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N-Acetyl cysteine reduces the levels of reactive oxygen species and improves *in vitro* maturation of oocytes from medium-sized bovine antral follicles

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Research Article

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Summary

This study aims to evaluate the effects of *N*-acetylcysteine (NAC) on bovine oocyte maturation, mitochondrial activity and transzonal projections (TZP), as well as on the levels of reactive oxygen species (ROS) and messenger RNA (mRNA) for catalase (*CAT*) superoxide dismutase (*SOD*), periredoxin-6 (*Prdx6*), glutathione peroxidase (*GPx*), growth and differentiation factor-9 (*GDF9*), histone *H1Foo*, cyclin B1 (*CCNB1*) and c-*Mos*. Bovine cumulus–oocyte complexes (COC) of medium-sized antral follicles (3.0–6.0 mm) were prematured in TCM-199 for 8 h at 38.5°C in 5% CO_2. After prematuration in the presence of forskolin and C-type natriuretic peptide, COCs were matured in TCM-199 alone or with 0.1, 0.5 or 2.5 mM NAC. Then, oocytes were classified according to the stage of chromatin. Furthermore, mitochondrial activity and intracellular levels of ROS and TZP were also evaluated. The levels of mRNAs for *CAT*, *SOD*, *Prdx6*, *GPx*, *GDF9*, *H1Foo*, *CCNB1* and c-*Mos* were evaluated using real-time polymerase chain reaction (RT-PCR). The results showed that NAC significantly increased the percentages of oocytes with resumption of meiosis when compared with those oocytes matured in control medium. Oocytes had homogeneous mitochondrial distribution, and those cultured with 0.1 and 0.5 mM NAC had lower levels of ROS when compared with the control. In addition, 0.5 mM NAC reduced TZP and the levels of mRNA for *CCNB1*. In contrast, NAC did not influence the expression of *CAT*, *GPx*, *Prdx6*, *SOD*, *GDF9*, *H1Foo*, and c-*Mos*. In conclusion, 0.5 mM NAC reduced the levels of ROS, TZP and mRNA for *CCNB1*, and improved *in vitro* resumption of meiosis in oocytes from medium-sized bovine antral follicles.

Introduction

The efficiency of *in vitro* embryo production still needs to be improved in domestic animals. For example, in bovine species, oocytes aspirated from slaughterhouse ovaries have heterogeneous cellular and molecular features and, consequently, the rate of oocytes that reach the blastocyst stage is ~35% (Lonergan and Fair 2008). As recently reviewed by Bezerra *et al.* (2021), there is a clear relationship between follicle size and the quality of the enclosed oocyte. Bovine oocytes from small antral follicles (<3.0 mm) still do not have the capacity to undergo meiotic maturation (Hyttel *et al.*, 1989; Lequarre *et al.*, 2005). Chromatin morphology of bovine oocytes from small-sized and mid-sized antral follicles (3.0–6.0 mm) have four discrete stages of germinal vesicle (GV), that is from GV0 to GV3 (Dieci *et al.*, 2016). According to these authors, approximately one-third of oocytes obtained from slaughterhouse ovaries have GV2 and GV3 status, whereas two-thirds of the oocytes are at the GV0 and GV1 stages. GV0 oocytes are transcriptionally active and unable to progress through metaphase II of the meiotic division (Luciano *et al.*, 2011). Therefore, many oocytes from mid-sized follicles (3.0–6.0 mm) may not have completed cytoplasmic maturation, which can negatively influence the blastocyst rate after *in vitro* fertilization, showing the importance of studying this oocyte population.

The presence of functional transzonal projections (TZP) mediating bidirectional communication between oocyte and cumulus cells is responsible for keeping high levels of cAMP in the oocyte, as well as its chromatin uncondensed (GV0). When this communication is interrupted, the chromatin rapidly condenses and RNA synthesis suddenly ceases (Luciano *et al.*, 2011). Therefore, an *in vitro* delay in meiotic resumption during a prematuration period has been used to increase the efficiency of oocyte development, the oocyte then gains more time to accumulate important molecules (Lima *et al.*, 2018). Among the transcripts that are stored are kinase c-*MOS*

