A Dissertation for the Degree of Master of Science

Study on Antioxidant Capacity of Paralichthys Olivaceus Egg Extracts in Oxidative Stress Exposed Porcine Oocyte Maturation Development

Hyo-Jin Park

Department of Biotechnology

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

June, 2021

산화 스트레스에 노출 된

돼지 난모세포 성숙에서

광어알 추출물의 항산화 효과 연구

지도교수 박세필

박 효 진

이 논문을 이학 석사학위 논문으로 제출함

2021 년 6 월

박효진의 이학 석사학위 논문을 인준함

심사위원장 _____ ㊞

위 원 _____ ㊞

위 원 _____ ㊞

제주대학교 대학원

Study on Antioxidant Capacity of Paralichthys Olivaceus Egg Extracts in Oxidative Stress Exposed Porcine Oocyte Maturation Development

Hyo-Jin Park

(Supervised by Professor Se-Pill Park)

A thesis submitted as a Qualified Dissertation

for the Degree of Master of Science

June, 2021

Department of Biotechnology

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

Approved as a Qualified Dissertation

for the Degree of Master of Science

Dissertation Committee:

Chairperson of the supervising committee

Professor Key-Zung Riu, Ph.D., College of Applied Life Sciences, Jeju National University

Professor Eun-Young Kim, Ph.D., College of Applied Life Sciences, Jeju National University

Professor Se-Pill Park, Ph.D., College of Applied Life Sciences, Jeju National University

Department of Biotechnology

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

In vitro maturation and embryonic development of the egg, compared to the body, the oocyte is damaged by reactive oxygen species (ROS) generated inside the cell. In this study, we investigated the effect of treatment with paralichthys olivaceus egg extract (POEE) on the maturation and development of oocytes exposed to oxidative stress during in vitro maturation of pig oocytes. As a result of analyzing the components of POEE, the content of vitamin B9 (folic acid, FA) was higher than that of other components, and the IC50 of POEE was calculated using the radical scavenging ability of DPPH. To create a peroxidative environment in pig eggs, 200 μM of H_2O_2 was treated, and immature pig eggs were treated with 0, 600, 650, 700, and 750 ppm POEE concentrations. As a preliminary test, FAs were treated with 0, 0.1, 0.2, 0.3, 0.4 mM (0 FA, 0.1 FA, 0.2 FA, 0.3 FA, 0.4 FA). The rate of cleavage and blastocyst formation in pig embryos was significantly increased at 0.3 FA. In the 650 POEE group, the survival rate and in the 700 POEE group, the cleavage rate increased significantly. In addition, the 650 POEE group significantly increased glutathione (GSH) levels compared to the control group, and the 0.3 FA group significantly reduced reactive oxygen species (ROS) activity compared to the control group. The 650 POEE group and the 0.3 FA group prevented abnormal spindle organization and chromosomal misalignment compared to the control group, and also significantly increased the total number of cells in the blastocyst phase and decreased apoptotic cells compared to the control group. These results show that POEE and FA reduce oxidative stress in pig eggs and improve their quality and developmental potential.

Key words: Porcine; Paralichthys olivaceus egg; Folic acid; Oocyte; In Vitro Maturation

1. INTRODUCTION

In the livestock industry, the in vitro production technology of embryos is considered as one of the important technologies, and it is being widely studied in the field of transgenic animals for the development of xenotransplantation animals or human disease models. It is damaged by reactive oxygen species (ROS) generated inside the cell for reasons such as supplementation of the culture medium (Won-Hee Lee, Ji-Eun Park & Choon-Keun Park. 2017; Mozafar Khazaei & Faranak Aghaz. 2017). Also, oocyte aging prevents the growth of mature mammalian oocyte processes in the metaphysics of secondary meiosis (MII) after ovulation. In addition, spindle abnormalities, chromosomal condensation disorders, mitochondria and changes in gene and protein expression negatively affect fertilization and subsequent embryonic development. Aging in vitro is associated with an increase in ROS levels such as hydrogen peroxide, peroxide and hydroxyl radicals (Young Do Yoo. 2013; Hideki Igarashi, Toshifumi Takahashi, & Satoru Nagase. 2015).

To reduce oxidative stress during in vitro maturation of the egg and embryonic development, antioxidants are used in the culture medium. There's a lot of research going on, so I've been looking for useful ingredients, including antioxidants. There are useful ingredients for plant and animal tissues, and the types are composed of antioxidants, anti-inflammatory and anticancer etc (R S Barberino, V R P Barros & M H T Matos. 2016). First, research on antioxidants involves the study of an extract of Salvia africana-lutea, suggesting that the effect on this extract could be used for both prevention and/or amelioration of diabetic symptoms. In another study, Dienaite et al. Polyphenol extracts extracted from the roots and leaves of Paeonia officinalis showed free-radical scavenging activity and inhibited α-amylase, suggesting its potential as an antidiabetic agent (Dimitrios Stagos 2020). In addition, radical scavenging activity of various plants such as Common walnut Juglans regia, Maidenhair tree Ginkgo biloba, Horse-chestnut Aesculus hippocastanum, and Ginger Zingiber officinale was measured. Overall, the plant extracts exhibit remarkable antioxidant properties, from 0.44 (Allium sativum) to 44.18 (Juglans regia) AAEQ values (Adél Szerlauth,

Szabolcs Muráth, Sándor Viski, Istvan Szilagyi. 2019). Studies on the anti-inflammatory properties of extracts from Tribulus terrestris (TT) and Cuscuta pedicellata (CP) in extracts of these plants showed that CP stem extract and TTF and TTL extract were major contributors to antioxidant, antibacterial, antifungal, anti-inflammatory and antiproteinase activity. was found to contain significant phenolic content as suggested by In addition, as an anticancer study of the extract, Sansevieria liberica Gerome and Labroy (Agavaceae) The hydroethanol root extract of this plant has significant anticancer activity warranting more extensive research (Abidemi J. Akindele, Dilip M. Mondhe, and Ajit K. Saxena. 2015). Also compared to the Xenopus extract treatment control in the current study, the combined treatment of Xenopus extract of donor cells and scriptaid treatment of reconstructed embryos additively increased blastocyst formation, which indicates that both treatments could be used together to improve the embryo development in pigs (Xiaoyu Yang, Jiude Mao & Randall S Prather. 2012).

Among these various extract components, samples were found with antioxidants as main components. In the course of the investigation, when research on fish eggs was investigated, the expression of POU5f1 and NANOG was confirmed in OFEC-17FEN cells, which developed an embryonic cell line of paralichthys olivaceus (Ju-Won Kim, JaeHun Cheong, and Hee Jeong Kong 2018). Another study looked at compounds derived from marine and plant organisms that exhibit cosmetically useful antioxidant and anti-aging activities, including vitamins, proteins, and unsaturated fatty acids. Based on this, when human dermal fibroblasts were treated with chum salmon eggs (CSE), cells treated with CSE extract showed concentration-dependent upregulation of several antioxidant genes, including OXR1, TXNRD1 and PRDX family genes, and collagen type I genes. The results showed that the overall antioxidant activity of cells was improved. Germline alpha factor (FIGLA), a bHLH transcription factor, is one of the factors involved in the early stages of ovarian development in Mus musculus, which performed a potentially important function in ovarian differentiation and development in paralichthys olivaceus (Shaoshuai Liang, Wenxiang Wang, Feng You 2020).

