Pakistan J. Zool., vol. 51(3), pp 1085-1092, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.1085.1092

Pioglitazone Mediated Reduction in Oxidative Stress and Alteration in Level of PPARγ, NRF2 and Antioxidant Enzyme Genes in Mouse Preimplantation Embryo during Maternal to Zygotic Transition

Xu Lijie, Obaid Ullah, Liu Haixing, Ihsan Ali, Zhongshu Li* and Nan-Zhu Fang*

Laboratory of Animal Genetic Breeding and Reproduction, Agriculture College of Yanbian University, Yanji 133002, China

Xu Lijie, Obaid Ullah and Hai Xing Liu have contributed equally to this study.

Article Information
Received 02 February 2018
Revised 25 March 2018
Accepted 11 April 2018
Available online 15 April 2019

Authors' Contribution
XL and NZF perceived and designed the study. XL, OU, LH and IA performed the experiments. IA and ZL wrote the manuscript. NZF prepared the draft version of the article for submission.

Key words
Peroxisome proliferator-activated receptor gamma (PPARγ), Nuclear regulatory factor 2 (NRF2), Oxidative stress, Developmental block, Maternal-to-zygotic transition.

ABSTRACT

Excessive accumulation of reactive oxygen species (ROS) is one of the reasons for the slow growth of mammalian preimplantation embryos. Peroxisome proliferators-activated receptor gamma (PPARγ) functions in the nuclear regulatory factor 2 (Nrf2) antioxidant pathways by binding to the promoters of antioxidant genes and regulates the expression of genes that associate with NADPH oxidase. To understand the role of PPARγ in the development of early embryos and define the mechanism responsible for the arrest in development during the maternal-to-zygotic transition (MZT), we used an embryo model of oxidative damage by H_2O_2. We found that H_2O_2 exposure significantly decreased embryo development, increased the intracellular ROS level, and upregulated the expression levels of the NADPH oxidase genes NOX2, DUOX1, and NOXA1. By contrast, embryo treatment with pioglitazone after H_2O_2 exposure promote embryo development, significantly decreased the ROS level, downregulates the expression levels of NOX2, DUOX1, and NOXA1, and upregulated the expression levels of PPARγ, Nrf2, and the antioxidant enzyme genes GPx3, GPx4, SOD1, SOD2, and SOD3. In conclusion, pioglitazone can reduce intracellular oxidative stress during in vitro development by promoting the expression of antioxidant genes and suppressing the expression of NADPH oxidant genes.

INTRODUCTION

Embryo cultures are valuable in the study of preimplantation embryonic development, and they are often used to produce transferable embryos, although culture conditions are not optimal (Rizos *et al*., 2008). The maternal-to-zygotic transition (MZT) is an important period of mammalian embryonic development. Concomitant with this cellular event, zygotic genome activation (ZGA) is triggered, and oocyte-specific components degrading by ubiquitin-mediated proteasomal pathway (Roest *et al*., 2004), and modulating control of maternal transcripts is switched to zygotic transcripts ultimately (Lee *et al*., 2014). These changes are crucial to ensure a successful transition from the early embryonic stage to the late embryonic stage. If there is a delay in ZGA, embryos will arrest during development (Qiu *et al*., 2003).

Mouse embryos generally arrest at the 2-cell stage during the G2 phase, a phenomenon referred to as the 2-cell block (Flach *et al*., 1982). During the 2-cell stage, there is an increase in the hydrogen peroxide (H_2O_2) level in cultured mouse embryos at metaphase, which remains elevated until they enter the early 4-cell stage (Naser-Esfahani *et al*., 1990). An elevated reactive oxygen species (ROS) level can lead to oxidative stress, which damages DNA, lipids, and proteins. Therefore, the 2-cell block is believed to associate with oxidative stress in mouse embryos.

Peroxisome Proliferator-activated receptor gamma (PPARγ), a nuclear receptor of the ligand-activated transcription factor family, can regulate gene transcription. PPARγ binds the retinoid X receptor (RXR) (Varga *et al*., 2011), and the heterodimer binds PPARγ response elements (PPREs), which are located in the promoter region of target genes. A previous study reported that

* Corresponding authors: nzfang@ybu.edu.cn; lizhongshu@ybu.edu.cn
0030-9923/2019/0003-1085 $ 9.00/0

PPARγ can induce the expression of antioxidant enzymes and suppress the expression and activity of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase through PPARγ. PPARγ then regulates oxidative stress by acting as a ROS scavenger and ROS generator (Jung *et al*., 2007). It is believed that PPARγ is a novel antioxidant stress factor (Polvani *et al*., 2012).

Pioglitazone, a derivative of thiazolidinedione, is a PPARγ agonist used in the treatment of individuals with type 2 diabetes (Kota *et al*., 2005). Accumulating evidence indicates that pioglitazone can also protect cells from oxidative stress (Chen *et al*., 2004).

