Review Report

Role of N-acetylcysteine in animal reproduction: a comprehensive review

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Abstract

N-acetylcysteine (NAC), a thiol-containing acetylated derivative of L-cysteine, has emerged as a biologically versatile compound with substantial relevance to animal reproductive physiology. Its principal mechanism of action is replenishment of intracellular glutathione through cysteine donation; secondary actions include direct scavenging of reactive oxygen species, modulation of redox-sensitive signalling pathways, hydrogen sulfide generation, metal chelation, and disruption of mucin disulfide bonds. Oxidative stress is a central disruptor of gamete quality, fertilization, implantation and early embryonic development across species. In vitro and in vivo evidence from cattle, sheep, goats, horses, dogs, buffaloes, and laboratory animals consistently demonstrates that NAC improves oocyte maturation and developmental competence, protects granulosa cells from apoptosis via PI3K/AKT signalling, supports early pregnancy establishment, attenuates endometrial inflammation and improves clinical fertility outcomes in mares with endometritis. NAC also preserves sperm motility, viability and mitochondrial function during semen extension and cryopreservation, and exhibits direct antibacterial and antiinflammatory activity relevant to mastitis-associated infertility in dairy cattle. These findings collectively established NAC as a promising antioxidant strategy for improving fertility and reproductive efficiency in domestic animals. This review consolidates current mechanistic and applied evidence on NAC in animal reproduction and identifies priority areas for future research.

Keywords: N-acetylcysteine, oxidative stress, glutathione, animal reproduction, sperm quality, endometritis

Introduction

Reproductive efficiency is a cornerstone of livestock productivity and its optimization remains a central goal of animal science. Despite considerable advances in assisted reproductive technologies, conception rates continue to be compromised by a range of pathophysiological factors, among which oxidative stress has attracted growing recognition as a universal disruptor of fertility.[1-3] Reactive oxygen species (ROS), generated during normal cellular metabolism, serve physiological roles at low concentrations (participating in oocyte maturation, sperm capacitation and implantation signalling) but become damaging to virtually every stage of reproductive biology once their production exceeds the capacity of endogenous antioxidant defenses.[1,2,4]

Sperm are most sensitive to oxidative injury; their plasma membranes are rich in polyunsaturated fatty acids and their limited cytoplasmic volume leaves minimal intrinsic antioxidant reserves, so that elevated ROS readily cause lipid peroxidation, mitochondrial dysfunction, DNA strand breaks and apoptosis-like changes that reduce motility and fertilizing ability.[4-6] Oocytes and early embryos are also vulnerable, particularly during in vitro maturation and the first cleavage divisions, when endogenous antioxidant systems are not fully established.[1,2,7] In the female reproductive tract, oxidative stress disrupts uterine receptivity, endometrial cell viability, follicular granulosa cell survival and placental function, and has been mechanistically linked to chronic endometritis in mares, early embryonic loss in small ruminants and ovarian follicular atresia in cattle.[2,3,8-11] The practical need for safe, effective antioxidant interventions that can be used in field conditions therefore remains considerable.

N-acetylcysteine (NAC) has attracted substantial interest as exactly such an intervention. A thiol-containing derivative of the amino acid L-cysteine, NAC was originally developed as a mucolytic agent and later as an antidote to acetaminophen-induced hepatotoxicity in clinical medicine.[12,13] Its subsequent characterization as a precursor for

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intracellular glutathione (GSH) synthesis, a direct scavenger of highly reactive oxygen radicals, a modulator of redox-sensitive signalling cascades and a generator of the cytoprotective gasotransmitter hydrogen sulfide (H_2S) has expanded its biological relevance considerably.[12,14-16] These combined antioxidant, antiinflammatory, mucolytic and cytoprotective properties make NAC a compound of considerable interest for reproductive medicine, where oxidative stress, inflammation and impaired cellular survival are recurrent contributors to subfertility.[13,15,17] The present review consolidates evidence on the biochemistry of NAC, its mechanisms of action relevant to animal reproduction and its documented effects across reproductive compartments and animal species, with the aim of providing a coherent framework for its practical application in veterinary reproductive management. Key mechanistic and applied findings are illustrated (Figures 1-3) and a cross-species summary of major outcomes is provided (Table).

