*, 1965) and (iv) NO donors inhibit uterine contractions in pregnant rhesus monkeys, sheep and rats as well as humans (Jennings *et al.*, 1993; Yallampalli *et al.*, 1993; Heymann *et al.*, 1994; Buhimschi *et al.*, 1995b), provide compelling evidence that NO differentially controls uterine contractility during pregnancy and labour (Figure 4).

Data with regard to the regulation of NO synthesis in pregnancy and labour suggest that iNOS, but not eNOS, is the key player in regulating contractile function. It has been shown that NOS activity increases in rat uterus during pregnancy and falls at term (Natuzzi *et al.*, 1993). Expression of rat myometrial iNOS has been shown to be up-regulated in pregnancy and fall at term (Riemer *et al.*, 1997). Similar up-regulation of NOS activity in the uterus during pregnancy has also been demonstrated in rabbits (Sladek *et al.*, 1993). Moreover, in a recent study, Bansal *et al.* (1997) demonstrated that in humans expression of myometrial iNOS activity was highest in pre-term not-in-labour patients, fell by 75% at term and was barely detectable in pre-term in-labour or term in-labour patients. They also demonstrated that, compared to pregnant subjects, there was no positive staining for iNOS in myometrial myocytes in non-pregnant subjects (Figure 5). Together, these findings provide convincing evidence that NO plays a key role during pregnancy and labour.

Which factors regulate iNOS activity in pregnancy are still unclear. In this regard, the role of cytokines is of most importance as they simultaneously increase during pregnancy (Das *et al.*, 1992; Bry and Hallman, 1993). Cytokines such as IL-1β and TNF-α are known to increase iNOS expression and induce NO synthesis, whereas cytokines such as IL-4, IL-10 and transforming growth factor (TGF)-β are known to inhibit iNOS activity (Davies *et al.*, 1995). It is feasible that the increases in NOS activity during pregnancy are due to positively regulating cytokines and the subsequent decrease during labour is

Figure 5. Percentage of myocytes containing inducible calcium-independent form of nitric oxide synthase (iNOS) within each group of myometrial biopsies during pregnancy (mean ± SEM). The number of patients within each group was as follows: preterm no labour, $n = 5$; preterm labour, $n = 3$; term labour, $n = 9$; term labour, $n = 5$. * = significantly greater than each of the other three groups ($P < 0.0001$). Reprinted with the permission of Bansal *et al.* (1997) and *J. Clin. Invest.*

largely due to inhibitory cytokines. An interrelationship between the cyclo-oxygenase pathway, nitric oxide and cytokines has also been demonstrated within the rat uterus and may importantly regulate uterine function in pregnancy (Dong and Yallampalli, 1996). Ovarian hormones have also been shown to induce iNOS in the uterus and may regulate uterine function (Yallampalli *et al.*, 1994; Huang *et al.*, 1995). Although the role of iNOS is evident, the role of eNOS in the endometrial epithelial and stromal cells is unclear. It is feasible that continuous generation of NO into the lumen facilitates processes such as menstruation and implantation via prostaglandin synthesis and modulation of anchoring proteins respectively. eNOS-derived NO may act as an alternative inhibitor of endometrial platelet aggregation via activation of soluble guanylyl cyclase generation or via cyclo-oxygenase catalysis. Moreover, local synthesis of NO within the uterus may be important for regulating myometrial activity, i.e. spontaneous contraction of the uterus. The role of NO in pregnancy has recently been reviewed by Sladek *et al.* (1997).

Cervix and vagina

Buhimschi *et al.* (1996) demonstrated that rat cervix expresses all three isoforms of NOS, i.e. iNOS, nNOS and eNOS. Furthermore, they demonstrated that expression of iNOS is increased in the cervix, and decreased in the uterus, during parturition and preterm labour (Figure 4). Moreover, nNOS, which was not expressed in the uterus during gestation, increased in the cervix during labour. In contrast to nNOS and iNOS, no significant changes were observed in eNOS expression during labour at term. These findings suggest that NOS activity in the uterus and cervix is differentially regulated during labour and may be involved in connective tissue remodelling during cervical ripening. The physiological and biological relevance of NO in pregnancy and labour comes from their finding that the inhibition of NO synthesis by administration of L-NAME prolonged the duration of delivery, the interval between each pup's expulsion (Figure 6), as well as decreasing cervical extensibility. These findings not only suggest the importance of NO synthesis in the uterus and cervix during labour and pregnancy, but also point to the role of various isoforms of NOS in regulating these effects. Which factors regulate the induction of iNOS may have important therapeutic implications, as administration of agents which selectively induce iNOS activity may induce cervical ripening and labour in patients with difficult labour. Indeed, cytokines as well as matrix factors are known to induce iNOS activity (Dubey *et al.*, 1997), whereas NO modulates connective tissue-degrading enzymes, i.e. matrix metalloproteases (Evans *et al.*, 1995; Kolpakov *et al.*, 1995). Since these factors are increased during pregnancy and cervical ripening, they may be modulating iNOS activity, which in turn helps in labour. Changes in hormones and their receptors are known to occur during pregnancy and labour, and they could regulate cervical NOS activity. In a recent study, Ali *et al.* (1997) provide evidence that changes in uterine and cervical NOS activity are progesterone dependent. Further studies are needed to study the effects of various matrix factors, hormones and cytokines on cervical and uterine NOS activity.

NOS activity has also been localized in the vagina. eNOS activity is localized in the stratified squamous epithelial lining and in smooth muscle cells (Chatterjee *et al.*, 1996). The expression of eNOS was maximal during oestrus and pro-oestrus, suggesting that NO may stimulate vaginal secretion. NOS immunoreactive axons and neurons have also been shown to be present in the vagina, moreover the most abundant NO-releasing innervation has been shown to be present in the cervix and vagina (Grozdanovic *et al.*, 1994).

Placenta and pre-eclampsia

The relationship between the placenta and NO has recently been reviewed by Buhimschi *et al.* (1997) and is discussed only briefly here. Although uterine cell-derived NO is important in pregnancy and labour, vascular NO seems to be important in pathological conditions such as pre-eclampsia. Moreover, the placental vasculature seems to be

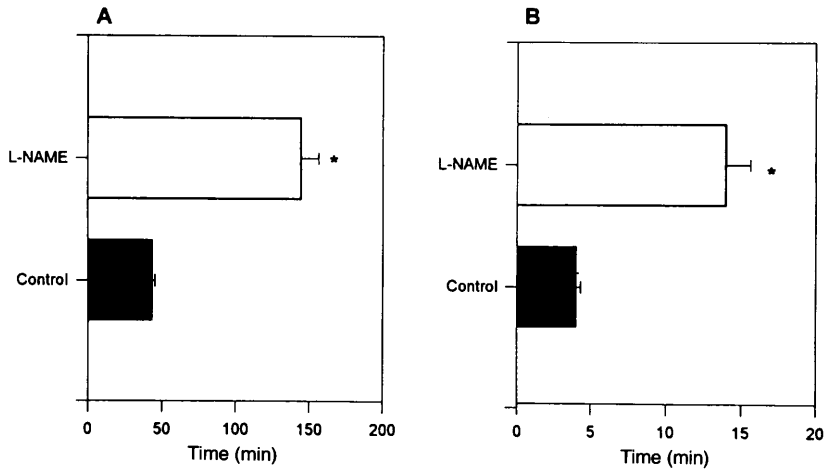

Figure 6. (**A**) Duration of delivery (in minutes) in rats infused with N-nitro-L-arginine methyl ester (L-NAME; 50 mg/day) versus control animals. Means (±SEM) marked with an asterisk were significantly different from control at a value of $P < 0.05$ (unpaired *t* test). (**B**) The interval between each pup's expulsion (pup interval) in rats infused with L-NAME (50 mg/day) and controls. Means (±SEM) marked with an asterisk were significantly different from control at a value of $P < 0.01$ (unpaired *t* test); $n = 18$ in each group. Reprinted with the permission of Buhimschi *et al.* (1996) and *Hum. Reprod.*

more important in the pathophysiology of pre-eclampsia. Both iNOS and eNOS are expressed within the placenta (Myatt *et al.*, 1993). Indirect evidence for the role of NO in pre-eclampsia comes from the studies of Buhimschi *et al.* (1995a), who demonstrated that administration of L-NAME (competes with L-arginine and inhibits NO synthesis) to pregnant rats resulted in a pre-eclampsia-like condition. Moreover, administration of L-arginine to rats infused with L-NAME reversed both the increase in blood pressure and the decrease in fetal weights observed with L-NAME alone. Although these findings suggest that inhibition of NO causes pre-eclampsia-like conditions, they do not prove that NO deficiency causes pre-eclampsia. Indeed, a recent study by Yallampalli *et al.* (1996) demonstrated that calcitonin gene-related peptide reduces fetal mortality and reverses hypertension in a L-NAME-induced pre-eclampsia-like condition. Taken together, these findings suggest that vasoconstriction caused by decreased synthesis of NO within the vasculature can cause pre-eclampsia and that agents that induce vasodilatation via NO generation, or independent of NO generation, may be therapeutically important in preventing pre-eclampsia-associated disorders.

Although the above findings suggest a role of NO in pre-eclampsia, direct evidence for this association is still lacking. Myatt *et al.* (1997) recently demonstrated that expression of eNOS, and hence NO synthesis, is increased in the fetal–placental vasculature obtained from patients suffering from pre-eclampsia with or without intrauterine growth restriction. This implies that the increase in eNOS expression in the fetal–placental vasculature of pre-eclamptic subjects may be an adaptive response to the increased resistance, poor perfusion and hypoxia. Indeed, shear stress and hypoxia are known to induce eNOS activity (Kelly *et al.*, 1996). Moreover, an increase in NO synthesis as a compensatory mechanism to reduce blood pressure has shown to occur in rats with '1 Kidney 1 Clip'-induced hypertension, as well as in spontaneously hypertensive rats (Arnal *et al.*, 1993; Dubey *et al.*, 1996). In a recent study, Myatt *et al.* (1996) demonstrated the presence of intense NOS staining and the presence of nitrotyrosine residues in the endothelial lining of placental villus of pre-eclamptic placenta. Moreover, they provided evidence for peroxynitrite formation and action in mediating endothelial damage (Myatt *et al.*, 1996)

In the light of the findings that L-NAME induces pre-eclampsia-like conditions in rats (Buhimschi *et al.*, 1996) whereas increased eNOS activity was observed in the placental–fetal microcirculation in humans, how do we explain the role of NO synthesis in pre-eclampsia? Moreover, most emphasis has been focused on the vasodilatory effects of NO and very little is known about the effects of NO on the growth of smooth muscle cells in the placental–fetal microcirculation. Indeed, abnormal growth of smooth muscle cells contributes to the remodelling process associated with the vaso-occlusive disorders observed in the placental–fetal microvessels. Since NO has been shown to regulate smooth muscle cell growth differentially, i.e. inhibit as well as induce growth, the regulatory effects of NO on placental–fetal microvascular smooth muscle cells may be important in pre-eclampsia and need to be investigated. Alternatively, it is feasible that these

differences in NO synthesis may be due to species differences.

Decreased NO synthesis within the vasculature may be a consequence of either damaged/dysfunctional endothelium, decreased substrate (L-arginine) availability, or an increased presence of asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of NO synthesis known to increase in pre-eclampsia (Fickling *et al.*, 1993). It is plausible that L-arginine substitution may increase NO synthesis by improving endothelial function as well as by competing with endogenous NO inhibitors such as ADMA. Hence, future studies should aim to evaluate whether administration of L-arginine protects against the disorders associated with pre-eclampsia. Moreover, it needs to be demonstrated whether lack of NO synthesis alone or other vasodilators, such as adenosine, prostaglandins etc., also cause pre-eclampsia-like conditions. From the therapeutic point of view, agents which induce vasodilation or counteract vasoconstriction and prevent endothelial dysfunction may prove to be useful in treating patients with pre-eclampsia. In this regard, endothelin antagonists, adenosine agonists and vasodilatory hormones (oestrogen, progesterone) may also be beneficial and they need to be investigated. Finally, since most vasoconstrictors induce and vasodilators inhibit the growth of vascular smooth muscle cells, the role of NO in regulating mitogen/vasoconstrictor-induced growth of fetal–placental microvascular smooth muscle cells needs to be evaluated.

