



Nicotinamide Mononucleotide Restores the Meiotic Competency of Porcine Oocytes Exposed to Ethylene Glycol Butyl Ether

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Ethylene glycol butyl ether (EGBE), a type of glycol ethers, is a common chemical used in both industrial and household products. Increasing animal studies have indicated that it produces reproductive problems, such as testicular damage, reduced female fertility, death of embryos, and birth defects. However, how it influences the female germ cells has not yet determined. Here, we found that EGBE exposure resulted in the defective porcine oocyte maturation via disruption of cytoskeleton dynamics, showing the abnormal spindle assembly, chromosome alignment, and actin organization. Meanwhile, EGBE exposure perturbed the mitochondrial distribution and function, leading to the accumulation of reactive oxygen species (ROS) and generation of DNA damage and apoptosis. Of note, nicotinamide mononucleotide (NMN) supplementation rescued the meiotic defects caused by EGBE exposure via restoring NAD⁺ level and mitochondrial function and thus eliminating the excessive ROS. Taken together, our observations illustrate that NMN supplementation is an effective strategy to protect oocyte quality against environmental pollutant-induced deterioration, contributing to improve the animal and human fertility.

Keywords: EGBE, NMN, oocyte quality, cytoskeleton, mitochondrial function

INTRODUCTION

The glycol ethers (GEs) consists of a class of organic solvents widely applied in the household and industrial products (Browning and Curry, 1994). At present, the chemical industry has synthesized more than 30 different GEs (Multigner et al., 2005), and ethylene glycol monobutyl ether (EGBE), ethylene glycol monoethyl ether (EGEE), and ethylene glycol monomethyl ether (EGME) are the most widely consumed products (Fort et al., 2018). Due to their potential toxicity and widespread availability, it has attracted more attentions. A previous study on the EGME-exposed female workers shows the extended menstrual cycles and the time to pregnancy (Hsieh et al., 2005). Furthermore, *in vitro* investigation of meiotic resumption in *Xenopus* oocytes has indicated that EGME influences the critical process of oocyte maturation prior to fertilization (Fort et al., 2002). EGEE is soluble in water or other solvents and absorbed through the skin of the body. EGEE in

the body is then converted to ethoxy acetaldehyde (EAA) and ethoxy acetic acid (EAA), two toxic metabolites, by ethanol dehydrogenase and aldehyde dehydrogenase (Cheever et al., 1984; Kennedy et al., 1993). Recent studies have found that EGEE exposure causes the health risks including the damage to the liver, blood, and reproductive systems (Welsch, 2005; Scofield et al., 2006; Wang et al., 2006). Repeated exposure to EGEE causes testicular atrophy, reduced sperm count, and sperm motility retardation (Melnick, 1984; Wang et al., 2006, 2007). EGBE is rapidly absorbed through dermal, pulmonary, and gastrointestinal routes (Bauer et al., 1992). The acute exposure to EGBE not only results in renal, neurologic, hematologic, and metabolic disorders but also leads to acute severe respiratory failure (Bauer et al., 1992). The reproductive toxicity of EGBE has been also reported in female mice by showing the subfertility (Cicoletta, 2006). Nevertheless, the underlying mechanisms concerning how EGBE impacts the female fertility and the quality of oocytes remain largely unknown.

Nicotinamide adenine dinucleotide (NAD⁺) is a coenzyme for multiple redox reactions involved in glycolysis, oxidative phosphorylation and tricarboxylic acid cycle in cells (Camacho-Pereira et al., 2016). In particular, NAD⁺ has effects on a variety of cellular endpoints directly or indirectly as a rate-limiting substrate for sirtuin proteins, the critical enzymes to regulate the mitochondrial homeostasis and pro-survival pathways (Anastasiou and Krek, 2006; Csiszar et al., 2008, 2009a,b; Das et al., 2018). It has been reported that decrease of intracellular NAD⁺ changes the NAD⁺/SIRT1 pathway and compromises the mitochondrial function, thereby leading to the ROS accumulation, DNA damage and apoptosis in neurons (Croteau et al., 2017). Notably, NMN, a key precursor to NAD⁺, has been implicated in replenishing NAD⁺ levels and restoring the expression of genes related to circadian rhythm, inflammatory response, and oxidative stress by activation of Sirt1 in both HFD-induced and aged mice (Yoshino et al., 2011).

In the present report, we investigated the potential toxicity of EGBE on the oocyte meiotic progression and cytoskeleton dynamics in pigs. We also validated the beneficial impact of NMN supplementation on the developmental competency of EGBE-exposed oocytes.

MATERIALS AND METHODS

Antibodies

Mouse monoclonal α -tubulin-FITC (fluorescein isothiocyanate) and acetyl- α -tubulin (Lys40) antibodies were obtained from Sigma-Aldrich (St Louis, MO, United States; Cat# F2168, ABT241); rabbit monoclonal GAPDH and Phospho-Histone H2A.X (Ser139) antibodies were obtained from Cell Signaling Technology (Danvers, MA, United States; Cat# 2118, 9718).

Collection of Porcine Oocytes

Porcine ovaries were obtained from a local abattoir and transported to the laboratory in a physiological saline containing streptomycin sulfate and penicillin G within 2 h after slaughtering. Cumulus-oocyte complexes (COCs) were

aspirated from the follicles using a disposable syringe. COCs with a compact cumulus cells were selected for *in vitro* maturation (IVM). The maturation medium is TCM-199 (Thermo Fisher Scientific, Waltham, MA, United States; Cat# 11150059) supplemented with 10% porcine follicular fluid, 5 μ g/mL insulin, 10 ng/mL EGF, 0.6 mM cysteine, 0.2 mM pyruvate, 25 μ g/mL kanamycin, and 10 IU/mL of each eCG and hCG. 20 germinal vesicle (GV) COCs were cultured in a drop of 100 μ L maturation medium covered with mineral oil at 38.5°C, 5% CO₂ for 26–28 h to metaphase I stage and for 42–44 h to metaphase II stage.