Currently, the 2-cell block is the main barrier to the mass production of embryos *in vitro*. Although studies show that supplementing media with antioxidants can reduce the rate of the developmental block (Goto *et al*., 1992), the effect of pioglitazone as an antioxidant in mouse embryo development is not yet known. To our knowledge, this is the first study to elucidate the mechanism of action of pioglitazone in mouse preimplantation embryos at cellular and molecular levels.

MATERIALS AND METHODS

All chemicals and reagents used in this study were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) unless otherwise indicated.

Superovulation and zygote collection

Kunming mice were purchased from the Experimental Animal Center of Yanbian University. Female mice 8-10 weeks old were housed under conditions of 12 h light: 12 h dark at approximately 24°C. Each female mouse received an intraperitoneal injection of 10 IU pregnant mare serum gonadotropin (PMSG; Ninbo Hormone Co., Ltd., Ninbo China), followed by 10 IU human chorionic gonadotropin (hCG; Ninbo Hormone Co., Ltd.) 48 h later. Immediately after the administration of hCG, each female mouse was mated with a male mouse. Mating was confirmed after 12 h by the presence of vaginal plugs. Approximately 22 h after the administration of hCG, the mice with vaginal plugs were sacrificed. Their oviducts were harvested and placed in the M_2 medium. Zygotes were released from the oviducts under a stereo microscope. Cumulus cells were removed with 0.03%. The embryos were washed and then cultured at 37°C in a humidified atmosphere of 5% CO_2 in the air.

Pioglitazone antioxidant capacity assay

Zygotes without cumulus cells were washed in M_{16} medium containing 25mM H_2O_2 three times and then cultured in the same medium at 37°C in a humidified atmosphere of 5% CO_2 in air for 30 min. After H_2O_2 treatment, the zygotes were randomly divided into two groups; one group was cultured in an M_{16} medium, while the other group was cultured in M_{16} medium containing 5uM pioglitazone. Pioglitazone was removed after 48 h, and the embryos were cultured until day 4. Embryo development up to the blastocyst stage was assessed. Non-treated embryos served as the control.

Measurement of the ROS level

Mouse embryos from control and treated groups were washed twice in poly vinyl alcohol (PVA) (1 mg/ml) and then placed in a 50 µl drop of 2', 7'-dichlorodihydro-fluoroscein diacetate (DCHFDA) (10 mol/L). The embryos were incubated for 15 min in dark at 37°C in a humidified atmosphere of 5% CO_2. Embryos were then washed twice with phosphate-buffered saline (PBS) and examined under an epifluorescent microscope (Leica DM IRM, Leica, Wetzlar, Germany) equipped with blue-light (535 nm) excitation. The images were analyzed by ImageJ 1.49 software-(Pro plus 6.0) (National Institutes of Health, Bethesda, MD, USA). The experiment was performed three times.

RNA isolation and cDNA synthesis

For PCR analysis, 200 embryos at the 2-cell stage were harvested at 42 h after hCG injection as described previously (Zhang *et al*., 2015). Total RNA was isolated from whole embryos using the Qiagen RNeasy Mini Kit (Qiagen, Hiden, Germany) according to the manufacturer's instructions. cDNA was synthesized by reverse transcription using the Prime Script™ RT Reagent Kit with gDNA Eraser (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's instructions. PCR was carried out in 25 µl reaction volumes containing 13 µl of 2× Taq PCR Master Mix (Tiangen Biotech Co., Ltd., Beijing, China), 2 µl of cDNA, 0.5 µl of forward and reverse primers (5 mM) (Table I), and 9 µl of sterile H_2O (Tiangen Biotech Co., Ltd.). Following the initial preincubation step at 95°C for 3 min, the reaction consisted of 35 cycles of denaturation at 95°C for 30 sec, annealing at 56-62°C for 30 sec, and extension at 72°C for 30 sec. The final extension was performed at 72°C for 5 min. The PCR products were separated by electrophoresis on 2% agarose gels (Wenmin *et al*., 2017). The band intensities were analyzed by Lane 1D Analysis Software (Beijing Sage Creation Science Co., Ltd., Beijing, China). Three biological replicates were performed.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 software (IBM Corp., New York, USA). Data are expressed as percentages (mean ± SEM). $P < 0.05$ was considered statistically significant.

Table I.- Sequences of primers used for quantitative real-time PCR analysis of gene expression in mouse embryos.

