From Nitric Oxide Toward S-Nitrosylation: Expanding Roles in Gametes and Embryos

Ješeta Michal, Marketa Sedmikova, Jean-Francois Bodart

Chapter 9

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Abstract

Nitric oxide (NO) is a gasotransmitter involved in various aspects of reproduction. The observational data from different species, such as sea urchin, ascidians, amphibians, rodents, porcine, bovine, and human, suggest that NO might have a significant role in reproduction through several mechanisms. This proposed role might appear preserved through evolution; however, the effects of NO also depend on the species or stages considered. There has been debate over the physiological relevance of NO, though the benefits of its use in assisted reproduction are now widely recognized. Over the past years, S-nitrosylation has provided a new angle to decipher the mechanisms through which NO exerts its actions. This chapter summarizes, in a nonexhaustive manner, research that explores the role of NO in gametes and embryos.

Keywords: nitric oxide, gamete, meiosis, oocyte, spermatozoa, nitrosylation, cell cycle, embryo, reproduction

1. Introduction

Nitric oxide (NO) is a gaseous free radical that plays a key role both in intra- and extracellular signaling pathways in a wide variety of organisms. The role of NO has been emphasized in many physiological processes including reproduction. NO is generated by nitric oxide synthases (NOS), whose isoforms have been detected in a variety of mammalian reproductive tissues such as ovary, uterus, testis, or epididymis. Nitric oxide has been involved in the regulation of follicle growth and ovulation in mice, spermatogenesis in humans, embryo implantation in rats, and meiosis in pigs and in mice. Data collected from the various abovementioned

species suggest that NO might have a significant role in reproduction through mechanisms preserved through evolution; however, one cannot discard that the effects of NO may also be dependent on the species or stages considered. Therefore, nitric oxide could be considered as a gasotransmitter ruling out several aspects of reproduction, from gametes to early embryogenesis, though there have been a debate over the physiological relevance of NO. This chapter summarizes, in a nonexhaustive manner, the research that explores the role of NO in gametes and embryos.

2. NO in sea urchin

Nitric oxide was first reported to trigger parthenogenetic activation in sea urchin oocytes and was suggested as a potential physiological regulator for egg activation. Twenty years ago, an increase in NO levels at fertilization was reported [1, 2], and NO was hypothesized as the primary activator for sea urchin egg activation [3]. Enthusiasm has been lately shaded by a report of NO increases occurring lately during fertilization in comparison to the rise of intracellular calcium level [4, 5]. From this observation, it has been suggested that the role of NO could be limited to sustaining the duration of the calcium transient [4]. NO increases are likely correlated to the calcium increases but not by being its primary activator [4, 5]. Nevertheless, NO increases may play a role in the hardening of the fertilization envelope surrounding the fertilized egg, thereby protecting the embryo from severe environmental conditions during its early development [5].

3. NO in ascidians oocytes, eggs, and embryos

In contrast to the observations performed in sea urchins, Hyslop et al. [6] reported that NO was not likely to be involved in the physiological process of fertilization of *Ascidiella aspersa* eggs. This report was in contrast to a previous study examining the inward current induced by NO, sharing similarities with the ascidians sperm current, which suggested that fertilization in ascidians could use NO as a second messenger [7]. Though nitric oxide donors induce increases in free calcium in ascidians and sea urchin eggs at the intracellular level, NOS inhibitor L-NAME (N(G)-nitro-L-arginine methyl ester) did not prevent sperm-induced fertilization, and not so much as a discrete increase in NO preceded the calcium wave [6].

However, NO pathways and production were related to metamorphosis, notochord, and tail regression in *Ciona intestinalis*, which are correlated to caspase-dependent apoptosis [8]. The nitric oxide synthase spatial pattern expression was highly dynamic during this larval development [8]. Experimental increases and decreases in NO levels can drive delays or accelerations in tail regression, respectively, with NO changes being related to the modulation of Caspase 3-like activity [8]. In most of the considered marine larvae of ascidians, NO acted as a repressor of the initiation of metamorphosis [9, 10]. Further works had been undertaken in *C. intestinalis* to unravel the role of NO during larval development. Mitogen-activated

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protein kinase (MAPK) and extracellular-regulated kinase (ERK) phosphorylations levels appeared closely related to NO levels [11].

In conclusion, the fine tuning of NO pathways and levels are physiologically involved in ascidian larvae development and metamorphosis. However, NO did not seem to be required at very early steps of development.