NO in the male reproductive system

Regulation of penile erection

In 1990, Ignarro *et al.* (1990) first demonstrated that electrical field stimulation of isolated strips of rabbit corpus cavernosum promotes the endogenous formation and release of NO. Based on this observation, they postulated that penile erection is mediated by NO generated in response to non-adrenergic–non-cholinergic neurotransmission. Subsequently, Holmquist *et al.* (1991) provided in-vivo evidence that the arginine/NO pathway induces penile erection in rabbits. Following this finding, the endogenous regulation of NO and the mechanisms by which it regulates penile erection have been a subject of intense research. NOS activity was localized in the rat penile neurons innervating the corpora cavernosa and to neuronal plexuses in the adventitial layer of penile arteries (Burnett *et al.*, 1992), suggesting that NO is a physiological mediator of erectile function. Apart from the nerves, eNOS is also abundantly present in the endothelium of penile vasculature and sinusoidal endothelium within the corpora cavernosa (Burnett *et al.*, 1996). Moreover, pineal cells have also been shown to express NOS activity (Maronde *et al.*, 1995).

Neuronal NOS seems to play a decisive role in mediating penile erection (Burnett *et al.*, 1996). In humans, NOS activity has been identified in discrete neuronal locations, including the pelvic plexus, cavernous nerves and their terminal endings within the corporeal erectile tissue, branches of the dorsal penile nerves and nerve plexuses in the adventitia of the deep cavernous arteries (Burnett *et al.*, 1993). NOS-containing innervation of the sympathetic preganglionic neurons from the spinal cord to the penis has also been shown recently (Vanhatalo and Soinila, 1995). The above findings suggest that NO affects penile erection at several neuronal levels (Vanhatalo *et al.*, 1996). Additionally, direct injection of L-NAME into the paraventricular nucleus, but not into the caudate nucleus, medial septum, pre-optic area and the CA1 field of the hippocampus, inhibited apomorphine- and oxytocin-induced penile erection. These findings suggest that actions of NO in the brain, and distal from the peripheral nerves, can also influence penile erection (Melis *et al.*, 1994a). Moreover, in mice lacking nNOS, eNOS has been shown to mediate NO-dependent penile erection (Burnett *et al.*, 1996). Together, these findings suggest that both nNOS and eNOS are capable of regulating penile erection. Because NO acts as a mediator of penile erection, factors regulating nNOS or eNOS may differentially induce penile erection.

Neuronal NOS from the rat penile corpora cavernosa has been cloned by Magee *et al.* (1996). The penile cNOS differs from the cerebral nNOS by the presence of a 102 nucleotides stretch. The mRNA is expressed in the rat penis, urethra, prostate and skeletal muscle, coexists with the cerebellar nNOS in the pelvic plexus and bladder, and is detectable in the cerebellum. The features of penile nNOS suggest that it may be regulated differentially from the cerebellar nNOS. In addition to eNOS and nNOS, iNOS is expressed in the cavernosal smooth muscle cells of the penis (Garban *et al.*, 1997). Both the rat and the human penile iNOS have recently been cloned and have several amino acid differences from their analogous isoform in nonpenile tissues (Garban *et al.*, 1997). Because erectile dysfunction is associated with reduction in nNOS activity as well as reduction in penile smooth muscle cell compliance (Regazzi *et al.*, 1996; Carrier *et al.*, 1997), the cloning of these NOS isoforms may open the way for their possible use in the management of erectile dysfunction. Indeed, induction of iNOS locally by injecting a mixture of iNOS inducers corrected the erectile dysfunction observed in old rats. Moreover, administration of rat penile iNOS

cDNA mitigated the ageing-associated erectile dysfunction (Garban *et al.*, 1997).

Several lines of evidence suggest that the NO–cGMP pathway is largely responsible for mediating the erectile response to administration of *N*-methyl-D-aspartic acid (Melis *et al.*, 1994b), acetylcholine (Champion *et al.*, 1997), oxytocin (Melis *et al.*, 1994a), apomorphine (Melis *et al.* 1994a), cocaine (Chan *et al.*, 1996), androgens (Penson *et al.*, 1996), testosterone (Zvara *et al.*, 1995), dihydrotestosterone (Lugg *et al.*, 1995), electrical stimulation (Holmquist *et al.*, 1991) and dopamine (Melis *et al.*, 1996). In contrast, vasoactive intestinal peptide- and adrenomedullin-induced penile erections were not reversed by L-NAME, a NO synthesis inhibitor, suggesting that NO-independent pathways also participate in penile erection (Hayashida *et al.*, 1996; Champion *et al.*, 1997). Together, these findings reaffirm the importance and the possible use of NO donors as a therapeutic agent in correcting penile erection. Because the effects of NO are largely local within the penis, and NO is a labile substance with an ultrashort half-life, application of the pharmacologically active substances directly to the end organ may enable the achievement of time-dependent high local drug concentrations without severe systemic side effects. Indeed, linsidomine (SIN-1), a NO donor, has been successfully used to correct erection disorders in men (Stief *et al.*, 1992).

Regulation of penile erection by androgens and the pituitary and its relationship with NOS activity is of special physiological interest. Deficiency of androgens, such as testosterone, has long been associated with impotence and dysfunction of penile erection (Garban *et al.*, 1995; Penson *et al.*, 1996). Indeed, castration results in a significant decrease in eNOS and cNOS activity in the penis and reduces electrical stimulation-induced penile erection by 70% (Lugg *et al.*, 1996; Penson *et al.*, 1996). Administration of the anti-androgen flutamide to intact rats similarly reduced eNOS and nNOS activity and penile erection. In castrated rats treated with flutamide, the erectile response was abolished completely; however, NOS activity was not decreased below the values observed in castrated rats. Oestradiol administration to castrated rats, hypophysectomy or GnRH antagonist treatment in intact rats diminished the erectile response, below the level in castrated animals. Moreover, in hypophysectomized rats, penile NOS activity fell below levels in castrated animals. These data suggest that penile erection in the rat is completely dependent on androgens, presumably because of their role in the maintenance of penile NOS activity and of other ancillary factors (Penson *et al.*, 1996).

Erectile dysfunction occurs in diseases such as diabetes I and II and is accompanied by hypogonadism, as well as a significant decrease in nNOS content and activity in the penis (Vernet *et al.*, 1995; Rehman *et al.*, 1997). It is feasible that administration of androgens or NO donors to diabetic rats may be therapeutically important to improve NOS content/activity and improve penile erection. Oxygen tension is known to regulate NO synthesis, and a low pO_2 inhibits NOS activity in the corpus cavernosum cytosol (Kim *et al.*, 1993). In the same manner, smoking leads to considerable decreases in penile NOS activity and nNOS content that are not reflected in a reduction of the erectile response to electrical stimulation (Xia *et al.*, 1997). Lack of effect on penile erection could largely be due to compensatory synthesis of NO by other NOS isoforms. Indeed, eNOS activity in rats exposed to smoke was not influenced (Xia *et al.*, 1997), and eNOS has been shown to induce penile erection in nNOS-deficient mice (Burnett *et al.*, 1996). Erectile function is also decreased in old and senescent rats. As compared to young rats, soluble penile NOS activity as well as NOS-containing fibres decreased in old rats (Garban *et al.*, 1995; Carrier *et al.*, 1997). Moreover, although the erectile mechanism appears to remain intact as rats age, the response to central and peripheral stimulation decreases, and the reduction in NOS-containing fibres might account for these observations. In conclusion, NO plays a decisive role in regulating penile erection, and local application of NO donors as well as gene therapy may prove to be of clinical and therapeutic benefit in the treatment of penile erection disorders.

Testis

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). This finding was subsequently confirmed by immunohistochemical localization (Burnett *et al.*, 1995). 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 (Milstien *et al.*, 1996); (ii) arginosuccinate synthetase as well as argininosuccinate lyase, the enzymes responsible for L-arginine generation, and they are abundant in the testis (Yu *et al.*, 1995) and (iii) haem oxygenase isozymes HO-1 and HO-2, which oxidatively cleave the haem molecule to produce NOS (Ewing and Maines, 1995). In addition, in rats treated with lipopolysaccharide, NOS-synthase is co-induced with

argininosuccinate synthetase and argininosuccinate lyase (Nagasaki *et al.*, 1996). To evaluate the physiological relevance for the presence of NOS activity, the distribution of NOS activity was subsequently characterized in various testicular cells. In this regard, cNOS activity was identified in the Leydig cells, Sertoli cells and endothelial cells of the human testis (Davidhoff *et al.*, 1995; Zini *et al.*, 1996). 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 (Davidhoff *et al.*, 1997). Testosterone, calmodulin, aspartate, glutamate and Ca^{2+}/calmodulin- dependent protein kinase II have been shown to co-localize with NOS-I in Leydig cells (Davidoff *et al.*, 1995). Moreover, cytokine-induced NO synthesis and iNOS expression have also been demonstrated in cultured Sertoli cells (Stephan *et al.*, 1995) as well as Leydig cells (Tatsumi *et al.*, 1997). These findings suggest that the testicular cells are well equipped with a NO–cGMP pathway, which may importantly participate in the regulation of testicular functions, 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. eNOS activity was absent in normal germ cells but present in degenerating or apoptotic intra-epithelial germ cells. 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 (Adams *et al.*, 1994; Davidhoff *et al.*, 1995). More recently, Wang *et al.* (1997) have identified a testis-specific variant of nNOS (TnNOS) in the testis. Because autocrine–paracrine factors are known to regulate steroidogenesis in the testis, the role of NO in regulating this process has been a subject of intense research and the findings are discussed in the steroidogenesis section of this review.

Sperm motility and fertilization

NO also regulates sperm motility, with low concentrations of NO enhancing (Hellstrom *et al.*, 1994) and medium/high concentrations of NO decreasing sperm motility (Rosselli *et al.*, 1995; Figure 7A). It is tempting to speculate that, under physiological conditions, small amounts of NO are generated and neutralize free radicals which inhibit sperm

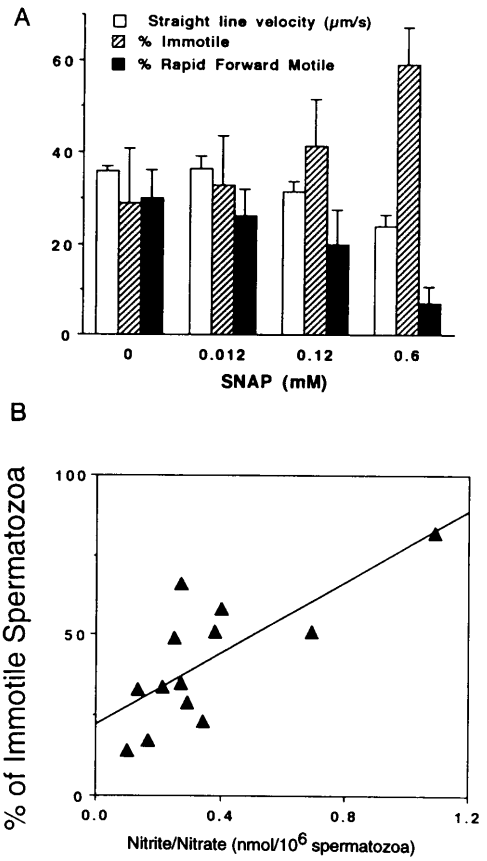

Figure 7. (**A**) Effect of *S*-nitroso-*N*-acetylpenicillamine (SNAP) on human sperm motility. (**B**) The correlation between nitrite/nitrate concentrations (nmol/10^6 spermatozoa) in seminal plasma and the percentage of immotile spermatozoa ($r = 0.74$, $P < 0.01$, Spearman rank coefficient = 0.677, $P < 0.05$). Reprinted with the permission of Rosselli *et al.* (1995) and *Hum. Reprod.*

motility. Thereby low concentrations of NO may protect against O^{2-}-mediated reduction of sperm motility. In contrast, excessive generation of NO 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. Indeed, in semen collected from different subjects a positive correlation was observed between the concentrations of NO and the percentage of immotile spermatozoa (Rosselli *et al.*, 1995; Figure 7B). Hence, the concentration of NO determines the effects on sperm motility and viability, and the concentration of NO in turn depends on the amount of NO generated, i.e. low (physiological conditions) and high (pathological conditions).