Primer name	Access No.	Sequence	Annealing Temp.	Size (bp)
GAPDH	BC023196	F: 5'-CATCACCATCTTCCAGGAGCG-3'	59ºC	357
		R: 5'-GAGGGGCCATCCACAGTCTTC-3'		
PPARγ	NM001127330	F: 5'-CTGATGCTTTATCCCCACAGACTCGG-3'	62ºC	253
		R: 5'-CCCTTTACCACAGTTGATTTCTCCAG-3'		
NRF2	NM010902	F: 5'-CAGCATGTTACGTGATGAGG-3'	62ºC	152
		R: 5'-GCTCAGAAAAGGCTCCATCC-3'		
GPX3	NM008161	F: 5'-CTCCTGAGACCAGCCAAGAC-3'	59ºC	235
		R: 5'-ATGGGGGTGTTGAGATACCA-3'		
GPX4	NR110342	F: 5'-ATGCCCGATATGCTGAGTGT-3'	59ºC	336
		R: 5'-GCTAGAGATAGCACGGCAGG-3'		
SOD1	NM011434	F: 5'-TTCGAGCAGAAGGCAAGCGGTGAA-3'	59ºC	396
		R: 5'-AATCCCAATCACACCACAAGCCAA-3'		
SOD2	NM013671	F: 5'-ATTAACGCGCAGATCATGCAG-3'	59ºC	243
		R: 5'-TTTCAGATAATCAGGTCTGACGTT-3'		
SOD3	NM011435	F: 5'-ATGTTGGCCTTCTTGTTCTACGG-3'	62ºC	756
		R: 5'-TTAAGTGGTCTTGCACTCGCTCT-3'		
NOX2	NM007807	F: 5'-ACCTTACTGGCTGGGATGAA-3'	59ºC	137
		R: 5'-TGCAATGGTCTTGAACTCGT-3'		
NOX4	NM015760	F: 5'-CCCAAGTTCCAAGCTCATTTCC-3'	59ºC	112
		R: 5'-TGGTGACAGGTTTGTTGCTCCT-3'		
DUOX1	NM001099297	F: 5'-GCGATTTGATGGATGGTAT-3'	59ºC	516
		R: 5'-TAGGCAGGTAGGGTTCTTT-3		
NOXA1	BC047532	F: 5'-CATCACCATCTTCCAGGAGCG-3'	59ºC	68
		R: 5'-GAGGGGCCATCCACAGTCTTC-3'		

Table II.- Effect of PIO on early mouse embryos development induced by H_2O_2.

Concentration (μM/L)	Zygote number	n	Cleavage rate	4-cell rate	Blastocyst rate
M16 (Control)	183	5	88.47±4.46[a]	63.74±8.01[a]	32.96±2.60[a]
H_2O_2 -treated	210	5	87.80±3.46[a]	39.53±2.88[b]	15.78±5.62[b]
H_2O_2 and PIO	158	5	91.86±4.33[a]	68.57±3.87[a]	35.94±3.97[a]

Data are the proportions of zygotes reaching the indicated stages within each treatment. Different letters in the same column means significant difference between the treatments ($P < 0.05$). PIO, pioglitazone.

RESULTS

Effects of H_2O_2 and pioglitazone on the development of mouse embryos

Mouse embryo development was investigated by culturing zygotes in the presence of 25mM H_2O_2 for 30 min, followed by 5μM pioglitazone (Table II). The cleavage rate did not differ significantly between groups ($P > 0.05$). By contrast, the 4-cell stage and the blastocyst rate of embryos exposed to H_2O_2 were significantly lower ($P < 0.05$) than in the control and those treated with H_2O_2 and pioglitazone.

Effects of H_2O_2 and pioglitazone on the ROS level in mouse embryos

The intracellular ROS level plays an important role in

the development of embryos (Ali *et al*., 2017). To quantify the ROS level by fluorescent microscopy, embryos were collected at the blastocyst stage and stained with DCHFDA (Fig. 1A). The ROS level in embryos treated with $H_2O_2$was significantly higher ($P < 0.05$) than the control and those treated with H_2O_2 and pioglitazone (Fig. 1B).

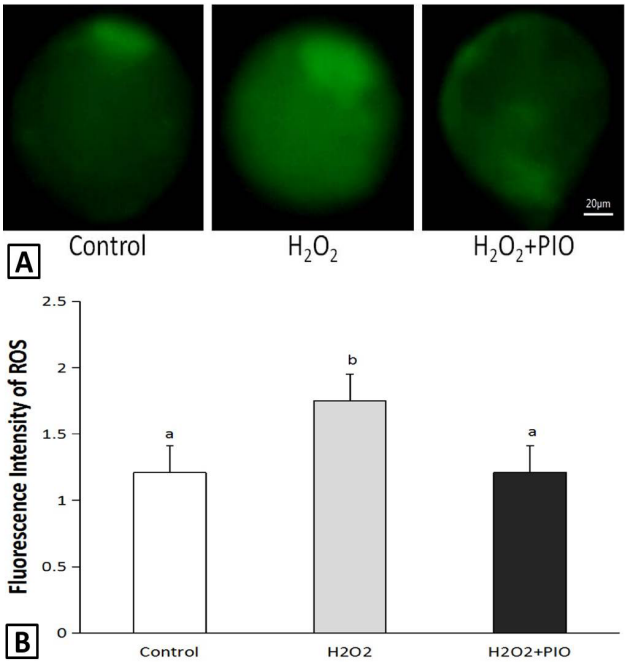

Fig. 1. Evaluation of ROS content in blastocysts: **A**, representative photographs of ROS levels in mouse embryos after staining with DCHFDA; **B**, Fluorescence intensity was quantified by Image J. Data are expressed as a mean value ± SEM of three independent experiments. Different letters in the same picture mean the significant difference between the treatments ($P < 0.05$).