4. NO in amphibian oocytes, eggs, and embryos

Amphibian oocytes offer a typical playground for deciphering the biochemical mechanisms underlying cell cycle transition. Meiosis or the M-phase–promoting factor (MPF) was discovered in amphibian oocytes [12] and characterized in this model to be made up of at least two subunits: Cdk1 (catalytic) and Cyclin B (regulatory) [13]. In *Xenopus* oocytes, NO-scavenging did not appear to impair the progression of M-phase entry and meiotic maturation because CPTIO (nitric oxide scavenger)-treated oocytes resumed and completed meiosis after hormonal stimulation by progesterone [14]. If NO is not required for meiotic progression, an excess in NO provided by a donor, such as S-nitroso-N-acetyl penicillamine (SNAP), leads to meiotic maturation inhibition. Under these conditions, SNAP lead heterogeneous response at the biochemical level with respect to the two pathways involved in meiotic resumption (MPF and MAPK). SNAP hindered the all-or-none response of MPF and MAPK pathways, especially in *Xenopus* oocytes; most oocytes exhibited partially active MPF and MAPK in the absence of any external signs of meiotic resumption [15]. Noticeably, SNAP altered meiotic spindle formation, therefore impairing proper genomic transmission [15]. This observation corroborates reports of mitosis inhibition through Tyrosine residue nitrosylation in plant models, and Alteration of cross walls orientation, presumably by impairing microtubules organization [16, 17]. Nevertheless, the mechanisms hindered by NO in vertebrate oocyte spindle formations remain undetermined.

Studies carried out in amphibians reported parthenogenetic activation of *Xenopus* eggs with nitric oxide donor SNAP. This parthenogenetic activation induced M-phase exit through an atypical mechanism involving calcium-dependent pathway and MAPK inactivation, while MPF activity was maintained [14] (**Figure 1A**).

During early development, the *Xenopus* nitric oxide synthase 1 (XNOS1) accounts for most of the NOS detected, being expressed in oocytes and eggs during segmentation [18]. At later stages, XNOS1 expression is restricted to the notochord, eyes, and developing neural system [18]. Exploration of NO function in *Xenopus* oocytes indicates that NO increased cell proliferation but impaired cell movements at gastrulation. Inhibition of NOS affected cell movements both in neural and mesodermal extension, through cGMPS-independent pathways involving dishevelled (Dsh) and the central components of the planary cell polarity (PCP) pathway [18]. Cell division during early development has been proposed according to this model to be impacted by NO through the ROCK-cGMP pathway (**Figure 1B**).

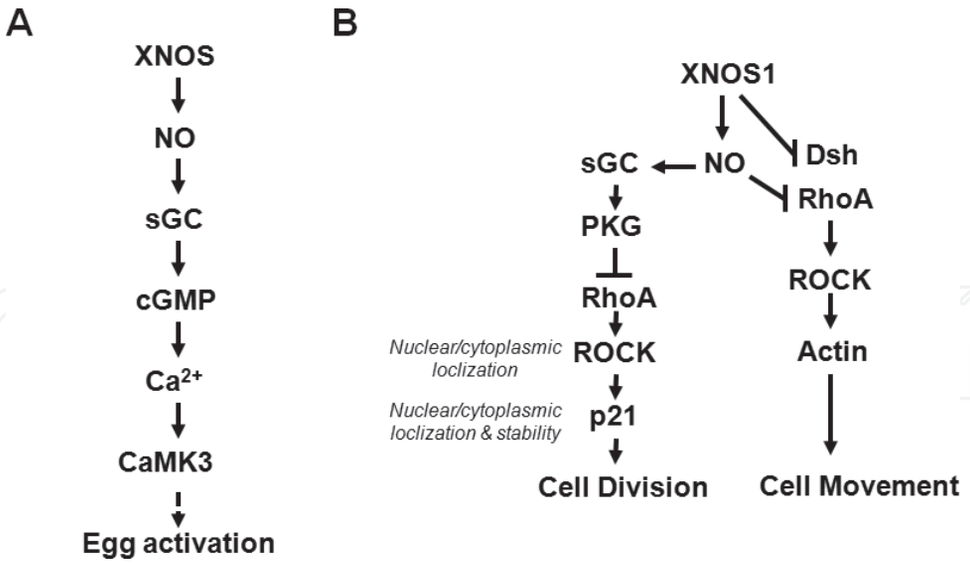

Figure 1. (A) Proposed mechanisms for NO action for parthenogenetic activation in *Xenopus* eggs. (B) Schematic representation of the pathways mediating the action of nitric oxide during early development in *Xenopus* embryos. Adapted from reference [18]. NO, nitric oxide; XNOS, *Xenopus* nitric oxide synthase; sGC, soluble guanylyl cyclase; cGMP, cyclic guanosine monophosphate; CaMK3, calmodulin kinase 3; Dsh, disheveled; PKG, protein kinase G.

5. NO in rodent oocytes, eggs, and embryos

Observations gathered in *Xenopus* oocytes may be to put in perspective with earlier reports endothelial NOS (eNOs) knock-out mice, in which meiotic abnormalities suggested that eNOS-derived NO is a modulator of oocyte meiotic maturation [19]. Indeed, the ovulation rates decreased in eNOS nullizygous mice, and oocytes often exhibited blocks during metaphase I or indicated various meiotic abnormalities with degenerative/atypical morphology of meiotic stages [19]. One should also note that in such conditions, oocytes from eNos nullizygous mice exhibit a higher rate of cell death than those in control ones. The importance of NOS for rodent oocyte meiotic maturation was confirmed by the expression of NOS isoforms in mouse or rat oocytes [20–22]. If NO was acknowledged to be important for meiotic maturation, high concentrations of NO can damage mouse oocyte and impair their further development [23]. Positive effects of low doses of NO donor SNAP during *in vitro* maturation was observed in mouse [24] and rat [25] oocytes. NO has been reported to play a dual role in oocyte meiotic maturation in mice, depending on its concentrations; however, the mechanism by which it influences oocyte maturation has not been fully clarified. In addition to these results, reports have shown that NO was most likely not essential for mouse oocyte fertilization [6].