So, based on various studies, we predicted that paralichthys olivaceus egg (POE) could also function in mammalian oocytes and selected it as a sample. Although many experiments have been

conducted on the culture or embryonic development of paralichthys olivaceus, they have not been used directly in mammalian oocytes or cells. In addition, Paralichthys Olivaceus accounts for 65.6% of the production of flatfish on Jeju Island as of 2015, and it is estimated that 9,000 tons of POE are wasted annually. Through this, it is expected that Jeju's resources can be utilized to contribute to the local community.

This study investigated the increase the development of embryos by improving the maturation rate of porcine oocytes by treating POEE by creating a peroxidation environment during in vitro maturation to confirm the antioxidant effect of components of POEE. We analyzed the level of ROS and GSH and spindle morphology in peroxidized porcine oocytes treated with or without POEE & FA. In addition, we determined the developmental competence and quality of embryos derived from these oocytes. Our results suggest that POEE has antioxidant properties and protects oocytes from oxidative stress in vitro and thereby prevents deterioration in their quality.

2. MATERIALS & METHODS

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma (St. Louis, MO, USA) unless stated otherwise.

2.2. Preparation of Paralichthys olivaceus eggs extract

EtOH extract was prepared to of Paralichthys olivaceus eggs. Fish eggs are added to 100 ml of EtOH 30% EtOH and tertiary distilled water, and then reacted on a shaker at 25 °C for 30 minutes. EtOH is evaporated with a rotary vacuum concentrator. It was filtered through filter paper and then freeze-dried.

The total phenol content (TPC) of the extract of paralichthys olivaceus eggs (POE) was obtained by adding 10 ml of 10% Folin Ciocalteu reagent and 8 ml of sodium carbonate (7.5% w/v) solution to 1 ml of extract (1000 μg/ml) in Singleton methanol and adjusting the reaction volume. It was made to 20 ml with distilled water. After incubation for 2 h at room temperature, absorbance was measured at 765 nm in a UV/Vis spectrophotometer. Total phenolics were quantified with a calibration curve of gallic acid (25-300 μg/ml).

2.3. Analysis of DPPH radical scavenging ability

DPPH exhibits strong absorption at 517 nm due to its own odd number of stable radical electrons, while when it reacts with an electron donor that provides electrons to hydrogen such as polyphenol, the electrons receive hydrogen radicals and become phenoxy. It forms a radical and turns into a molecule of a stable form, thus reducing the absorbance at that wavelength. At this time, the donated electrons are irreversibly bonded, and in proportion to the number, the

progressive purple color of DPPH becomes pale and the absorbance decreases. The Paralichthys Olivaceus egg, extracted with 30% EtOH and DW, harvested the day, one day old, two days old, and hatched were measured by concentration from diluted in DMSO and serial dilutions were prepared 0.01 g/ml (1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000ppm). 20 μL of the samples were diluted in DMSO and added to 180 μL of a 167 μM DPPH working solution. The absorbance of the solution was measured at 517 nm. The DPPH free radical scavenging activity was calculated using the following equation

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

The DPPH free radical scavenging activity was expressed as an inhibition concentration at 50% of activity (IC50), value was obtained by creating a trend line using the equation.

2.4. IVM and peroxidized of porcine oocytes

Prepubertal porcine ovaries were collected from a local slaughterhouse and transported to the laboratory in saline supplemented with 75 μg/ml penicillin G and 50 μg/ml streptomycin sulfate within 2 hr at 30–33°C. Cumulus□oocyte complexes (COCs) were aspirated from follicles with a diameter of 2–8 mm using an 18□gauge needle and a disposable 10 ml syringe. COCs were washed three times in tissue culture medium (TCM)□199□HEPES containing 0.1% (w/v) bovine serum albumin (BSA). Thereafter, COCs were matured in groups of 50 in 500 μl TCM□199 (Gibco, Grand Island, NY) containing Earle's salts, 0.57 mM cysteine, 10 ng/ml epidermal growth factor, 0.5 μg/ml follicle□stimulating hormone, 0.5 μg/ml luteinizing hormone, and 10% (v/v) porcine follicular fluid under mineral oil for 44 hr at 38.8°C in 5% CO2 and 95% air. Peroxidized oocyte was induced by treating 200μM H2O2 for an 44 hr in TCM□199 (Sigma).

2.5. Folic acid treatment

Before maturation, the oocytes were covered with mineral oil and incubated in a 4-well dazu well containing 500 μl of TCM-199 and Folic acid at 38.8 ° C in a humid atmosphere of 5% CO2 and 95% air. Prior to maturation, GV stage oocytes were transferred to TCM-199 containing 0.1, 0.2, 0.3 and 0.4 mM folic acid (Sigma), including was treated at the same time with H2O2 and incubated for 44 h as described above. After treatment, oocytes were collected and Peroxidation was evaluated.

2.6. POEE treatment

Prior to maturation, GV stage oocytes were transferred to TCM-199 containing 600, 650, 700 and 750 ppm POEE, including was treated at the same time with H2O2 and incubated for 44 h as described above. After treatment, oocytes were collected and Peroxidation was evaluated.

2.7. Parthenogenetic activation and embryo culture

Following maturation, cumulus cells were removed by pipetting in the presence of 1 mg/ml hyaluronidase for 2–3 min. PA was induced by treating oocytes in porcine zygote medium (PZM)□5 containing 0.4% (w/v) BSA (IVC medium) with 5 μM Ca2+ ionomycin (Sigma) for 5 min. After 3 hr of culture in IVC medium containing 7.5 μg/ml cytochalasin B (Sigma), embryos were washed three times in the same medium and cultured for 7 days at 38.8°C in a humidified atmosphere of 5% CO2 and 95% air. Oocytes and embryos were washed in Dulbecco's phosphate□buffered saline (DPBS) and either fixed in 3.7% (w/v) paraformaldehyde for 20 min and stored at 4°C, or snap□frozen in liquid nitrogen and stored at 70°C, depending on the experiment.

2.8. Measurement of intracellular ROS and GSH levels

DCFHDA and CMF2HC were used to determine the intracellular levels of ROS and GSH, respectively, as previously described (H. W. Yang et al., 1998; You, Kim, Lim, & Lee, 2010) with slight modifications. Briefly, cumulus cells were removed from COCs by pipetting in the presence of 0.1% (w/v) hyaluronidase. Denuded oocytes were incubated in DPBS containing 50 μM DCFHDA or 100 μM CMF2HC in the dark for 20 min at 38.8°C. Thereafter, oocytes were washed more than five times with DPBS containing 0.1% (w/v) BSA to completely remove excess dye and immediately analyzed by epifluorescence microscopy (Olympus, Tokyo, Japan). The ROS level was measured using excitation and emission wavelengths of 450–490 nm and 515–565 nm, respectively. The excitation and emission wavelengths of CMF2HC are 371 and 464 nm, respectively. Grayscale images were acquired with a digital camera (Nikon, Tokyo, Japan) attached to the microscope, and mean grayscale values were calculated using Image J software (NIH, Bethesda, MD). Background fluorescence values were subtracted from the final values before statistical analysis. The experiment was independently repeated 6–7 times with 10–20 oocytes per experiment.

2.9. Immunofluorescence

Meiotic spindles and nuclei of oocytes were visualized after maturation. Cumulus cells were removed from porcine COCs matured for 44 h, and then oocytes were fixed overnight at 4°C with 4.0% (w/v) paraformaldehyde prepared in PBS. Fixed oocytes were incubated for 30 min at 38.8°C with 0.5% (v/v) Triton X-100. After blocking for 1 h with 1% BSA (w/v) prepared in PBS (blocking solution I), oocytes were incubated overnight at 4°C with an Alexa Fluor 488-conjugated anti-α-tubulin antibody (Sigma, diluted 1:200 in blocking solution I). Nuclei were stained with Hoechst 33342 (1 μg/mL) for 30 min. Finally, oocytes were washed three times with

PBS containing 0.1% (w/v) BSA, mounted onto glass slides, and examined under an inverted Olympus IX-71 microscope. At least 20 oocytes were examined per group.