PPARγ and NRF2 expression in mouse embryos during the MZT

Compared to the respective controls, there were no significant changes in PPARγ and nuclear regulatory factor 2 (NRF2) expression levels in embryos treated with H_2O_2. However, PPARγ and NRF2 levels increased significantly ($P <0.05$) in embryos treated with H_2O_2 and pioglitazone (Fig. 2B).

Expression of antioxidant genes downstream of NRF2 in mouse embryos during the MZT

To determine the role of pioglitazone in H_2O_2-induced oxidative damage, the expression levels of several antioxidant enzymes in mouse embryos treated with H_2O_2 or H_2O_2 and pioglitazone were measured. Compared to the respective controls, there were no significant changes in the expression levels of GPX4 in embryos treated with H_2O_2. By contrast, the expression levels of GPX3, SOD1, and SOD3 increased in embryos treated with H_2O_2, while those of GPX4 and SOD2 decreased compared with the respective controls (Fig. 3B). After the treatment of embryos with H_2O_2 and pioglitazone, the expression levels of GPX3, GPX4, SOD1, SOD2, and SOD3 increased compared to H_2O_2 treatment alone.

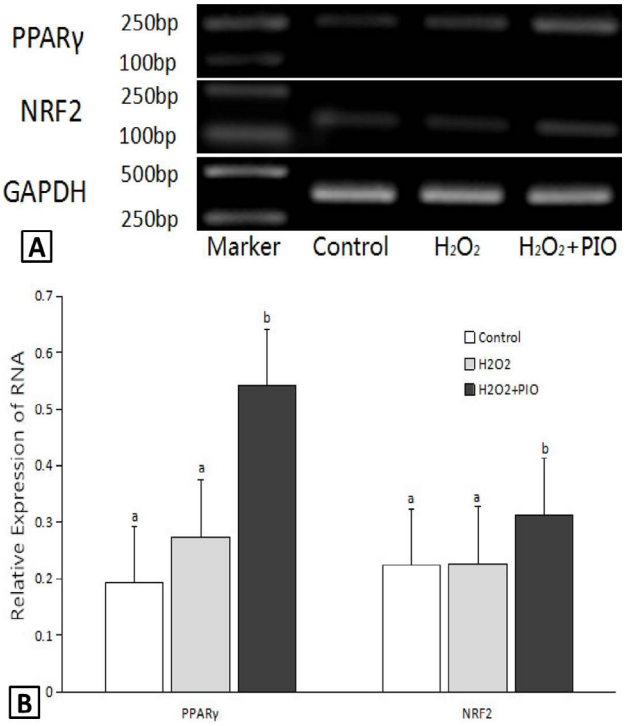

Fig. 2. The expression of PPARγ and Nrf2 in cultured mouse embryos, which were exposed to H_2O_2 of 30 min and then treated with pioglitazone, with untreated embryos as a control. **A**, PCR results of PPARγ and Nrf2 RNA in mouse embryos; **B**, relative expression of PPARγ and Nrf2 RNA normalized with the internal marker GAPDH. Different letters in the same picture mean the significant difference between the treatments ($P < 0.05$).

NADPH oxidase expression in mouse embryos during the MZT transition

To further investigate the role of pioglitazone in H_2O_2-induced oxidative damage, the NADPH oxidase expression level in mouse embryos treated with H_2O_2 or H_2O_2 and pioglitazone was measured. As shown in Figure 4B, the expression levels of NOX2, NOX4, DUOX1, and NOXA1 increased in 2-cell stage embryos treated with H_2O_2. After the treatment of embryos with H_2O_2 and pioglitazone, the NOX2, NOX4, DUOX1 and NOXA1 level decreased compared to H_2O_2 treatment alone.