Though NO is not a primary stimulus for oocyte activation through calcium mobilization, as observed in sea urchin and ascidians, it has been reported to play several potential roles during embryogenesis. The NO donor SNP brought about the arrest of embryonic development at early stages in mice; only half of the treated embryos reached blastocysts stages, with concentrations ranging from 10 nM to 1 mM [26]. Inhibition of NO production also suggested that NO plays a role in preimplantation embryos, which can be achieved through oxygen consumption

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limitation and mitochondrial activity or apoptosis modulation [27, 28]. Recently, it has been suggested that NO, through the use of the NO donor SNP and NOS inhibitor L-NAME, may regulate blastocyst hatching, which is a crucial step for embryo survival and implantation [29].

6. NO in porcine oocytes, eggs, and embryos

The production of low concentrations of NO concentrations has been reported to be necessary for meiotic maturation in porcine oocytes, whereas high concentrations of NO damage oocytes integrity [30]. Inhibition of NO synthase suppressed *in vitro* maturation of porcine oocytes, as attested by the absence of GVBD or meiosis I to meiosis II transitions [31]. NO synthases were detected in porcine oocytes [32, 33], but the involvement of NO/NOS in oocyte development has not been fully elucidated. Previously, it was assumed that NO acted *via* the cGMP cascade, similar to mechanisms observed for muscle contractions [19], or *via* the cascade-activating kinases, which are necessary for the resumption of meiotic maturation [34] (**Figure 2**). Nitric oxide had been reported to act on NO-sensitive guanyl cyclases, but new articles suggest that NO could also exert its functions by non-cGMP-mediated pathways by protein S-nitrosylation. It was reported that NOS inhibition decreases the amount of S-nitrosylated proteins in porcine oocytes [35].

Figure 2. Scheme of NO/GMPc/PKG pathway in porcine oocytes and likely impact on basic regulation factor of meiosis, MPF. Nitric oxide synthase (NOS) increase enhances the concentration of nitric oxide (NO) in oocyte. NO stimulates the activity of soluble guanylyl cyclase (sGC), which catalyzes the production of cyclic guanosine monophosphate (cGMP). cGMP is necessary for protein kinase G (PKG) activation, which in turn suppresses MPF activation, and therefore, impairs meiosis resumption and maturation.

In porcine oocytes, NO donors are potent to induce parthenogenetic activation, resulting in early embryonic development [36]. NO production is also important for embryonic development since the NOS inhibitor L-NAME strongly decreases the proportion of porcine embryo blastocysts after 6 days of *in vitro* cultivation [37].

7. NO in bovine oocytes, eggs, and embryos

As well as in porcine oocytes, nitric oxide synthases (NOS) were detected in bovine oocytes [38]. Oocyte maturation was noticeably inhibited by the use of NOS inhibitors [39], which strengthens the hypothesis that NO plays a role during oocyte meiosis in this model. Regarding the molecular mechanisms involved, Bilodeau-Goeseels was first to suggest that the inhibitory effect of NO on bovine oocyte meiotic resumption did not appear to be mediated by the cGMP/PKG pathway; this is in contrast to previous observations gathered in mice [40].

Inhibition of NOS during maturation of bovine oocytes affected the quality of resulting bovine embryos by increasing the number of apoptotic blastomeres [41]. The importance of NO for correct embryonic development was observed, mainly for transition from morulae to the blastocyst stage. Treatment of embryos with the NOS inhibitor L-NAME reduced blastocysts numbers and hatching rates [42]. Contrarily, exposing bovine mature oocytes to a nitric oxide donor short term did not induce stress tolerance and had no positive effect on the *in vitro* embryo production of bovine embryos, as was expected [43].

8. A role for NO in human follicles?

Only a limited number of studies have addressed NO in human reproduction. Anteby et al. [44] observed an increase in NO concentrations in follicular fluid from bigger follicles, which positively correlated follicular volume and oestradiol concentrations. NO most likely acts as an important endocrinological regulator of ovulation. NO may be involved in an autocrine/paracrine regulation of the developing follicle and have a direct effect on granulosa cells, theca cells, and the developing oocyte. In a recent study, a possible association between idiopathic recurrent spontaneous abortion and variations in the gene encoding endothelial nitric oxide synthase was proposed [45].