(Wu *et al*., 1997), histone *H1Foo* (Fu *et al*., 2003; Yun *et al*., 2015), cyclin B1 (*CCNB1*; Wu *et al*., 1997) and growth and differentiation factor 9 (*GDF9*) (Biase and Kimble, 2018). These transcripts play important roles during oocyte maturation and early embryo development (Wu *et al*., 1997). It is also important to consider that oxidative stress during *in vitro* culture of oocytes can cause negative effects, mainly due to the accumulation of reactive oxygen species (ROS). To minimize the damage caused by oxidative stress, non-enzymatic antioxidants are commonly added to culture medium. For example, *N*-acetylcysteine (NAC) is a precursor of intracellular cysteine and glutathione that reduces oxidative stress by eliminating free oxygen radicals. Mahmoodi *et al.* (2015) reported that NAC regulates the expression of antioxidant enzymes. Among these enzymes, superoxide dismutase (SOD) promotes a dismutation of superoxide radicals to H_2O_2, which is further detoxified to water and oxygen by catalase (CAT) or glutathione peroxidase. CAT is responsible for the conversion of H_2O_2 into water and oxygen, whereas glutathione peroxidase (GPx) catalyzes the degradation of lipid peroxides and H_2O_2 (Kala *et al*., 2016). Periredoxin-6 (Prdx6) is another enzyme with the ability to bind and reduce phospholipid hydroperoxides (Fisher *et al*., 2018). However, it is still not known if NAC reduces the levels of ROS during bovine oocyte maturation and if it influences TZP, mitochondrial activity, and gene expression.

The aims of this study were to evaluate the effects of NAC on oocyte maturation, mitochondrial activity and TZP distribution, as well as on the levels of ROS and mRNA for *CAT*, *SOD*, *Prdx6*, *GPx*, *GDF-9*, *H1Foo*, *CCNB1* and c-*Mos* in bovine oocytes matured *in vitro*.

Material and methods

All chemicals were purchased from Sigma Chemicals Company, St. Louis, MO, USA unless otherwise stated in the text.

Ovaries and cumulus–oocyte complexes (COC) recovery

Cow ovaries ($n = 200$) were collected in slaughterhouses and transported to the laboratory in TCM-199 containing antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin) at 32°C, for a maximum period of 1 h. The COC of mid-sized antral follicles (3.0–6.0 mm) were recovered and, after morphological evaluation, destined for *in vitro* prematuration. This study was approved and carried out in accordance with the rules and guidelines of the Ethics and Animal Welfare Committee of the Federal University of Ceará (no. 016/20).

Prematuration and maturation of COC

For *in vitro* prematuration, COC were cultured in TCM-199 containing Earle salts and L-glutamine (Sigma) supplemented with 0.2 mM pyruvic acid, 5.0 µg/ml luteinizing hormone (LH) (Lutropin®-V, Bioniche, Belleville, ON, Canada), 0.5 µg/ml follicle-stimulating hormone (FSH) (Follitropin®-V, Bioniche, Belleville, Canada), 0.4% BSA, 100 IU/ml penicillin, 50 µg/ml streptomycin, 100 µM forskolin (FSK) and 100 nM C-type natriuretic peptide (CNP). The COC were cultured in four-well plates for 8 h at 38.5°C, with 5% CO_2 in air, and then used for *in vitro* maturation (IVM).

For IVM, the culture medium was the same as used during prematuration, but without FSK and CNP. For the treatments, COC were cultured in control medium alone or supplemented with 0.1, 0.5 or 2.5 mM NAC. The NAC concentrations were chosen according to previous studies (Whitaker *et al*., 2012). COC were matured *in vitro* for 22 h (Bezerra *et al*., 2016), and then, chromatin configuration, TZP, mitochondrial activity, and the levels of ROS and RNAs were evaluated. This experiment was repeated eight times.

Assessment of chromatin configuration of oocytes

To assess meiotic progression after IVM, the cumulus cells were removed with the aid of a vortex and the oocytes were fixed in 4% paraformaldehyde for 15 min and transferred to 0.1% Triton X-100. The chromatin configuration was evaluated after the addition of 10 µg/ml of Hoechst 33342 under an inverted epifluorescence microscope (Nikon, TS100). Oocytes with a nuclear membrane were considered to be at the GV stage, whereas those without a nuclear membrane, that is that were at germinal vesicle break-down (GVBD), metaphase I, anaphase I, telophase I, or metaphase II stages, were considered to have resumed meiosis (Bezerra *et al*., 2016).

Assessment of mitochondrial activity in oocytes

After *in vitro* culture in the different treatments, the oocytes were recovered and stained for mitochondrial evaluation. Briefly, oocytes were incubated in PBS with 100 nM Mitotracker Red (Mitotracker® Red, CMXRos, Molecular Probes, Melbourne, Victoria, Australia) at 37°C for 20 min. After this period, the oocytes were washed in PBS and evaluated with the aid of an epifluorescence microscope (TS100; Nikon Corp.), as described by Lima *et al.* (2018). Fluorescence intensity was measured using ImageJ software (version 1.46; National Institutes of Health, Bethesda, MD).