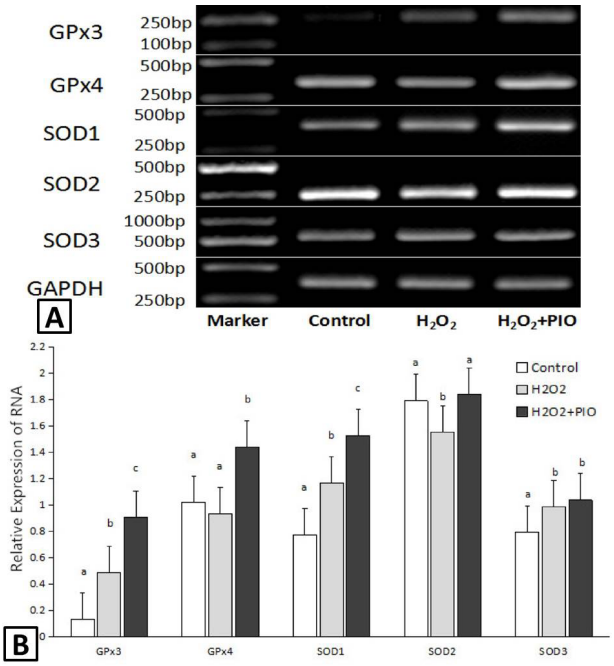

Fig. 3. The expression of GPX and SOD in cultured mouse embryos, which were exposed to H_2O_2 of 30 min and then treated with pioglitazone, with untreated embryos as a control. **A**, PCR results of GPX and SOD RNA in mouse embryos; **B**, Relative expression of GPX and SOD RNA normalized with the internal marker GAPDH. Different letters in the same picture mean the significant difference between the treatments ($P < 0.05$).

DISCUSSION

In vitro, culture conditions create a suboptimal environment for mouse preimplantation embryos (Ecker *et al*., 2004), which are extremely susceptible to oxidative stress. Cultured embryos, whose defense mechanisms are insufficient, tend to arrest during development (Qiu *et al*., 2003). Therefore, altering the response of cultured embryos to oxidative stress might prevent their developmental arrest.

Several studies have reported that PPARγ agonists can activate antioxidant pathways. For instance, the PPARγ agonist GW1929 stimulated the activity of human dopaminergic neurons and protected them from oxidative stress induced by H_2O_2 and the mitochondrial toxin rotenone (Makela *et al*., 2016). Similar results were reported in human umbilical vein endothelial cells; H_2O_2 decreased the PPARγ expression level, which was reversed by catalase (CAT) treatment (Blanquicett *et al*., 2010). However, these findings are inconsistent with the results of this study. We demonstrated that H_2O_2 had no effect on the PPARγ expression level in mouse embryos. This discrepancy in results might be due to differences in cell culture conditions, the H_2O_2 concentration, the length of incubation, and the time of termination after treatment. Next, we determined whether pioglitazone can affect H_2O_2-induced oxidative stress and promote development. H_2O_2 treatment impaired embryo development, while pioglitazone promoted embryo development by partially alleviating the oxidative stress. This effect was independent from that of PPARγ on oxidative stress, because the Nrf2 antioxidant pathway was activated.

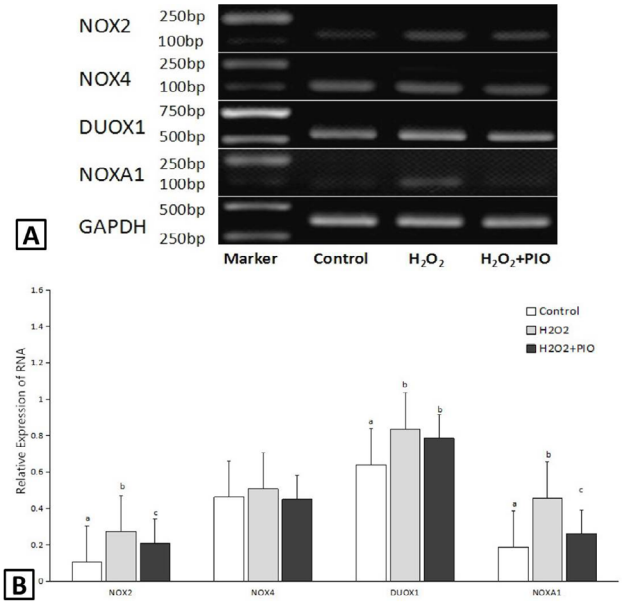

Fig. 4. The expression of NADPH oxidase in cultured mouse embryos, which were exposed to H_2O_2 of 30 min and then treated with pioglitazone, with untreated embryos as a control. **A**, PCR results of NADPH oxidase RNA in mouse embryos; **B**, relative expression of NADPH oxidase RNA normalized with the internal marker GAPDH. Different letters in the same picture mean the significant difference between the treatments ($P < 0.05$).

Under physiological conditions, Nrf2 is inactive in the cytosol, where it rapidly degrades, When cells encounter oxidative stress, active Nrf2 shuttles to the nucleus, where it binds to the antioxidant reactive element (ARE) in the promoter of antioxidant genes (Polvani *et al*., 2012). In this study, oxidative stress failed to induce Nrf2 expression in 2-cell stage embryos. These results agree with those of Amin *et al*. (2014) who reported that the Nrf2 mRNA level in bovine embryos cultured under high-oxygen conditions (*i.e.,* 20% vs. 5%, the normal) decreased moderately. However, the Nrf2 expression level in late-stage embryos cultured under high-oxygen conditions was higher than in embryos cultured under low-oxygen conditions. It might

be because the activation of Nrf2 takes much longer time in oxidative stress environment in embryos development (Nasr-Esfahani *et al*., 1990).