9. NO roles in spermatozoa

In spermatozoa, NO was described to be involved in the regulation of viability, motility, capacitation, hyperactivation, acrosome reaction, and fusion with oocytes. Thus nitric oxide appears to be crucial for the processes driving successful fertilization (reviewed in [46, 47]). NOS isoforms were found in sperm of different mammal species such as mice [48], bulls [49], boars [50], and humans [51]. The abovementioned and well-known duality of NO's effects, depending upon concentrations, was also described in spermatozoa. Low levels of NO stimulate hyperactivation and increase motility of cryopreserved sperm after thawing [52]; conversely, high concentrations of NO decrease sperm motility [46, 53, 54] and inhibit sperm-oocyte fusion [55].

NO was reported to have a positive impact on sperm motility [55], whereas NO donor increased sperm motility [56, 57], inhibition of nitric oxide synthases by L-NAME negatively affected this motility [58]. Miraglia and colleagues [53] observed the existence of NO signaling pathways in human spermatozoa. NO stimulates sperm motility *via* the activation of soluble guanylate cyclase (sGC), the subsequent synthesis of cGMP, and the activation of cGMP-dependent protein kinase. The level of cGMP is modulated by cGMP-dependent phosphodiesterase (PDE). These observations concur with a former report that PDE inhibitor sidenafil citrate increased sperm motility [59]. NO is, on the other hand, considered a major free radical involved in sperm damage at sperm motility level. Nitrosative stress produced by high levels

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of reactive nitrogen species decreases progressive and total motility, as well as several sperm kinetic parameters, meanwhile, sperm viability is not affected [60, 61].

Sperm capacitation and acrosome reaction of mammal spermatozoa are essential for the fertilization process to occur. Both of them are NO-dependent. The final step in spermatozoa maturation, capacitation, involves a cascade of events such as the removal of cholesterol from plasma membrane, an influx of Ca^{2+} followed by an increase in intracellular cAMP levels, change of pH, and hyperactivation of sperm [62] (**Figure 3**). Acrosome reaction is a precondition for sperm fusion with the oocyte. It encompasses the release of proteolytic enzymes from the acrosome cap of the spermatozoa and an influx of Ca^{2+} and phosphorylation of tyrosine residues at the molecular level [63]. It has been reported that NO donors support acrosome reaction and accelerate capacitation and hyperactivation, whereas NOS inhibitors such as L-NAME significantly decrease or block this processes [46, 56, 58]. L-Arginin has similar effects as NO donors. Supplementation by L-arginin increases intracellular NO levels and supports sperm capacitation and acrosomal reaction without decreasing sperm viability [64]. Herrero et al. [65] also reported that capacitation was regulated by NO *via* cAMP levels and protein tyrosine phosphorylation. Moreover, it was proven that exogenous NO induces acrosomal reactions in human spermatozoa, and the process was mediated by the stimulation of a NO-sensitive sGC, cGMP synthesis, and the activation of PKC. However, the presence of extracellular Ca^{2+} was required for PKC activation in such conditions [66].

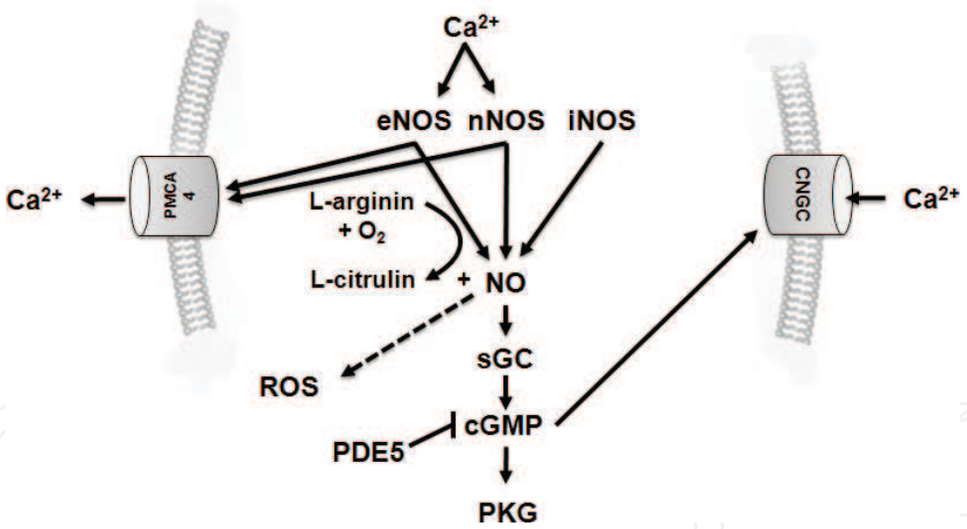

Figure 3. Scheme of NO/NOS role in sperm capacitation and acrosome reaction; eNOS—endothelial nitric oxide synthase; nNOS—neuronal nitric oxide synthase; iNOS—inducible nitric oxide synthase; sCG—soluble guanylyl cyclase; cGMP—cyclic guanosine monophosphate; PKG—protein kinase G; PDE5—phosphodiesterase 5; CNGC—cyclic nucleotide-gated channels; PMCA4—plasma membrane calcium ATPase 4; ROS—reactive oxygen species.