Although the above findings suggest a paracrine role of NO in regulating sperm motility, a recent study by Lewis *et al.* (1996) provides evidence for the presence of eNOS and bNOS in the human spermatozoon that regulates (increases)

sperm motility in an autocrine fashion. Moreover, they demonstrated that, as compared to normozoospermic samples, the expression of eNOS and concentrations of NO generated were lower in asthenozoospermic samples, suggesting that decreased endogenous NO may influence sperm motility and hence fertilization. However, in contrast to these findings, Schaad *et al.* (1996) found no evidence for the presence of NOS activity in human spermatozoa. It is possible that these differences are due to the differences in procedures used to assay NO synthesis, and further studies will be required to confirm whether the spermatozoa contain NOS and whether endogenous sperm-derived NO regulates sperm motility.

Schaad *et al.* (1996) also reported that human seminal plasma inhibits brain NOS activity and characterized it to be a heat-stable non-competitive inhibitor of bNOS. The physiological relevance of the presence of such an endogenous NO inhibitor is unclear. It is possible that ejaculation of spermatozoa into the female reproductive tract triggers an immune reaction, similar to that observed during bacterial infection or organ transplantation. This would induce iNOS activity and consequently result in the generation of large amounts of NO that can induce sperm toxicity, as reported by Rosselli *et al.* (1995). Hence, presence of endogenous NO inhibitor in the seminal plasma could play a physiological role in inhibiting NOS activity (iNOS) and maintaining NO at low concentrations to prevent toxic damage to the spermatozoa and surrounding cells or to prevent the hypermotility of sperm associated with capacitation process. Moreover, presence of endogenous NO inhibitor may also prevent generation of $ONOO^-$ by inhibiting the generation of NO that would otherwise react with O^{2-} to form $ONOO^-$.

Mechanisms by which NO can directly influence sperm motility may involve inhibition of mitochondrial respiration, as NO disrupts electron transfer in the respiratory chain (Szabo *et al.*, 1996). Apart from NO, L-arginine, the substrate for NOS, has also been shown to influence sperm viability. In men with abnormal spermatozoa the arginine content is lower than in normal men. Moreover, concentrations of arginase, the enzyme that catabolizes arginine, have been shown to increase in the seminal plasma (Papp *et al.*, 1979) and treatment with L-arginine improves sperm motility (Jungling and Bunge, 1976) because physiological concentrations of L-arginine are needed to generate NO, whereas in the presence of low arginine, NOS generates O_2^- and $ONOO^-$ (Xia *et al.*, 1996). It is feasible that O_2^- and $ONOO^-$ eventually cause sperm toxicity/dysfunction in patients with low L-arginine (Tomlinson *et al.*, 1992). However, this possibility needs to be investigated further. Moreover, NO has been shown to stimulate prostaglandin E_2 (PGE_2), and 5-hydroxy-eicosatetraenoic acid in mouse spermatozoa (Herrero *et al.*, 1995); however, the significance of this finding remains unclear.

Steroidogenesis

The observations that (i) luteinizing hormone-releasing hormone (LHRH) is generated in the pre-optic area of the hypophysis, (ii) neurotransmitters regulate the synthesis of LHRH, (iii) the hypophysis is well endowed with neurons and nerve supply, and (iv) NO acts as a neurotransmitter and NOS is present in the brain as well as neurons, lead to the possible conclusion that NO regulates LHRH synthesis. Direct evidence supporting the notion that NO regulates LHRH synthesis comes from the in-vitro studies of Rettori *et al.* (1994), who demonstrated that treatment of arcuate nucleus–median eminence explants (ANME) with sodium nitroprusside, a NO donor, increased LHRH release into the medium in a concentration-dependent manner. Moreover, the NO synthesis inhibitor L-NMMA inhibited norepinephrine-induced, but not basal release of LHRH. Following this observation, the mechanisms by which NO induces LHRH release have been explored in detail and the multiple pathways involved are shown in Figure 8. In 1979, Ojeda *et al.* demonstrated that PGE_2 stimulates LHRH release both *in vitro* and *in vivo*. Subsequently, using radioimmunoassays as well as [^{14}C]arachidonate, Rettori *et al.* (1992) provided evidence that NO induces the release as well as the synthesis of PGE_2 from the hypothalamus. These findings also suggested that NO must activate the enzymes, i.e. cyclo-oxygenase which converts arachidonate to prostanoids. Subsequently, it was shown that NO activates both cyclo-oxygenase I and II (Salvemini, 1997). Together, these findings provided evidence that NO induces LHRH release by increasing PGE_2 synthesis.

Similar to NO, norepinephrine induces LHRH release both *in vivo* and *in vitro* via α_1-adrenergic receptors (Ojeda *et al.*, 1979, 1986; Negro-Vilar *et al.*, 1986). Norepinephrine-induced LHRH synthesis is accompanied by an increase in PGE_2 concentration, and NOS synthesis inhibitors (L-NAME) as well as NO scavengers block the effects of norepinephrine on LHRH synthesis (Rettori *et al.*, 1992; McCann and Rettori, 1996a). These findings provide evidence that norepinephrine-induced LHRH synthesis is also mediated via NO-induced PGE_2 generation. Similar to norepinephrine, excitatory amino acids such as glutamic acid also induce LHRH release from the hypothalamus (Brann and Mahesh, 1994). Because glutamic acid induces norepinephrine release in the brain,

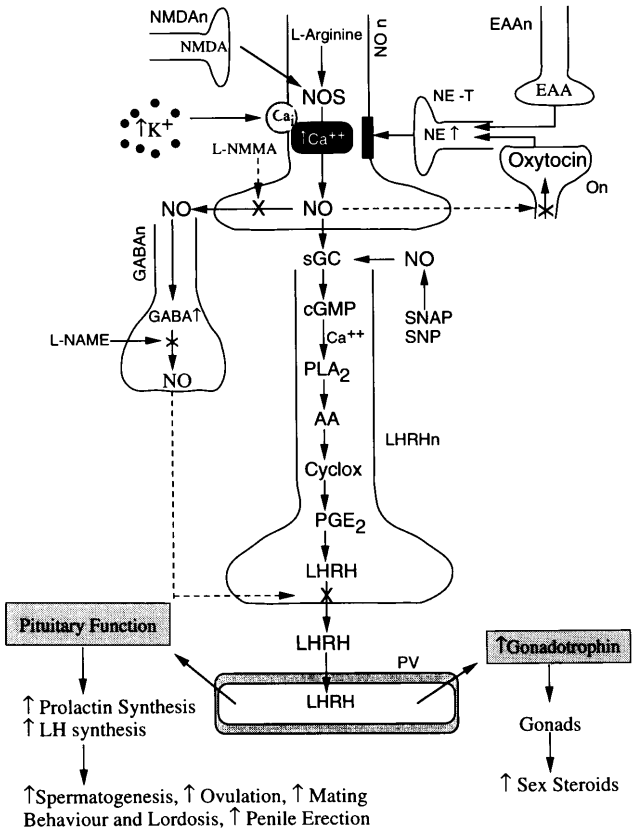

Figure 8. Multiple mechanisms / pathways by which NO can regulate steroidogenesis. NO regulates the release of luteinizing hormone-releasing hormone (LHRH) in response to oxytocin, norepinephrine (NE), excitatory amino acids (EAA) such as glutamic acid, γ-amino butyric acid (GABA), *N*-methyl-D-aspartate (NMDA) and extracellular potassium (K^+). LHRH in turn stimulates the pituitary and triggers the synthesis of prolactin and luteinizing hormone (LH) which subsequently regulate multiple reproductive functions. LHRH also stimulates the gonads via gonadotrophin release and this regulates the synthesis of sex steroids. PV, Portal vein; SNP, sodium nitroprusside; SNAP, *S*-nitroso-*N*-acetylpenicillamine; Ca_i, calcium channel; NOS, nitric oxide synthase; sGC, soluble guanylyl cyclase; cGMP, cyclic GMP (cGMP); PLA_2, phospholipase A_2; AA, arachidonic acid; Cyclox, cyclooxygenase; PGE_2, prostaglandin E_2; NMDAn, NMDA nerve; NOn, NOergenic neuron; EAAn, EAA nerve; On, oxytocin nerve; LHRHn, LHRH nerve, GABAn, GABA nerve; NE-T, norepinephrine nerve terminal; ↑ increase; X, blockade; and ——, inhibitor. Modified with permission from McCann and Rettori (1996b) and *Soc. Exp. Biol. Med.*

it was postulated that the effects of glutamic acid were mediated via norepinephrine-induced NO generation (Navarro *et al.*, 1994). In this regard, it has been shown that glutamic acid-induced LHRH release is inhibited by L-NMMA as well as by haemoglobin, a NO scavenger (Rettori *et al.*, 1994). Moreover, phentolamine blocked glutamic acid-, but not sodium nitroprusside-induced release of LHRH (Kamat *et al.*, 1995). The above findings, together with the observation that NO synthesis inhibitors abrogated, whereas NO-donors enhanced, *N*-methyl-D-aspartate (NMDA; stimulates NO release via NMDA receptors on NOergic neurons)-induced LHRH synthesis, suggests that NO derived from NO generating neurons induces LHRH release (Bonavera *et al.*, 1993; Figure 8). In addition to regulating norepinephrine- and excitatory amino acid-induced LHRH release, NO has also been shown to mediate KCl-induced LHRH release (Bonavera *et al.*, 1993; Figure 8).

Release of oxytocin in the hypothalamus by oxytocin neuronal endings is known to induce mating behaviour. Because progesterone is known to induce mating in ovariectomized, oestrogen-primed rats by sequentially stimulating the release of NO and LHRH, Rettori *et al.* (1997) recently postulated that oxytocin may also induce mating behaviour via NO-induced LHRH release. Using medial basal hypothalamic explants, they demonstrated that oxytocin stimulated LHRH, PGE_2 as well as NO release in the absence, but not in the presence, of L-NMMA. Moreover, they demonstrated that oxytocin-induced NO release was inhibited by prazocine, an α_1 receptor blocker, thus providing evidence that oxytocin activates NOS by stimulating the release of norepinephrine (Figure 8). Another interesting observation made by this group was that incubation of neuronal lobe explants with sodium nitroprusside inhibited oxytocin release, thus suggesting that NO generated by oxytocin induces LHRH as well as inhibits oxytocin release by a negative feedback loop (Figure 8).

The release of LHRH is not continuous but pulsatile. Mechanisms regulating the pulsatile release of LHRH are still unclear; however, recent studies suggest that γ-aminobutyric acid (GABA) may importantly regulate this phenomenon. In contrast to the other neuroactive factors, GABA can both induce and inhibit LHRH release from the hypothalamus (McCann and Rettori, 1996a). Via LHRH neurons in the medial pre-optic area GABA inhibits LHRH release, whereas via the neurons in the arcuate nucleus–median eminence region it stimulates LHRH release. In this regard, it has been shown that L-NMMA and haemoglobin prevent the inhibitory effects of GABA on LHRH release, suggesting that the inhibitory effects of GABA are NO mediated (Seilicovich *et al.*, 1995; McCann and Rettori, 1996a; Figure 8). Interestingly, the NO donor sodium nitroprusside stimulated the release of GABA from nucleus–median eminence explants, and these effects were largely blocked by the NO scavenger haemoglobin (McCann and Rettori, 1996a). NO has also been shown to mediate potassium-induced GABA synthesis in this tissue. Together, these findings suggests NO is an important

regulator of GABA synthesis (Figure 8), and may be important in regulating the pulsatile release of LHRH. It is feasible that pulsatile release of norepinephrine from the noradrenergic terminals induces NO release which simultaneously increases both LHRH and GABA synthesis. Once the concentrations of GABA reach a threshold value it inhibits LHRH release, leading to a pulsatile profile; however, other mechanisms may also participate (for review, see McCann and Rettori, 1996b).