Biochemistry and mechanisms of action of N-acetylcysteine

NAC (N-acetyl-L-cysteine; molecular formula $C_5H_9NO_3S$; MW 163.19 g/mol) is an acetylated derivative of L-cysteine.[12] Following absorption, it is rapidly deacetylated to free cysteine, the rate-limiting substrate for de novo synthesis of reduced GSH, the most abundant nonprotein intracellular thiol in mammalian cells.[12,18-20] In its reduced form, GSH detoxifies electrophilic compounds, neutralizes hydrogen peroxide (H_2O_2) and lipid peroxides via glutathione peroxidase (GPx) and glutathione reductase (GR), and regulates redox-sensitive signal transduction.[19-21]

Primary mechanism: glutathione precursor activity

Replenishment of intracellular GSH is NAC's principal and best-characterized mechanism of action, underlying most of its secondary antioxidant, antiinflammatory and cytoprotective effects described below.[12,18-20]

Secondary mechanisms

Direct radical scavenging and mucolytic action

In addition to GSH replenishment, NAC reacts directly (though comparatively slowly) with highly oxidizing free radicals such as hydroxyl radical, nitrogen dioxide and carbonate radical through its free thiol group, an action considered secondary to its role in thiol homeostasis and redox modulation.[12,14,22] The same thiol group's strong reducing potential toward protein disulfide bonds explains NAC's well-characterized mucolytic action: by cleaving disulfide cross-links within mucin glycoprotein networks, NAC reduces mucus viscoelasticity, a property exploited in the management of equine endometritis and experimentally in canine biliary mucoceles.[12,17,23]

Hydrogen sulfide-mediated cytoprotection

NAC also acts as an indirect H_2S donor; NAC-derived cysteine is desulfurated to H_2S by cystathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase.[16,24] H_2S, a gasotransmitter alongside nitric oxide and carbon monoxide, exerts antioxidant, antiinflammatory and mitochondria-protective effects partly through persulfidation of cysteine residues on target proteins

Table. Summary of major studies evaluating N-acetylcysteine in animal and reproductive biology

Animals	Compartment	Dose/route	Key outcomes
Mare	Endometrium	3.3% intrauterine infusion	77% first-cycle pregnancy rate; ↓ pathological mucus; ↓ neutrophil ROS; safe endometrial histology[63]
Mare	Endometrium	10 mg/kg oral × 4 days	↓ PMN infiltration; ↓ COX-2; altered glandular mucus; no systemic mucolytic effect[64]
Nubian goat	Early pregnancy	0.07% dietary, days 0-30	↑ Litter size; ↑ serum NO, FSH, LH, E_2, P_4; ↑ SOD, GPx; 207 DEGs enriched in PI3K-Akt, PPAR, angiogenesis[56]
Nubian goat	Uterine proteome	0.07% dietary	125 DEPs; enriched in Jak-STAT, RNA surveillance, tight junctions, steroid biosynthesis pathways[59]
Sheep	Granulosa cells	100 µmol/l in vitro	↓ ROS; ↑ SOD1/CAT; ↑ Bcl-2/Bax; ↑ E_2 and P_4 via CYP19A1/3β-HSD; PI3K/AKT activation[52]
Pig	IVM oocyte	3.5 mM in IVM medium	Restored spindle; corrected H3K27me3; ↓ ROS; ↑ blastocyst rate under heat stress[45]
Mouse	IVM oocyte	NAC in IVM medium	↑ Nuclear maturation; ↓ oxidative damage; ↑ fertilization and blastocyst rates (BPA model)[46]
Mouse	Postovulatory oocyte	NAC in culture medium	Delayed oocyte aging; preserved spindle and mitochondrial function[22]
Cattle	IVM/embryo	Thiol compounds in IVM	↑ Intracellular GSH; ↑ cleavage and blastocyst rates; ↑ postfertilization GSH retention[41-43]
Cattle	Mammary gland	In vitro (MIC-based)	> 50% biofilm inhibition; dose-dependent antioxidant and antiinflammatory activity[79]
Dog	Biliary system	20% NAC, in vitro (1:4 dilution)	Significant ↓ bile viscosity versus water controls; mucolytic via disulfide bond cleavage[23]