Assessment of ROS levels in oocytes

The oocytes were washed in 0.1% polyvinyl alcohol in phosphate-buffered saline (PBS-PVA) and incubated with 10 mM 6-carboxy-2,7-dichlorodihydrofluorescein diacetate (H2DCFDA, Molecular Probes®, Eugene, OR, USA), which measures singlet oxygen, superoxide, hydroxyl radical, peroxide, hydroperoxides and other types of ROS. After incubation at 38.5°C for 30 min, in the dark, the oocytes were washed with PBS-PVA and placed on glass slides in ProLong® Gold (Molecular Probes, Eugene, OR, USA), as described by Sovernigo *et al.* (2017). The slides were evaluated using an epifluorescence microscope (Nikon, TS100) under 460 nm wavelength. The fluorescence intensity of ROS staining was analyzed individually using ImageJ software (version 1.46; National Institutes of Health, Bethesda, MD, USA). Relative fluorescence intensity was considered to be directly proportional to ROS concentration.

Analysis of TZP in COC

After IVM, oocytes were fixed in 4% paraformaldehyde for up to 1 h at room temperature. Then, they were transferred to a solution composed of PBS plus 0.1% BSA and 0.1% Tween 20 for 8–12 h at 4°C. After this period, COC were incubated in blocking solution composed of PBS plus 0.5% BSA, 0.2% sodium azide, 1% milk powder, 10% goat serum, 1% of donkey serum, 0.1 M glycine and 0.1% Triton X-100 for 1 h, under agitation and protected from light. Then, the oocytes were incubated for 2 h at room temperature with Alexa 488 Phalloidin antibody (Invitrogen, cat. no. T6199, 1:50 dilution) also under shaking and protected from light. After this incubation period, the oocytes were subjected to three washes in blocking solution, for 5 min, as described by Lopes *et al.* (2020).

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Table 1. Primer pairs used for quantification of messenger RNAs

Target gene	Primer sequence (5′→3′)	Strand	GenBank accession number
CCNB1	CTCCAGTGCTCTCCTCCTCACT	S	NM_001045872. 1
	CTAATCTTCGTGTTCCTGGTGATCC	As	
GDF9	ACAACACTGTTCGGCTCTTCACCC	S	GI: 51702523
	CCACAACAGTAACACGATCCAGGTT	As	
c-MOS	CTGCAAGATCGGGGACTTCG	S	AY_168496.1
	CTCGGTGAGTGTAGGTGCCA	As	
H1Foo	CCCAAGAAGCCGAGTGAGTC	S	NM_001035372.1
	CTTGGTATCTGCTTGGCGGC	As	
Prdx6	GCACCTCCTCTTACTTCCCG	S	GI: 59858298
	GATGCGGCCGATGGTAGTAT	As	
GPx1	AACGTAGCATCGCTCTGAGG	S	GI: 156602645
	GATGCCCAAACTGGTTGCAG	As	
SOD	GTGAACAACCTCAACGTCGC	S	GI: 31341527
	GGGTTCTCCACCACCGTTAG	As	
CAT	GGGTTCTCCACCACCGTTAG	S	GI: 402693375
	GGGGCCCTACTGTCAGACTA	As	
GAPDH	TGTTTGTGATGGGCGTGAACCA	S	GI: 402744670
	ATGGCGCGTGGACAGTGGTCATAA	As	

As, Anti-sense; S, sense.

Finally, oocytes were placed on 60-well slides (μ-Slide Angiogenesis IbiTreat, Ibidi GmbH, Germany) containing 10 μl of mounting medium (50% glycerol and 50% PBS) with DAPI (ABCAM-104139) and examined under a confocal laser scanning microscope (Zeiss LSM 700 META, Weimer, Germany). The TZP distribution throughout the zona pellucida of oocytes was evaluated.

Quantification of mRNA in oocytes

Quantification of mRNA was performed in oocytes cultured with 0.5 mM NAC, and had higher rates of resumption of meiosis when compared with those culture in control medium alone or supplemented with 0.1 mM NAC. Total RNA isolation and DNA synthesis were performed using TRIzol (Invitrogen, São Paulo, Brazil), according to the manufacturer's instructions. For this, 1 ml of TRIzol was added to each sample, and the lysate was then aspirated through 20G needles before being centrifuged at 10,000 *g* for 3 min. Then, the lysates were diluted 1:1 in 70% ethanol and placed in a mini-column. After binding the RNA to the column, DNA digestion was performed using RNase-free DNase (340 units/ml) for 15 min. After washing the columns three times, RNA was collected in 30 μl of ultrapure water. Total RNA concentration was assessed using a spectrophotometer (Instrutherm) and 1 μg of total RNA was used for reverse transcription. Before the reverse transcription reaction, the RNA samples were incubated for 5 min at 70°C, and then cooled in ice. Reverse transcription was performed in a total volume of 20 μl, composed of 10 μl of sample RNA, 4 μl of reverse transcriptase buffer (Invitrogen, São Paulo, Brazil), 8 RNasin units, 150 units of Superscript reverse transcriptase, 0.036 U random primers, 10 mM DTT and 0.5 m of each dNTP (Invitrogen, São Paulo, Brazil). The mixture was incubated at 42°C for 1 h, then at 80°C for 5 min, and then stored at −20°C. A negative control was prepared under the same conditions, but without the addition of reverse transcriptase.