The physiological importance of this regulation is that LHRH stimulates the pituitary and causes release of gonadotrophins, which in turn activates the gonads to produce sex steroids. In females, LHRH induces ovulation, whereas in males it induces spermatogenesis (McCann, 1982). Moreover, LHRH not only regulates gonadal function but also secretion of steroids by both males and females (for review, see McCann, 1982; Jennes and Conn, 1994). In-vivo studies with castrated male rats have shown that infusion of L-NMMA caused a cessation of LH but not FSH pulses, suggesting that NO was required for pulsatile LHRH release but not for the putative FSH releasing factor, which possibly controls the release of FSH (Rettori *et al.*, 1993, 1994). The physiological role of NO in mediating the steroid-induced LH surge has been determined by Aguan *et al.* (1996), who demonstrated that administration of eNOS and bNOS, but not iNOS, antisense oligonucleotides into the third ventricle of ovariectomized rats attenuated the oestrogen+progesterone-induced surge in LH.

The pituitary is known to control the release of prolactin, a hormone required in reproduction and lactation. Interestingly, NOS has been localized in anterior pituitary cells, i.e. folliculostellate cells and gonadotropes (Ceccatelli *et al.*, 1993). Similar to LH, abrupt increases in the rate of prolactin release occur during the afternoon of pro-oestrus. It is feasible that NO or NO-induced pulsatile release of LHRH regulates the release of prolactin. Incubation of isolated hemipituitaries with the NO donor sodium nitroprusside suppressed prolactin release and this suppression was completely blocked by the NO scavenger haemoglobin. Incubation with NOS-synthesis inhibitors (L-NMMA) increased prolactin release, whereas treatment with cGMP inhibited it (Duvilanski *et al.*, 1995). These findings suggest that NO, via the cGMP pathway, inhibits prolactin release from the pituitary. Interestingly, dopamine, which is known to inhibit prolactin release, was ineffective in the presence of L-NMMA and haemoglobin, suggesting that its inhibitory effects were also mediated via NO. As compared to prolactin, NO did not alter basal release of LH, but blocked LHRH-induced LH release (Cecccatelli *et al.*, 1993). In contrast to these findings, Bonavera *et al.* (1994) have shown that oestradiol benzoate as well as oestradiol benzoate plus progesterone induced prolactin and LH, but not corticosterone surge in ovariectomized rats was significantly blocked by co-administration of L-NMMA, suggesting that NO induces both LH and prolactin release. It is feasible that differences between the two studies are due to the in-vitro and in-vivo systems used. Indeed, increases in prolactin in the in-vivo system may have been mediated via NO-induced release of factors known to regulate prolactin release.

NO has also been shown to regulate granulosa–luteal cell steroidogenesis (Van Voorhis *et al.*, 1994). NOS has been localized in the granulosa cell layer of follicles, theca cells, ovarian stroma, surface of oocytes, endothelium and adventitia of ovarian blood vessels. In cultured granulosa–luteal cells, treatment with *S*-nitroso-*N*- acetylpenicillamine (SNAP, a NO donor), but not cGMP, decreased the basal synthesis of both oestrogen and progesterone. Incubation of the same cells with L-NAME resulted in a significant increase in oestrogen and progesterone concentrations. These findings suggest that NO mediates its inhibitory effects via a cGMP-independent pathway. Indeed, SNAP inhibited the activity of aromatase, one of the enzymes responsible for steroid synthesis.

NOS activity has been demonstrated in both the Leydig and Sertoli cells of the testis (Davidoff *et al.*, 1997). Because these cells importantly regulate testosterone synthesis, it is feasible that NO also regulates testosterone synthesis. Most studies to date have provided evidence that NO is a negative regulator of testosterone synthesis (Adams *et al.*, 1992; Welch *et al.*, 1995). In rats, alcohol-induced suppression of testosterone synthesis was reversed in the presence of L-NAME, suggesting that ethanol induces its effects via NO generation (Adams *et al.* 1993). Administration, of L-NAME to male rats also increased testosterone concentrations, suggesting that NO down-regulates testosterone synthesis. In a recent study, Adams *et al.* (1996) showed that L-NAME abrogated morphine-induced suppression and HCG-induced release of testosterone synthesis, without altering LH secretion. Furthermore, L-NAME attenuated morphine's suppression of HCG-stimulated testosterone secretion, indicating that the effects occur directly in the testis and are not dependent on LH secretion. Because NO donors and L-NAME did not enhance the effects of morphine and the opioid antagonist naltrexone respectively, it is possible that the NO and opioids suppressed testosterone release via separate mechanisms. With regard to the mechanism by which NO suppresses testosterone release, Welch *et al.* (1995) have recently shown that HCG-induced testosterone synthesis in cultured Leydig cells was increased by L-NMMA and L-NAME, and accompanied by a decrease in intracellular

cGMP. This finding suggests that NO mediates its effects via the cGMP pathway. Interestingly, Adams *et al.* (1994) also showed that in-vivo administration of vasodilators that do not generate NO, i.e. hydralazine and nicardipine, inhibited the basal release of testosterone as well as L-NAME-induced testosterone release. Together, these findings suggest that inhibitory effects of NO may also be regulated by changes in the perfusion within the vasculature; however, further studies are required to investigate this possibility.

Sexual behaviour

Ample evidence suggests that LHRH not only triggers the synthesis of gonadotrophins and gonadal steroids but also participates in regulating mating behaviour in vertebrates (Moss and MaCann, 1973; McCann, 1982; Sakuma and Pfaff, 1983; Figure 8). In ovariectomized rats given oestrogen, injection of progesterone induced mating within 30 min, and this effect was blocked by antisera directed against LHRH (Sakuma and Pfaff, 1983). Progesterone- as well oestrogen+progesterone-induced mating behaviour (judged by lordosis) was also blocked by L-NMMA and L-NAME, but not by D-NMMA (inactive in inhibiting NOS; Mani *et al.*, 1994). In contrast to NO synthesis inhibitors, administration of NO donors significantly induced lordosis and these effects were blocked by antisera against LHRH, suggesting that the NO-induced mating was LHRH mediated (Mani *et al.*, 1994). Recently, it was shown that oxytocin, which induces mating behaviour in both males and females, induced LHRH release via NO generation, suggesting that the sexual effects of oxytocin are NO mediated (Rettori *et al.*, 1997). NOS activity was also recently identified in the lordosis-relevant neurons of the ventromedial hypothalamus of female rats (Rachman *et al.*, 1996). NO also regulates mating behaviour in males, where NO via neuronal regulation mediates penile erection. Inhibition of NO synthesis has been shown to impair copulation and decrease the number of ex-copula erections, increase the number of seminal emissions, and decrease the latency to the first seminal emission, possibly by decreasing sympathetic nervous system activity (Hull *et al.*, 1994). In contrast to the above studies, excess and inappropriate sexual behaviour was observed in mice lacking nNOS (Nelson *et al.*, 1995). Together, these findings provide evidence that NO importantly regulates sexual as well as mating behaviour. This may have important clinical implications for patients with depressed mating behaviour, as therapeutic means of inducing NOS activity may correct these abnormalities.

Finally, NO synthesis may in part be controlled by the reproductive system and may contribute to sexual dimorphism. Oestradiol induces NOS mRNA expression in different tissues (Weiner *et al.* 1994), and circulating concentrations of NO_2/NO_3 directly correlate with oestradiol concentrations in ovulating patients (Rosselli *et al.*, 1994a). Clinical studies demonstrate that ovarian dysfunction is associated with cardiovascular disease and decreased endothelium-derived NO synthesis, whereas oestrogen substitution has cardioprotective effects and circulating concentrations of NO_2/NO_3 are increased in postmenopausal women treated with oestrogen (Rosselli *et al.*, 1995; Imthurn *et al.*, 1997). Elucidation of the precise role of NO in reproductive biology may provide an important link between the reproductive system and other systems in which NO is involved, such as the cardiovascular system.

In summary, the fact that NO is involved in the physiology, biology and pathophysiology of the reproductive system may have great clinical implications in developing therapeutic strategies to prevent NO-related reproductive disorders. In this regard, use of NO donors such as L-arginine (substrate for NO synthesis), as well as NO synthesis inhibitors (L-NAME etc.) may prove to be therapeutically important in correcting impotence due to dysfunction in penile erection, infertility due to infection, labour, pre-eclampsia, abortion, extrauterine pregnancy, etc. Future studies should aim to evaluate the use of NO synthesis modulators for treating specific reproductive disorders.

Acknowledgements

This work was supported by a grant from the Swiss National Foundation and EMDO Foundation.

References

Abu-Soud, H.M., Feldman, P.L., Clark, P. and Stuehr, D.J. (1994) Electron transfer in the nitric oxide syntheses: characterization of L-arginine analogs that block heme iron reduction. *J. Biol. Chem.*, **269,** 32318–32326.

Abu-Soud, H.M., Loftus, M. and Stuehr, D.J. (1995a) Subunit dissociation and unfolding of macrophage NO synthase: relationship between enzyme structure, prosthetic group binding, and catalytic function. *Biochemistry*, **34,** 11167–11175.

Abu-Soud, H.M., Wang, J., Rousseau, D.L. *et al.* (1995b) Neuronal nitric-oxide synthase self-inactivates by forming a ferrous–nitrosyl complex during aerobic catalysis. *J. Biol. Chem.*, **270,** 22997–23006.

Adams, M.L., Nock, B., Truong, R. and Cicero, T.J. (1992) Nitric oxide control of steroidogenesis: endocrine effects of NG-nitro-L-arginine and comparisons to alcohol. *Life Sci.*, **50,** PL35–PL40.

Adams, M.L., Forman, J.B., Kalicki, J.M. *et al.* (1993) Antagonism of alcohol-induced suppression of rat testosterone secretion by an inhibitor of nitric oxide synthase. *Alcohol Clin. Exp. Res*., **17,** 660–664.

Adams, M.L., Meyer, E.R., Sewing, B.N. and Cicero, T.J. (1994) Effect of nitric oxide-related agents on rat testicular function. *J. Pharmacol. Exp. Ther.*, **269,** 230–270.

Adams, M.L., Meyer, E.R. and Cicero, T.J. (1996) Effects of nitric oxide-related agents on opioid regulation of rat testicular steroidogenesis. *Biol. Reprod.*, **54,** 1128–1134.

Adashi, E.Y. (1989) Cytokine-mediated regulation of ovarian function: encounters of a third kind. *Endocrinology,* **124,** 2043–2045.

Aguan, K., Mahesh, V.B., Ping, L. *et al.* (1996) Evidence for a physiological role for nitric oxide in the regulation of the LH surge: effect of central administration of antisense oligonucleotides to nitric oxide synthase. *Neuroendocrinology,* **64,** 449–455.

Albakri, Q.A. and Stuehr, D.J. (1996) Intracellular assembly of inducible NO synthase is limited by nitric oxide-mediated changes in heme insertion and availability. *J. Biol. Chem.*, **271,** 5414–5421.

Albina, J.E., Mills, C.D., Barbul, A. *et al.* (1988) Arginine metabolism in wounds. *Am. J. Physiol.*, **254,** E459–E467.