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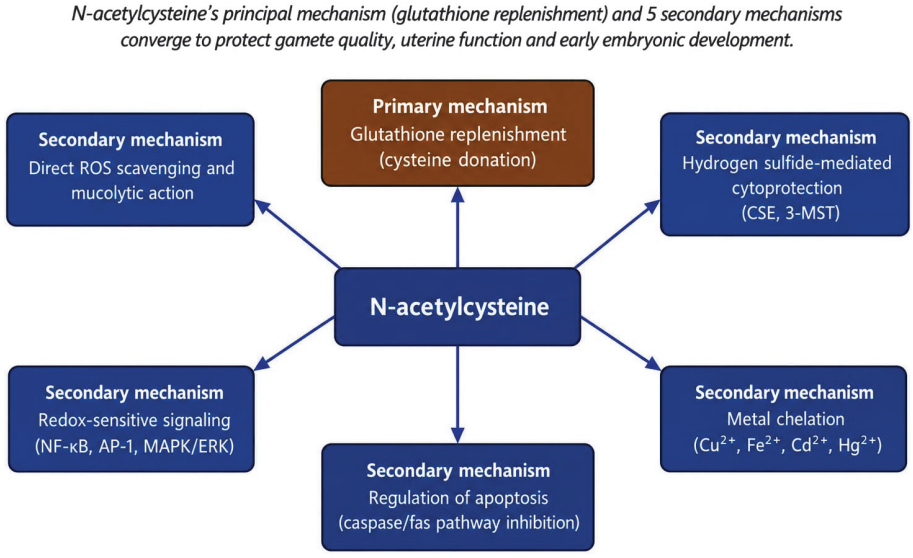


Figure 1. Biochemical mechanisms of N-acetylcysteine (NAC) relevant to animal reproductive function; NAC exerts antioxidant and cytoprotective effects through 6 interconnected mechanisms, collectively supporting gamete quality, uterine function and early embryonic development

such as the transcription factors NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and nuclear factor erythroid 2-related factor 2, suppressing inflammation and enhancing antioxidant gene expression.[25,26]

Redox-sensitive signalling, metal chelation and antiinflammatory action

At the signalling level, NAC inhibits the redox-sensitive transcription factors NF-κB and activator protein 1 that drive expression of proinflammatory cytokines, cyclooxygenase-2 (COX-2) and proapoptotic genes when excessively activated by ROS.[15,27] It also modulates the mitogen-activated protein kinase (MAPK) cascade, inhibiting the stress-activated c-Jun N-terminal kinase while promoting extracellular signal-regulated kinase 1/2 phosphorylation, shifting the balance from pro-apoptotic toward pro-survival signalling.[15,27] Finally, its thiol and carboxyl groups confer metal-chelating properties that bind redox-active transition metals (Cu^{2+}, Fe^{2+}, Cd^{2+}, Hg^{2+}), preventing metal-catalyzed Fenton-type ROS generation, a mechanism of particular relevance in heavy metal-induced reproductive toxicity.[12]

Regulation of apoptosis

Oxidative stress activates apoptotic cascades in reproductive cells through both the extrinsic (death receptor-dependent) and intrinsic (mitochondria-dependent) pathways, involving mitochondrial membrane depolarization, cytochrome-c release and sequential caspase-9/caspase-3 activation, alongside Fas receptor/ligand upregulation that engages caspase-8 and Bid cleavage.[28-31] In a cadmium-induced oxidative stress model, NAC pretreatment substantially blocked ROS generation and suppressed these downstream apoptotic events in both pathways, an effect reinforced by NAC-driven catalase upregulation that sustains suppression of H_2O_2 accumulation beyond the period of direct NAC action.[32] These mechanisms translate directly to reproductive cell biology; apoptosis is the principal trigger of follicular atresia in granulosa cells, contributes to oxidative stress-induced motility and DNA-integrity loss in sperm, and impairs cleavage and blastocyst formation in early embryos through mitochondrial dysfunction and caspase activation.[5,33,34] By interrupting apoptotic signalling at multiple nodes (upstream ROS suppression, Fas pathway blockade and mitochondrial stabilization) NAC provides a mechanistic basis for its protective role across these reproductive cell types (Figure 1).[29,32]

Role of N-acetylcysteine in female reproduction

Glutathione and oocyte developmental competence

Intracellular GSH is the principal antioxidant protecting oocytes and early embryos from oxidative damage, and its concentration during oocyte maturation is a key determinant of developmental competence.[35-38] GSH is required for cytoplasmic maturation and for sperm chromatin decondensation and male pronucleus formation after fertilization, since GSH reduces disulfide bonds in sperm chromatin; inhibiting GSH synthesis during oocyte maturation impairs these events,[37,39] a role that is conserved across species (documented in mouse, hamster, pig and cattle oocytes)[35,36,40]