NO has the ability to improve the quality of freshly ejaculated sperm as well as thawed sperm. The NO donor sodium nitroprusside (SNP) was found to increase motility and viability of sperm after thawing and reduced membrane lipid peroxidation levels [57, 67].

10. NO effects on oocyte aging

Mammal oocytes are normally fertilized soon after the completion of meiotic maturation during the MII stage. If ovulated *in vitro,* matured oocytes are not fertilized, they undergo a process called aging, which is characterized by numerous changes. Oocyte aging rapidly decreases their quality and capacity to undergo embryonic development after fertilization. Functional and morphological changes associated with oocyte aging include decreased fertilization rates, polyspermy, parthenogenetic activation, apoptosis, chromosomal abnormalities, cortical granules exocytosis, ooplasmic microtubule dynamics, zona pellucida hardening, decreases in MPF and MAPK activities, epigenetic changes, and abnormal or delayed embryo development [24, 68–71]. Pathological conditions of oocyte aging impose limits for assisted reproduction technologies in animals as well as in humans [72].

It has been described that nitric oxide plays a part in oocyte aging, but it appears to do so by mobilizing more than one signaling pathway. NO can act either as a decelerating factor in oocyte aging [24] or, conversely, as an important cause of unwholesome aging-associated changes, which are caused by high ROS production [73].

Although high levels of NO are related mostly to pathological conditions, e.g., poor oocyte quality, increased protein nitration, and resistance to IVM in women with endometriosis [74], supplementing culture medium with low doses of the NO donor S-nitroso acetyl penicillamine (SNAP) delays manifestation of oocyte aging, *i.e.*, decrease of spontaneous cortical granule exocytosis, zona pellucida hardening, and the rate of spindle abnormalities [24]. The significance of NO in sustaining oocyte quality was demonstrated by Goud et al. [75]. Exposure of aged oocytes to L-NAME resulted in a significant disruption of fertilization and apoptosis during early embryonic development.

Different NOS isoforms could play different roles in aging. Lower NO levels produced by eNOS and nNOS could participate in delaying oocyte aging through the activation of sGC, which leads to an increased production of cyclic guanosine monophosphate [76]. However, high NO levels generated by iNOS were associated with higher $O_2^{\bullet-}$ production and promoted oocyte fragmentation and apoptosis [75]. Contrarily, Tripathi and colleagues [73] reported that the generation of NO through iNOS-mediated pathways was associated with the maintenance of meiotic arrest in diplotene-arrested oocytes and the sustained reduction of iNOS expression. Furthermore, they reported that intracellular NO level may induce apoptosis in aged rat oocytes cultured *in vitro*. Similarly, Nevoral et al. [77] described the suppression of apoptosis and lysis after prolonged cultivation of porcine oocytes in media supplemented by the NOS nonspecific inhibitor L-NAME. The decrease of intracellular levels of NO interrupts intracellular signal transduction pathways, especially Ca^{2+}-mediated pathways [75]. Premkumar and Chaube [78] reported that NO increases levels of cytosolic free Ca^{2+}, cGMP, and Wee 1 through an iNOS-mediated pathway. High levels of these signaling molecules trigger parthenogenetic activation of aged oocytes *via* the accumulation of phosphorylated Cdk1 (pThr-14/Tyr-15), a catalytic subunit of MPF. These findings indicate that NO can influence changes associated with oocyte aging in various manners.

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11. S-nitrosylation as a posttranslational modification potentially regulating cell cycle

Though cGMP pathway has been reported to be the main road for NO's involvement, evidences have been raised for c-GMP independent pathway, through protein S-nitrosylation (i.e., in porcine oocytes [35]). S-nitrosylation is an established posttranslation modification, whose potential spectra of involvements in cancer cell lines, oocytes, and embryos ranges from cell cycle regulation to embryo implantation [18, 79–81]. The effects of NO may differ among the cellular models considered. For instance, whereas low concentrations of NO donors DETA-NO promote cell proliferation in promyelotic HL-60 cells [82], nitric oxide synthase inhibition drives cell proliferation at blastula stages in *Xenopus* embryos [18]. Such a duality in the effects certainly rely on the diversity of S-nitrosylated proteins.

S-nitrosylation has been reported to modify several regulators of cell cycle progression (**Figure 4**), such as cyclin-dependent kinases (CDKs). CDKs' S-nitrosylation was observed for CDK2, CDK5, and CDK6. While CDK2-nitrosylation enhances its activity independently of any effects on protein levels expression [82], the effect of S-nitrosylation on CDK5 and CDK6 remains elusive. Though G2/M arrests might be observed associated with NO release [83, 84], no S-nitrosylation has been so far reported for Cdk1, the catalytic subunit of MPF. S-nitrosylation of cyclin B was seek in HL-60 cells, but not observed [82]. As well, no S-nitrosylations have yet been reported for polo-like kinases (PLKs), anaphase promoting factor/cyclosome (APC/C), Wee1, and Myt1, which are MPF regulators. Nevertheless, the dual specificity phosphatase Cdc25, which is the main activator of MPF, is clearly impacted by its S-nitrosylation because it annihilates its phosphatase activity [82, 87]. Recently, an