2.10. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and hoechst staining

At 7 days after PA, blastocysts were fixed, washed more than three times with PBS containing 0.1% BSA, and then incubated with 0.1% Triton X□100 at 38.8°C for 30 min. Blastocysts were incubated with fluorescein□conjugated dUTP and terminal deoxynucleotidyl transferase (In Situ Cell Death Detection Kit; Roche, Manheim, Germany) in the dark for 1 hr at 38.8°C. Mitotic and apoptotic cells were scored. Nuclei were stained with Hoechst 33342 (1 μg/ml) for 30 min, and embryos were washed with PBS containing 0.1% BSA. Blastocysts were mounted onto glass slides and examined under an inverted Olympus IX□71 fluorescence microscope. The experiment was independently repeated four times.

2.11. Statistical analysis

Data from all experiments were analyzed using the general linear model procedure within Statistical Analysis System software (SAS User's Guide 1985, Statistical Analysis System Inc., Cary, NC, USA). The paired Student's *t*-test and Tukey's multiple range test were used to determine significant differences. *P*-values less than 0.1 were considered significant.

3. Results

3.1. Analysis of POE Extract (POEE) Components

The result of analyzing the components of the POE extract (POEE), it consisted of Collagen (280 mg/100g), Lutein (9 μg/100g), Coenzyme Q10 (2 mg/100g), Vitamin E-α (26 mg/100g) and Vitamin B9 (330 mg/100g) (Table 1). Among them, the Vitamin B9 (folic acid, FA) was found to contain a larger amount than other components, and was confirmed to be 1,500 times higher than the spinach (211 μg/100g). We extracted the POEE using 30% ethanol, and 3rd distilled water (30E and DW).

Table 1. Content of the ingredients in the Paralichthys olivaceus egg through high-performance liquid chromatography (HPLC)

Analysis article	**Result**
Collagen	280 mg/100g
Lutein	9 μg/100g
Coenzyme Q10	2 mg/100g
Vitamin E-α	26 mg/100g
Vitamin B9(Folic acid)	330 mg/100g

3.2. Extraction of Paralichthys olivaceus egg and DPPH analysis of folic acid

The status of Paralichthys olivaceus eggs were 1 day, 1 day, 2 days, and hatched, extracted with 30E and DW, respectively. The 9000ppm radical scavenging rates were 31.9%, 33.3%, 27.0%, and 71.6% in 30E respectively, in the order of 1 day, 1 day, 2 days, hatching, and the hatching rate was significantly higher than that of other days ($p < 0.05$). In DW, it was 36.5%, 53.5%, 39.5%, and 67.3% in the above order, and although it was not significantly high in hatching, it showed a high pattern like 30E. Based on this, when the IC50 value of hatching POEE was calculated 30E 5,830 mg/mL, and DW 6,143 mg/mL (Figure 1). The final sample concentration by DPPH reaction by IC 50 value is diluted 10-fold by adding it with the reagent of DPPH. Therefore, the final concentration of POEE was determined to be 600, 650, 700, 750 diluted 10 times from the IC 50 value.

Table 2. DPPH antioxidant analysis of POEE

Sample	Extraction solvent	Conc. (ppm)	The day	One day	Two day	hatching	EC_{50}
Paralichthys olivaceus eggs extract (POEE)	30% EtOH	1000	4.6 ± 1.4	4.7 ± 0.7	-0.1 ± 4.5	5.3 ± 2.9	IC_{50} of hatching = 5,830
		2000	8.8 ± 1.7	12.5 ± 0.2	4.1 ±2.4	25.5 ± 12.6	
		3000	14.0 ± 1.3	12.9 ± 2.3	4.1 ± 1.9	27.0 ± 1.8	
		4000	23.3 ± 1.8	14.6 ± 0.9	12.5 ± 0.3	39.6 ± 2.6	
		5000	24.2 ± 0.5	20.9 ± 6.0	15.4 ± 1.6	46.6 ± 3.4	
		6000	28.8 ± 0.8	22.1 ± 4.6	17.7 ± 1.2	55.1 ± 3.4	
		7000	38.8 ± 2.3	34.8 ± 9.8	21.8 ± 0.9	62.7 ± 0.3	
		8000	34.9 ± 2.3	35.3 ± 13.5	22.0 ± 1.1	60.4 ± 5.3	
		9000	31.9 ± 9.5	33.3 ± 6.0	27.0 ± 0.7	71.6 ± 1.5	
	3rd D.W	1000	-1.6 ± 2.5	6.5 ± 0.3	2.2 ± 1.7	4.7 ± 0.6	IC_{50} of hatching = 6,143
		2000	4.8 ± 0.4	16.9 ± 2.0	7.8 ± 2.6	19.9 ± 2.3	
		3000	7.0 ± 1.0	22.4 ± 2.1	12.9 ± 5.1	28.7 ± 3.9	
		4000	15.0 ± 2.6	31.5 ± 5.2	19.4 ± 5.8	37.3 ± 3.7	
		5000	18.0 ± 6.5	35.3 ± 11.5	22.1 ± 8.1	47.6 ± 5.9	
		6000	21.0 ± 6.8	39.5 ± 5.9	28.8 ± 8.2	52.7 ± 5.9	
		7000	26.2 ± 7.7	47.7 ± 5.7	34.3 ± 8.2	57.7 ± 3.0	
		8000	30.4 ± 9.8	46.9 ± 4.9	33.1 ± 9.3	57.1 ± 6.3	
		9000	36.5 ± 13.8	53.5 ± 8.6	39.5 ± 11.0	67.3 ± 5.2	

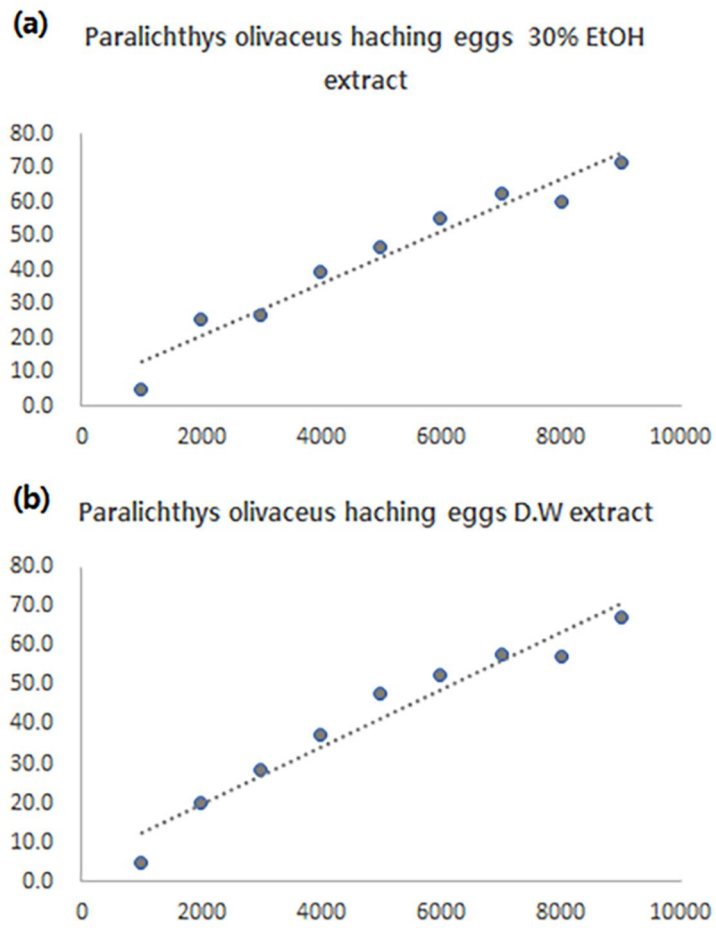


Fig. 1. Each Paralichthys Olivaceus eggs extract extracted with 30%, and 0% EtOH was chemically analyzed for its antioxidant function.