Supplementation of in vitro maturation (IVM) media with thiol-containing compounds, including NAC, cysteine and cystine, significantly increases intracellular GSH in bovine oocytes, and elevated GSH persists into presumptive zygotes following fertilization.[41-43] Higher GSH established during IVM is associated with improved cleavage rates, morula formation and blastocyst development compared to oocytes matured in unsupplemented media, reflecting enhanced resistance to oxidative damage during the first cleavage divisions, when endogenous antioxidant capacity is inherently low.[36,42-44]

NAC specifically protects oocytes against stressors encountered during in vitro handling. In porcine oocytes exposed to heat stress during IVM, 3.5 mM NAC improved spindle organization, corrected aberrant H3K27me3 histone modifications,

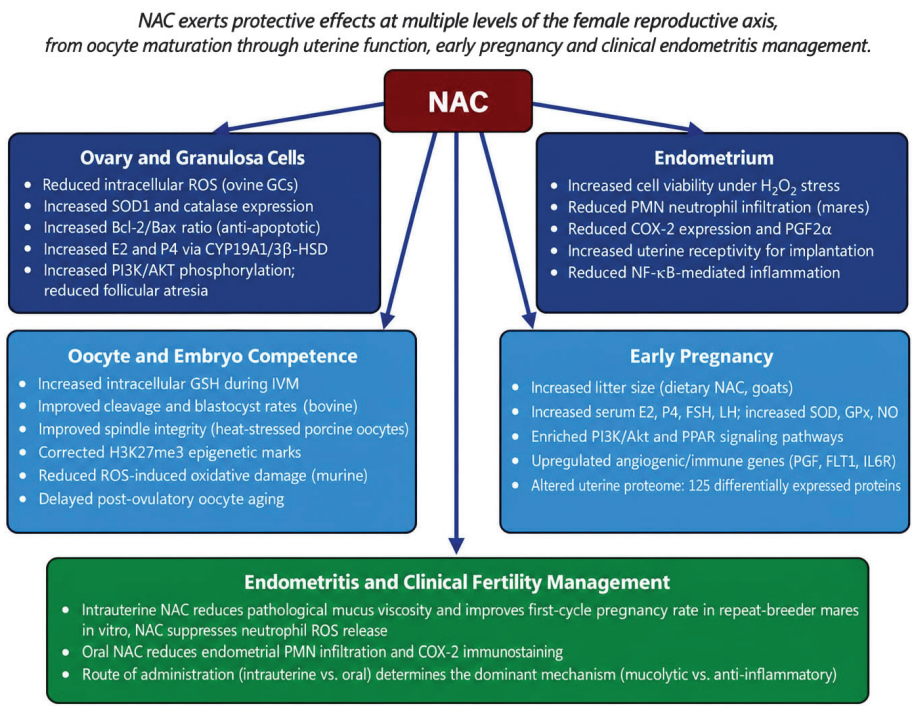


Figure 2. Role of N-acetylcysteine (NAC); NAC exerts protective effects at multiple levels of the female reproductive axis, from oocyte maturation through uterine function, early pregnancy and clinical endometritis management

reduced intraoocyte ROS and improved subsequent fertilization and blastocyst formation.[45] In murine oocytes exposed to bisphenol A, NAC supplementation reduced oxidative damage and increased fertilization and blastocyst rates, and NAC has also delayed postovulatory oocyte aging in mice by preserving mitochondrial function and spindle integrity.[22,46,47] At the molecular level, NAC-mediated GSH replenishment enhanced the GPx and GR enzyme systems, supporting mitochondrial function and embryo developmental kinetics under in vitro conditions, establishing thiol-mediated GSH restoration as a fundamental mechanism underlying NAC's benefits on oocyte competence and embryo quality in domestic animal species (Figure 2).[21,45,48-50]

Granulosa cell proliferation, survival and steroidogenesis

Granulosa cells (GCs) are the primary somatic constituents of ovarian follicles and are responsible for estradiol synthesis, oocyte nurturing and the provision of growth factors that govern follicular fate.[33,34] Their proliferation and survival directly determine whether a follicle progresses to ovulation or undergoes atresia, a process driven principally by GC apoptosis.[33,34,51] Oxidative stress within the follicular microenvironment accelerates GC apoptosis by depleting intracellular GSH, impairing mitochondrial function and activating caspase-dependent cell death pathways, thereby suppressing estrogen secretion and impairing oocyte competence.[7,51]