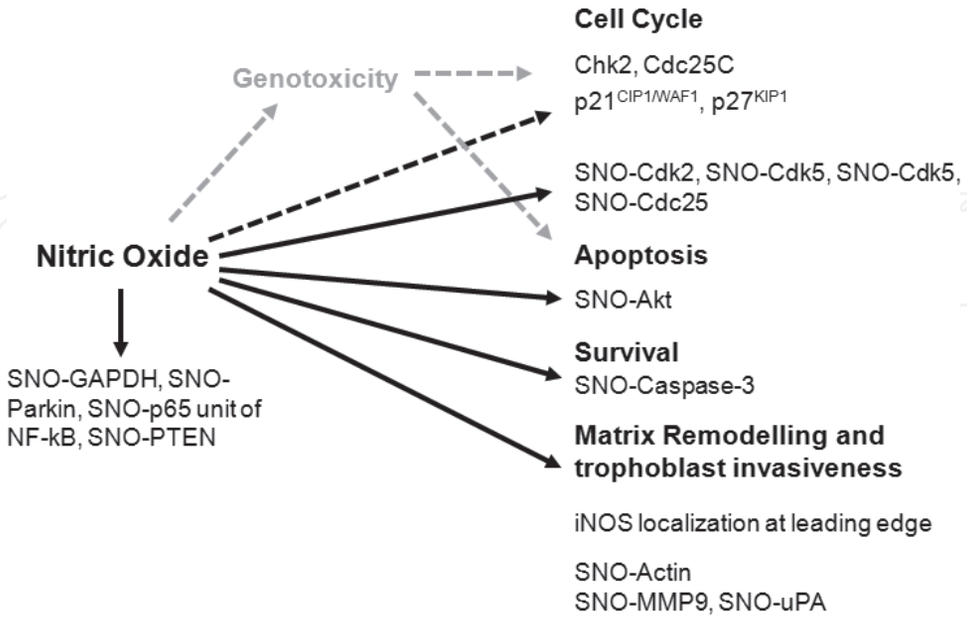

Figure 4. Comprehensive scheme of nitrosylation pivotal role in cell cycle, apoptosis, survival, matrix remodelling, and trophoblasts invasiveness.

alternate mechanism than direct S-nitrosylation of Cdc25C for its inhibition was proposed since NOAD, a nitric oxide-releasing derivative of oleanolic acid, induced activation of Chk2, resulting in an increase of the inhibitory phosphorylation of Cdc25C on its residue Serine 216 [84]. However, genotoxicity of nitric oxide might account for the activation of Chk2, the latter being involved in DNA damage response machinery. In the same study, the arrest in G2/M was associated with the upregulation of Cdk inhibitors $p21^{WAF1/CIP1}$ and $P27^{KIP1}$, without providing evidence for direct S-nitrosylation of these proteins. $p21^{WAF1/CIP1}$ downregulation by NO was also reported in *Xenopus* embryos, but through nitric oxide modulation of the RhoA-ROCK pathway [18] (**Figure 1B**). Thus, though there are converging evidences for role of NO in cell cycle regulation [85], the exact mechanisms remain to be fully deciphered.

12. S-nitrosylation plays pivotal role in modulating trophoblast motility and survival

As mentioned above, S-nitrosylation has been also called to play a role in preimplantation embryos and implantation (**Figure 4**). Microenvironnemental presence of NO was reported to contribute to the pathologic effects of endometriose on the development potential of embryos. In this context, NO effects on embryos survival could either rely upon S-nitrosylation, NO/GC/cGMP, or peroxynitrite formation. Lee and collaborators [28] suggested that the apoptotic effects of NO excess on mice embryos could be related to S-nitrosylation, in exclusion to other any mechanisms. The latter effects were closely associated with lipid-rich organelles (mitochondria and endoplasmic reticulum) [28, 86]. On the other hand, trophoblasts might also be protected from apoptosis *via* S-nitrosylation of caspase 3 [87].

Moreover, NO was shown to influence trophoblasts motility [88, 89]: it was further proposed that NO effects trophoblasts migration and invasion, which are critical processes for the successful embryonic development. In human trophoblasts, NO was required for outgrowth since L-NAME prevented this phenomenon in a dose-dependent manner [90]. Nevertheless, one shall keep in mind that high concentrations of NO in the environment have deleterious effects on trophoblasts outgrowth [90]. Effects of NO on trophoblasts motility has been proposed to be mediated by nitrosylation of the matrix metalloprotease MMP9 [79], based on (1) iNOS and MMP9 colocalization in front migration in trophoblast (leading edge with lamellipodium) and (2) observations of MMP9 being S-nitrosylated [91]. The dynamics of iNOS and S-nitrosylated proteins accumulation at the leading edge in trophoblasts led the authors to conclude that iNOS was not likely to be passively piling up [79] (**Figure 5**). In these conditions, iNOS also accumulates in aggregates in the cytoplasm. Taken together, colocalization of Actin, MMP9, and iNOS at the leading edge suggested indeed an active role for S-nitrosylation in cell migration. Invasiveness of cytotrophoblast-derived cell lines induced by adrenomedullin was also associated with an increase in S-nitrosylated protein rate [92]. S-nitrosylation of urokinase plasminogen activator (uPA), whose involvement in extracellular matrix is acknowledged, was reported to increase in these conditions [92]; the mechanisms through which S-nitrosylation increased this enzyme activity remain to be clarified.