3.3. Effect of POEE & Folic acid treatment on maturation of porcine oocytes

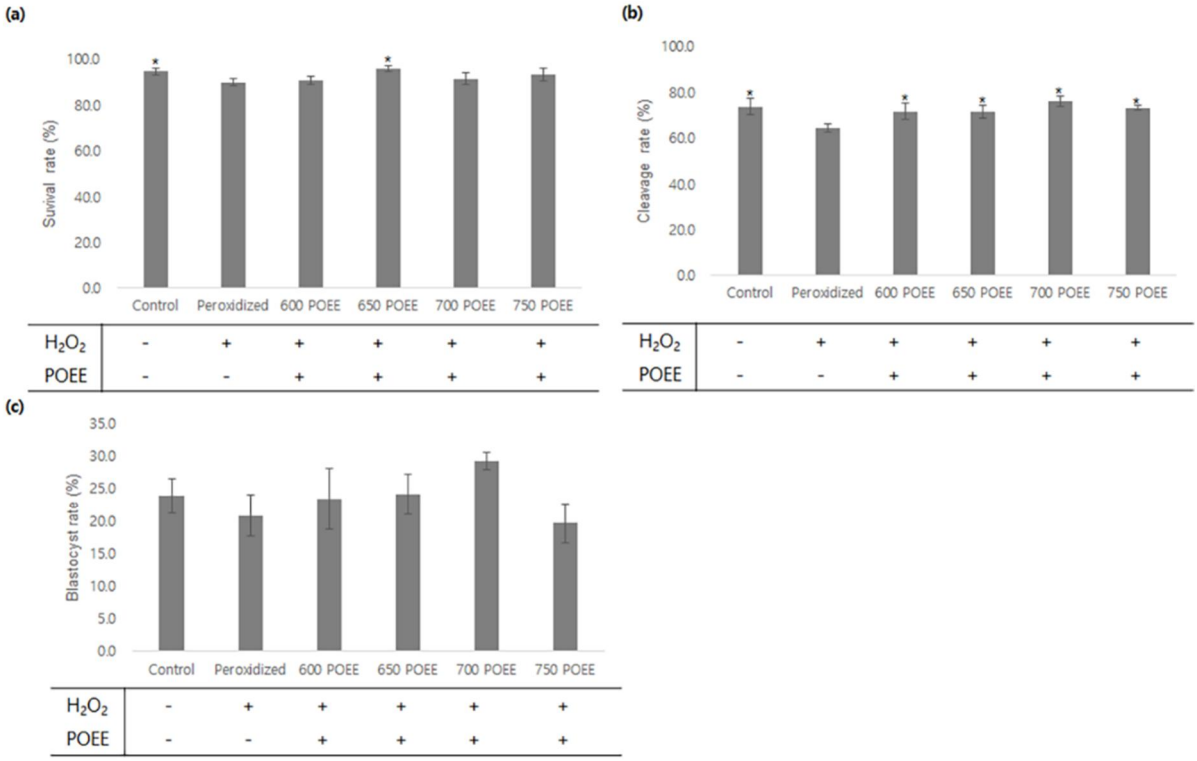
This is the result of confirming the rate of egg development by processing POEE during the IVM period. in the control group, the ovarian maturation rate was observed in the peroxidized group and the folic acid-treated group peroxidized with H_2 O_2 , the egg was activated through unit generation, and finally cultured until the blastocyst period to measure the fertility and blastocyst of the embryo.

We checked antioxidant effects of the POEE and FA in porcine oocyte on peroxidative stress (200 μM H2O2). As preliminary test, the FA was treated with 0, 0.1, 0.2, 0.3, 0.4 mM (0 FA, 0.1 FA, 0.2 FA, 0.3 FA and 0.4 FA) (Table 3). The rates of cleavage and blastocyst formation were significantly increased at 0.3 FA ($p < 0.05$). When the experiment to set the concentration of POEE was conducted, POEE at concentrations of 0, 600, 650, 700, and 750 were treated (Figure 2). The oocyte survival rate showed a significant increase from 650 ppm to 95.6% compared to the control group, and the cleavage rate was the highest at 700 ppm at 76.1%, but compared to the control group, it was confirmed that there was a significant increase in all treatment groups except the control group. Also, the blastocyst rate was 700 ppm was higher than that of the control group, but did not increase significantly.

Additionally, GSH assay was performed on porcine oocytes to confirm the effect of each concentration. As a result, the level of GSH was significantly increased at the concentrations of 600 and 650 ppm compared to the control, confirming that the POEE treatment restored as much as the level of the normal group (Figure 3).

Table 3. Effect of folic acid treatment with H_2O_2 of porcine oocytes *In vitro* on embryo development

Conc. (mM)	No. (%) of MII Survival	No. (%) of	
		Cleaved at day 2	Blastocyst at day 7
0 FA	86.9 ± 2.0	68.8 ± 2.1	21.8 ± 1.6
0.1 FA	85.1 ± 2.0	67.2 ± 3.6	23.9 ± 2.1
0.2 FA	83.9 ± 2.2	70.3 ± 5.4	22.1 ± 4.0
0.3 FA	87.7 ± 1.9	78.7 ± 2.5*	27.8 ± 3.0*
0.4 FA	82.1 ± 3.2	72.6 ± 5.0	26.9 ± 3.2



*** H_2O_2 concentrate : 200ul**

Fig. 2. Effects of POEE on subsequent embryo development in porcine blastocysts. (a) The percentage of survived oocytes , (b) cleaved embryos, (c) and formed blastocysts during in vitro maturation and subsequent culture for 7 days. Relative expression of the developmental genes; H_2O_2 concentrate, 200uM; POEE concentrate (ppm).