EGBE and NMN Treatment

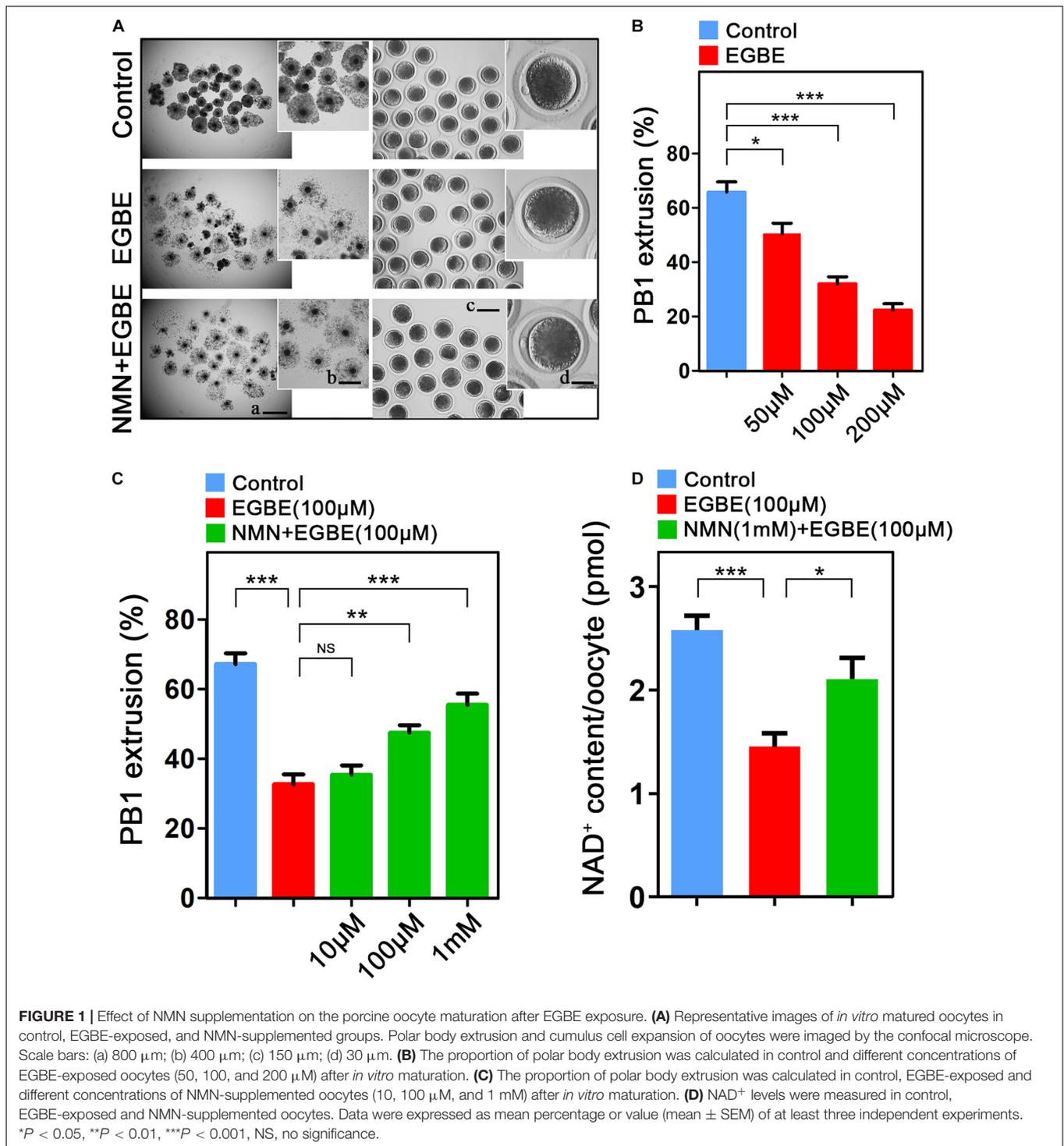
Ethylene glycol butyl ether (EGBE) was obtained from Sigma-Aldrich (Cat# 53071) and dissolved in the water to 500 mM for a stock solution, which was further diluted with maturation medium to a working concentration of 50, 100, or 250 μ M, respectively. Nicotinamide mononucleotide (NMN; GeneHarbor Biotech, Hong Kong, China) was dissolved in the water to 1 M for a stock solution and diluted with maturation medium to a working concentration of 100 nM, 10 μ M, and 1 mM, respectively.

Measurement of NAD⁺ Levels

NAD⁺ levels were measured with a NAD/NADH Quantitation Kit (Sigma-Aldrich; Cat# MAK037) according to the manufacturer's instruction. In brief, 120 oocytes were harvested for total NAD⁺ extraction and quantification based on the procedure described by Pantazi et al. (2015). The NAD⁺ concentration was calculated by subtracting the NADH values from NAD_{total} (NAD⁺ and NADH). NAD_{total} and NADH levels were quantified in a colorimetric assay at 450 nm using iMark™ Microplate Absorbance Reader (Bio-Rad, Hercules, CA, United States).

Fluorescence Staining and Confocal Microscopy

Denuded oocytes (DOs) were obtained by transferring COCs in 300 μ L of 10 mg/mL hyaluronidase solution for 15 min. Then DOs were incubated in the fixation solution (4% paraformaldehyde/PBS) for 30 min, in the permeabilization solution (1% Triton X-100/PBS) for 1 h, and in the blocking solution (1% BSA-supplemented PBS) for 1 h at room temperature (RT), followed by incubation with α -tubulin-FITC antibody (1:200), acetyl- α -tubulin antibody (1:100), γ H2A.X antibody (1:100) or phalloidin-TRITC (1:100; Sigma-Aldrich; Cat# P1951) overnight at 4°C. After washes in PBST, oocytes were incubated with the corresponding secondary antibodies for 1 h and counterstained with 10 μ g/mL Hoechst 33342 or propidium iodide (PI) for 10 min at RT. In addition, oocytes were stained at 38.5°C for 30 min with 500 nM MitoTracker Red CMXRos (Thermo Fisher Scientific; Cat# M7512) for mitochondrion staining, with 2 μ M MitoProbe JC-1 (Thermo Fisher Scientific; Cat# M34152) for mitochondrial membrane potential assessment, with 10 μ M dichlorofluorescein diacetate (DCFHDA; Beyotime, Huangzhou, China; Cat# S0033S) for ROS staining, and with Annexin-V-FITC (1:10; Beyotime; Cat#



C1062) for apoptosis assessment. Lastly, oocytes were mounted on the glass slides and imaged under a confocal microscope (LSM 700 META, Zeiss, Germany).

Immunoblotting

Porcine oocytes were collected in the lysis buffer (4 \times LDS sample buffer, Thermo Fisher Scientific; Cat# NP0007) with

protease inhibitor and heated at 95°C for 5 min. Proteins were separated on 10% precast gels (Bis-Tris) and transferred to PVDF membranes. The blots were then incubated in the blocking buffer (5% low fat dry milk/TBST) for 1 h at RT and probed with acetyl- α -tubulin antibody (1:1,000) or GAPDH antibody (1:5,000) overnight at 4°C. After washes in TBST, the blots were incubated with the corresponding secondary antibodies for 1 h

at RT. Chemiluminescence signals were acquired with ECL Plus (ThermoFisher Scientific; Cat# 32132) and protein bands were detected by Tanon-3900 Imaging System.