Ali, M., Buhimschi, I., Chwalisz, K. and Garfield, R.E. (1997) Changes in expression of nitric oxide synthase isoforms in rat uterus and cervix during pregnancy and parturition. *Mol. Hum. Reprod.*, **3**, 995–1003.

Anteby, E.Y., Hurwitz, A., Korach, O. *et al.* (1996) Human follicular nitric oxide pathway: relationship to follicular size, oestradiol concentration and ovarian blood flow. *Hum. Reprod.*, **11,** 1947–1951.

Archer, S. (1993) Measurement of nitric oxide in biological models. *FASB J.*, **7,** 349–360.

Arnal, J.F., Battle, J.T., Menard, J. and Michel, J.B. (1993) The vasodilatatory effect of endogenous nitric oxide is a major counter regulatory mechanism in the hypertensive rats. *J. Hypertension*, **11,** 945–950.

Assreuy, J., Cunha, F.Q., Liew, F.Y. and Moncada, S. (1993) Feedback inhibition of nitric oxide synthase activity by nitric oxide. *Br. J. Pharmacol.*, **108,** 833–837.

Azuma, H., Obayashi, S., Hamasaki, H. *et al.* (1995) Role of endothelium in human uterine arteries during normal menstrual cycle. *Br. J. Phamacol.*, **114,** 902–908.

Baek, K.J., Thiel, B.A., Lucas, S. and Stuehr, D.J. (1993) Macrophage nitric oxide synthase subunits: purification, characterization and role of prosthetic groups and substrate in regulating their association into a dimeric enzyme. *J. Biol. Chem.*, **268,** 21120–21129.

Bansal, R.K., Goldsmith, P.C., Youping, H. *et al.* (1997) A decline in myometrial nitric oxide synthase expression is associated with labor and delivery. *J. Clin. Invest.*, **99,** 2502–2508.

Baydoun, A.R., Bogle, R.G., Pearson, J.D. and Mann, G.E. (1993) Selective inhibition by dexamethasone of induction of NO synthase, but not of induction of L-arginine transport, in activated murine macrophage J774 cells. *J. Biol. Chem.*, **110,** 1401–1406.

Beckman, J.S. and Crow, J.P. (1993) Pathological implications of nitric oxide, superoxide and peroxynitrite formation. *Biochem. Soc. Trans.*, **21,** 330–334.

Beckman, J.S., Beckman, T.W., Chen, J. *et al.* (1990) Apparent hydroxyl radical production of peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA*, **87,** 1620–1624.

Ben-Shlomo, I., Adashi, E.Y. and Payne, D.W. (1994) The morphogenic/cytotoxic and prostaglandin activities of interleukin-1 beta in the rat ovary are nitric oxide independent. *J. Clin. Invest.*, **94,** 1463–1469.

Blachier, F., M'rabet-Touil, H., Darcy-Vrillon, B. *et al.* (1991) Stimulation by D-glucose of the direct conversion of arginine to citrulline in enterocytes isolated from pig jejunum. *Biochem. Biophys. Res. Commun.*, **168,** 1034–1040.

Bogle, R.G., Baydoun, A.R., Pearson, J.D. *et al.* (1992) L-Arginine transport is increased in macrophages generating nitric oxide. *Biochem. J.*, **284,** 15–18.

Bonavera, J.J., Sahu, A., Kalra, P.S. and Kalra, S.P. (1993) Evidence that nitric oxide may mediate the ovarian steroid-induced luteinizing hormone surge. Involvement of excitatory amino acids. *Endocrinology,* **131,** 2481–2487.

Bonavera, J.J., Sahu, A., Kalra, P.S. and Kalra, S.P. (1994) Evidence in support of nitric oxide (NO) involvement in the cyclic release of prolactin and LH surges. *Brain. Res.*, **660,** 175–179.

Bonello, N., Mckie, K., Jasper, M. *et al.* (1996) Inhibition of nitric oxide effects on interleukin-1 beta-enhanced ovulation rate, steroid hormone and ovarian leukocyte distribution at ovulation in the rat. *Biol. Reprod.*, **54,** 436–445.

Brann, D.W. and Mahesh, V.B. (1994) Excitatory amino acids: function and significance in neuroendocrine regulation. *Front. Neuroendocrinol.*, **15,** 3–50.

Bredt, D.S., Hwang, P.M., Glatt, C.E. *et al.* (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature*, **351,** 714–718.

Bredt, D.S., Ferris, C.D. and Snyder, S.H. (1992) Nitric oxide synthase regulatory sites. Phosphorylation by cyclic AMP-dependent protein kinase, protein kinase C, and calcium/calmodulin protein kinase, identification of flavin and calmodulin binding sites. *J. Biol. Chem.*, **267,** 10976–10981.

Bry, K. and Hallman, M. (1993) TGF-beta 2 prevents preterm delivery induced by IL-1 alpha and TNF-alpha in the rabbit. *Am. J. Obstet. Gynecol.*, **168,** 1318–1322.

Bryant, E.C., Tomlinson, A., Mitchell, J.A. *et al.* (1995) Nitric oxide synthase in the rat fallopian tube is regulated during the oestrous cycle. *J. Endocrinol.*, **146,** 149–157.

Buga, G.M., Griscavage, J.M., Rogers, N.E. and Ignarro, L.J. (1993) Negative feedback regulation of endothelial cell function by nitric oxide. *Circ. Res.*, **73,** 808–812.

Buhimschi, I., Yallampalli, C., Chwalisz, K. and Garfield, R.E. (1995a) Pre-eclampsia-like conditions produced by nitric oxide inhibition: effect of L-arginine, D-arginine and steroids hormones. *Hum. Reprod.*, **10,** 2723–2730.

Buhimschi, I., Yallampalli, C., Dong, I.L. and Garfield, R.E. (1995b) Involvement of a nitric oxide–cGMP pathway in control of human uterine contractility during pregnancy. *Am. J. Physiol.*, **172,** 1577–1584.

Buhimischi, I., Ali, M., Jain, V. *et al.* (1996) Differential regulation of nitric oxide in the rat uterus and cervix during pregnancy and labour. *Hum. Reprod.*, **11,** 1755–1766.

Buhimschi, I., Gaade, G.R. and Garfield, R.E. (1997) The nitric oxide pathway in pre-eclampsia: pathophysiological implication. *Hum. Reprod. Update*, **4**, 24–42.

Burnett, A.L., Lowenstein, C.J., Bredt, D.S. *et al.* (1992) Nitric oxide: a physiological mediator of penile erection. *Science*, **257,** 410–413.

Burnett, A.L., Tillman, S.L., Chang, T.S. *et al.* (1993) Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *J. Urol.*, **150,** 73–76.

Burnett, A.L., Ricker, D.D., Chamness, S.L. *et al.* (1995) Localization of nitric oxide synthase in the reproductive organs of male rat. *Biol. Reprod.*, **52,** 1–7.

Burnett, A.L., Nelson, R.J., Calvin, D.C. *et al.* (1996) Nitric oxide-dependent penile erection in mice lacking nitric oxide synthase. *Mol. Med.*, **2,** 288–296.

Busconi, L. and Michel, T. (1993) Endothelial nitric oxide synthase: N-terminal myristoylation determines subcellular localization. *J. Biol. Chem.*, **268,** 8410–8413.

Carrier, S., Nagaraju, P., Morgan, D.M. *et al.* (1997) Age decrease nitric oxide synthase-containing nerve fibres in the rat. *J. Urol.*, **157,** 1088–1092.

Cecatelli, S., Hulting, A.L., Zhang, X. *et al.* (1993) Nitric oxide synthase in rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. *Proc. Natl. Acad. Sci. USA*, **90,** 11292–11296.

Champion, H.C., Wang, R., Shenassa, B.B. *et al.* (1997) Adrenomedullin induces penile erection in the cat. *Eur. J. Pharmacol.*, **319,** 71–75.

Chang, J.Y., Huang, C.L. and Chn, S.H. (1996) Nitric oxide as a mediator of cocaine-induced penile erection in the rat. *Br. J. Pharmacol.*, **118,** 155–161.

Charles, I.G., Palmer, R.M.J. and Hickery, M.S. (1993) Cloning, characterization, and expression of a cDNA encoding an inducible nitric oxide synthase from the human chondrocyte. *Proc. Natl. Acad. Sci. USA*, **90,** 11419–11423.

Chatterjee, S., Gandula, P.R., Dong, Y.L. and Yallampalli, C. (1996) Immunocytochemical localization of nitric oxide-II in reproductive organs of female rats during the oestrous cycle. *Histochem. J.*, **28,** 715–723.

Cho, H.J., Xie, Q., Calaycay, J. *et al.* (1992) Calmodulin is a subunit of nitric oxide synthase from macrophages. *J. Exp. Med.*, **176,** 599–604.

Cho, H.J., Martin, E., Xie, Q.-W. *et al.* (1995) Inducible nitric oxide synthase identification of amino acid residues essential for dimerization and binding of tetrahydrobiopterin. *Proc. Natl. Acad. Sci. USA*, **92,** 11514–11518.

Chun, S.Y., Eisenhauer, K.M., Kubo, M. and Hsueh, A.J.W. (1995) Interleukin beta suppresses apoptosis in rat ovarian follicles by nitric oxide production. *Endocrinology*, **136,** 3120–3127.

Clancy, R.M., Levartovsky, D., Leszczynska-Piziak, L. *et al.* (1993) Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for S-nitrosoglutathione as a bioactive intermediary. *Proc. Natl. Acad. Sci. USA*, **91,** 3680–3684.

Cook, J.P. and Tsao, P.S. (1993) Cytoprotective effects of nitric oxide. *Circulation*, **88**, 2451–2454.

Corraliza, I.M., Campo, M.L., Fuentes, J.M. *et al.* (1993) Parallel induction of nitric oxide and glucose-6-phosphate dehydrogenase in activated bone marrow-derived macrophages. *Biochem. Biophys. Res. Commun.*, **196,** 342–347.

Das, S.K., Flanders, K.S.C., Andrews, G.K. and Dey, S.K. (1992) Expression of transforming growth factor-beta isoform (beta 2 beta 3) in the mouse uterus: analysis of the preimplantation period and effect of ovarian steroids. *Endocrinology*, **130,** 3459–3466.

Davidoff, M.S., Middendorff, R., Mayer, B. and Holstein, A.F. (1995) Nitric oxide synthase (NOS-I) in Leydig cells of human testis. *Arch. Histol. Cytol.*, **58,** 17–30.

Davidoff, M.S., Middendorff, R., Mayer, B. *et al.* (1997) Nitric oxide /cGMP pathway components in the Leydig cells of the human testis. *Cell Tissue Res.*, **287,** 161–170.

Davies, M.G., Fulton, G.J. and Hagen, P.O. (1995) Clinical biology of nitric oxide. *Br. J. Surg.*, **82,** 1598–1610.

Dinerman, J.L., Lowenstein, L.J. and Snyder, S.H. (1993) Molecular mechanisms of nitric oxide regulation: potential relevance to cardiovascular disease. *Circ. Res.*, **73,** 217–222.

Dong, Y.L. and Yallampalli, C. (1996) Interaction between nitric oxide and prostaglandin E2 pathways in pregnant rat uteri. *Am. J. Physiol.*, **270,** E471–E476.

Dubey, R.K., Boegehold, M., Gillespie, D. and Rosselli, M. (1996) Increased nitric oxide activity in early renovascular hypertension. *Am. J. Physiol.*, **270,** R118–R124.

Dubey, R.K., Jackson, E.K., Rupprecht, H.D. and Sterzel, R.B. (1997) Factors controlling growth and matrix production in vascular smooth muscle and glomerular mesengial cell. *Curr. Opin. Nephrol. Hypertension*, **6,** 88–105.

Duvilanski, B.N.H., Zambruno, N., Rettori, V. and McCann, S.M. (1995) Role of nitric oxide in control of prolactin release by adenohypophysis. *Proc. Natl. Acad. Sci. USA*, **92,** 170–174.