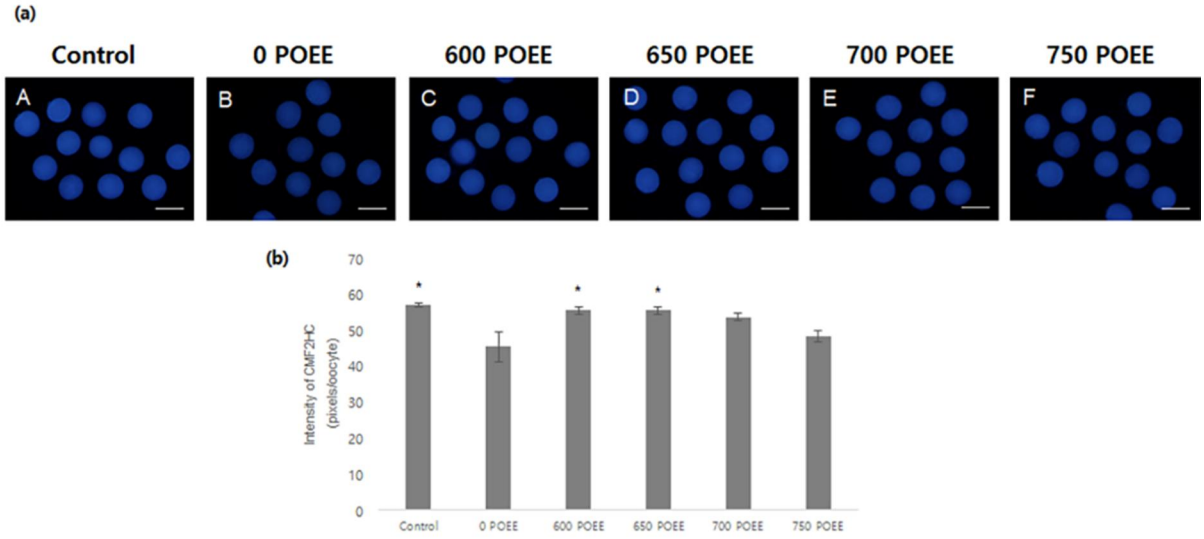


Fig. 3. Antioxidant effect of POEE on the level of oxidative stress during porcine oocyte maturation in peroxidation environments. Images of oocytes stained with CMF2HC (blue). (A-F) GSH staining (scale bar, 100μm). (b) Quantification of the fluorescence intensity of CMF2HC.

3.4. POEE and FA reduces the level of ROS and increase the level of GSH in peroxidized porcine oocytes in vitro

The effects of POEE and FA on the levels of ROS and glutathione (GSH) were analyzed by staining oocytes with dichlorohydrofluorescein diacetate (DCFHDA) and CellTracker™ Blue 4-chloromethyl-6,8-difluoro-7-hydroxycoumarin (CMF2HC), respectively. The staining intensity of ROS was significantly lower ($p<0.05$) in the control and 0.3 FA groups than in the peroxidized group (control, 29.1±0.4 pixels/oocyte; peroxidized, 33.5±1.4 pixels/oocyte; and 0.3 FA, 29.9±0.5 pixels/oocyte.). The staining intensity of GSH was significantly higher ($p<0.05$)in the control and 650 POEE groups than in the peroxidized group (control, 57.0±0.7 pixels/oocyte; peroxidized, 45.3±4.1 pixels/oocyte; and 650 POEE, 55.4±1.0 pixels/oocyte) (Figure 4).

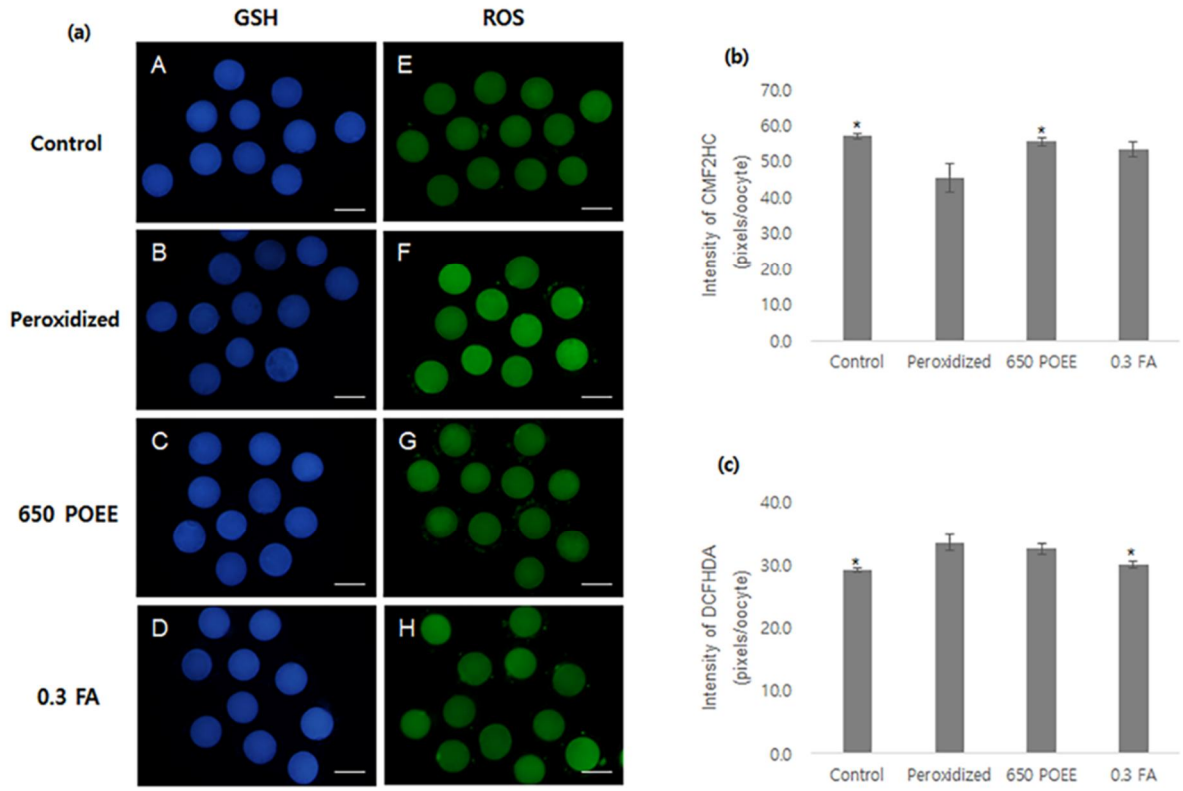


Fig. 4. Antioxidant effect of FA on the level of oxidative stress during porcine oocyte maturation in peroxidation environments. Images of oocytes stained with DCFHDA (green) and CMF2HC (blue). (A-D) ROS staining; (E-H) GSH staining (scale bar, 100μm). (b) Quantification of the fluorescence intensity of DCFHDA and (c) Quantification of the fluorescence intensity of CMF2HC.

3.5. POEE & FA prevents aberrant spindle organization and chromosome misalignment in peroxidized porcine oocytes in vitro

To further investigate the effect of POEE & FA on spindle organization, spindles without abnormalities were classified as normal, whereas those in which chromosomes failed to align at the metaphase plate were classified as abnormal (Lenie, Cortvrindt, Eichenlaub-Ritter, & Smitz, 2008). The percentage of oocytes with normal meiotic spindles was significantly higher ($p<0.05$) in the 650 POEE group and 300 FA group than in the peroxidized group, and was similar in the control and 650 POEE group and 300 FA group (control, 57.9±4.6%; peroxidized, 48.1±1.9%; 650 POEE group, 60.7±3.6%; and 300 FA group, 57.8±2.2%) (Figure 5).