Evaluation of Total ATP Content

Total ATP content in a pool of 20 oocytes was determined using the Bioluminescent Somatic Cell Assay Kit (Sigma-Aldrich; Cat# FLASC), following the procedure described by Combelles and Albertini (2003) and the manufacturer's instruction. A 5-point standard curve (0, 0.1, 0.5, 1.0, 10, and 50 pmol of ATP) was generated in each assay and the ATP content was calculated by using the formula derived from the linear regression of the standard curve.

RNA Extraction and Quantitative Real-Time PCR

Fifty oocytes were collected to extract the total RNA using RNeasy Mini Kit (Qiagen, Germantown, MD, United States; Cat# 74104) which was then reversed to cDNA with PrimeScript RT Master Mix (Takara, Kusatsu, Shiga, Japan; Cat# RR036A). Quantitative real-time PCR was performed using Real-Time PCR System (QuantStudio 7 Flex, Thermo Fisher Scientific) with SYBR Green PCR Master Mix (Thermo Fisher Scientific; Cat# 4344463). Data were normalized with *GAPDH* and the fold change was quantified by the comparative CT method. The primers were listed as follows:

GSR (F: ACAGTGGGACTCACAGAAGA/R: AGGTAGGATGAATGGCAAC);

GPX1 (F: CCAAGTTTATCACCTGGTCTCC/R: AGGCACTGCTAGGCTCCTG); *GPX4* (F: TGTGGTTTACGGATTCTGG/R: CCTTGGGCTGGACTTTCA);

SOD1 (F: GGTCCTCACTTCAATCCTG/R: CTTCATTTCCACCTCTGC);

SOD2 (F: TATCCGTCGGCGTCCAAG/R: GCGGCGTATCGCTCAGTT);

GAPDH (F: TGGGCTACACTGAGGACC/R: TACCAGGAATGAGCTTGA).

Statistical Analysis

Data from at least three independent replicates were designated as mean percentage or value (mean \pm SEM). Differences between two groups were analyzed by paired-samples *t*-test using GraphPad Prism 6 statistical software. *P* < 0.05 was accepted to be significant.

RESULTS

NMN Recovers the Meiotic Maturation of EGBE-Exposed Porcine Oocytes

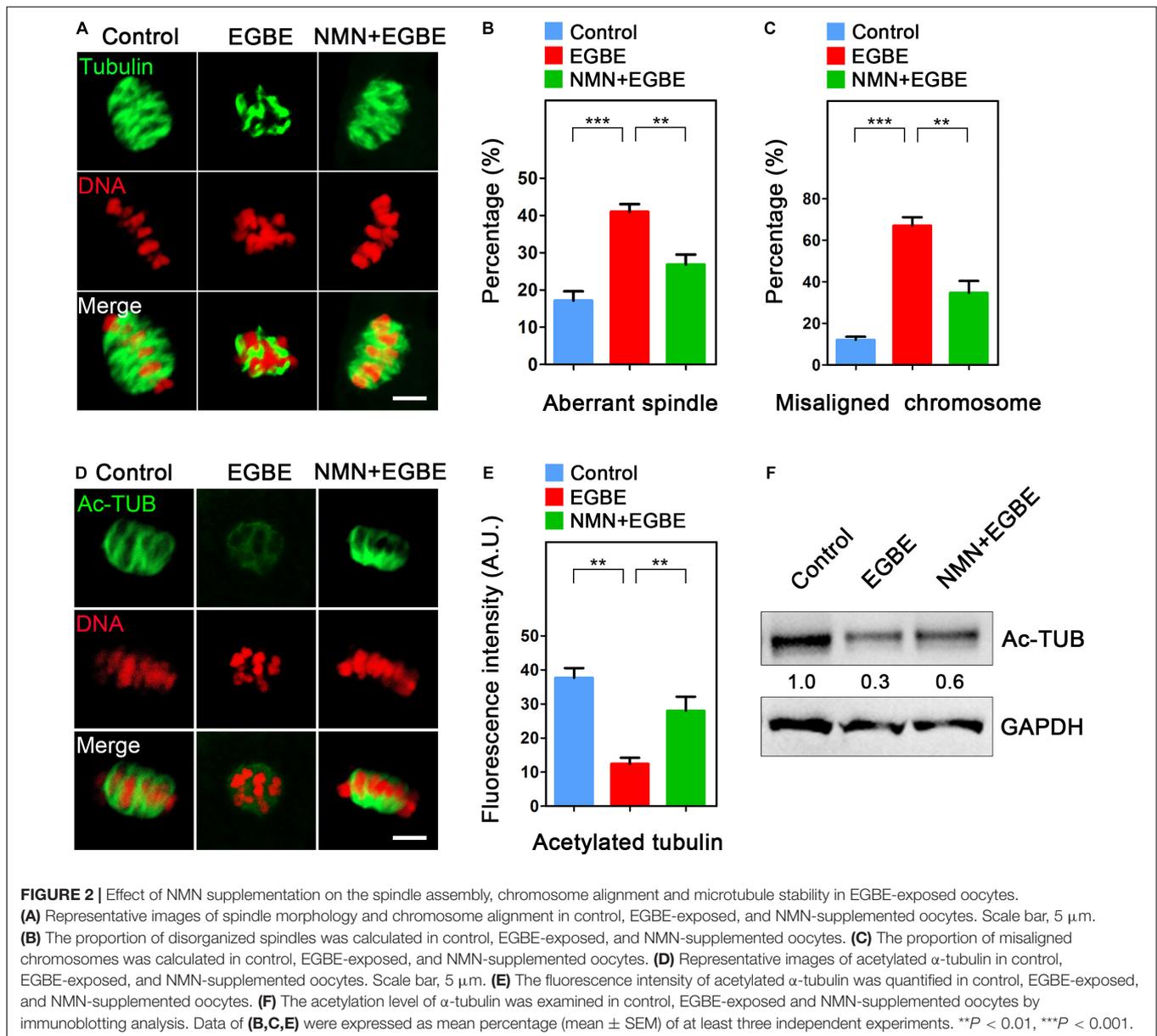
To examine the influence of EGBE on the meiotic progression of porcine oocytes, increasing concentrations of EGBE (50, 100, and 200 μ M) were added to the *in vitro* maturation medium. We observed that EGBE exposure prominently led to the oocyte meiotic failure by showing the defective expansion of cumulus cells and the reduced proportion of oocytes that completed