The relationship between PPARγ, a protein of the Nrf2 antioxidant pathway, and Nrf2 is direct (Park *et al*., 2004); in the absence of Nrf2, PPARγ expression decreases, and vice versa. For example, PPARγ expression was markedly reduced in Nrf2 null mice compared to wild-type mice (Cho *et al*., 2010). Furthermore, PPARγ agonists, such as rosiglitazone and 15d-PGJ2, upregulated Nrf2 expression, PPARγ might also act together with Nrf2 in the activation of antioxidant genes (Park *et al*., 2004), such as CAT, glutathione peroxidase (GPx), and superoxide dismutase (SOD). During oxidative stress, PPARγ regulates the expression of CAT (Girnun *et al*., 2002; Gray *et al*., 2012), one of the two most important enzymes involved in the degradation of H_2O_2. The activation of PPARγ also markedly increased CAT expression and activity. In rat cortical neurons, for example, pioglitazone activated PPAR, thereby inducing CAT activity (Gray *et al*., 2012). Furthermore, the protection of endothelial cells against H_2O_2-induced oxidative stress by rosiglitazone is dependent on CAT (Girnun *et al*., 2002).

GPx is the other important enzyme involved in the degradation of H_2O_2. Studies with PPARγ agonists report PPARγ to regulate GPx in different cell lines (Chung *et al*., 2009; Pei *et al*., 2013). In human skeletal muscle cells treated with H_2O_2, the PPARγ agonist troglitazone upregulated GPx3 mRNA and protein levels (Chung *et al*., 2009). Similar results were reported by Pei *et al*. (2013) who showed that rosiglitazone prevented the decrease in GPx expression and activity in rat cardiomyocytes exposed to infrasound, while $O_2^{(-)}$ and H_2O_2 levels decreased. In the same study, rosiglitazone also upregulated SOD1 and SOD2 expression and activity (Pei *et al*., 2013). Results from transient transfixion assays, promoter analysis, and knockout mice reveal that PPARγ regulates SOD (Flach *et al*., 1982; Yu *et al*., 2008). For example, SOD2 mRNA and protein levels decreased in the heart of PPARγ knockout mice (Yu *et al*., 2008). The PPARγ agonist pioglitazone also elevated the mRNA levels of all three SOD isoforms. Our finding was in the line with these results.

In addition to up regulating the expression levels of antioxidant enzymes, PPARγ downregulates NADPH oxidase expression. The different NADPH oxidase isoforms NOX1–NOX5 and DUOX1/2 play important roles in ROS generation because oxidative stress induced by H_2O_2 can activate NADPH oxidase (Wu *et al*., 2010). Hwang *et al*. (2005) reported that PPARγ, a negative regulator of NADPH oxidase, can reduce the production of $O_2^{(-)}$, Similar results were reported in vascular endothelial cells, supporting the concept that PPARγ agonists decrease p22-phox gene expression in HUVECs. Another study reported that PPARγ agonist ciglitazone down regulated the NOX regulatory subunit of p47-phox oxidase in phagocytes (Von *et al*., 2002). We found that pioglitazone inhibits not only the NOX catalytic subunit but also the NOX regulatory subunit of NOXA1, crucial components of NADPH oxidase. The mechanism responsible for the decrease in NADPH oxidase expression is not clear. Based on the evidence that the PPARγ agonist rosiglitazone decreased NADPH oxidase expression in endothelial cells via AMPK activation (Ceolott *et al*., 2007).

CONCLUSION

In conclusion, pioglitazone functions as an antioxidant in mouse embryos by increasing the PPARγ level, activating the Nrf2 anti-oxidative pathway, and inhibiting NADPH oxidase. These cellular events led to the suppression of H_2O_2-induced ROS production, which improved embryonic development. PPARγ, resulting in an increase in antioxidant enzyme activity but a decrease in the NADPH oxidase level, mediated the protective effects of pioglitazone.

ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (No. 31360546).

Statement of conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

Ali, I., Shah S.Z.A., Yi, J., Li, Z.S., Obaid, U. and Fang, N.Z., 2017. Reactive oxygen species-mediated unfolded protein response pathways in preimplantation embryos. *J. Vet. Sci,* **18**: 1-9. https://doi.org/10.4142/jvs.2017.18.1.1

Amin, A., Gad, A., Salilew-Wondim, D., Prastowo, S., Held, E., Hoelker, M., Rings, F., Tholen, E., Neuhoff, C., Looft, C., Schellander, K. and Tesfaye, D., 2014. Bovine embryo survival under oxidative-stress conditions is associated with activity of the NRF2-mediated oxidative-stress-response pathway. *Mol. Reprod. Dev.*, **81**: 497-513. https://doi.org/10.1002/mrd.22316

Blanquicett, C., Kang, B.Y., Ritzenthaler, J.D., Jones, D.P. and Hart, C.M., 2010. Oxidative stress modulates PPAR gamma in vascular endothelial

cells. *Free Radic. Biol. Med.*, **48**: 1618-1625. https://doi.org/10.1016/j.freeradbiomed.2010.03.007

