)JOEBXJ 1VCMJTIJOH $PSQPSBUJPO
0YJEBUJWF .FEJDJOF BOE $FMMVMBS -POHFWJUZ
7PMVNF "SUJDMF *% QBHFT
IUUQ EY EPJ PSH

Review Article

Gasotransmitters in Gametogenesis and Early Development: Holy Trinity for Assisted Reproductive Technology—A Review

Jan Nevoral,[1,2] Jean-Francois Bodart,[3] and Jaroslav Petr[4]

Laboratory of Reproductive Medicine of Biomedical Center, Faculty of Medicine in Pilsen, Charles University in Prague, Alej Svobody / , Pilsen, Czech Republic
Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University in Prague, Alej Svobody / , Pilsen, Czech Republic
Team Regulation des Signaux de Division, UMR CNRS, Université de Lille , Sciences et Technologies, FR CNRS, Villeneuve d•Ascq Cedex, France
Institute of Animal Science, Pratelstvi , Praha Uhrineves, Czech Republic

Correspondence should be addressed to Jan Nevoral; jan.nevoral@lfp.cuni.cz

Received April ; Accepted July

Academic Editor: Michael D. Pluth

Creation of both gametes, sperm and oocyte, and their fusion during fertilization are essential step for beginning of life. Although molecular mechanisms regulating gametogenesis, fertilization, and early embryonic development are still subjected to intensive study, a lot of phenomena remain unclear. Based on our best knowledge and own results, we consider gasotransmitters to be essential for various signalisation in oocytes and embryos. In accordance with nitric oxide (NO) and hydrogen sulfide (H_2S) physiological necessity, their involvement during oocyte maturation and regulative role in fertilization followed by embryonic development have been described. During these processes, NO- and H_2S-derived posttranslational modi cations represent the main mode of their regulative e ect. While NO represent the most understood gasotransmitter and H_2S still intensively studied gasotransmitter, appreciation of carbon monoxide (CO) role in reproduction is still missing. Overall understanding of gasotransmitters including their interaction is promising for reproductive medicine and assisted reproductive technologies (ART), because these approaches contend with failure of in vitro assisted reproduction.

1. Introduction

Human reproductive medicine and assisted reproductive technologies (ART) have been gaining increasing signi cance, dealing with human reproduction failure. Doubtlessly, the oocyte and sperm are crucial cells for assisted reproduction because these haploid gametes are required to build a diploid zygote, capable of further development. Female and male gametes exhibit di erent morphological features and, excluding brought genome, they di erently contribute to embryo formation. While centrosomes, small noncoding RNAs, and posttranslationally modi ed residual histones are sperm-inherited, oocytes provide mitochondria, mRNAs (distributed according to a speci c pattern), histones, metabolic enzymes, and cytoplasmic factors to sustain development, as summarized elsewhere […]. Hence, one has to consider the oocyte as a microenvironment lled with a precisely balanced cocktail of the numerous factors that are essential for embryonic development. Also, oocytes o er physical environments favourable to self-organizing process like division spindle assembly. Upon fertilization, a succession of mitotic divisions is triggered; the transition from maternal to zygotic mRNAs transcription and transformation of a low organized cellular mass into a blastocyst will occur prior to implantation. Together with gametogenesis and DNA integrity maintenance, these events are of high interest for ART. Indeed, any failure in these processes will impact severely the embryo•s fate. Untangling the processes at the molecular and cellular levels is crucial for ART and we should underline that the e ects of many contributors, besides the main regulators of gametogenesis and early embryogenesis, remain uncovered.

Oocyte maturation, which can be simulated in vitro conditions, deserves particular attention because meiotic division and achievement of developmental competence are nalized during this short and extremely important period (summarized in [ ]). e quality of matured oocytes is decisive for the fertilization rate, as a result of sperm penetration and complex oocyte changes including cortical granule exocytosis-prevented polyspermy and oocyte activation for embryonic development […]. In fact, the early embryonic development, where high-quality blastocyst is optimal for embryo transfer into the recipient body, is decisive for the success of ART [,]. Numerous factors have been identi ed to play di erent roles in chromosome segregation and developmental competence achievement, regulating kinases, structural cytoskeletal proteins, enough histones, and second messengers (cAMP, cGMP, and Ca^{2+} ions) […]. In addition to these known key factors, gaseous molecules with signal transduction ability, hence named gasotransmitters […], have been involved in the oogenesis as well [,]. eir impact is acknowledged along with a better understanding of gasotransmitters• signalling pathways. Moreover, recent observations point out imperfect in vitro imitation [ ] and some gasotransmitter signalisation seems to be lacking in incomplete gametes• maturation and early embryogenesis.