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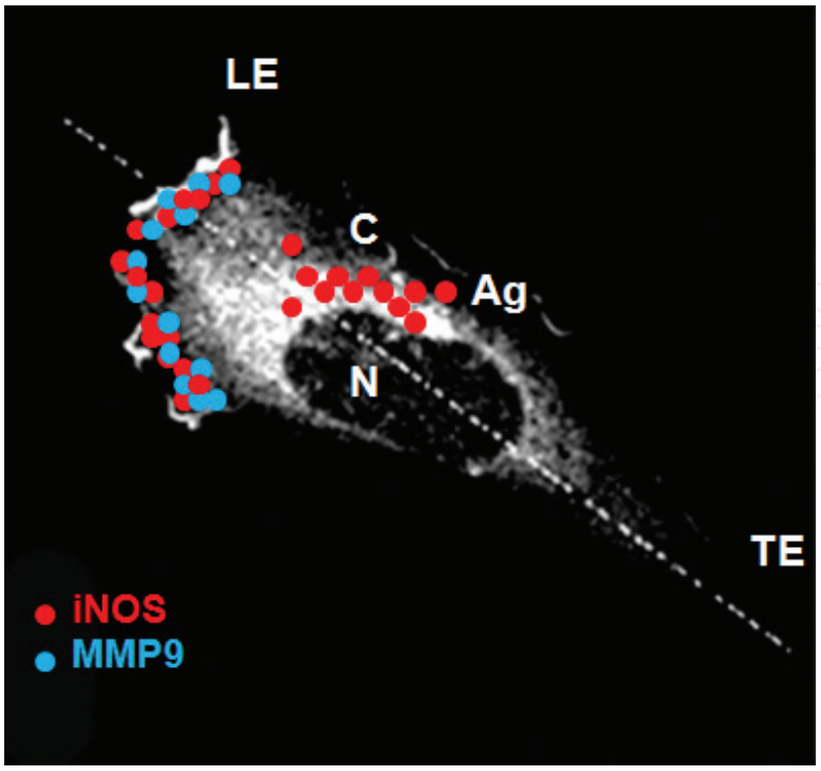

Figure 5. Schematic view of iNOS and MMP9 localization in trophoblasts. Adapted from reference [79]. LE, leading edge; TE, trailing edge; N, nucleus; C, cytoplasm; Ag, aggresome (iNOS particle). Actin polymerisation sites were also detected in LE. Noteworthy, Harris and colleagues noted a colocalization of eNOS and actin in trophoblasts.

13. A yin-yang relationship for S-nitrosylation and S-sulfhydration?

Along with NO, hydrogen sulfide (H_2S) is another gasotransmitter involved in regulating various aspects of cellular life. Many protein sites have been reported to undergo both S-nitrosylation and S-sulfhydration (brought by H_2S), such as Actin [93, 94], GAPDH [95], Parkin [96], PTEN [97], and the p65 subunit of NF-κB [98]. If S-sulfhydration and nitrosylation may occur on the same residue [99], reactive site cysteine, they generally promote different and opposing effects. Indeed, S-nitrosylation typically reduces cysteine thiols reactivity, while S-sulfhydration increases cysteine thiols reactivity, thereby making them more nucleophilic. If one wants to compare S-sulhydration and nitrosylation, it has mainly to outline that (1) proteins are rather S-sulhydrated than S-nitrosylated, and (2) nitrosylation rather inhibits and impairs protein functions. In this regard, the case of tensin (PTEN) provides an example of yin-yang relationship for S-nitrosylation and S-sulfhydration (**Figure 6**). Gene suppressor PTEN acts as an inhibitor of the PI-3 kinase/Akt signaling pathway, attenuating cellular growth and survival. S-nitrosylation enabled Akt hyperactivity, and thereby is associated with observed neuroprotective effects [100]. Oxidation of the active site cysteine is acknowledged as a common mechanism for regulating protein tyrosine phosphatases [101]. In addition, a NO-mediated PTEN degradation mechanism has been suggested to be common in neurodegenerative conditions where NO exerts a critical physiopathological role [102]. Finally, S-sulfhydration was