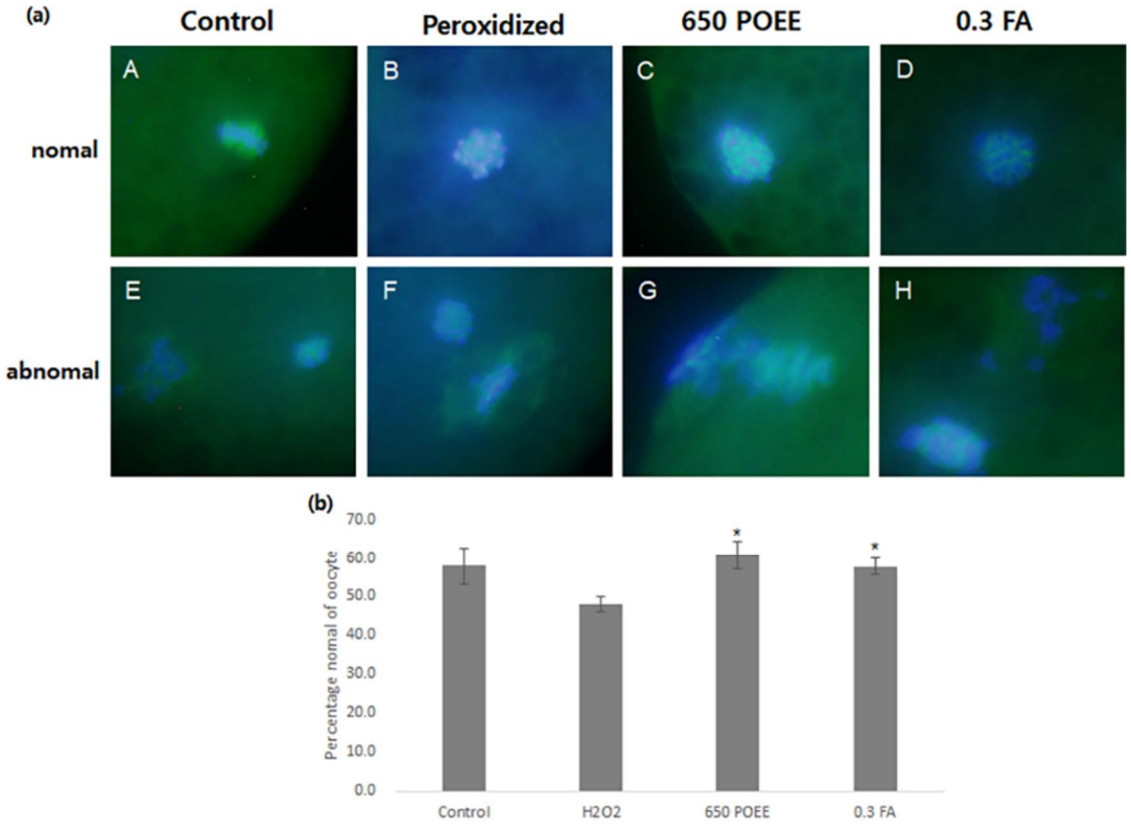


Fig. 5. Morphological classification of meiotic spindles and nucleus. (A-D) nomal; (E-H) abnomal (a) Normal and abnormal chromosome alignment and meiotic spindle formation in oocytes. (b) Percentage of oocytes showing normal morphology of chromosomes and meiotic spindle.

3.6. POEE & FA improves the developmental capacity and quality of embryos derived from peroxidation porcine oocytes in vitro

To investigate whether POEE and FA treatment during IVM of oocytes influences subsequent embryo development and quality, oocytes were matured in the presence or absence of 650 ppm POEE and 0.3mM FA then parthenogenetically activated. The blastocyst formation rates were calculated at 7 days after PA (Fig. 5). The total number of cells per blastocyst was significantly higher ($p<0.05$) in the control and 650 POEE groups than in the aging group (control,). Genomic DNA fragmentation was assessed by terminal deoxynucleotidyl transferase dUTP nick-end labeling to detect apoptotic cells in blastocysts. The percentage of apoptotic cells was significantly higher ($p<0.05$) in the aging group than in the control and 650 POEE groups (control) (Figure 6).

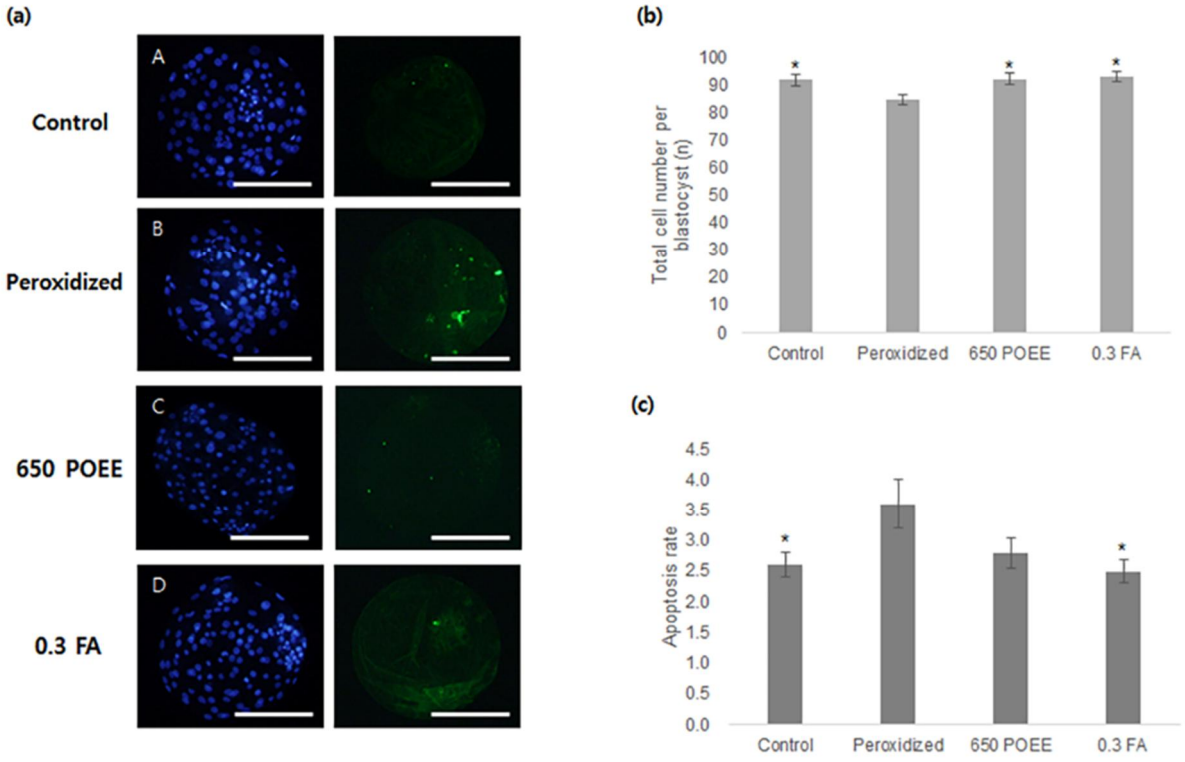


Fig. 6. Effects of FA on IVM Peroxidized with H_2O_2 on the quality of parthenogenetically activated embryos. (a) Morphology of blastocyst Total cell and apoptotic cells. (b) Total cell number per blastocyst. (c) Frequency of apoptotic cells per blastocyst.

4. DISSCUSTION

The present study investigated to make extracts using large amounts of paralichthys olivaceus eggs (POE) that are being discarded in Jeju, and to find the main active ingredient in these extracts. In addition, we investigated the improve the maturation rate of pig oocytes by adding these substances during maturing in vitro, thereby increasing the development of embryos. When the ingredients of POEE were analyzed, it was found that vitamin B9 (folic acid, FA) contained a higher amount than other ingredients, and was 1,500 times higher than that of spinach (211 μg/100 g). After calculating the IC50 using the radical scavenging ability of DPPH and setting the concentration, treatment with 0, 600, 650, 700, or 750 ppm POEE during in vitro maturation in a peroxidative environment significantly increased the GSH level and the proportion of oocytes with normal spindle. Additionally, the treatment of these POEEs showed an increase in the quality and an improved rate of development.