The quantification of messenger RNAs was performed using SYBR Green. Each real-time reaction (15 μl) contained 7.5 μl of SYBR Green Master Mix (PE Applied Biosystems, Foster City, CA), 5.5 μl of ultrapure water, 1 μl of cDNA and 0.5 μM of each primer. Real-time PCR was performed on a thermocycler (Master Cycler, Eppendorf, Germany) using oocytes samples from four repetitions of the experiment. The primers (Table 1) were designed to amplify specifically messenger RNAs for CAT, SOD, Prdx6, GPx, GDF9, H1Foo, CCNB1 and c-Mos. The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used to normalize mRNA levels. The specificity of each primer pair was confirmed by analyzing the melting curve of PCR products. The thermal cycling profile for the first round of PCR was initially denaturation and activation of the polymerase for 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C. The final extension was performed at 72°C for 10 min. All reactions were performed in a Step One Plus instrument (Applied Biosystems, Foster City, CA, USA). The $2^{-\Delta\Delta Ct}$ method was used to transform the C_t values into mRNA expression relative levels (Livak and Schmittgen, 2001).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (5.0) software. The percentages of oocyte at the GV stage and with resumption of meiosis in the different treatments were evaluated using the chi-squared test. The data of expression of mRNAs *for CAT*, *SOD*, *Prdx6*, *GPx*, *GDF-9*, *H1Foo*, *CCNB1* and c-*Mos*, as well as those of ROS and mitochondria in the oocytes, were analyzed using the Kruskal–Wallis test, followed by Dunn's multiple

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Table 2. Percentages of oocytes in germinal vesicle (GV) stage and that had meiotic resumption after IVM in the presence of different concentrations of NAC

Treatments	Total (*n*)	Oocyte chromatin configuration	
		GV (%)	Resumption of meiosis (%)
Control	127	48.8^{A} (62/127)	51.2^{A} (65/127)
NAC 0.1	146	32.9^{B} (48/146)	67.1^{B} (98/146)
NAC 0.5	136	12.5^{C} (17/136)	87.5^{C} (119/136)
NAC 2.5	147	16.3^{C} (24/147)	83.7^{C} (123/147)

^{A,B,C}Differences between treatments in each column, $P < 0.05$.