the maturation (**Figure 1A**). Quantification data revealed that exposure to different doses of EGBE all caused a decline in the polar body extrusion (PBE) after IVM for 44 h, and addition with 100 and 200 μ M EGBE considerably lowered the rate of PBE from 67% in controls to 32 and 22%, respectively ($66.7 \pm 2.8\%$, *n* = 112, control vs. $32.1 \pm 2.4\%$, *n* = 101, 100 μ M EGBE, *P* < 0.001 vs. $22.3 \pm 2.3\%$, *n* = 109, 200 μ M EGBE, *P* < 0.001; **Figure 1B**). We then used 100 μ M EGBE for subsequent studies. To test whether NMN can improve the defective oocyte development induced by EGBE exposure, we supplemented different doses of NMN (10, 100 μ M, and 1 mM) with 100 μ M EGBE in the maturation medium. As expected, 1 mM NMN increased the proportion of PBE in EGBE-exposed oocytes to the level indistinguishable from the controls ($67.2 \pm 2.1\%$, *n* = 117, control, *P* < 0.001 vs. $32.7 \pm 2.8\%$, *n* = 120, EGBE vs. $55.5 \pm 2.1\%$, *n* = 110, 1 mM NMN, *P* < 0.001; **Figure 1C**). We further detected the NAD⁺ levels after EGBE exposure and NMN supplementation in oocytes. The results displayed that NMN supplementation significantly elevated the NAD⁺ levels that were reduced in EGBE-exposed oocytes (2.58 ± 1.13 , *n* = 120, control, *P* < 0.001 vs. 1.45 ± 1.55 , *n* = 120, EGBE vs. 2.11 ± 1.24 , *n* = 120, NMN, *P* < 0.05; **Figure 1D**). Altogether, these results indicate that NMN can recover the failure of porcine oocyte meiotic maturation induced by EGBE exposure via restoring the NAD⁺ level.

NMN Restores the Spindle/Chromosome Structure and Microtubule Dynamics in EGBE-Exposed Porcine Oocytes

Given that oocyte maturational arrest is usually correlated with the disruption of spindle assembly, we then tested this in EGBE-exposed oocytes by staining them with α -tubulin-FITC antibody. The immunofluorescence results in **Figure 2A** displayed that most of oocytes in the control exhibited a typical barrel-shaped spindle apparatus with well-aligned chromosomes. By contrast, EGBE-exposed oocytes showed various disorganized spindle morphologies with misaligned chromosomes (**Figure 2A**). Conversely, NMN supplementation reduced the incidence of aberrant spindle/chromosome structure induced by EGBE exposure (disorganized spindle: $17.2 \pm 2.5\%$, *n* = 37, control, *P* < 0.001 vs. $41.0 \pm 2.1\%$, *n* = 36, EGBE vs. $26.9 \pm 2.6\%$, *n* = 38, NMN, *P* < 0.01; misaligned chromosome: $12.1 \pm 1.5\%$, *n* = 37 control, *P* < 0.001 vs. $67.1 \pm 2.1\%$, *n* = 36, EGBE vs. $34.7 \pm 2.8\%$, *n* = 38, NMN, *P* < 0.01; **Figures 2A–C**).

One of the most important indicators for the microtubule stability is the acetylation level of α -tubulin in both somatic and germ cells (Montagnac et al., 2013; Xu et al., 2017). Thus, we asked whether the defective spindle organization in EGBE-exposed oocytes results from the perturbed microtubule dynamics through evaluating the acetyl- α -tubulin level. As assessed in **Figure 2D**, the acetyl- α -tubulin signals were dramatically disappeared in EGBE-exposed oocytes compared to the controls. The quantification of fluorescence intensity and immunoblotting analysis further verified this observation (**Figures 2E,F**). On the contrary, NMN supplementation significantly increased the acetyl- α -tubulin signals in EGBE-exposed oocytes (37.7 ± 2.9 , *n* = 31, control, *P* < 0.01 vs.



12.4 ± 2.7 , $n = 29$, EGBE vs. 28.1 ± 2.1 , $n = 30$, NMN, $P < 0.01$; **Figures 2D–F**), suggesting that NMN is able to restore the abnormal spindle formation caused by EGBE exposure by maintaining the microtubule stability.

NMN Rescues the Actin Dynamics in EGBE-Exposed Porcine Oocytes

In normal oocytes, the actin cytoskeleton takes critical parts in the establishment of cortical polarization and asymmetric spindle positioning. To test whether the actin dynamics is involved in the EGBE effect on oocyte quality, we applied phalloidin-TRITC to display the actin filaments. The data as judged by fluorescence imaging and quantification in **Figure 3** validated the impairment of actin integrity in EGBE-exposed oocytes, showing the remarkable decrease of signals on the plasma membrane

in comparison with the controls (**Figures 3A–C**). While, NMN supplementation substantially raised the actin signals in EGBE-exposed oocytes (48.1 ± 1.3 , $n = 29$, control, $P < 0.001$ vs. 13.6 ± 1.2 , $n = 31$, EGBE vs. 32.7 ± 1.3 , $n = 33$, NMN, $P < 0.01$; **Figures 3A–C**). Overall, all above results illustrate that NMN improves the oocyte maturation via maintaining the integrity of cytoskeleton.

NMN Improves the Mitochondrial Function in EGBE-Exposed Porcine Oocytes

As an energy factory of cells, mitochondria produce enough ATP for various cellular events, including the cytoskeleton dynamics. We therefore examined if mitochondrial function