Only matured oocytes are able to go through in vitro fertilization, a key technique of assisted reproduction [ ]. Fertilization consists in the interactions of male and female gametes leading to embryonic development. e high cell division rate, typical of this period, is highly sensitive to well-orchestrated cell cycle regulation [, ,]. Oocyte maturation and early embryonic development persist as delicate steps for in vitro approaches, calling for ART improvement. Nevertheless, gasotransmitters rise expectations due to their broad physiological e ect and promising results of gasotransmitters supplementation.

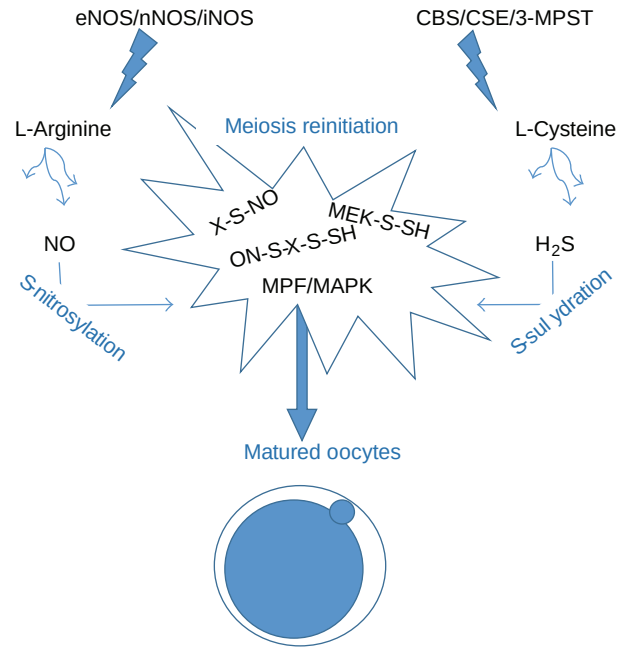
e aim of this review is to compare the biological necessity of all three gasotransmitters in the oocyte and embryo, observing their in vitro culture in ART, as a key factor for creating a new individual. is comparison highlights protein posttranslational modi cations as crucial molecular action of gasotransmitters during oogenesis and preimplantation embryonic development.

2. Gasotransmission in Female Reproductive Processes

. . NO as a •Yes SignalŽ for Fertilization and Early Development. Only matured gametes, which underwent adequate changes, are capable of fertilization. ese changes involve especially oocyte maturation, sperm capacitation, and acrosome reaction and are an essential prerequisite for both successful fertilization and further embryonic development. Biochemical changes regulate gametes• changes and their interactions during fertilization process. Originally, these changes were believed to be exclusively regulated via kinase signalling, such as protein kinase A- (PKA-) M-phase/maturation promoting factor- (MPF) mitogen-activated protein kinase (MAPK) and calmodulin-dependent protein kinase II (CaMKII), either directly dependent or indirectly regulated by molecules of second messengers, Ca^{2+} and cAMP. In addition to these two messengers, the involvement of NO, a small gaseous molecule, in cell signalling of physiological processes has been described [,]. Along with NO, gasotransmitters H_2S and CO were suggested to participate in the above-mentioned processes as well [, …] (see Figure).