Ehren, I., Adolfsson, J. and Wiklund, N.P. (1994) Nitric oxide synthase in the human urogenital tract. *Urol. Res.*, **22,** 287–290.

Ekerhovd, E., Braennstroem, M., Alexandersson, M. and Norstroem, A. (1997) Evidence for nitric oxide mediated contractile activity in isolated strips of human Fallopian tube. *Hum. Reprod.*, **12,** 301–305.

Ellman, C., Corbett, J.A., Misko, T.P. *et al.* (1993) Nitric oxide mediates interleukin-1 induced cellular cytotoxicity in the rat ovary. *J. Clin. Invest.*, **92,** 3053–3056.

Evans, C.M., Stevanovi-Racic, M. and Lancaster, J. (1995) Nitric oxide and its role in orthopaedic disease. *Clin. Orthop.*, **312,** 275–294.

Ewing, J.F. and Maines, M.D. (1995) Distribution of constitutive (HO-2) and heat-inducible (HO-1) heme oxygenase isozymes in rat testes: HO-2 displays stage-specific expression in germ cells. *Endocrinology*, **136,** 2294–2302.

Fickling, S.A., Williams, D., Vallance, P. *et al.* (1993) Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and preeclampsia. *Lancet*, **41,** 1447–1451.

Forstermann, U., Closs, E.I., Pollock, J.S. *et al.* (1994) Nitric oxide synthase isozymes, characterization, purification, molecular cloning, and functions. *Hypertension*, **23,** 1121–1131.

Furchgott, R.F. and Zawadski, J.V. (1980) The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373–376.

Gangula, P.R.R., Dong, Y.L. and Yallampalli, C. (1997) Rat myometrial smooth muscle cells express nitric oxide synthase. *Hum. Reprod.*, **12,** 561–568.

Garban, H., Arquez, D., Cai, L. *et al.* (1995) Restoration of normal adult penile erection response in aged rats by-long term treatment with androgens. *Biol. Reprod.*, **53,** 1365–1372.

Garban, H., Marquez, D., Magee, T. *et al.* (1997) Cloning of rat and human inducible penile nitric oxide synthase: application for gene therapy of erectile dysfunction. *Biol. Reprod.*, **56,** 954–963.

Garthwaite, J., Charles, S.L. and Chess-Williams, R. (1988) Endothelium-derived relaxing factor release on activation of NMDA receptor suggests role as intercellular messenger in the brain. *Nature*, **336**, 385–388.

Geller, D.A., Lowenstein, C.J., Shapiro, R.A. *et al.* (1993) Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes. *Proc. Natl. Acad. Sci. USA*, **90,** 3491–3495.

Ghosh, D.K., Abu-Soud, H.M. and Stuehr, D.J. (1996) Domains of macrophage NO synthase have divergent roles in forming and stabilizing the active dimeric enzyme. *Biochemistry*, **35,** 1444–1449.

Giovanelli, J., Campos, K.L. and Kaufman, S. (1991) Tetrahydrobiopterin, a cofactor for rat cerebellar nitric oxide synthase, does not function as a reactant in the oxygenation of arginine. *Proc. Natl. Acad. Sci. USA*, **88,** 7091–7095.

Griffith, O.W. and Stuehr, D.J. (1995) Nitric oxide synthase properties and catalytic mechanism. *Annu. Rev. Physiol.*, **57**, 707–736.

Gross, S.S. and Levi, R. (1992) Tetrahydrobiopterin synthesis: an absolute requirement for cytokine-induced nitric oxide generation by vascular smooth muscle. *J. Biol. Chem.*, **267,** 25722–25729.

Grozdanovic, Z., Mayer, B., Maumgarten, H.G. and Bruning, G. (1994) Nitric oxide synthase-containing nerve fibres and neurons in the genital tract of female mouse. *Cell Tissue Res.*, **225,** 355–360.

Hattori, M., Sakamoto, K., Fujihara, N. and Kojima, I. (1996) Nitric oxide a modulator for the epidermal growth factor receptor expression in developing ovarian granulosa cells. *Am. J. Physiol.*, **270,** C812–C818.

Hayasahida, H., Okamura, T., Tomoyoshi, T. and Toda, N. (1996) Neurogenic nitric oxide mediates relaxation of canine corpus cavernosum. *J. Urol.*, **155,** 1122–1127.

Heinzel, B., John, M., Klatt, P. *et al.* (1992) Ca^{2+}/calmodulin-dependent formation of hydrogen peroxide by brain nitric oxide synthase. *Biochem. J.*, **281,** 627–630.

Hellstrom, W.J.G., Bell, M., Wang, R. and Sikka, S.C. (1994) Effects of sodium nitroprusside on sperm motility, viability, and lipid peroxidation. *Fertil. Steril.*, **61,** 1117–1122.

Herrero, M.B., Franci, A.M. and Gimeno, M.A. (1995) Mouse spermatozoa can synthesize PGE_2, and 5-HETE in vitro: stimulatory action of nitric oxide. *Prostaglandins Leukocytes Essential Fatty Acids*, **53,** 347–350.

Heymann, M.A., Bootstaylor, B., Roman, C. *et al.* (1994) Glyceryl trinitrate stops labour in sheep. In Moncada, S., Feelisch, M., Busse, R. and Higgs, E.A. (eds), *Biology of Nitric Oxide*, vol. 3. Portland Press, London, pp. 201–203.

Hibbs, J.B. Jr., Taintov, R.R. and Varzin, Z. (1987) Macrophage cytotoxicity: role for L-arginine deaminase and imino nitrogen oxidation to nitrite. *Science*, **235,** 473–476.

Holmquist, F., Stief, C.G., Jonas, U. and Andersson, K.E. (1991) Effect of the nitric oxide synthase inhibitor NG-nitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit. *Acta Physiol. Scand.*, **143,** 299–304.

Huang, J., Roby, K.F., Pace, J.L. *et al.* (1995) Cellular localization and hormonal regulation of inducible nitric oxide synthase in mouse uterus. *J. Leukocyte Biol.*, **57,** 27–35.

Hull, E.M., Lumley, L.A., Matuszewich, L. *et al.* (1994) The roles of nitric oxide in sexual function of male rats. *Neuropharmacology*, **33,** 1499–1504.

Hurshman, A.R. and Marletta, M.A. (1995) Nitric oxide complexes of inducible nitric oxide synthase: spectral characterization and effect on catalytic activity. *Biochemistry*, **34,** 5627–5634.

Hurwitz, A., Loukides, J., Ricciarelli, E. *et al.* (1992) Human intraovarian interleukin-1 (IL-1): highly compartmentalized and hormonally dependent regulation of the genes encoding IL-1, its receptor and its receptor antagonist. *J. Clin. Invest.*, **89,** 1746–1754.

Hurwitz, A., Finci-Yeheskel, Z., Milwidsky, A. *et al.* (1997) In-vitro modulation of plasminogen activator activity, prostaglandin E and nitric oxide production by interleukin-1 in pregnant mare serum gonadotrophin-primed theca-interstitial cells. *Hum. Reprod.*, **12,** 774–779.

Ignarro, L.J., Buga, G.M., Wood, K.S. *et al.* (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA*, **84,** 9265–9269.

Ignarro, L.J., Bush, P.A., Buga, G.M. *et al.* (1990) Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem. Biophys. Res. Commun.*, **170,** 843–850.

Imthurn, B., Rosselli, M., Jaeger, A.W. *et al.* (1997) Differential effects of hormone-replacement therapy on endogenous nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17β-estradiol valerate and cyproterone acetate or medroxyprogesterone acetate. *J. Clin. Endocrinol. Metab.*, **82,** 388–394.

Inoue, Y., Bode, B.P., Beck, D.J. *et al.* (1993) Arginine transport in human liver. Characterization and effects of nitric oxide synthase inhibitors. *Ann. Surg.*, **218,** 350–363.

Jablonkashariff, A. and Olson, L.M. (1997) Hormonal regulation of nitric oxide synthase and their cell specific expression during follicular development in the rat ovary. *Endocrinology*, **138,** 460–468.

Jaing, Y., Singh, A.K., Kannan, M.S. *et al.* (1996) Isolation of nitric oxide synthase in the rat uterus. *Life Sci*., **58,** 1009–1014.

Jennes, L. and Conn, P.M. (1994) Gonadotropin-releasing hormone and its receptors in rat brain. *Front. Neuroendocrinol*., **15,** 51–71.

Jennings, E.R.W., MacGillivray, T.E. and Harrison, M.R. (1993) Nitric oxide ablates preterm labor in the rhesus monkey. *J. Maternal Fetal Med*., **2,** 170–175.

Jungling, M.L. and Bunge, R.G. (1976) The treatment of spermatogenic arrest with arginine. *Fertil. Steril.*, **27,** 282–283.

Kaipia, A. and Hsueh, A.J.W. (1997) Regulation of ovarian follicle atresia. *Annu. Rev. Physiol.*, **59,** 349–363.

Kamat, A., Yu, W.H., Rettori, V. and McCann, S.M. (1995) Glutaminic acid stimulated luteinizing hormone-releasing hormone release is mediated by alpha adrenergic stimulation of nitric oxide release. *Brain Res. Bull*., **37,** 233–235.

Kaufman, S. (1993) New tetrahydrobiopterin-dependent systems. *Annu. Rev. Nutr.*, **13,** 261–286.

Keaney, J.F., Jr., Simon, D.I., Stamler, J.S. *et al.* (1993) NO forms an adduct with serum albumin that has endothelium-derived relaxing factor like properties. *J. Clin. Invest.*, **91,** 1582–1589.

Kelly, R.A., Balligand, J.-L. and Smith, T.N. (1996) Nitric oxide and cardiac function. *Circ. Res.*, **79,** 363–380.

Kim, N., Vardi, Y., Padma-Nathan, H. *et al.* (1993) Oxygen tension regulates the nitric oxide pathway. Physiological role in penile erection. *J. Clin. Invest*., **91,** 437–442.

Klatt, P., Schmidt, K., Lehner, D. *et al.* (1995) Structural analysis of porcine brain nitric oxide synthase reveals a role for tetrahydrobiopterin and L-arginine in the formation of SDS-resistant dimer. *EMBO J.*, **14,** 3687–3695.

Klatt, P., Pfeiffer, S. and List, B.M. (1996) Characterization of heme-dependent neuronal nitric oxide synthase reveals a role for heme in subunit dimerization and binding of the amino acid substance and tetrahydrobiopterin. *J. Biol. Chem.*, **271,** 7336–7342.

Kol, S., Benshlomo, I., Ruutiainen, K. *et al.* (1997) The midcycle increase in ovarian glucose uptake is associated with enhanced expression of glucose transporter 3: possible role for interleukin 1, a putative intermediary in ovulatory process. *J. Clin. Invest.*, **99,** 2274–2283.

Kolpakov, V., Gordon, D. and Kulik, T.J. (1995) Nitric oxide-generating compound inhibits fetal preterm and collagen synthesis in cultured vascular smooth muscle cells. *Cell. Res.*, **76,** 305–309.

Koshland, D.E. (editorial) (1992) Nitric oxide: the molecule of the year. *Science*, **258,** 1861.

Kumar, D., Zourlas, P.A. and Barnes, A.C. (1965) In vivo effect of amylnitrite on human pregnant uterine contractility. *Am. J. Obstet. Gynecol.*, **91,** 1066–1068.

Lancaster, J.R. Jr., Langrehr, J.M., Bergonia, H.A. *et al.* (1992) EPR detection of heme and non-heme iron-containing protein nitrosylation by nitric oxide during rejection of rat heart allograft. *J. Biol. Chem.*, **267,** 10994–10998.

Lees, C.S., Campbell, E., Jauniax, E. *et al.* (1994) Arrest of preterm labor and prolongation of gestation with glyceryl trinitrate, a nitric oxide donor. *Lancet*, **343,** 1325–1326.