The folic acid content of other plants except spinach was (180.5 μg/100 g) in the transgenic tomato and (188.5 μg/100 g) in lettuce. The folic acid content of POEE was 180,000 times and 170,000 times higher than that of transgenic tomato and lettuce, respectively (M J I Shohag, Yan-yan Wei, Xiao-e Yang 2011). In another plant, in Eleutherococcus senticosus (146.9 μg/100 g), Aster glehni F. Schmidt (142.8 μg/100 g), and Ledebouriella seseloides H. Wolff (140.4 μg/100 g), when compared to the folic acid content of POEE, It shows 220,000 ~230,000 times difference, indicating that the folic acid content of POEE is higher than that of other plants (Bo Min Kim, So-Min Kim, Youngmin Choi 2014).

DPPH is a stable free radical that can be used to measure the radical scavenging activity of antioxidants. The DPPH assay is a short and simple method to measure the electron-donating ability of antioxidants during lipid oxidation (Kikuzaki, Hisamoto, Hirose, Akiyama, & Taniguchi, 2002). To compare chemical antioxidant activity, we calculated the IC 50 value

representing the concentration of the sample required to inhibit 50% of the DPPH radical. To calculate an approximate IC 50 value with a 95% confidence level, a linear regression line was generated against antioxidant concentration, based on the guidelines by Sebaugh JL (Sebaugh, 2011; Blois, M. S 1958). To confirm the antioxidant effect of POEE through the radical scavenging ability of DPPH, the status of Paralichthys olivaceus eggs were 1 day, 1 day, 2 days, and hatched, extracted with 30E and DW, respectively. At this time, incubation showed the highest radical scavenging ability (Table 2), and the IC50 value was calculated based on these results (Figure 1). The final sample concentration is dilute 10-fold when adding DPPH reagent. Therefore, the final concentrations of POEE were determined to be 600, 650, 700, and 750 diluted 10-fold from their IC 50 values.

The easiest way to assess the quality of oocytes in vitro is to identify the developmental rate. Developmental rate determines the efficiency with which embryos are produced in vitro. Peroxidation negatively affects oocyte competency and embryo development , and reduces the cleavage rate. The survival rate was significantly increased in the 650 POEE group compared to the control group, and the 700 POEE group had the highest cleavage rate, but the 650 POEE group also showed a significant increase compared to the control group. Many factors increase the developmental rate of oocytes (R. Li, Liu, Pedersen, & Callesen, 2015; Park, So, & Hyun, 2017; Shin et al., 2018).

Oxidative stress arises in postovulatory aging oocytes, in which ROS production is increased and antioxidant protection is concomitantly decreased (Lord & Aitken, 2013). Intracellular GSH protects oocytes against oxidative damage; however, its level gradually decreases during aging (Boerjan & de Boer, 1990; Gerard-Monnier, D., & Chaudiere, J 1996). One of the most important roles of GSH is to maintain a redox state in cells that protects them from the harmful effects of oxidative damage. The protective action of GSH against ROS is facilitated by its interaction with related enzymes such as GPx and GSH reductase (Yang HW, Choi KW and Oh KS 1998; Mozafar Khazaei and Faranak Aghaz 2017).

Treatment of 650 POEE prevents a decrease in oocyte maturation efficiency during in vitro peroxidation (Shi S, Grothe S, Roughley PJ and Mort JS 2004). The chromatin fibers of the nucleus condense into chromosomes during mitosis and meiosis cell division. Spindle microtubules extend from centrosome to centripetal and arrange chromosomes to regulate the cell cycle (Trumbo P, Yates AA, Schlicker S and Poos M 2001). If mature oocytes are not fertilized, the superstructure of the centrosome and microtubules is destroyed, leading to spindle abnormalities and chromosomal loosening (Sun GW, Kobayashi H and Terao T 1999). We found that the percentage of oocytes with normal spindles decreased with peroxidation in vitro, and that 650 POEE treatment prevented this reduction. In this study, when oocyte peroxidation in vitro, treatment with 650 POEE retains chromosomes and spindles in MII.

Oocyte quality is a key factor in determining embryonic capacity regardless of whether maturation occurs under normal or stress conditions. Oocytes that are degraded due to exposure to oxidative stress from peroxidation have a reduced developmental potential (Guérin P, El Mouatassim S and Ménézo Y 2001). Accordingly, in the control group, the total number of cells decreased slightly, while the frequency of apoptosis per blastocyst increased (Eichenlaub-Ritter, U 2012; Bomfim, M. M., Andrade, Perecin, F 2017). We showed a slight improvement in the quality of POEE-treated embryos during in vitro peroxidation.

In conclusion, this study demonstrate that supplementation of IVM media with POEE significantly improves the maturation of porcine oocytes by regulating oxidative stress, nuclear and cytoplasmic maturation. Moreover, POEE treatment enhances the development and quality of embryos derived from these oocytes. Therefore, the antioxidant POEE can prevent oxidative stress in oocytes and embryos cultured in vitro during the production. Thus, POEE suggests that it can contribute to the local economy by improving assisted reproductive technology and leveraging Jeju's resources.

REFERENCE

Won-Hee Lee, Ji-Eun Park, Yong Hwangbo, Hwa-Young Kim, Ji-Eun Lee, Byeong-Buhm Kang, Hee-Tae Cheong, Boo-Keun Yang and Choon-Keun Park. (2017). Effects of reactive oxygen species and antioxidants during in vitro maturation oocytes and embryo development in pigs. Reproductive & Developmental Biology. 41(1): 17-23. doi: 10.12749/RDB.2017.41.1.17.

Mozafar Khazaei and Faranak Aghaz. (2017). Reactive oxygen species generation and use of antioxidants during in vitro maturation of oocytes. Int J Fertil Steril. 11(2): 63–70. doi: 10.22074 / ijfs.2017.4995.

Young Do Yoo. (2013). Mitochondrial reactive oxygen species production mediated by romo1 expression. hanyang medical reviews. 33(2): 90-96. doi: 10.7599/hmr.2013.33.2.90.

Hideki Igarashi, Toshifumi Takahashi and Satoru Nagase. (2015). Oocyte aging underlies female reproductive aging: biological mechanisms and therapeutic strategies. Reprod Med Biol. 14(4): 159–169. doi: 10.1007/s12522-015-0209-5.

R S Barberino, V R P Barros, V G Menezes, L P Santos, V R Araújo, M A A Queiroz and M H T Matos. (2016). Amburana cearensis leaf extract maintains survival and promotes in vitro devel opment of ovine secondary follicles. Zygote. 24(2):277-85. doi: 10.1017/S0967199415000179.

Dimitrios Stagos. (2020). Antioxidant activity of polyphenolic plant extracts. Antioxidants (Basel). 9(1): 19. doi: 10.3390/antiox9010019.

Adél Szerlauth, Szabolcs Muráth, Sándor Viski and Istvan Szilagyi. (2019). Radical scavenging activity of plant extracts from improved processing. Heliyon. 5(11):2763. doi: 10.1016/ j.heliyon. 2019. e02763.

Xiaoyu Yang, Jiude Mao, Eric M Walters, Ming-Tao Zhao, Jennifer Teson, Kiho Lee and Randall S Prather. (2012). Xenopus egg extract treatment reduced global DNA methylation of donor cells and enhanced somatic cell nuclear transfer embryo development in pigs. Biores Open Access. 1(2):79-87. doi: 10.1089/biores.2012.0214.