In cultured ovine GCs, NAC treatment at concentrations of 50-1,000 µmol/l significantly reduced intracellular ROS and upregulated antioxidant enzymes superoxide dismutase 1 (SOD1) and catalase in a concentration-dependent manner, with 100 µmol/l producing the strongest antioxidant response.[52] Furthermore, NAC markedly promoted GC proliferation by increasing expression of cell-cycle regulatory genes CCND1 and CDK4 and the antiapoptotic gene Bcl-2, while concurrently downregulating the proapoptotic gene Bax, yielding an increased Bcl-2/Bax ratio indicative of enhanced cell survival.[52] Proliferative effects were most pronounced after 48 hours of culture at 100 µmol/l NAC.[52]

Beyond its antiapoptotic effects, NAC directly stimulated steroid hormone synthesis. Estradiol and progesterone secretion increased in a dose-dependent manner, accompanied by upregulation of the steroidogenic enzymes CYP19A1 (aromatase) and 3β-hydroxysteroid dehydrogenase (3β-HSD).[52] Mechanistic investigation confirmed that NAC activated the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signalling pathway; NAC significantly increased phosphorylation of both PI3K and AKT and inhibition of PI3K with LY294002 abolished NAC-induced GC proliferation and hormone secretion, confirming PI3K/AKT as the central effector of NAC action in the ovarian follicular microenvironment.[52]

Endometrial protection and uterine cellular integrity

Excessive oxidative stress within the endometrium compromises uterine receptivity and implantation success by inducing apoptosis and necrosis of endometrial epithelial and stromal cells.[8,53,54] In cultured endometrial cells exposed to H_2O_2-induced oxidative injury, NAC significantly improved cell viability and demonstrated superior antioxidant activity relative to other tested antioxidants including flavonoids and α-tocopherol.[53] Critically, NAC was protective only when present during the oxidative insult, indicating that its primary endometrial action is immediate ROS scavenging rather than delayed repair, a finding with practical implications for periimplantation antioxidant timing.[53]

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Through inhibition of redox-sensitive NF-κB activation and downstream COX-2 expression, NAC reduced prostaglandin $F_{2\alpha}$ production and limited inflammatory cell recruitment within the endometrium.[15,53] These combined cytoprotective and antiinflammatory actions support endometrial cellular viability, glandular integrity and the immunological milieu required for embryo implantation and early pregnancy maintenance.[8,53,54]

Early pregnancy support

Early pregnancy is one of the most oxidatively vulnerable phases of reproduction; ∼ 30% of conceptus losses in goats occur during early pregnancy, attributed to impaired uterine environment, elevated ROS and hormonal insufficiency.[11,55] Uterus must simultaneously undergo rapid vascular remodelling, modulate immune responses to tolerate the semiallogeneic embryo and provide a nutrient-rich microenvironment for embryo implantation, all processes sensitive to oxidative perturbation.[9,10]

Dietary supplementation with 0.07% NAC in Nubian goats from days 0-30 of pregnancy significantly increased litter size compared to unsupplemented controls whereas birth weight remained unaffected, indicating that NAC primarily improved embryo survival rather than modifying fetal growth trajectory.[56] Maternal serum nitric oxide (NO) concentrations were significantly elevated following NAC supplementation, a mechanistically important finding since NO promotes uterine angiogenesis, regulates vascular tone, modulates immune tolerance at the fetomaternal interface and directly supports embryo implantation.[56,57]

Transcriptomic analysis of endometrial tissue from NAC-supplemented goats identified 207 differentially expressed genes, enriched in pathways related to oxidoreductase activity, cytokine-receptor interactions, PI3K-Akt signalling, peroxisome proliferator-activated receptor (PPAR) signalling and angiogenesis.[56] Key genes involved in angiogenesis (PGF, FLT1), immune modulation (IL6R) and lipid metabolism (PPARG, FABP3, CD36) were substantially upregulated, indicating that NAC orchestrates a coordinated molecular program of endometrial vascular development, immune regulation and metabolic support for the developing embryo.[56,58]