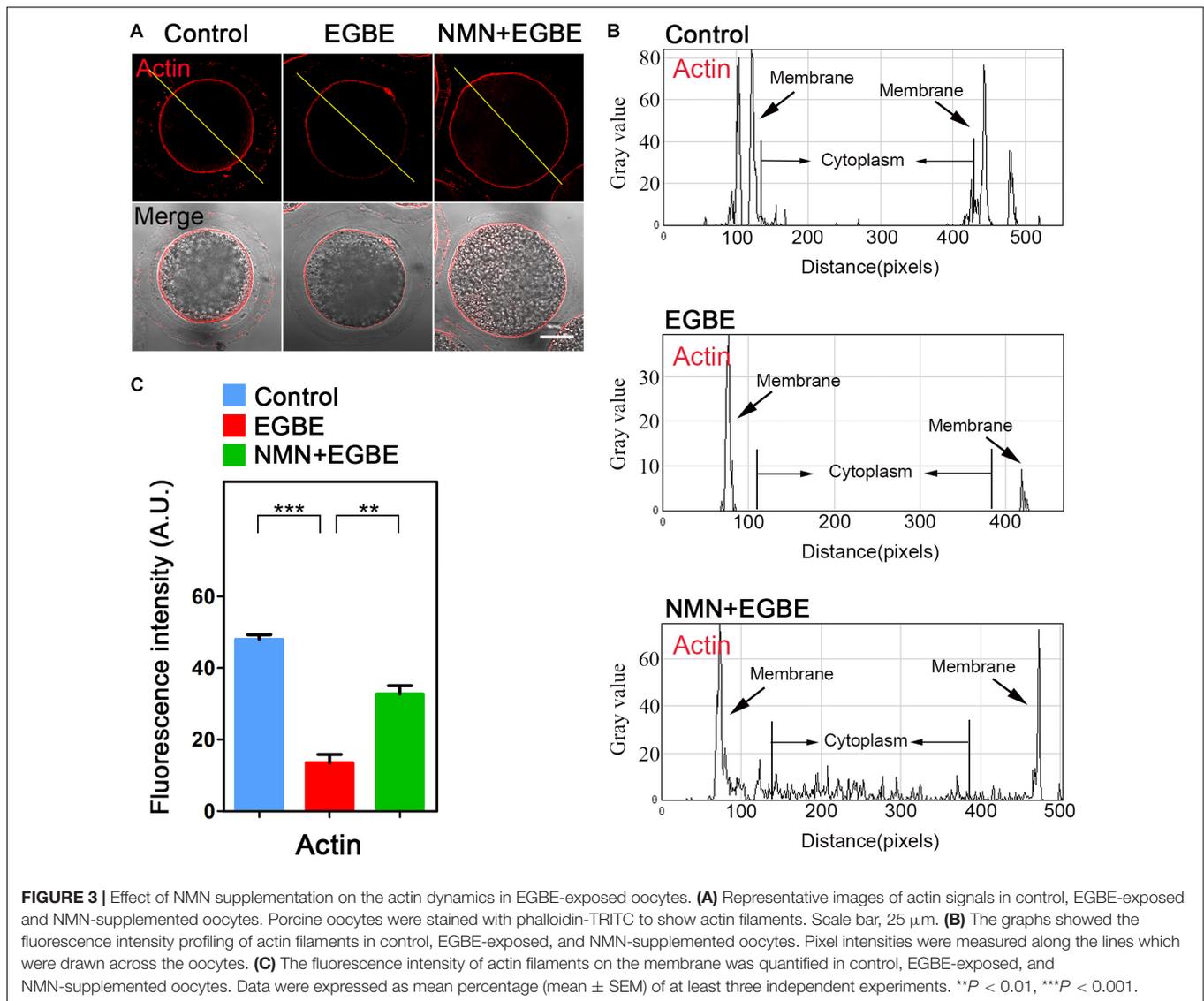


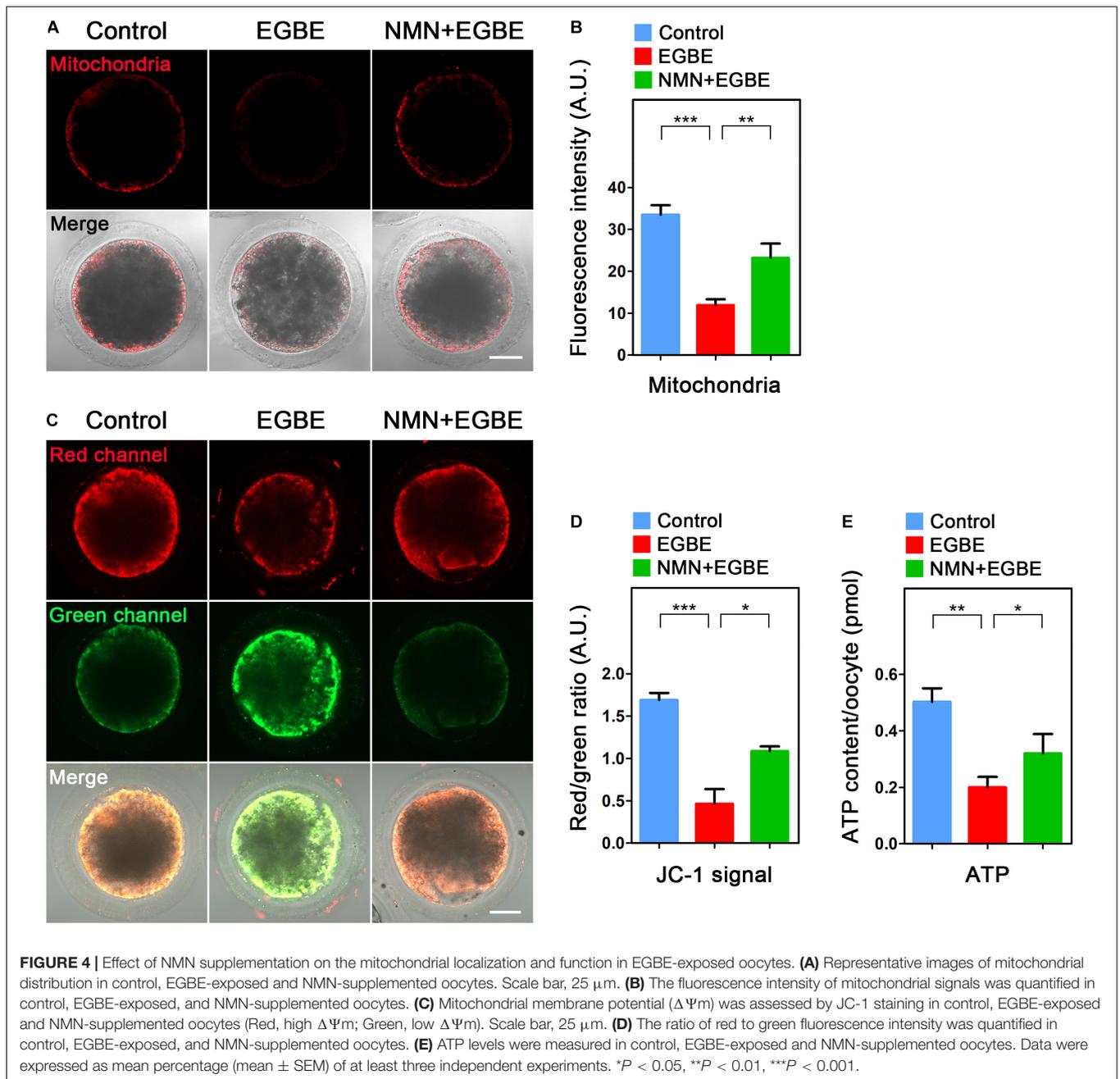
FIGURE 3 | Effect of NMN supplementation on the actin dynamics in EGBE-exposed oocytes. **(A)** Representative images of actin signals in control, EGBE-exposed and NMN-supplemented oocytes. Porcine oocytes were stained with phalloidin-TRITC to show actin filaments. Scale bar, 25 μm . **(B)** The graphs showed the fluorescence intensity profiling of actin filaments in control, EGBE-exposed, and NMN-supplemented oocytes. Pixel intensities were measured along the lines which were drawn across the oocytes. **(C)** The fluorescence intensity of actin filaments on the membrane was quantified in control, EGBE-exposed, and NMN-supplemented oocytes. Data were expressed as mean percentage (mean \pm SEM) of at least three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