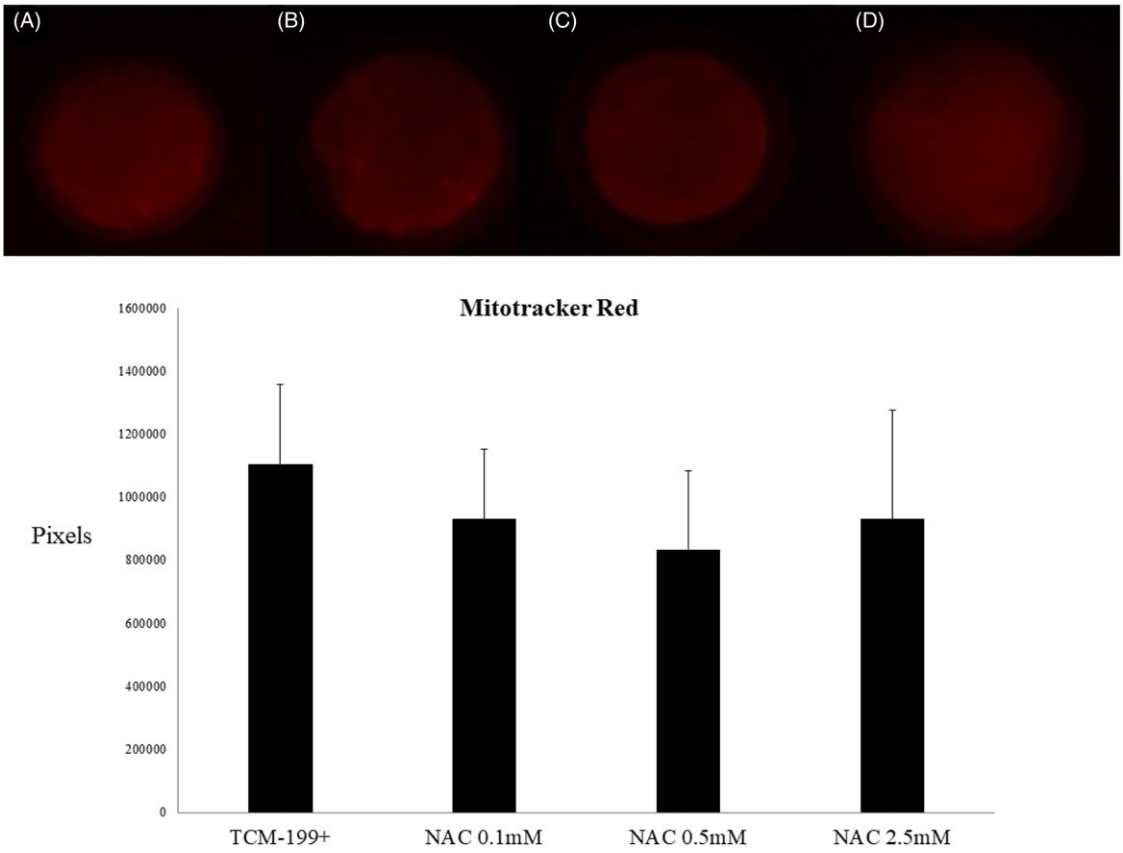

Figure 1. Fluorescence staining intensity and distribution of mitochondria in oocytes after IVM in control medium alone (A) or supplemented with 0.1 mM (B), 0.5 mM (C), or 2.5 mM (D) NAC.

comparison test. Differences were considered significant when the *P*-value was < 0.05.

Results

Assessment of meiotic progression in the oocytes

During IVM, the presence of different concentrations of NAC significantly reduced the percentages of GV oocytes when compared with control medium. Additionally, lower percentages of GV oocytes were observed after using culture medium supplemented with 0.5 and 2.5 mM NAC, when compared with those oocytes matured in the presence of 0.1 mM NAC. The presence of 0.5 and 2.5 mM NAC increased the percentages of oocytes with resumption of meiosis when compared with those cultured in control medium alone or supplemented with 0.1 mM NAC (Table 2).

Mitochondrial activity in the oocytes

Oocytes matured in control medium alone or containing NAC had mitochondria uniformly distributed throughout the cytoplasm. No significant differences in fluorescence intensity were observed between the treatments (Figure 1).

Intracellular levels of ROS

The intracellular levels of ROS in oocytes matured with 0.1 and 0.5 mM NAC were lower ($P < 0.05$) than those observed in oocytes matured in control medium (Figure 2). No significant differences were observed in ROS levels between oocytes matured in the presence of 2.5 mM NAC and those from the control group ($P > 0.05$) (Figure 2).

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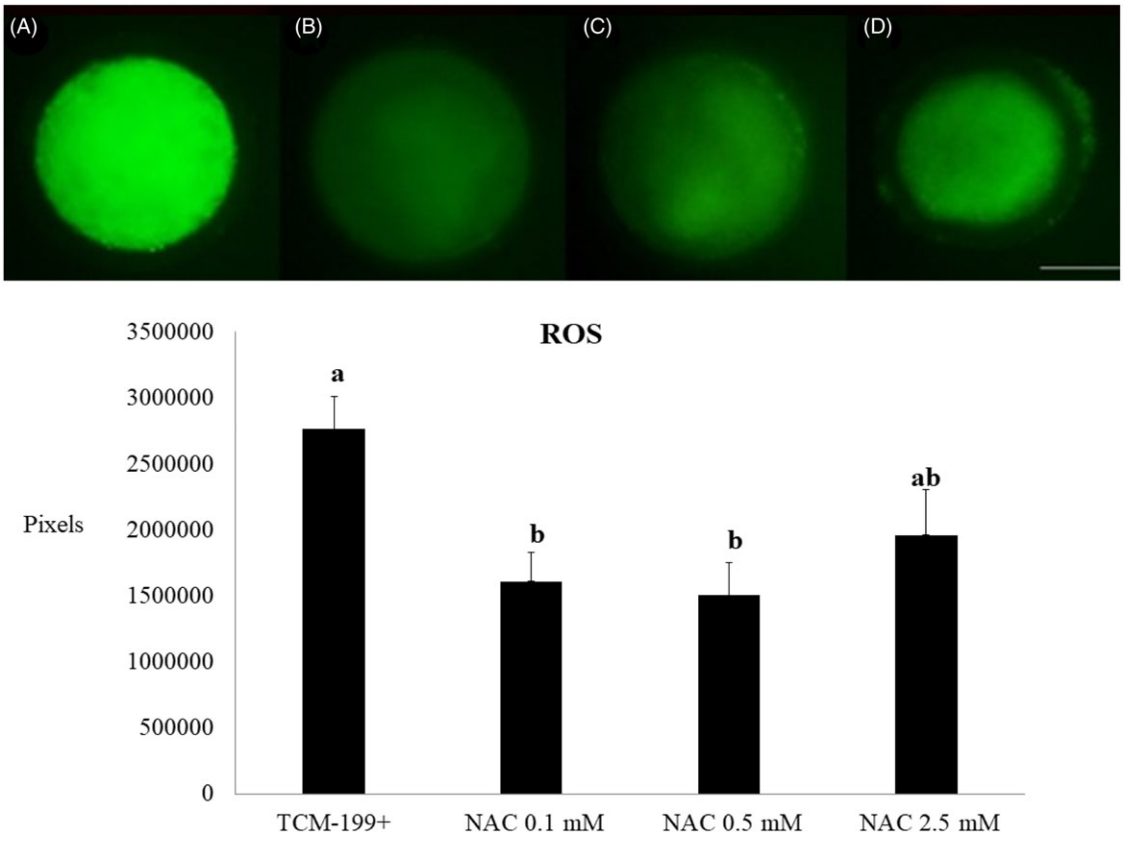