Complementary proteomic analysis of uterine horn tissue from early-pregnant goats supplemented with 0.07% NAC identified 125 differentially expressed proteins, with 47 upregulated and 78 downregulated.[59] These proteins were predominantly associated with Janus kinase-signal transducer and activator of transcription (Jak-STAT) signalling, RNA surveillance pathways, tight junction integrity, steroid hormone biosynthesis and amino acid biosynthesis, pathways essential for endometrial structural integrity, hormonal responsiveness and appropriate uterine immune responses.[59] NAC supplementation additionally increased serum concentrations of FSH, LH, estradiol and progesterone, alongside enhanced activities of SOD and GPx and elevated NO, confirming improved systemic antioxidant capacity and reproductive endocrine function during early pregnancy.[59]

Endometritis and clinical fertility management

Persistent mating-induced endometritis and chronic endometritis are the principal causes of subfertility in mares, affecting ∼15-20% of mares presented for breeding.[60,61] These conditions are characterized by excessive intrauterine mucus accumulation, sustained neutrophil infiltration and perpetuated inflammatory responses that impair uterine clearance, sperm transport and the penetration of intrauterine antibiotics.[60-62] Activated neutrophils recruited to the uterine lumen following breeding generate substantial quantities of ROS, creating an oxidative environment hostile to sperm and endometrial tissue alike.[62]

NAC's mucolytic action in the equine uterus derives from its reduction of disulfide cross-links within mucin glycoprotein networks, reducing viscoelasticity and improving mucus fluidity.[12,17] Histological evaluation of endometrial biopsies following intrauterine infusion of 3.3% NAC in fertile mares confirmed that NAC significantly reduced extracellular mucus thickness and staining intensity without inducing endometrial irritation or increasing inflammatory cell infiltration, establishing its safety for intrauterine use.[63] In vitro evaluation of serum-derived equine neutrophils demonstrated that a 3% NAC solution significantly inhibited reactive oxygen radical release whereas lower concentrations were ineffective, providing a direct mechanism by which intrauterine NAC protects endometrial epithelium and sperm from neutrophil-mediated oxidative damage.[63]

Clinical reproductive evaluation demonstrated that repeat-breeder mares with excessive mucus production but no active uterine infection achieved a first-cycle pregnancy rate of 77% following prebreeding intrauterine infusion of 3.3% NAC combined with postmating uterine lavage and oxytocin therapy.[63] This rate was comparable to that of reproductively normal control mares and substantially higher than rates achieved with antibiotic treatment alone in mares with bacterial endometritis.[63] Early embryonic death rates in NAC-treated mares remained within acceptable clinical limits, confirming that intrauterine NAC has no adverse effect on early pregnancy maintenance.[63]

Oral NAC treatment (10 mg/kg body weight for 4 consecutive days) in estrous mares produced various effects. Oral NAC did not reduce intrauterine mucus viscosity; indeed, mean effective viscosity increased between days 1 and 5 in treated mares compared to controls.[64] However, oral NAC produced a significant antiinflammatory response; endometrial biopsies collected on day 5 had significantly fewer polymorphonuclear (PMN) neutrophils and markedly reduced nuclear immunostaining for COX-2 in NAC-treated mares.[64] Histochemical evaluation further demonstrated greater mucus accumulation within deep uterine glands, without adverse effects on luminal epithelial integrity or Ki-67-positive cellular proliferation.[64] These findings revealed that route of treatment fundamentally determines NAC's pharmacological action in the equine reproductive tract; intrauterine infusion exerts primarily mucolytic effects, whereas systemic oral administration acts through antiinflammatory and immunomodulatory mechanisms.[63,64]

Role of N-acetylcysteine in male reproduction and sperm quality

Oxidative stress is one of the most important and prevalent etiological factors in male subfertility, negatively affecting spermatogenesis, sperm maturation and postejaculatory sperm function.[4-6,65,66] Physiological ROS concentrations are required for normal sperm capacitation, hyperactivation, acrosome reaction and zona pellucida binding; however,

pathological ROS overproduction disrupts redox homeostasis and initiates lipid peroxidation of the sperm plasma membrane that is particularly vulnerable owing to its high content of docosahexaenoic acid and other polyunsaturated fatty acids.[4,6,67] The downstream consequences include mitochondrial dysfunction, chromatin denaturation, DNA strand breaks, and apoptosis-like changes that reduce motility, impair morphology and compromise the fertilizing potential of affected sperm.[5,6,68]

Much of the intervention evidence linking NAC to improved sperm quality originates from human andrology trials; veterinary evidence base is more limited and centres on semen extension and cryopreservation, summarized below.