Lewis, S.E., Donnelly, E.T., Sterling, E.S.L. *et al.* (1996) Nitric oxide synthase and nitric oxide production in human sperm: evidence that endogenous nitric oxide is beneficial to sperm motility. *Mol. Hum. Reprod.*, **2**, 873–878.

Lugg, J.A., Rajfer, J. and Gonzalez-Cadavid, N.F. (1995) Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. *Endocrinology*, **136,** 1495–1501.

Lugg, J., Ng, C., Rajfer, J. and Gonzalez-Cadavid, N. (1996) Cavernosal nerve stimulation in the rat reverses castration-induced decrease in penile NOS activity. *Am. J. Physiol.*, **271,** E354–E361.

Magee, T., Fuentes, A.M., Garban, H. *et al.* (1996) Cloning a novel neuronal nitric oxide synthase expressed in penis and lower urinary tract. *Biochem. Biophys. Res. Commun.*, **226,** 145–151.

Magness, R.R., Rosenfeld, C.R., Hassan, A. and Shaul, P.W. (1996) Endothelial vasodilator production by uterine and systemic arteries. Effects of AngII on PGl_2 and NO in pregnancy. *Am. J. Physiol.*, **270,** H1914–H1923.

Magness, R.R., Shaw, C.E., Pherretton, T.M. *et al.* (1997) Endothelial vasodilator production by uterine and systemic arteries. II. Pregnancy effects on NO synthase expression. *Am. J. Physiol.*, **272,** H1730–H1740.

Mani, S.K., Allen, J.M.C., Rettori, V. *et al.* (1994) Nitric oxide mediates sexual behavior in female rats by stimulating LHRH release. *Proc. Natl. Acad. Sci. USA*, **91,** 6468–6472.

Marletta, M.A., Yoon, P.S., Iyengar, R. *et al.* (1988) Macrophage oxidation of L-arginine to nitrite and nitrate: nitric acid is an intermediate. *Biochemistry*, **27,** 8706–8711.

Maronde, E., Middendorff, R., Mayer, B. and Olcese, J. (1995) The effect of NO-donors and rat pineal cells: stimulation of cGMP and cGMP-independent inhibition of melatonin synthesis. *J. Neuroendocrinol*., **7,** 207–214.

McCann, S.M. (1982) Physiology and pharmacology of LHRH and somatostatin. *Annu. Rev. Pharmacol. Toxicol.*, **22,** 491–515.

McCann, S.M. and Rettori, V. (1996a) Gamma amino butyric acid (GABA) control anterior pituitary hormone secretion. In Racagni, G. and Donoso, O.A. (eds), *Adv. Biochem. Psycopharmacol.*, vol. 42. Raven Press, New York, pp. 173–189.

McCann, S.M. and Rettori, V. (1996b) The role of nitric oxide in reproduction. *Soc. Exp. Biol. Med.*, **211**, 7–15.

McDonald, L.J. and Moss, J. (1993) Stimulation by nitric oxide of an NAD linkage to glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. USA*, **90,** 6238–6241.

Melis, M.R., Stancampiano, R. and Argiolas, A. (1994a) Nitric oxide synthase inhibitors prevent N-methyl-D-aspartic acid-induced penile erection and yawning in the male rat. *Neurosci. Lett.*, **179,** 9–12.

Melis, M.R., Stancampiano, R. and Argiolas, A. (1994b) Prevention by NG-nitro-L-arginine methyl ester of apomorphine and oxytocin-induced penile erection and yawning: site of action in the brain. *Pharmacol. Biochem. Behav.*, **48,** 799–804.

Melis, M.R., Succu, S. and Argiolas, A. (1996) Dopamine agonist increase nitric oxide production in the paraventricular nucleus of the hypothalamus: correlation with penile erection and yawning *Eur. J. Neurosci.*, **10,** 2056–2063.

Michel, T., Li, G.K. and Busconi, L. (1993) Phosphorylation and subcellular translocation of endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. USA*, **90,** 6252–6256.

Milstein, S., Jaffe, H., Kowlessur, D.N. and Bonner, T.I. (1996) Purification and cloning of the GTP cyclohydrolase I feedback regulatory protein GFRP. *J. Biol. Chem.*, **271,** 19743–19751.

Mitchell, H.H., Shonle, H.A. and Grindley, H.S. (1916) The origin of the nitrates in the urine. *J. Biol. Chem.*, **24,** 461–490.

Mohr, S., Stamler, J.S. and Brune, B. (1996) Post translational modification of glyceraldehyde-3-phosphate dehydrogenase by S-nitrosylation and subsequent NADH attachment. *J. Biol. Chem.*, **271,** 4209–4214.

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991) Nitric oxide physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43,** 109–142.

Mori, M. (1996) Coinduction of nitric oxide synthase, arginosuccinate synthetase, and arginosuccinate lyase in lipolisaccharide-treated rats. RNA blot, immunoblot, and immunochemical analyses. *J. Biol. Chem.*, **271,** 2658–2662.

Morris, S.M. Jr. (1992) Regulation of enzymes of urea and arginine synthesis. *Annu. Rev. Nutr.*, **12,** 81–101.

Morris, S.M. and Billiar, T.R. (1994) New insights into the regulation of inducible nitric oxide synthesis. *Am. J. Physiol.*, **266,** E829–E839.

Moss, R.L. and McCann, S.M. (1973) Induction of mating behavior in rat by luteinizing hormone releasing factor. *Science*, **181,** 177–179.

Mulsch, A., Mordvintcev, P.I., Vanin, A.F. and Busse, R. (1993) Formation and release of dinitrosyl iron complexes by endothelial cells. *Biochem. Biophys. Res. Commun.*, **196,** 1303–1308.

Murad, F. (1994) Regulation of cytosolic guanylyl cyclase by nitric oxide: the NO–cyclic GMP signal transduction system. *Adv. Pharmacol.*, **26,** 19–33.

Murphy, M.E., Piper, H.M., Watanabe, H. and Sies, H. (1991) Nitric oxide production by cultured aortic endothelial cells in response to thiol depletion and replenishment. *J. Biol. Chem.*, **266,** 19378–19383.

Myatt, L., Brockman, D.E., Eis, A.L.W. and Pollock, J.S. (1993) Immunohistochemical localization of nitric oxide synthase in the human placenta. *Placenta*, **14,** 487–495.

Myatt, L., Rosenfield, R.B., Brockman, D.E. *et al.* (1996) Nitrotyrosine residues in placenta. Evidence of peroxynitrite formation and action. *Hypertension*, **28,** 488–493.

Myatt, L., Eis, A.L.W., Brockman, D.E. *et al.* (1997) Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies. *Hum. Reprod.*, **12,** 167–172.

Nagasaki, A., Gotoh, T., Takeya, M. *et al.* (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J.*, **6,** 3051–3064.

Nagasaki, A., Gotoh, T., Takeya, M. *et al.* (1996) Coinduction of nitric oxide synthase, arginosuccinate synthetase, and arginosuccinate lyase in lipopolysaccharide-treated rats. RNA blot, immunoblot, and immunohistochemical analyses. *J. Biol. Chem.*, **271,** 2658–2662.

Nakayama, D.K., Geller, D.A., Di Silvio, M. *et al.* (1994) Increased activity of de novo tetrahydrobiopterin synthesis in pulmonary artery smooth muscle cells stimulated to produce nitric oxide. *Am. J. Physiol.*, **266,** L455–L460.

Nathan, C. (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J.*, **6,** 3051–3064.

Nathan, A.K. and Xie, Q.W. (1994) Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.*, **269,** 13275–13278.

Natuzzi, E.S., Ursell, P.C., Harrison, M. *et al.* (1993) Nitric oxide synthase activity in pregnant uterus decreases at parturition. *Biochem. Biophys. Res. Commun.*, **15,** 1–8.

Navarro, C.E., Cabrera, R.J. and Donoso, A.O. (1994) Release of H3-noradrenaline by excitatory amino acids from rat mediobasal hypothalamus and the influence of aging. *Brain Res. Bull.* **33,** 677–682.

Negro-Vilar, S.R., Conte, D. and Valenca, M. (1986) Transmembrane signals mediating neural peptide secretion. *Endocrinology*, **119,** 2796–2802.

Nelson, R.J., Demas, G.E., Huang, P.L. *et al.* (1995) Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature*, **23,** 336.

Nussler, A.K. and Billiar, T.R. (1993) Inflammation, immunoregulation and inducible nitric oxide synthase. *J. Leukocyte Biol.*, **54,** 171–178.

Nussler, A.K., Billiar, T.R., Liu, Z.-Z. and Morris, S.M. (1994) Coinduction of nitric oxide synthase and arginosuccinate synthetase in a murine macrophage cell line. Implications for regulation of nitric oxide production. *J. Biol. Chem.*, **269,** 1257–1261.

Ojeda, S.R., Negro-Vialr, A. and McCann, S.M. (1979) Release of prostaglandins Es by hypothalamic tissue: evidence for their involvement in catecholamine-induced LHRH. *Endrocrinology*, **104,** 617–624.

Ojeda, S.R., Urbanski, H.F., Katz, K.H. *et al.* (1986) Activation of two different but complementary pathways stimulates release of LHRH. *Proc. Natl. Acad. Sci. USA*, **83,** 4932–4936.

Palmer, R.M.J., Ferrige, A.G. and Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxation factor. *Nature*, **327,** 524–526.

Palmer, R.M.J., Ashton, D.S. and Moncada, S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **33,** 664–668.

Papp, G., Graf, J., Molnar, J. and Jambor, E. (1979) Importance of arginine content and arginase activity in infertility. *Andrologia*, **27,** 282–283.

Peng, A.T., Gorman, S.M., Shulman, S.M. *et al.* (1989) Intravenous nitroglycerin for uterine relaxation in the postpartum patient with retained placenta. *Anesthesiology*, **71,** 172–173.

Penson, D.F., Ng, C., Cai, L. *et al.* (1996) Androgen and pituitary control penile nitric oxide synthase and erectile function in rat. *Biol. Reprod.*, **55,** 567–574.

Pou, S., Pou, W.S., Bredt, D.S. *et al.* (1992) Generation of superoxide by purified brain nitric oxide synthase. *J. Biol. Chem.*, **267,** 173–176.

Powers, R.W., Chen, L., Russel, P.T. and Larse, W.J. (1995) Gonadotropin-stimulated regulation of blood–follicle barrier is mediated by nitric oxide. *Am. J. Physiol.*, **269,** E290–E298.

Rachman, I.M., Pfaff, D.W. and Cohen, R.S. (1996) NADPH diaphorase activity and nitric oxide synthase immunoreactivity in lordosis relevant neurons of the ventromedial hypothalamus. *Brain Res.*, **740,** 291–306.

Regazzi, E., Chinellato, A., Italiano, G. *et al.* (1996) Characterization of in vitro relaxant mechanism in erectile tissue from rabbits of different ages. *Urol. Res.*, **24,** 317–322.

Rehman, J., Chenven, E., Brink, P. *et al.* (1997) Diminished neurogenic but not pharmacological erections in the 2 to 3 month experimentally diabetic F344 rat. *Am. J. Physiol.*, **41,** H1960–H1971.

Rettori, V., Gimeno, M., Lyson, K. and McCann, S.M. (1992) Nitric oxide mediates norepinephrine-induced prostaglandin E2 release from the hypothalamus. *Proc. Natl. Acad. Sci. USA*, **89,** 11543–11546.

Rettori, V., Belova, N., Dees, W.L. *et al.* (1993**)** Role of nitric oxide in the control of luteinizing hormone-releasing hormone release in vivo and in vitro. *Proc. Natl. Acad. Sci. USA*, **90,** 10130–10134.

Rettori, V., Kamat, A. and McCann, S.M. (1994) Nitric oxide mediates the stimulation of luteinizing-hormone releasing hormone releasin induced by glutamic acid in vitro. *Brain Res. Bull.*, **33,** 501–503.