is disturbed in EGBE-exposed oocytes. Firstly we examined the mitochondrial localization by MitoTracker Red staining and found that a majority of mitochondria distributed around lipid droplets in the subcortex of control porcine oocytes, but lost this normal distribution pattern in EGBE-exposed oocytes (Figure 4A). The quantification of fluorescence intensity revealed that mitochondrial signals dramatically declined in the EGBE-exposed oocytes compared to the controls (Figure 4B). On the contrary, this abnormality was protected by the treatment with NMN (33.5 ± 2.3 , $n = 27$, control, $P < 0.001$ vs. 11.9 ± 2.3 , $n = 25$, EGBE vs. 23.3 ± 2.4 , $n = 28$, NMN, $P < 0.01$; Figures 4A,B). We also applied JC-1 staining to evaluate the mitochondrial membrane potential ($\Delta\Psi\text{m}$). A red fluorescence represented the high membrane potential while a green fluorescence indicated the low membrane potential (Figure 4C). The quantification results displayed that the ratio of red to green signals was considerably decreased in EGBE-exposed oocytes compared to the controls, but elevated in NMN-supplemented oocytes (1.69 ± 0.08 , $n = 29$,

control, $P < 0.001$ vs. 0.46 ± 0.17 , $n = 31$, EGBE vs. 1.09 ± 0.06 , $n = 28$, NMN, $P < 0.05$; Figures 4C,D). As production of ATP is the main function of mitochondria, we lastly quantified the ATP levels in three groups of oocytes. The data revealed that ATP content was prominently reduced in EGBE-exposed oocytes in comparison with the controls, but restored after supplementation of NMN (0.5 ± 0.048 , $n = 120$, control, $P < 0.01$ vs. 0.2 ± 0.037 , $n = 120$, EGBE vs. 0.32 ± 0.068 , $n = 120$, NMN, $P < 0.05$; Figure 4E). Altogether, these observations suggest that NMN supplementation improves the EGBE-induced mitochondrial dysfunction in oocytes.

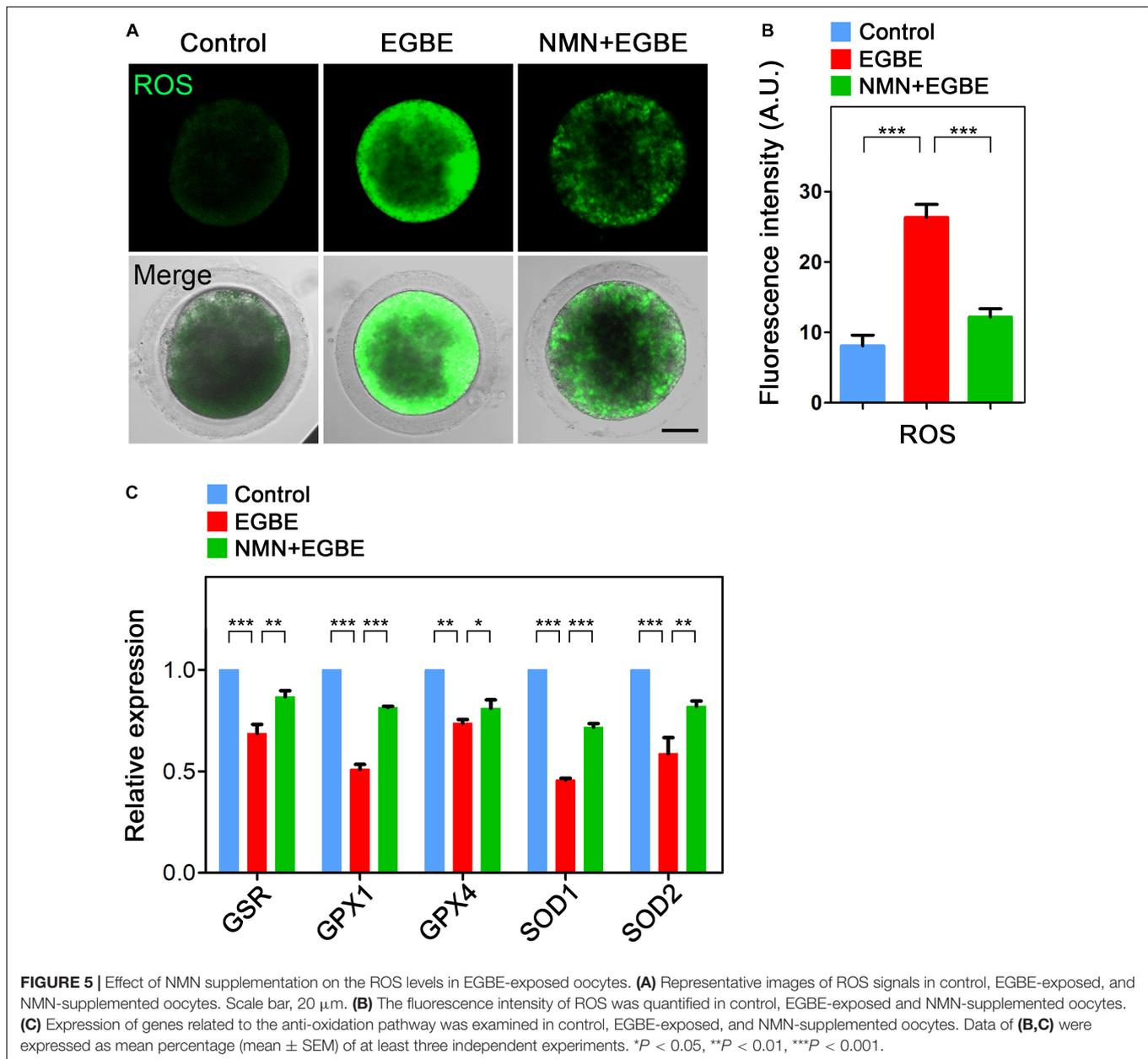
NMN Removes the Accumulated ROS to Suppress DNA Damage and Apoptosis in EGBE-Exposed Porcine Oocytes