In the context of semen cryopreservation, oxidative stress generated during centrifugation, cooling and freezing causes progressive membrane lipid peroxidation and mitochondrial damage that reduce postthaw sperm viability.[69,70] Supplementation of semen extenders with NAC or cysteine significantly reduced ROS production, preserved mitochondrial membrane potential and improved motility and viability in chilled canine semen and postthawed buffalo bull semen, supporting incorporation of thiol antioxidants into extender formulations for cryopreservation in these species.[69,70] At the testicular level, NAC protected spermatogonial cells from oxidative stress-induced apoptosis by suppressing caspase activation and maintaining mitochondrial integrity, thereby safeguarding the spermatogenic stem cell pool under conditions of testicular oxidative stress (Figure 3).[71]

More recent work extended these findings to additional livestock species and to in vivo application. Oral NAC supplementation in heat-stressed goat bucks improved testicular hemodynamics, seminal antioxidant capacity, testosterone concentrations and semen quality, indicating benefits beyond in vitro extender use.[72] In cryopreserved goat semen, NAC supplementation increased antioxidant enzyme activity and postthaw motility while reducing lipid peroxidation and sperm apoptosis in a concentration-dependent manner.[73] Comparable protection has been reported in cryopreserved boar sperm, where NAC supplementation reduced ROS-mediated impairment of motility, plasma membrane integrity and mitochondrial function.[74] In poultry (previously identified as a species group with sparse NAC evidence) NAC supplementation of cryopreservation extenders improved postthaw motility, viability, membrane integrity and mitochondrial membrane potential in rooster semen in a concentration-dependent manner.[75] Reported effects were not uniformly positive across species and doses; in ram semen, only modest NAC concentrations improved postthaw membrane integrity whereas higher concentrations were detrimental to motility, underscoring the need for species and dose-specific optimization before field application.[76]

N-acetylcysteine, mammary health and reproductive efficiency

Bovine mastitis is the most economically damaging disease in dairy cattle and exerts well-documented adverse effects on reproductive performance, including delayed resumption of cyclicity, reduced conception rates and prolonged calving intervals.[77,78] The condition is characterized by intramammary infection, excessive inflammatory responses and local oxidative stress, mediated most commonly by *Staphylococcus aureus* and *Escherichia coli* that evade host immune clearance through biofilm formation.[77,78]

In vitro evaluation of mastitis-associated *Staphylococcus aureus* and *Escherichia coli* isolates demonstrated that NAC exhibits direct antibacterial activity, with minimum inhibitory concentration (MIC) values of 32-128 mg/ml across strains.[79] Critically, NAC at both MIC and subMIC concentrations inhibited biofilm biomass by more than 50-60%, a mechanism of considerable importance for controlling chronic and recurrent infections refractory to conventional antibiotic monotherapy.[79] NAC further demonstrated concentration-dependent free-radical scavenging capacity and dose-dependent antiinflammatory activity, suggesting its potential to simultaneously limit oxidative tissue damage and attenuate excessive inflammatory responses in the mammary parenchyma.[79]

The reproductive implications of these findings operate primarily through the systemic consequences of mammary

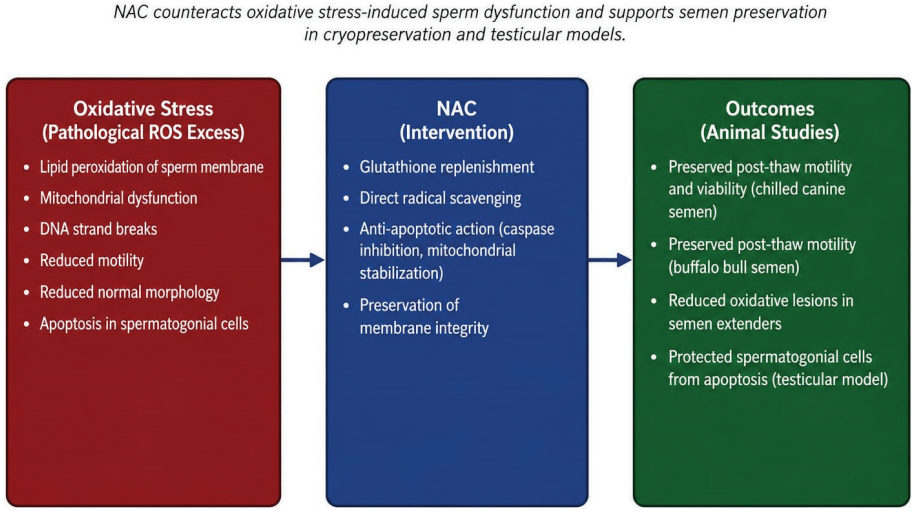


Figure 3. Role of N-acetylcysteine (NAC) in male reproductive biology; NAC counteracts oxidative stress-induced sperm dysfunction at the cellular and molecular level and supports semen quality and viability in cryopreservation and testicular models