Ceolotto, G., Gallo, A., Papparella, I., Franco, L., Murphy, E., Iori, E., Pagnin, E., Fadini, G.P., Albiero, M., Semplicini, A. and Avogaro, A., 2007. Rosiglitazone reduces glucose-induced oxidative stress mediated by NADPH oxidase via AMPK-dependent mechanism. *Arterioscler. Thromb. Vasc. Biol.*, **27**: 2627-2633. https://doi.org/10.1161/ATVBAHA.107.155762

Chen, J., Li, D., Zhang, X. and Mehta, J.L., 2004. Tumor necrosis factor-alpha-induced apoptosis of human coronary artery endothelial cells: modulation by the Peroxisome Proliferator-activated receptor-gamma ligand pioglitazone. *J. Cardiovasc. Pharmacol. Ther.,* **9**: 35-41. https://doi.org/10.1177/107424840400900i106

Chen, J., Li, D., Zhang, X. and Mehta, J.L., 2010. Nrf2-regulated PPARγ expression is critical to protection against acute lung injury in mice. *Am. J. Respir. Crit. Care Med.,* **182**: 170-182. https://doi.org/10.1164/rccm.200907-1047OC

Cho, H.Y., Glad, W.W., Wang, X., Chorely, B., Bell, D., Reddy, S.P. and Kleeberger, S.R., 2010. Nrf2-regulated PPARγ expression is critical to protection against acute lung injury in mice. *Am. J. Respir. Crit. Care Med.,* **182**: 170–182.

Chung, S.S., Kim, M., Youn, B.S., Lee, N.S., Park, J.W., Lee, I.K., Lee, Y.S., Kim, J.B., Cho, Y.M., Lee, H.K. and Park, K.S., 2009. Glutathione peroxidase 3 mediates the antioxidant effect of Peroxisome proliferator activated receptor γ in human skeletal muscle cells. *Mol. Cell Biol.*, **29**: 20-30. https://doi.org/10.1128/MCB.00544-08

Ecker, D.J., Stein, P., Xu, Z., Williams, C.J., Kopf, G.S., Bilker, W.B., Abel, T. and Schultz, R.M., 2004. Long-term effects of culture of preimplantation mouse embryos on behavior. *Proc. natl. Acad. Sci.*, **101**: 1595-1600. https://doi.org/10.1073/pnas.0306846101

Flach, G., Johnson, M.H., Braude, P.R., Taylor, R.A. and Bolton, V.N., 1982. The transition from maternal to embryonic control in the cell mouse embryo. *EMBO J.,* **1**: 681-686.

Girnun, G.D., Domann, F.E., Moore, S.A. and Robbins, M.E., 2002. Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter. *Mol. Endocrinol.*, **16**: 2793-2801. https://doi.org/10.1210/me.2002-0020

Goto, Y., Noda, Y., Narimoto, K., Umaoka, Y. and Mori, T., 1992. Oxidative stress on mouse embryo development *in vitro*. *Free Radic. Biol. Med.*, **13**: 47-53. https://doi.org/10.1016/0891-5849(92)90165-D

Gray, E., Ginty, M., Kemp, K., Scolding, N. and Wilkins, A., 2012. The PPAR-γ agonist pioglitazone protects cortical neurons from inflammatory mediators via improvement in peroxisomal function. *J. Neuroinflam.*, **9**: 63-73. https://doi.org/10.1186/1742-2094-9-63

Hwang, J., Kleinhenz, D.J., Lassègue, B., Griendling, K.K., Dikalov, S. and Hart, C.M., 2005. Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. *Am. J. Physiol. Cell Physiol.*, **288**: 899-905. https://doi.org/10.1152/ajpcell.00474.2004

Hwang, J., Kleinhenz, D.J., Rupnow, H.L., Campbell, A.G., Thulé, P.M., Sutliff, R.L. and Hart, C.M., 2007. The PPAR gamma ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice. *Vascul. Pharmacol.*, **46**: 456-462. https://doi.org/10.1016/j.vph.2007.01.007

Jung, T.W., Lee, J.Y., Shim, W.S., Kang, E.S., Kim, S.K., Ahn, C.W., Lee, H.C. and Cha, B.S., 2007. Rosiglitazone protects human neuroblastoma SH-SY5Y cells against MPP+ induced cytotoxicity via inhibition of mitochondrial dysfunction and ROS production. *J. neurol. Sci.,* **253**: 53-60. https://doi.org/10.1016/j.jns.2006.11.020

Kota, B.P., Huang, T.H. and Roufogalis, B.D., 2005. An overview on biological mechanisms of PPARs. *Pharmacol. Res.*, **51**: 85-94. https://doi.org/10.1016/j.phrs.2004.07.012

Lee, M.T., Bonneau, A.R. and Giraldez, A.J., 2014. Zygotic genome activation during the maternal-to-zygotic transition. *Annu. Rev. Cell. Dev. Biol*, **30**: 581-613. https://doi.org/10.1146/annurev-cellbio-100913-013027

Makela, J., Tselykh, T.V., Kukkonen, J.P., Eriksson, O., Korhonen, L.T. and Lindholm, D., 2016. Peroxisome proliferator-activated receptor-γ (PPARγ) agonist is neuroprotective and stimulates PGC-1α expression and CREB phosphorylation in human dopaminergic neurons. *Neuropharmacology*, **102**: 266-275. https://doi.org/10.1016/j.neuropharm.2015.11.020

Nasr-Esfahani, M.H., Aitken, J.R. and Johnson, M.H., 1990. Hydrogen peroxide levels in mouse oocyte and early cleavage stage embryos developed *in vitro* or *in vivo*. *Development*, **109**: 501-508.