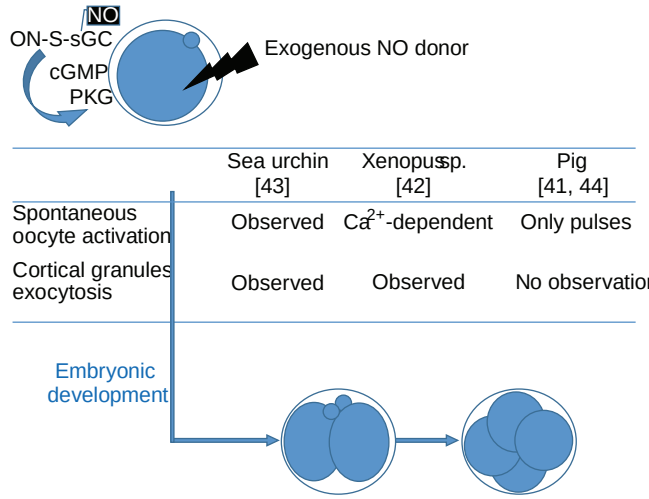
NO represents the most read-up gasotransmitter, with ability to regulate molecular processes in gametes and embryo […]. All NO synthases, that is, endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), are present in mammalian oocyte with various subcellular localization, where they are essential for endogenous production of NO and its cell signalisation [,]. NO action leads to ovulation of matured and fertilizable oocytes [,] as a result of reinitiation of oocyte meiosis and correct oocyte maturation [, ,]. Accordingly, NO level in oocytes of young mice is signi cantly higher than old animals and NO antiaging e ect is obvious [ ]. On the contrary, increased eNOS expression accompanies improved mouse oocyte quality a er estrogen administration [ ]. One of NO action modes, S-nitrosylation of proteins, has been observed in oocytes during meiotic maturation [ ]. However, NO is able to stimulate soluble guanylate cyclase (sGC), which is a NO-speci c receptor, in cGMP production and thus NO increases protein kinase G (PKG) activity […]. On the other hand, S-nitrosylation of sGC a ects the decreasing responsiveness to NO in somatic cells and molecular mechanism-dependent dual e ect of NO is obvious [ ]. In contrast to oocyte maturation [ ], NO-sGC-cGMP-PKG signal pathway is capable of inducing spontaneous oocyte activation and subsequent parthenogenetic development [ ]. NO-induced oocyte activation indicates a pulsation pattern of NO action in porcine oocytes [ ]. Based on an observation of Xenopus oocytes, the parthenogenetic NO e ect is Ca^{2+}-dependent and occurs due to MAPK inactivation [ ].

While NO might promote but is dispensable for Xenopus and mammals oocyte activation, NO is essential for oocyte activation event during the fertilization process in sea urchin oocytes [ ]. In accordance with this variable e ect, cortical granules exocytosis has been reported in Xenopus oocytes [ ] but not in porcine oocytes [ ]. e interspecies di erences of NO action during fertilization are obvious and NO seems to be even nonessential during mammalian fertilization (Figure). e ambiguous NO necessity could be a result of a more diverse NO e ect when NO is associated with in ammation and/or oxidative stress [,]. Accordingly, the role of NO during subsequent embryonic development a er fertilization remains controversial […] for in ammation (endometriosis), accompanying NO [,] and protein nitration [ ]. However, creation of secondary products of NO interactions seems to be one of possible mechanisms of NO negative action [ ]. e physiological role of NO in embryogenesis is still unexceptionable when NO is involvement in embryonic stem cell di erentiation through transcriptional factors [ ]. erefore, NO is able to be considered as trigger for oocyte maturation and fertilization as well as subsequent embryonic development.



F : Gasotransmission in oocytes, resulting in S-sulfhydration and nitrosylation of various factors. Both gasotransmitters NO and H_2S are enzymatically released, respectively, from L-arginine and L-cysteine. Subsequently, NO- and/or H_2S-posttranslationally modified proteins lead to MPF/MAPK-orchestrated meiotic maturation reinitiation (equal to GVBD, germinal vesicle breakdown) and completion (with extruded polar body and small particles visible in perivitelline space). S-sulfhydration of MEK, upstream MAPK kinase, is known [ ] and more S-sulfhydrated factors are considered. In addition to S-sulfhydration, S-nitrosylation seems to be exclusive mechanism of NO-regulated oocyte maturation [ ]. Disclosure of complete •S-sulfhydrationŽ and •S-nitrosylationŽ is still lacking (X-S-SH,X-S-NO) and we can assume wide protein index underwent this posttranslational modifications as well as NO-H_2S intraprotein cross-talking (HS-X-S-NO).

. . H S in Gametogenesis and Embryo Development. S-sulfhydration, another gasotransmitter-derived posttranslational modification, is supposed to be a prime way of H_2S molecular action [,] without known H_2S-specific receptors. In contrast to NO, little is known about H_2S and S-sulfhydration involvement in gametogenesis and embryonic development. Nevertheless, all three H_2S-releasing enzymes, CBS, CSE, and -MPST, were observed in porcine oocyte and surrounding cumulus cells [ ]. is observation is in accordance with earlier finding of H_2S involvement in folliculogenesis and oocyte maturation [,]. e necessity of H_2S in matured oocytes interferes with the contribution to developmental competence acquirement and subsequent embryonic development [ ]. In addition, there is the observation of a protective effect of H_2S against oocyte aging and H_2S-positively affected further embryonic development [ ]. Physiological action of endogenously released H_2S immediately in oocyte has been described and modified kinase activity of MPF and MAPK has been observed [, ,]. S-sulfhydration of these kinases and their upregulated factors are presumable. Activating S-sulfhydration of MEK, leading to MAPK signalling [ ], confirms this assumption and the findings mean that S-sulfhydration is crucial for enzyme activity and shift its significance to protein phosphorylation.