Mitochondrial dysfunction is usually correlated with the excessive ROS accumulation, which can result in the oxidative



damage to macromolecules in cells and thus induce the apoptosis. To verify this assumption in the context of EGBE exposure, we performed DCFH staining to assess the ROS levels in control and EGBE-exposed oocytes. The data revealed that ROS signals were hardly detected in the cytoplasm of control oocytes, but considerably elevated in EGBE-exposed oocytes (Figure 5A). Expectedly, NMN supplementation markedly lowered the ROS signals in EGBE-exposed oocytes (8.1 ± 1.5 , $n = 23$, control, $P < 0.001$ vs. 26.3 ± 1.9 , $n = 24$, EGBE vs. 12.2 ± 1.2 , $n = 23$, NMN, $P < 0.001$; Figures 5A,B). In addition, we validated the downregulated expression of a couple of genes implicated in the anti-oxidation pathways in EGBE-exposed oocytes, but

recovered in NMN-supplemented oocytes (Figure 5C). As the high level of ROS usually induces DNA damage and apoptosis (Ratan et al., 1994; Ozben, 2007), we next assessed DNA damage accumulation by $\gamma\text{H2A.X}$ antibody staining and early apoptosis occurrence by Annexin-V staining. The fluorescent images and intensity measurement analysis displayed that $\gamma\text{H2A.X}$ signals were enhanced on the chromosomes in EGBE-exposed oocytes compared to the controls (Figures 6A,B). Whereas supplementation with NMN significantly reduced the accumulation of DNA damage (10.7 ± 1.4 , $n = 21$, control, $P < 0.001$ vs. 33.0 ± 1.6 , $n = 23$, EGBE vs. 18.3 ± 1.6 , $n = 21$, NMN, $P < 0.01$; Figures 6A,B). Furthermore, we observed that

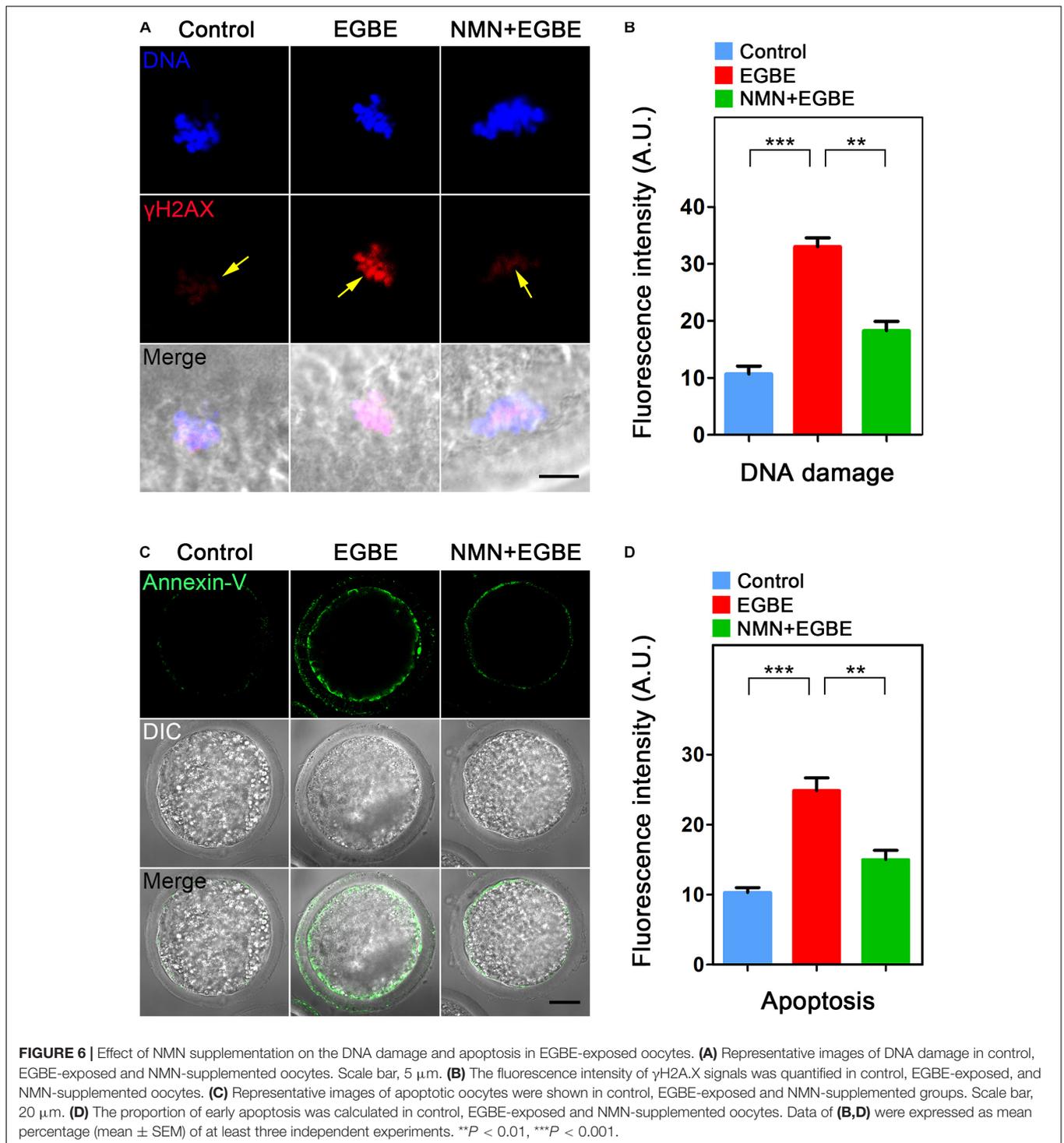


Annexin-V signals were hardly found in control oocytes, but robustly present on the membrane of EGBE-exposed oocytes (**Figure 6C**). Concordantly, the frequency of apoptosis was significantly elevated in EGBE-exposed group, but decreased in NMN-supplemented group (10.3 ± 0.7 , $n = 29$, control, $P < 0.001$ vs. 24.9 ± 1.8 , $n = 31$, EGBE vs. 15.1 ± 1.3 , $n = 27$, NMN, $P < 0.01$; **Figures 6C,D**). Collectively, our findings demonstrate that NMN.

DISCUSSION

Ethylene glycol butyl ether (EGBE), which belongs to the chemical family of glycol ethers, is a commonly used solvent for hydraulic fluids, varnishes and resins, and a component of