Park, E.Y., Cho, I.J. and Kim, S.G., 2004. Transactivation of the PPAR-responsive enhancer module in chemo-preventive glutathione S-stransferase gene by the Peroxisome proliferator-actuated receptor-gamma

and retinoid X receptor heterodimer. *Cancer Res.*, **64**: 3701-3713. https://doi.org/10.1158/0008-5472.CAN-03-3924

Pei, Z., Meng, R., Zhuang, Z., Zhao, Y., Liu, F., Zhu, M.Z. and Li, R., 2013. Cardiac Peroxisome proliferator-activated receptor-γ expression is modulated by oxidative stress in acutely infrasound-exposed cardiomyocytes. *Cardiovasc. Toxicol.,* **13**: 307-315. https://doi.org/10.1007/s12012-013-9211-5

Polvani, S., Tarocchi, M. and Galli, A., 2012. PPARγ and oxidative stress: Con (β) catenating NRF2 and FOXO. *Peroxsimone Proliferator-Activated Receptors Res.,* **2**: 641-657.

Qiu, J.J., Zhang, W.W., Wu, Z.L., Wang, Y.H., Qian, M. and Li, Y.P., 2003. Delay of ZGA initiation occurred in 2-cell blocked mouse embryos. *Cell Res.*, **13**: 179-185. https://doi.org/10.1038/sj.cr.7290162

Rizos, D., Clemente, M., Bermejo-Alvarez, P., de La Fuente, J., Lonergan, P. and Gutiérrez-Adán, A., 2008. Consequences of *in vitro* culture conditions on embryo development and quality. *Reprod. Domest. Anim.*, **43**: 44-50. https://doi.org/10.1111/j.1439-0531.2008.01230.x

Roest, H.P., Baarends, W.M., de Wit, J., van Klaveren, J.W., Wassenaar, E., Hoogerbrugge, J.W., van Cappellen, W.A., Hoeijmakers, J.H. and Grootegoed, J.A., 2004. The ubiquitin-conjugating DNA repair enzyme HR6A is a maternal factor essential for early embryonic development in mice. *Mol. Cell. Biol.*, **24**: 5485-5495. https://doi.org/10.1128/MCB.24.12.5485-5495.2004

Varga, T., Czimmerer, Z. and Nagy, L., 2011. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. biophys. Acta*, **10**: 1007-1022. https://doi.org/10.1016/j.bbadis.2011.02.014

Von Knethen, A. and Brüne, B., 2002. Activation of Peroxisome proliferator-activated receptor gamma by nitric oxide in monocytes/macrophages down regulates p47phox and attenuates the respiratory burst. *J. Immunol.*, **169**: 2619-2626. https://doi.org/10.4049/jimmunol.169.5.2619

Wenmin, C., Weirong, P., Yubo, Q,. Yingcho, L., Xingqin, Z., Yan, H., Jige, X., Hongjiang. and Yangzhi, Z., 2017. Effect of prokaryotic expressed nucleaplasmin on the efficiency of somatic cell nuclear transfer in Banna Mini-Pig Inbred line. *Pakistan. J. Zool.,* **49**: 313-320.

Wu, M., Wang, Q., Luo, J.Y., Jiang, B., Li, X.Y., Chen, R.K. and Lu, Y.B., 2010. Activation of phospholipase D involved in both injury and survival in A549 alveolar epithelial cells exposed to H_2O_2. *Toxicol. Lett.*, **15**: 168-174. https://doi.org/10.1016/j.toxlet.2010.04.014

Yu, X., Shao, X.G., Sun, H., Li, Y.N., Yang, J., Deng, Y.C. and Huang, Y.G., 2008. Activation of cerebral peroxisome proliferator-activated receptors gamma exerts neuroprotection by inhibiting oxidative stress following pilocarpine-induced status epilepticus. *Brain Res.,* **20**: 146-158. https://doi.org/10.1016/j.brainres.2008.01.047

Zhang, Y., Jiang, Y., Lian, X., Xu, S., Wei, J., Chu, C. and Wang, S., 2015. Effects of ER a-specific antagonist on mouse preimplantation embryo development and zygotic genome activation. *J. Steroid Biochem.*, **145**: 13-20. https://doi.org/10.1016/j.jsbmb.2014.09.023