F : NO action in oocyte activation is evolutionary inconsistent. e NO/sCG/cGMP/PKG signal pathways are presumed, where dual NO effect on sCG, resulting in its S-nitrosylation and NO binding, is expectable. Obviously, dependency of fertilization and oocyte activation, followed by cleavage and the second polar body extrusion, is shaded in evolutionary more developed organisms, where fulfilment of certain conditions (Ca^{2+} presence, pulsative character of NO) is necessary.

However, in contrast to the essential and protective effect of H_2S in mammalian oocytes, our own observation of oxidative stress-like effect of H_2S in Xenopus oocytes indicates less conservative evolutionary mechanism through species. Moreover, some findings support that H_2S action is at least comparable to reactive oxygen species (ROS) throughout reactive sulfide species (RSS) creation […].

Although the role of the third gasotransmitter, CO, remains uncovered, the necessity of gasotransmitters for male and female reproduction including fertilization and embryonic development is unquestionable. Accordingly, S-nitrosylation and sulfhydration of sulphur amino acid cysteine seem to be crucial protein posttranslational modifications for reproductive processes and their understanding brings relevant possibilities for ART.

3. An Increasing Attractiveness of S-Nitrosylation and S-Sulfhydration

Decades of research have established a high potential for NO and S-nitrosylation in controlling cellular mechanisms. Indeed, both NO and H_2S might engage in protein short-lived covalent reactions, which modulate proteins structure and functions. NO builds its signalling activity by binding to sulfhydryl groups of cysteine residues in target proteins. e latter process is called S-nitrosylation. In a similar manner, S-sulfhydration is a posttranslational modification of specific

residues, through the formation of persul de (-SSH) bonds. Both sul ydration and S-nitrosylation are reversible.

ere is a broad spectrum of S-nitrosylated proteins. An exhaustive list would be beyond the scope of this review. Nevertheless, it is to note that nitrosylated proteins include cytoskeleton, cell migration, cell cycle, and antiapoptotic proteins, as well as proteins involved in transcription and protein synthesis […]. In a similar way, protein-SSH formation is now admitted to mediate in a fundamental manner the cellular signalling by H_2S, based on the detection of S-sul ydrated proteins and on the demonstration of their perturbed functions [ ]. Spatial environments of the modi ed residues drive the impact of S-sul ydration on protein function. For example, it may protect residues from oxidation under oxidative stress and therefore may sustain protein activities.

. . From Cell Cycle to Implantation, Potential Roles for S-Nitrosylation. erefore, S-nitrosylation is a well-established posttranslation modi cation, whose potential involvements at physiological level in oocytes and embryos go from cell cycle regulation (meiotic transition, segmentation) to embryo survival and implantation.

Indeed, S-nitrosylation targets can be found within main modulators of meiosis progression or cell cycle progression and their regulators. ough the M-phase promoting factor, made up with cyclin B and cyclin-dependent kinase (CDK), was not reported to be itself S-nitrosylated, the S-nitrosylation of CDKs was observed for CDK , CDK , and CDK […]. While CDK -nitrosylation increases its activity independently of any e ects on protein levels expression, the e ect of S-nitrosylation on CDK and CDK remains elusive. S-nitrosylation of cyclin B was sought in HL- cells, but not observed [ ]. No S-nitrosylation was reported for polo-like kinases (PLKs), anaphase promoting factor/cyclosome (APC/C), WEE , and MYT , which are among the close regulators of MPF. Nevertheless, the dual speci city cell division cycle phosphatase (CDC), which is the main activator of MPF, is clearly impacted since its S-nitrosylation annihilates its phosphatase activity ([,]; Gelaude and Bodart: personal observations).