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inflammation. Prolonged oxidative stress and inflammatory cytokine release during mastitis impair hypothalamic-pituitary-ovarian axis function, suppress LH pulse frequency, delay ovarian follicular maturation and dysregulate corpus luteum function, collectively contributing to anestrus and reduced conception rates.[77,78] By mitigating the severity and persistence of mastitis through combined antibiofilm, antioxidant and anti-inflammatory mechanisms, NAC may indirectly restore reproductive cyclicity and improve fertility in affected dairy cows.[77,79]

Research gaps and conclusion

The body of evidence reviewed here establishes NAC as a biologically versatile compound with demonstrated reproductive benefits across a remarkable range of species and reproductive compartments.[12,13,15] Its capacity to simultaneously replenish intracellular GSH, scavenge ROS, inhibit apoptotic and inflammatory signalling via NF-κB and MAPK pathways, generate the cytoprotective gasotransmitter H_2S and reduce pathological mucus viscosity makes it applicable across conditions as diverse as equine endometritis, impaired oocyte maturation in cattle and pigs, early embryonic loss in goats, male subfertility and bovine mastitis.[12,15,16,63,64] Despite this breadth, several important research gaps remain that warrant systematic investigation.

First, the majority of evidence is derived from in vitro studies or small-scale in vivo experiments. Large-scale, randomized controlled field trials in commercial livestock populations are needed to establish species-specific effective dosing regimens, optimal routes and timing of treatment and dose-response relationships across reproductive applications. The contrasting effects of intrauterine versus oral NAC in mares illustrated that route of treatment fundamentally determines pharmacological outcome, a distinction requiring systematic investigation across species.

Second, while the PI3K/AKT and MAPK pathways have been identified as downstream mediators of NAC action in granulosa cells and Jak-STAT and PPAR pathways in uterine tissue, the precise protein targets of NAC-derived H_2S through persulfidation in reproductive cells remain incompletely characterized. Integrative omics approaches, including transcriptomics, proteomics and metabolomics applied to ovarian, uterine and seminal cells from NAC-supplemented animals would substantially deepen mechanistic understanding and identify novel biomarkers predictive of reproductive responses to NAC.

Third, the interaction between NAC and the reproductive tract microbiome is entirely unexplored. Given that NAC exhibits direct antibacterial and antibiofilm properties, its effects on the uterine or seminal microbiome and the consequences of such effects on reproductive outcomes represent a timely area for investigation, particularly given the growing appreciation for the role of commensal uterine bacteria in reproductive health.

Fourth, evidence for NAC in poultry and aquaculture reproduction is sparse. Systematic evaluation of NAC supplementation on reproductive efficiency in commercially important poultry species and fish would expand its translational relevance, particularly in the context of heat stress-induced reproductive dysfunction, a challenge common to both sectors.

Fifth, the long-term safety of chronic NAC supplementation in breeding animals requires careful characterization. NAC influences sulphur amino acid metabolism, methyl group availability and thiol-disulfide homeostasis, all of which have pleiotropic effects beyond redox regulation. Rigorous safety profiling across species and production systems, encompassing reproductive endocrine function, thyroid physiology and immune competence, is warranted before routine incorporation into reproductive management protocols.

In conclusion, N-acetylcysteine occupies a distinctive position among veterinary reproductive adjuncts by virtue of the mechanistic depth and species breadth of its documented effects. Its ability to simultaneously address the root cause of many reproductive failures (oxidative stress and its downstream inflammatory and apoptotic consequences) through multiple complementary mechanisms makes it uniquely valuable in reproductive medicine. A clearer understanding of optimal delivery strategies, molecular targets and long-term safety will be essential to translate the substantial mechanistic evidence accumulated to date into practical, evidence-based protocols for improving fertility and reproductive efficiency in domestic animals worldwide.

Conflict of interest

Author declares no conflict of interest.

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