Rettori, V., Canteros, G., Renoso, R. *et al.* (1997) Oxytocin stimulates the release of luteinizing hormone releasing hormone from medial basal hypothalamic explants by releasing nitric oxide. *Proc. Natl. Acad. Sci. USA*, **94,** 2741–2744.

Riemer, R.K., Buscher, C., Bansal, R.K. *et al.* (1997) Increased expression of nitric oxide synthase in the myometrium of pregnant rat uterus. *Am. J. Physiol.*, **272**, E1008–E1015.

Rogers, N.E. and Ignarro, L.J. (1992) Constitutive nitric oxide synthase from cerebellum is reversibly inhibited by nitric oxide formed from L-arginine. *Biochem. Biophys. Res. Commun.*, **189,** 242–249.

Rosselli, M., Imthurn, B., Macas, E. *et al.* (1994a) Circulating nitrite/nitrate increase with follicular development: indirect evidence for estradiol mediated release. *Biochem. Biophys. Res. Commun.*, **202,** 1543–1552.

Rosselli, M., Imthurn, B., Macas, E. *et al.* (1994b) Endogenous nitric oxide modulates endothelin-1 induced contraction in bovine oviduct. *Biochem. Biophys. Res. Commun.*, **201,** 143–148.

Rosselli, M., Dubey, R., Imthurn, B. *et al.* (1995) Effect of nitric oxide on human spermatozoa: evidence that nitric oxide decreases sperm motility and induces sperm toxicity. *Hum. Reprod.*, **10,** 1786–1790.

Rosselli, M., Dubey, R.K., Rossellli, M.A. *et al.* (1996) Identification of nitric oxide in human and bovine oviduct. *Mol. Hum. Reprod.*, **2,** 607–612.

Sakuma, Y. and Pfaff, D.W. (1983) Modulation of lordosis reflex of female rats by LHRH, its antiserum and analogs in mesencephalic central gray. *Neuroendocrinology*, **36,** 218–224.

Salvemini, D. (1997) Regulation of cyclooxygenase enzymes by nitric oxide. *Cell. Mol. Life Sci.*, **53,** 576–598.

Sax, H.C., Hasselgren, P.O., Talamini, M.A. *et al.* (1988) Amino acid uptake in isolated, perfused liver: effect of trauma and sepsis. *J. Surg. Res.*, **45,** 50–55.

Schaad, N.C., Zhang, X.Q., Campana, A. and Schroderte-Slatkine, S. (1996) Human seminal plasma inhibits brain nitric oxide synthase activity. *Hum. Reprod.*, **11,** 561–565.

Seilcovich, A., Duvilanski, B.H., Pisera, D. *et al.* (1995) Nitric oxide inhibits hypothalamic luteinizing hormone-releasing hormone release by releasing gamma-aminobutyric acid. *Proc. Natl. Acad. Sci. USA*, **92,** 3421–3424.

Sessa, W.C. (1994) The nitric oxide synthase family of proteins. *J. Vasc. Res.*, **31,** 131–143.

Shukorski, L. and Tsafriri, A. (1994) The involvement of nitric oxide in the ovulatory process in the rat. *Endocrinology,* **135,** 2287–2290.

Siddhanta, U., Wu, C., Abu-Soud, H.M. *et al.* (1996) Heme iron reduction and catalysis by a nitric oxide synthase heterodimer containing one reductase and two oxygenase domains. *J. Biol. Chem.*, **271,** 7309–7312.

Sladek, S.M., Regenstein, A.C., Lykins, D. and Roberts, J.M. (1993) Nitric oxide synthase in pregnant rabbit uterus decreases on the last day of pregnancy. *Am. J. Obstet. Gynecol*., **169,** 1285–1291.

Sladek, S.M., Magness, R.R. and Conrad, K.P. (1997) Nitric oxide and pregnancy. *Am. J. Physiol. Reg. Integ. Comp. Physiol.*, **41,** R441–R463.

Snyder, S.H. (1995) Nitric oxide: NO endothelial NO. *Nature*, **377,** 196–197.

Stamler, J.S., Jaraki, O., Osborne, J. *et al.* (1992) Nitric oxide circulates in mammalian plasma primarily as a S-nitroso adduct of serum albumin. *Proc. Natl. Acad. Sci. USA*, **89,** 7674–7676.

Stephan, J.P., Guillemois, C., Jegou, B. and Bauche, F. (1995) Nitric oxide production by Sertoli cells in response to cytokines and lipolisaccharide. *Biochem. Biophys. Res. Commun.*, **312,** 218–224.

Stief, C.G., Holmquist, F., Djamilian, M. *et al.* (1992) Preliminary results with the nitric oxide donor linsidomine chlorhydrate in the treatment of human erectile dysfunction. *J. Urol.*, **148,** 1437–1440.

Stuehr, D.J. and Marletta, M.A. (1985) Mammalian nitrate biosynthesis: mouse macrophages produce nitrate and nitrite in response to Escherichia coli lipopolysaccharide. *Proc. Natl. Acad. Sci. USA*, **82,** 7738–7742.

Su, Z., Blazing, M.A., Fan, D. and George, S.E. (1995) The calmodulin–nitric oxide synthase interaction: critical role of the calmodulin latch domain in enzyme activation. *J. Biol. Chem.*, **270,** 29117–29122.

Sugino, N., Takiguci, S., Ono, M. *et al.* (1996) Nitric oxide concentration in the follicular fluid and apoptosis of granulosa cells in human follicles. *Hum. Reprod*., **11,** 2484–2487.

Szabo, C., Zingarelli, B. and Salzman, A.L. (1996) Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ. Res.*, **78,** 1051–1063.

Tabraue, C., Penate, R.D., Gallardo, G. *et al.* (1997) Induction of guanosine triphosphate cyclohydrolase by follicle stimulating hormone enhances interleukin 1 beta stimulated nitric oxide synthase activity in granulosa cells. *Endocrinology,* **138,** 162–168.

Tannenbaum, S.R., Fett, D., Young, V.R. *et al.* (1928) Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science*, **200,** 1487–1489.

Tatsumi, N., Fujisawa, M., Kanzaki, M. *et al.* (1997) Nitric oxide production by cultured rat Leydig cells. *Endocrinology,* **138,** 994–998.

Telfer, J., Lyall, F., Norman, J.E. and Cameron, I.T. (1995) Identification of nitric oxide synthase in human uterus. *Hum. Reprod*., **10,** 19–23.

Telfer, J.F., Irvine, G.A., Kohnene, G. *et al.* (1997) Expression of endothelial and inducible nitric oxide synthase in non pregnant and decidualized human endometrium. *Mol. Hum. Reprod.*, **3,** 69–76.

Tomlinson, M.J., East, S.J., Barratt, C.L. *et al.* (1992) Preliminary communication: possible role of reactive nitrogen intermediates in leucocytes-mediated sperm dysfunction. *Am. J. Reprod. Immunol.*, **27,** 89–92.

Tzeng, E., Billiar, T.R., Robbins, P.D. *et al.* (1995) Expression of human inducible nitric oxide synthase in a tetrahydrobiopterin (H_4B)-deficient cell line: H_4B promotes assembly of enzyme subunits into an active dimer. *Proc. Natl. Acad. Sci. USA*, **92,** 11771–11775.

Vanhatalo, S. and Soinila, S. (1995) Direct nitric oxide-containing innervation from rat spinal cord to the penis. *Neurosci. Lett.*, **199,** 45–48.

Vanhatalo, S., Klinge, E., Sjostrand, N.O. and Soinila, S. (1996) Nitric oxide-synthesizing neurons originating at several different levels innervate rat penis. *Neuroscience*, **75,** 891–899.

Van Voorhis, B.J., Dunn, M.S., Snyder, G.A. and Weiner, C.P. (1994) Nitric oxide: an autocrine regulator of human granulosa–luteal cell steroidogenesis. *Endocrinology,* **135,** 1799–1806.

Van Voorhis, B.J., Moore, K., Strijbos, P.L.M. *et al.* (1995) Expression and localization of inducible and endothelial nitric oxide synthase in the rat ovary. *J. Clin. Invest.*, **96,** 2719–2726.

Veille, J.C., Li, P., Eisenach, J.C. *et al.* (1996) Effects of estrogen on nitric oxide biosynthesis and vasorelaxant activity in sheep uterine and renal arteries in vitro. *Am. J. Obstet. Gynecol.*, **174,** 1043–1049.

Vernet, D., Cai, L., Garban, H. *et al.* (1995) Reduction of penile nitric oxide synthase in diabetic BB/WORdp (type I) and BBZ/WORdp (type II) rats with erectile dysfunction. *Endocrinology,* **136,** 5709–5717.

Wang, Y., Goligorsky, M.S., Lin, M. *et al.* (1997) A novel, testis specific mRNA transcript encoding an NH2 terminal truncated nitric oxide synthase. *J. Biol. Chem.*, **272,** 11392–11401.

Weiner, C.P., Lizasoain, I., Baylis, S.A. *et al.* (1994) Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proc. Natl. Acad. Sci. USA*, **19,** 5212–5216.

Welch, C., Watson, M.E., Poth, M. *et al.* (1995) Evidence to suggest nitric oxide is an interstitial regulator of Leydig cell steroidogenesis. *Metabolism*, **44,** 234–238.

Werner-Felmayer, G., Werner, E.R., Fuchs, D. *et al.* (1990) Tetrahydrobiopterin-dependent formation of nitrite and nitrate in murine fibroblasts. *J. Exp. Med.*, **172,** 1599–1607.

Werner-Felmayer, G., Werner, E.R., Fuchs, D. *et al.* (1993) Pteridine biosynthesis in human endothelial cells. Impact on nitric oxide-mediated formation of cyclic-GMP. *J. Biol. Chem.*, **268,** 1842–1846.

Xia, Y., Dawson, V.L., Dawson, T.M. *et al.* (1996) Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc. Natl. Acad. Sci. USA*, **93,** 6770–6774.

Xia, Y., Garban, H., Ng-, C. *et al.* (1997) Effect of long-term passive smoking on erectile function and penile nitric oxide synthase in the rat. *J. Urol.*, **157,** 1121–1126.

Xu, X., Star, R.A., Tortorici, G. and Muallem, S. (1994) Depletion of intracellular Ca^{2+} stores activates NOS to generate cGMP and regulate Ca^{2+} influx. *J. Biol. Chem.*, **269,** 12645–12653.

Yallampalli, C., Garfield, R.E. and Byam-Shmith, M. (1993) Nitric oxide inhibits uterine contractility during pregnancy but not during delivery. *Endocrinol*ogy, **133,** 1899–1902.

Yallampalli, C., Izumi, H., Byam-Smith, M. and Garfield, R.E. (1994) An L-arginine-nitric oxide guanosine monophosphate system exists in the uterus and inhibits contractility during pregnancy. *Am. J. Obstet. Gynecol.*, **170,** 175–185.

Yallampalli, C., Dong, Y.L. and Wimalawansa, S.J. (1996) Calcitonin gene-related peptide reverses the hypertension and significantly decreases fetal mortality in pre-eclampsia rats induced by N-nitro-L-arginine methyl ester. *Hum. Reprod*., **11,** 895–899.

Yu, Y., Tereda, K., Nagasaki, A. *et al.* (1995) Preparation of recombinant arginosuccinate synthetase and arginosuccinate lyase: expression of the enzyme in rat tissue. *J. Biochem., Tokyo*, n, 952–957.

Zackrisson, U., Mikuni, M., Wallin, A. *et al.* (1996) Cell specific localization of nitric oxide synthases (NOS) in the rat ovary during follicular development, ovulation and luteal formation. *Hum. Reprod.*, **11,** 2667–2673.

Zini, A., O'Bryan, M.K., Magid, M.S. and Schlegel, P.N. (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.

Zvara, P., Sioufi, R., Schipper, H.M. *et al.* (1995) Nitric oxide mediated penile erection is a testosterone dependent event: a rat erection model. *Int. J. Impotence Res*., **7,** 209–219.

Received on August 11, 1997; accepted on January 7, 1998