Beyond the cell cycle regulators, S-nitrosylation has been called to play a role in preimplantation embryos and implantation. Microenvironmental presence of NO was reported to contribute to the pathologic e ects of endometriosis on the development potential of embryos. In this context, NO e ects on embryo survival could either rely upon S-nitrosylation, NO/GC/cGMP or peroxynitrite formation. Lee et al. [ ] suggested that the apoptotic e ects of excessive NO on embryos were related to S-nitrosylation rather than to any other mechanisms. ese e ects were closely associated with lipid-rich organelles (mitochondria and endoplasmic reticulum) [,]. Regarding implantation, NO was shown to in uence trophoblasts motility [,]. It was further suggested that the e ects of NO on trophoblast migration and invasion, which are critical processes for the successful embryonic development, were mediated by nitrosylation of the matrix metalloprotease MMP [ ]. Indeed, while MMP has been reported to be nitrosylated [ ], it was colocalized with iNOS and S-nitrosylated proteins at the leading edge in trophoblast [ ]. Finally, the trophoblast also appeared to be protected from apoptosis via S-nitrosylation of caspase [ ]. us, S-nitrosylation of proteins might play pivotal roles throughout the early development, modulating cell cycle, trophoblast motility, and embryo survival (Table).

. . S-Sul ydration as Another Modulator of Enzymatic Activities. e impacts of H_2S and S-sul ydration have been addressed and considered to a lesser extent, mainly due to the lack of methodologies [ ]. Since the speci - cation of protein S-sul ydration sites has been enabled, increasing evidence has come to underline the ability of S-sul ydration to enhance or impair an enzymatic activity. S-sul ydration was reported to impair the activity of KEAP [ ], while it increases the activity of K_{ATP} and Ca^{2+} channels, glyceraldehyde -phosphate dehydrogenase (GAPDH), nuclear factor B (NF- B), and MAPK/ERK kinase (MEK) [, , …]. In addition to the above-mentioned S-sul ydrated proteins, S-sul ydration of cystathionine -synthase (CBS) and cystathionine lyase (CSE), H_2S-releasing enzymes, has been observed [ ] and existence of feedback in H_2S production is supported.

Protein phosphatase serves as points of exibility and crucial regulation in network signalling. Evidence had raised the fact that it might be particularly subject to S-sul ydration. Among the phosphatase types involved in early embryogenesis and/or signalling pathways and whose activity might be modulated by S-sul ydration are phosphatase and tensin homolog (PTEN), protein-tyrosine phosphatase B (PTP B), and aforementioned CDC . Protein phosphatase PTEN is requested at early steps for proper embryonic development [ ]. In the case of PTEN, S-sul ydration was reported to maintain the activity of the phosphatase [ ], by preventing its S-nitrosylation, which would result in protein degradation [ ]. PTP B belongs to the family of ErbB, involved in numerous signalling pathways modulating proliferation, adherence, migration, or survival. PTP B was shown to be inactivated by S-sul ydration of cysteine C , located in its catalytic site [ ].

Also, CDC might be sulhydrated and inactivated presumably by modi cation of the cysteine in its active site [ ]. ere is no direct evidence for CDC sul ydration, but since organosulphur compounds inhibit CDC A and promote G /M arrest [ ] and CDC are targeted by ROS and S-nitrosylation, CDC are likely to be S-sul ydrated [ ]. Further studies are obviously needed to gather an exhaustive list of S-sul ydrated proteins, and one might rst focus on proteins, which have been already reported as being S-nitrosylated. MKP , ERK , CDK and CDK , CDC , and MMP appear as appealing candidate (Table). Indeed, evidences have been raised for cross-talk between S-sul ydration and S-nitrosylation for many proteins.

. . A Cross-Talk of S-Sul ydration and S-Nitrosylation? Many protein sites have been reported to undergo either S-nitrosylation or S-sul ydration. As an example, the residue cysteine C in GAPDH had been found either S-nitrosylated or S-sul ydrated [, …]. Susceptibility

T : Examples of S-nitrosylated and/or S-sul ydrated proteins.

Protein	Sul ydration site	Sul ydration e ect on function	Nitrosylation site	Nitrosylation e ect on function	References
MKP	n.d.	n.d.	C	Stability of protein	Guan et al., [ ]
ERK	n.d.	n.d.	C (potential)	Prevention of phosphorylation	Feng et al., [ ]
CDK	n.d.	n.d.	n.d.	Increase of kinase activity	Kumar et al., [ ]
CDK	n.d.	n.d.	n.d.	n.d.	Foster et al., [ ]
CDC	n.d.	n.d.	n.d.	Loss of phosphatase activity	Foster et al., [ ]; Majumdar et al., [ ]
MMP	n.d.	n.d.	n.d.	Increase of activity	Harris et al., [ ]
PTP B	C	Reduction of phosphatase activity	n.d.	n.d.	Krishnan et al., [ ]
PTEN	C , C	Maintenance of enzyme activity and prevention of further oxidation by NO	C	Promotion of survival signal and protein degradation	Kwak et al., [ ]; Ohno et al., [ ]
Actin	n.d.	Increase of polymerization activity	Cys	Decrease in polymerization activity and network formation	Dalle-Donne et al., [ ]; Mustafa et al. [ ]; om et al., [ ]
MEK	C	Facilitation of Parp activation	n.d.	Loss of kinase activity	Ben-Lulu et al., [ ]; Zhao et al., [ ]
Parkin	n.d.	Increase of activity	n.d.	Decrease of activity	Chung et al., [ ]; Vandiver et al., [ ]
GAPDH	C	Increase of the activity sevenfold	C	Inhibition of glycolytic activity	Greco et al., [ ]; Hao et al., [ ]; Hara et al., [ ]; Mustafa et al., [ ]