Figure 2. Fluorescence staining intensity for ROS in oocytes after IVM in control medium alone (A) or supplemented with 0.1 mM (B), 0.5 mM (C), or 2.5 mM (D) NAC. [a,b]Significant differences between treatments ($P < 0.05$).

Analysis of TZP in the oocytes

Fluorescence analysis showed that the distribution of TZP throughout the zona pellucida decreased in oocytes matured in the presence of 0.5 mM NAC when compared with those matured in control medium alone or supplemented with 0.1 or 2.5 mM NAC (Figure 3).

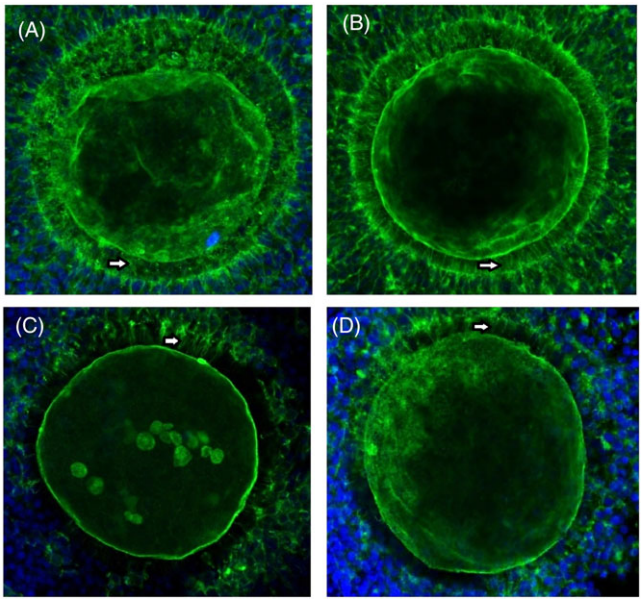

Figure 3. Confocal microscopy images showing TZP (arrows) distribution in oocytes after IVM in control medium alone (A) or supplemented with 0.1 mM (B), 0.5 mM (C), or 2.5 mM (D) NAC. Green staining: F-actin; blue DAPI staining: cellular nuclei.

Levels of mRNA for CAT, SOD, PRDX6, GPX, GDF-9, H1FOO, CCNB1 and *c*-MOS in the oocytes

Figure 4 shows that 0.5 mM NAC significantly reduced the levels of messenger RNA for *CCNβ1* after IVM. Despite an apparent reduction in the levels of mRNA for *CAT*, *SOD*, *Prdx6*, *GPx*, *c-MOS*, *GDF9* and *H1Foo* in oocytes matured in the presence of NAC, the differences were not significant when compared with the control group ($P > 0.05$).

Discussion

This study shows that NAC increases the meiotic progression and reduces the levels of ROS during oocyte maturation. It is known that during IVM, oocytes are exposed to high tensions of oxygen leading to increased production of ROS, which can damage DNA, RNA and proteins. Considering that oxidative stress in oocytes is often determined by measuring ROS levels (Li and Zhao, 2019), NAC contributed to maintain the redox balance during IVM and had a positive effect on oocyte meiotic resumption. These effects may be associated with the capacity of NAC to scavenge free radicals and to chelate metals (Atkuri *et al.*, 2007). The ability of NAC to control oxidative stress by glutathione (GSH), is well known (Güntürk *et al.*, 2019). Li and Zhao (2019) revealed that 0.1 mM NAC was able to reduce intracellular levels of bisphenol-induced ROS in