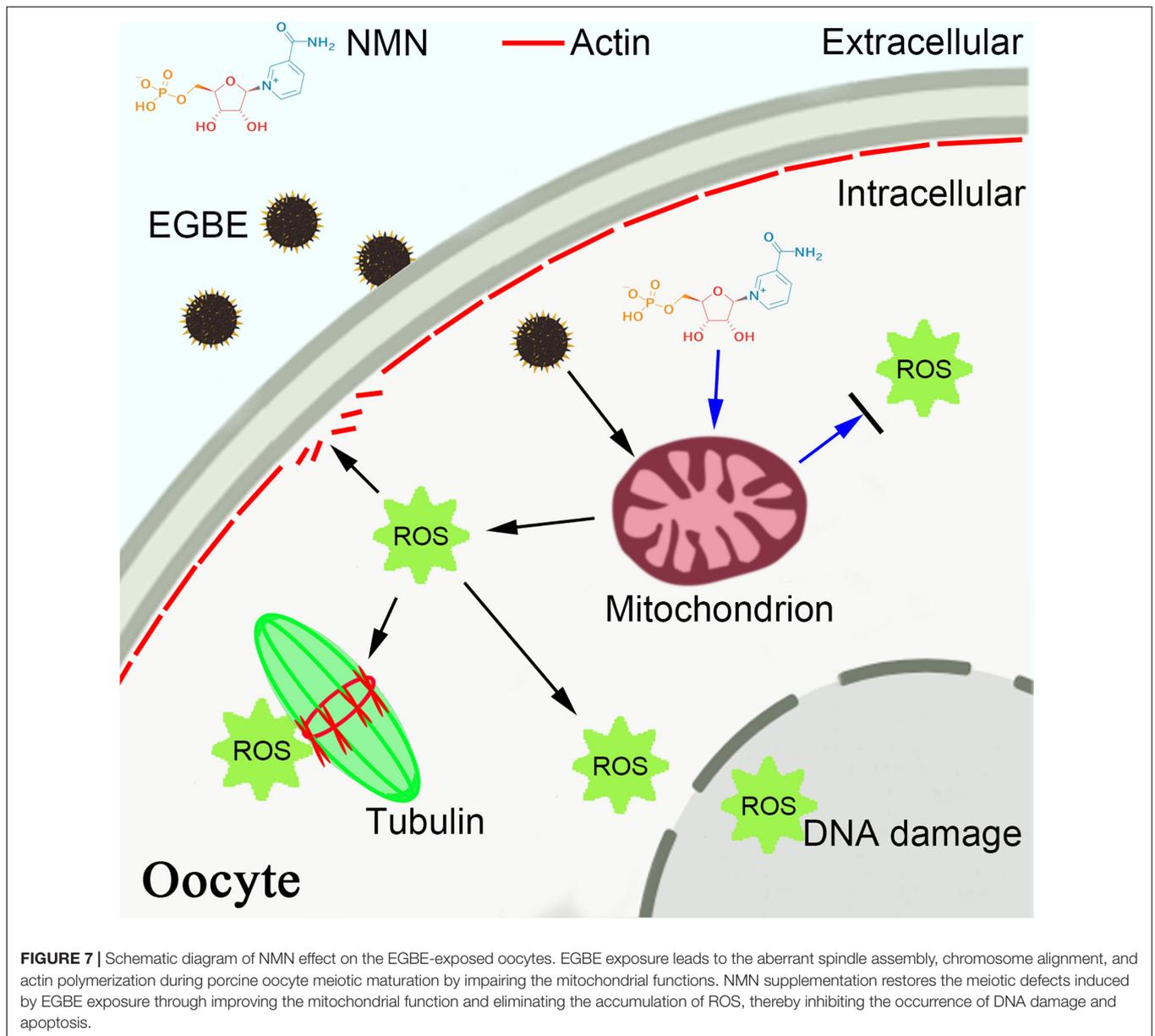
various household products such as floor polishes and waxes, as well as cleaning compositions for upholstery, leather and glass (Rambourg-Schepens et al., 1988; Browning and Curry, 1994). In recent years, an increasing amount of studies have reported that compounds in the glycol ethers have adverse impact on the reproductive system. Ethylene glycol monoethyl ether (EGEE) severely reduces the activities of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), and thus destroys the redox balance in the rat testes and sperm (Adedara and Farombi, 2010). The percentage of tetraploid sperm in male rats exposed to EGEE for 14 days (400 mg/kg/day) was significantly higher than that in controls (Yu et al., 1999). Ethylene glycol monomethyl ether (EGME) has been shown to induce the apoptosis of mouse oocytes and granulosa cells (Weng et al., 2010). Supplementation



of EGME during oocyte development also affected nuclear maturation and impaired the meiotic process of *Xenopus laevis* eggs (Fort et al., 2002). However, how EGBE affects the quality of female germ cells remains to be further explored.

To this end, we investigated the extrusion of polar body and the expansion of cumulus cells, two key indicators for the normal porcine oocyte maturation. EGBE-exposed oocytes

displayed a reduced frequency of polar body extrusion with weakened expansion of cumulus cells in a dose-dependent manner, suggesting that EGBE exposure leads to the failure of oocyte meiotic maturation. Since the impairment of cytoskeletal assembly often results in the defect of cell division in both meiotic and mitotic cells (Azoury et al., 2008; Heng and Koh, 2010), we further explored the negative



effect of EGBE exposure on the oocyte meiotic progression by testing the cytoskeleton dynamics. Our data showed that EGBE exposure disrupted the spindle organization through compromising the stability of microtubules, which was coupled with the chromosome misalignment. Moreover, EGBE exposure disturbed the integrity of actin, another essential component of cytoskeleton, on the oocyte membrane. Thus, these findings demonstrate that the perturbed oocyte maturation induced by EGBE exposure is attributed to the defective cytoskeleton dynamics.

Mitochondrion functions as a primary factory in oocytes to generate ATP source for cell development (Dumollard et al., 2007; Niu et al., 2019), and its distribution pattern has been considered as one of the critical indicators for evaluating the oocyte cytoplasmic maturation. Our findings revealed that

the localization, membrane potential, and ATP production of mitochondria were disturbed in EGBE-exposed oocytes, implying that EGBE exposure impairs the oocyte cytoplasmic maturation by destructing the mitochondrial distribution and function. Consequently, ROS levels were increased to induce the accumulation of DNA damage and occurrence of apoptosis in EGBE-exposed oocytes.

It has been reported that NMN, a key NAD^+ intermediate, recovers the function of cerebrovascular endothelial and neurovascular coupling responses in aged mice by attenuating mitochondrial production of ROS (Tarantini et al., 2019). In addition, we recently evidenced that *in vivo* supplementation of NMN restores the NAD^+ level to ameliorate the quality of oocytes from aged mice *via* maintaining the chromosome euploidy and fertilization ability

(Miao et al., 2020). In line with this protective effect NMN, we validated that NMN could recover the EGBE exposure-induced meiotic defects by restoring NAD⁺ level and removing excessive ROS, hence suppressing DNA damage accumulation and apoptosis occurrence in porcine oocytes.

To sum up, we provide a body of evidence documenting that NMN protects the oocytes from EGBE exposure by restoring the mitochondrial function and suppressing EGBE-induced production of ROS and apoptosis (Figure 7).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Research Institute Committee of Nanjing Agricultural University, China.

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AUTHOR CONTRIBUTIONS

BX and YF designed the research. YM, XL, XS, QG, JC, and RW performed the experiments. YM, XL, YF, and BX analyzed the data. YM, YF, and BX wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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