for both modi cations may strike root in the chemical properties of the involved thiols by S-nitrosylation and S-sul ydration [ ]. If S-sul ydration and nitrosylation can occur on reactive cysteine residues, they frequently involve the same residue, generally by promoting di erent and opposing e ects. Indeed, S-nitrosylation typically reduces cysteine thiols reactivity while S-sul ydration increases cysteine thiols reactivity, thereby making them more nucleophilic. For instance, S-sul ydration and nitrosylation on the same sites have been reported for GAPDH, Parkin, and the p subunit of NF- B (nuclear factor-B) (Table). e increase of GAPDH activity stimulated by S-sul ydration is antagonized by nitrosylation, which impairs the glycolytic activity of the enzyme [, …]. Similarly for Parkin, the S-nitrosylation impairs the enzyme activity whereas sul ydration stimulates it [,].

S-nitrosylation and sul ydration both regulate the p subunit of the antiapoptotic transcription factor NFB, which provided quite a school-case for the interplay of S-nitrosylation and sul ydration [ ]. S-sul ydration of NF- B has been reported to inhibit apoptosis. Persul dation of cysteine of p unit of NF- B promotes binding of NF- B to the coactivator ribosomal S , thereby increasing its binding to promoters of antiapoptotic genes. Also, cysteine persul dation might function as the molecular •keyŽ by which hydrogen sul de prevents NFB pathway activation in ox-LDL-induced macrophage in ammation by impairing NF- B p phosphorylation, nuclear translocation, and, therefore, DNA binding activity [ ]. One has to note that NF- B S-sul ydration may not account for all the protective e ects of H_2S towards in ammation. Subsequent to sul ydration, nitrosylation of p reversed the activation of NFB targets [,].

Similarly, actin, whose modi cations of properties are requested for the rapid cadence of cytokinesis during early embryogenesis, is nitrosylated or sul ydrated. While S-sul ydration of actin resulted in an increase of lament polymerization [ ], S-nitrosylated actin exhibited a decrease in polymerization activity and thus an impairment in actin network formation [,]. Actin-binding proteins such as pro lin [ ] and co lin [ ] are also subject to S-nitrosylation and may contribute through the latter modi-cations to modulate the remodelling of the actin network.

us, if we are to compare S-sul ydration and nitrosylation, we should mainly outline that ( ) proteins are rather S-sul ydrated than S-nitrosylated and ( ) nitrosylation is more likely to inhibit and impair protein functions (Table).

One may also hypothesize that the sequence of S-nitrosylation and sul ydration could provide a way for a ne tuning of signalling pathways and cellular functions regulation. Because protein S-nitrosylation can foster intramolecular disul de bond formation, a protein S-nitrosylation event might promote the formation of a more enduring S-sul ydration reaction. Moreover, S-sul ydration of eNOS and its increased activity have been described [ ]. Supervision of Ca^{2+} in ux and availability of eNOS, Ca^{2+}-dependent,

is another mechanism of H_2S-controlled NO creation [ ]. Likewise, reverse NO modulation e ect on H_2S releasing is assumed; however, it has not been uncovered so far.

4. Perspectives of Gasotransmitters for Assisted Reproductive Technologies

. . About Recent Reproductive Medicine. With respect to the above-described posttranslational modi cations, the causality of some of the phenomena is explained. Assisted reproductive technologies (ART), as a medicinal approach to the solution of human infertility, are a eld where the posttranslational modi cations and their consequences could be utilized.

Embryos by produced in vitro by ART show di erences compared to the in vivo grown embryos. Routinely used ART techniques, such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), may a ect embryonic development di erentially on cellular and molecular levels. Moreover, individual approaches are not equal where