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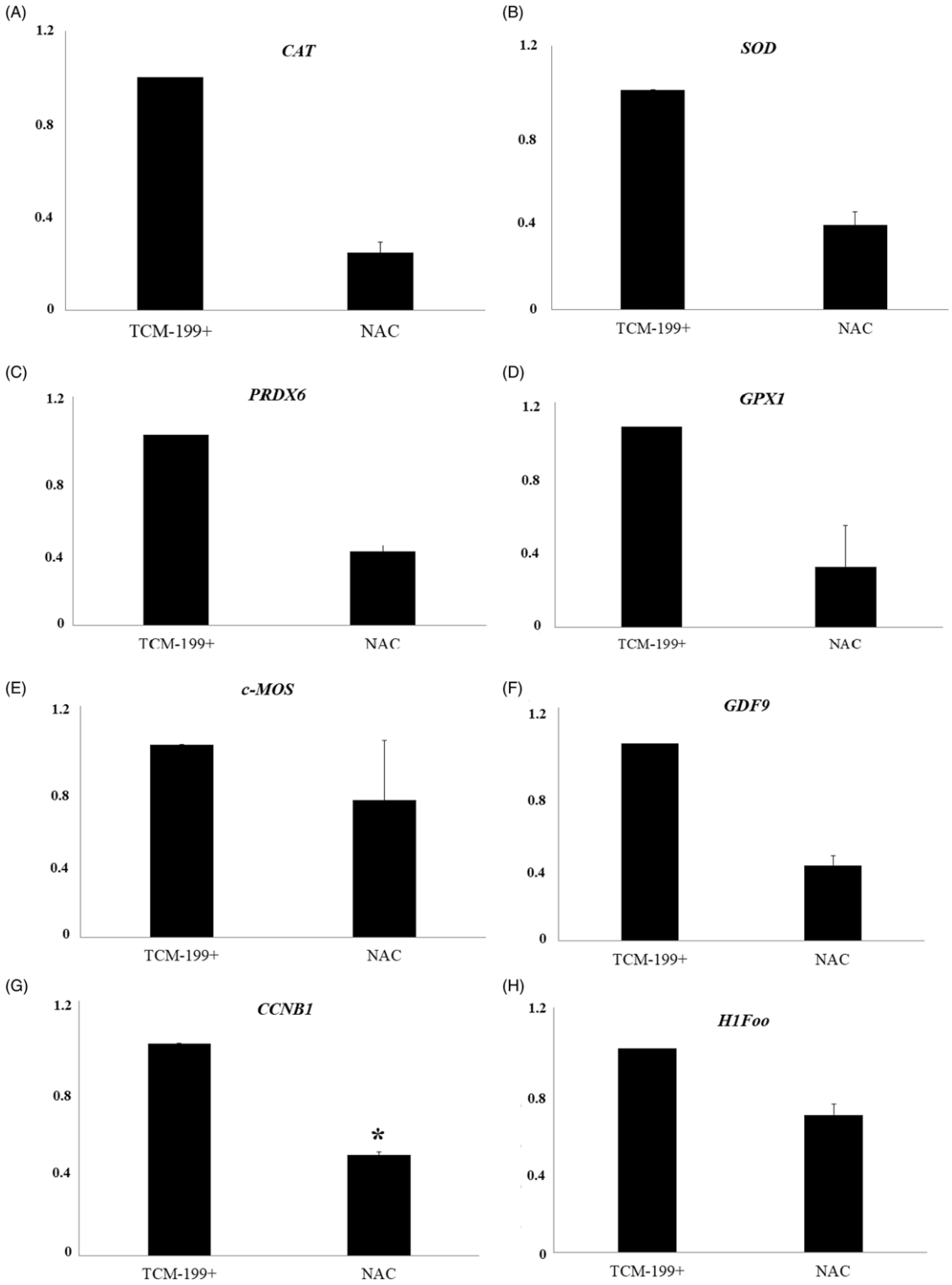

Figure 4. Levels of mRNA for *CAT* (A), *SOD* (B), *Prdx6* (C), *GPx1* (D), c-*MOS* (E), *GDF9* (F), *CCNB1* (G), and *H1FOO* (H) in bovine oocytes after maturation in TCM-199+ alone or supplemented with 0.5 mM NAC. *Statistically significant differences ($P < 0.05$).

in vitro matured mouse oocytes, restoring oocyte nuclear maturation and the subsequent competence of embryonic development after *in vitro* fertilization. Recently, Sun *et al.* (2021) reported that treatment of oocytes with 1.0 mM NAC for 8 h during IVM attenuated intracellular ROS. Considering that antioxidant enzymes are synthesized in response to oxidative stress, a reduction in ROS in oocytes matured in the presence of NAC can explain the tendency to reduce the levels of mRNAs for enzymes that control oxidative stress such as Prdx6, SOD, CAT and GPx. The mechanism of action of NAC to control oxidative stress and to improve oocyte IVM was recently reviewed by Barrozo *et al.* (2021).