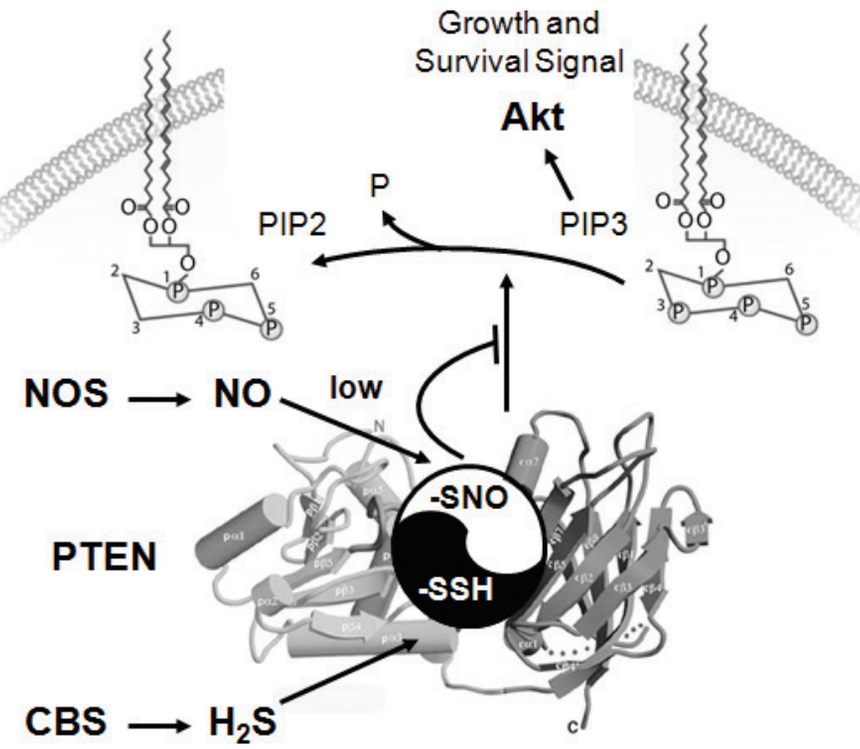

Figure 6. PTEN (left: phosphatase domain; right: C2 domain) is either S-nitrosylated (SNO) or S-sulfhydrated (SSH). When S-sulfhydrated, PTEN exerts its activity of phosphatase on PIP3 (phosphatidylinositol—3,4,5-triphosphate), in order to generate PIP2 (phosphatidylinositol-4,5-bisphosphate). In the absence of hydrogen sulfide, low concentrations of NO drive S-nitrosylation of PTEN, leading to its inactivation. In these conditions, Akt activity is maintained. If low concentration of NO drives SNO-PTEN, higher concentrations lead to SNO-Akt and abrogation of survival signal.

reported to maintain the activity of this lipid tyrosine-phosphatase, thereby preventing its S-nitrosylation [97]. PTEN structure reports disparate sites for S-nitrosylation (Cys 83 [100]), S-sulfhydration, and hydrogen peroxide-induced disulfide bond formation (Cys 71 and Cys 124 [103]). One should keep in mind that in this particular example, S-nitrosylation targets a different site than the Cys 124, mandatory in the phosphatase activity of PTEN.

It is tantalizing to hypothesize that sequence of S-nitrosylation and S-sulfhydration could provide a way for fine tuning of signaling pathways and cellular functions regulation. Because protein S-nitrosylation can foster intramolecular disulfide bond formation, a protein S-nitrosylation event might promote the formation of a more enduring S-sulfhydration reaction.

14. Conclusion

While NO is not necessary for meiotic resumption *per se* in amphibians, NO clearly influences meiotic processes in rodents, porcine, and bovine oocytes. During fertilization, the role of nitric oxide evolved from the hypothesis of being a primary activator to being solely correlated to fertilization, and its role was limited to a particular function such as hardening of the fertilization envelope in sea urchins. Therefore, the physiological relevance of NO has

been debated. Nevertheless, NO, together with its effects on spermatozoa (viability, motility, capacitation acrosome reaction, and fusion with oocytes) appeared as a modulator of oocyte aging. The benefits of nitric oxide use in assisted reproduction are now well-considered.

Though NO was not reported to be involved in early developmental processes in ascidians, NO positively affects cell proliferation in early *Xenopus* embryos and impairs cell movement during gastrulation in this model. The involvement of NO during segmentation is emphasized in mammalian models, where NO seemed to be requested for segmentation and blastocysts survival (rodents, porcine, and bovine) and for implantation through blastocysts hatching (rodents and bovine) and trophoblasts motility (humans).

One of the main difficulties when considering the effects of NO remains in the existence of the multiple pathways that can be activated by this gasotransmitter: cGMP-dependent pathway, calcium-related pathways, and reactive oxygen species production. Over the past few years, S-nitrosylation has offered a new angle to decipher NO's actions since S-nitrosylation modulates the activities of many key regulators such as members of the RhoA-ROCK pathway, Cdk2, Cc25, or PTEN. PTEN regulation by nitrosylation offers a new paradigm since sulfhydration and nitrosylation, both provided by gasotransmitters, appeared to play reciprocally in a yin-yang manner.

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Author details

Ješeta Michal[1], Marketa Sedmikova[2], and Jean-François Bodart[3]*

*Address all correspondence to: jean-francois.bodart@univ-lille1.fr

1 Department of Obstetrics and Gynaecology, Center of Assisted Reproduction, University Hospital Brno and Masaryk University, Brno, Czech Republic

2 Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Prague-Suchdol, Czech Republic

3 University of Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, Lille, France

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