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The present study shows that oocytes cultured *in vitro* had a homogeneous distribution of mitochondria in the cytoplasm, but NAC did not influence mitochondrial activity. During oocyte maturation, mitochondria are a key component of the metabolic machinery responsible for supplying energy that is consumed during the maturation process and is also the main generator of free radicals in mammals (Ferreira *et al.*, 2009; Brand *et al.*, 2013). The distribution of mitochondria to areas of high energy consumption is crucial for the oocyte and embryo during critical periods of the cell cycle. For this reason, the mitochondrial distribution pattern has been associated with the quality and developmental capacity of mammalian oocytes (Cajas *et al.*, 2020). Mastrorocco *et al.* (2020) demonstrated that dispersed mitochondrial distribution throughout the cytoplasm is associated with maturity and competence of oocytes. Furthermore, Takahashi *et al.* (2016) revealed that mitochondria in human oocytes at the GV stage have a heterogeneous distribution, but after GVBD this configuration quickly changed to a homogeneous distribution. In rats, Dumollard *et al.* (2006) found that mitochondria appeared uniformly distributed in the periphery of the cytoplasm in immature oocytes. Romek *et al.* (2011) also described that there was an accumulation of mitochondria in the periphery before maturation and in the formation of mitochondria clusters located in the centre after oocyte maturation in cattle.

During development, growing oocytes engage in a prolonged phase of intensive RNA synthesis necessary to produce essential transcripts for oocyte growth and early embryonic development (Bezerra *et al.*, 2019). In the follicular microenvironment, cell communication between oocytes and cumulus cells is complex, and both sides have active regulatory functions. Macromolecules, such as RNAs (Macaulay *et al.*, 2014), can also be transported from cumulus cells to oocytes. In the present study, the distribution of TZP throughout the zona pellucida was reduced in oocytes matured with 0.5 mM NAC. Recently, Abbassi *et al.* (2021) showed that TZP retraction is not regulated by oocyte-derived signals, but is instead controlled by epidermal growth factor receptor (EGFR) signalling within the granulosa cells. Previously, Wang *et al.* (2013) reported that NAC enhances mRNA levels for *EGF* and amphiregulin in porcine epithelial cells. Fully grown oocytes within antral follicles are held in meiotic arrest through the action of cyclic GMP (cGMP) secreted only by the mural granulosa cells (Jaffe and Egbert, 2017). Consequently, the reduction in the distribution of TZP in oocytes cultured with 0.5 mM NAC led to a drop in cGMP transported to the oocyte, enabling the latter to initiate meiotic maturation.

Bezerra *et al.* (2019) showed that prematuration of oocytes from mid-sized antral follicles increased the levels of mRNA for *CCNB1* and c-*Mos*, but did not influence the levels of mRNAs for *GDF9*, *PARN*, *eIF4E* and *H1Foo*. Conversely, these authors showed an increase in the levels of all this transcripts during *in vivo* and *in vitro* growth of preantral follicles, emphasizing that they were actively synthesized during follicular growth, but not at the late stages of oocyte maturation. Our study showed that NAC reduced the levels of *CCNB1* during oocyte maturation, which can be associated with the higher rate of oocyte meiotic resumption in oocytes matured in the presence of NAC. This reduction can be associated with the translation of *CCNB1* mRNA to proteins, which are required during meiosis (de Vantéry *et al.*, 1996). The apparent reduction in the levels of mRNA for c-*MOS*, *GDF9* and *H1Foo* in oocytes matured in the presence of NAC can also be associated with their translation to proteins that will have an important role during oocyte fertilization and early embryo development. Regarding the role of these proteins, c-*MOS* is a kinase expressed exclusively in germ cells that have an important role in oocyte maturation (Wu *et al.*, 1997). The c-*MOS*–MAPK1/3 pathway is important for maintaining oocytes arrested at the MII stage (Phillips *et al.*, 2002). GDF9 is involved in the process of oocyte maturation by regulating the function of cumulus cells from preovulatory follicles (Gui and Joyce, 2005). *H1Foo* plays a key role in the control of chromatin configuration and is essential for oocyte maturation (Furuya *et al.*, 2007).

In conclusion, the presence of 0.5 mM NAC in maturation medium increases the percentage of oocytes with resumption of meiosis and reduces the levels of ROS and the distribution of TZP in the oocyte, showing that supplementation of culture medium with NAC is very important to optimize oocyte IVM. NAC also reduces mRNA levels for *CCNB1*, but does not influence the expression of *CAT*, *GPX*, *Prdx-6*, *SOD*, *GDF9*, *H1Foo* and c-*Mos*. Understanding the mechanisms involved in oxidative stress control and how NAC is able to reduce the damage caused by oxidative stress can contribute to the improvement of oocyte IVM.

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Statement of interest. None of the authors have any conflict of interest to declare.

Ethical standards. The authors declare that all procedures were performed according to national and institutional guidelines on the care and use of animals.

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