Rabia Naz, Hafsa Ayub, Sajid Nawaz, Zia Ul Islam, Tayyaba Yasmin, Asghari Bano, Abdul Wakeel, Saqib Zia and Thomas H Roberts. (2017). Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC Complement Altern Med. 8;17(1):302. doi: 10.1186/s12906-017-1815-z.

Abidemi J. Akindele, Zahoor A. Wani, Sadhana Sharma, Girish Mahajan, Naresh K. Satti, Olufunmilayo O. Adeyemi, Dilip M. Mondhe, and Ajit K. Saxena. (2015). In vitro and in vivo anticancer activity of root extracts of sansevieria liberica gerome and labroy (Agavaceae). Hindawi Article ID 560404(11) doi:10.1155/2015/560404.

Shaoshuai Liang, Wenxiang Wang, Lijuan Wang, Zhihao Wu, Yuxia Zou, Xungang Tan, Yan Liu, Zhuangzhuang Peng and Feng You. (2020). Figla gene roles in the proliferation of oocytes in the olive flounder paralichthys olivaceus. Aquaculture. 528;15(735493). doi: 10.1016/ j.aquaculture.2020.735493.

Ju-Won Kim, Bang Geun Oh, Julan Kim, Dong-Gyun Kim, Bo-Hye Nam, Young-Ok Kim, Jung Youn Park, JaeHun Cheong and Hee Jeong Kong. (2018). Development and characterization of a new cell line from olive flounder paralichthys olivaceus. Dev Reprod. 22(3): 225–234. doi: 10.12717/ DR.2018.22.3.225.M.

Elizabeth Ross. (2010). Gene-environment interactions, folate metabolism and the embryonic nervous system. Wiley Interdiscip Rev Syst Biol Med. 2(4): 471–480. doi: 10.1002/wsbm.72.

Atsushi Yoshino, Natalia Polouliakh, Akira Meguro, Masaki Takeuchi, Tatsukata Kawagoe and Nobuhisa Mizuki. (2016). Chum salmon egg extracts induce upregulation of collagen type I and exert antioxidative effects on human dermal fibroblast cultures. Clin Interv Aging. 11: 1159–1168. doi: 10.2147/CIA.S102092.

Rima Obeid, Wolfgang Holzgreve and Klaus Pietrzik. (2013). Is 5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects?. J Perinat Med. 1;41(5):469-83. doi: 10.1515/jpm-2012-0256.

L B Bailey and J F Gregory. (1999). Folate metabolism and requirements. J Nutr.129(4):779-82. doi: 10.1093/jn/129.4.779.

Blois M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199-1200. doi:10.1038/1811199a0.

Wijesekara R. G. S. (2018). Antioxidant activities of traditional plants in sri lanka by DPPH free radical-scavenging assay. Data Brief, 17, 870-875. doi:10.1016/j.dib.2018.02.013.

Guérin P, El Mouatassim S and Ménézo Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Human Reproduction Update. 7(2). 175–189.

Eichenlaub-Ritter U. (2012). Oocyte ageing and its cellular basis. Int J Dev Biol, 56(10-12), 841-852. doi:10.1387/ijdb.120141ue

Bomfim M. M., Andrade G. M., Del Collado M., Sangalli J. R., Fontes P. K., Nogueira M. F. G and Pereci, F. (2017). Antioxidant responses and deregulation of epigenetic writers and erasers link oxidative stress and DNA methylation in bovine blastocysts. Mol Reprod Dev, 84(12), 1296-1305. doi:10.1002/mrd.22929.

M J I Shohag, Yan-yan Wei, Ning Yu, Jie Zhang, Kai Wang, Johan Patring, Zhen-li He and Xiao-e Yang. (2011). Natural variation of folate content and composition in spinach (Spinacia oleracea) germplasm. J Agric Food Chem. 14;59(23):12520-6. doi: 10.1021/jf203442h.

Bo Min Kim, So-Min Kim, Ji Yeon Oh, Young-Sook Cho, Se-Na Kim and Youngmin Choi. (2014). Total folate contents of 15 edible plants consumed in korea using trienzyme extraction method. Journal of the Korean Society of Food Science and Nutrition. 43(11):1796-1800. doi: 10.3746/jkfn.2014.43.11.1796.

Abeydeera L. R., Wang W. H., Cantley T. C., Prather R. S and Day B. N. (1998). Presence of beta-mercaptoethanol can increase the glutathione content of pig oocytes matured in vitro and the rate of blastocyst development after in vitro fertilization. Theriogenology, 50(5), 747-756. doi:10.1016/s0093-691x(98)00180-0.

Eppig J. J. (1989). Maintenance of meiotic arrest in mouse oocytes by purines: modulation of cAMP levels and cAMP phosphodiesterase activity. Gamete Res, 23(3), 323-334. doi:10.1002/mrd.1120230309.

Gerard-Monnier D. and Chaudiere J. (1996). Metabolism and antioxidant function of glutathione. Pathol Biol (Paris), 44(1), 77-85.

Francenia Santos-Sánchez, Salas-Coronado R., Villanueva-Cañongo C. and Hernández-Carlos B. (2019). Antioxidant compounds and their antioxidant mechanism. Antioxidants. doi:10.5772/intechopen.85270.

Halliwell B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med, 91(3C), 14S-22S. doi:10.1016/0002-9343(91)90279-7.

Jeong S. G., Lee S. E., Park Y. G., Son Y. J., Shin M. Y., Kim E. Y. and Park S. P. (2017). Treatment of allicin improves maturation of immature oocytes and subsequent developmental ability of preimplantation embryos. Zygote. 25(4), 480-488. doi:10.1017/S0967199417000302.

Hussein T. S., Thompson J. G. and Gilchrist R. B. (2006). Oocyte-secreted factors enhance oocyte developmental competence. Dev Biol, 296(2), 514-521. doi:10.1016/j.ydbio.2006.06.026.

Lee S. E., Kim E. Y., Choi H. Y., Moon J. J., Park M. J., Lee J. B. and Park S. P. (2014). Rapamycin rescues the poor developmental capacity of aged porcine oocytes. Asian-Australas J Anim Sci, 27(5), 635-647. doi:10.5713/ajas.2013.13816

Mihalas B. P., De Iuliis G. N., Redgrove K. A., McLaughlin E. A. and Nixon B. (2017). The lipid peroxidation product 4-hydroxynonenal contributes to oxidative stress-mediated deterioration of the ageing oocyte. Sci Rep, 7(1), 6247. doi:10.1038/s41598-017-06372-z

Menezo Y., Dale B. and Cohen M. (2010). DNA damage and repair in human oocytes and embryos: a review. Zygote. 18(4), 357-365. doi:10.1017/S0967199410000286

Marques M. G., Nicacio A. C., de Oliveira V. P., Nascimento A. B., Caetano H. V., Mendes C. M. and Visintin J. A. (2007). In vitro maturation of pig oocytes with different media, hormone and meiosis inhibitors. Anim Reprod Sci, 97(3-4), 375-381. doi:10.1016/j.anireprosci.2006.02.013

Papaioannou V. E. and Ebert K. M. (1988). The preimplantation pig embryo: cell number and allocation to trophectoderm and inner cell mass of the blastocyst in vivo and in vitro. Development. 102(4), 793-803.

Kikuzaki H., Hisamoto M., Hirose K., Akiyama K. and Taniguchi H. (2002). Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem. 50(7), 2161-2168. doi:10.1021/jf011348w.

Chaube S. K., Prasad P. V., Thakur S. C. and Shrivastav T. G. (2005). Hydrogen peroxide modulates meiotic cell cycle and induces morphological features characteristic of apoptosis in rat oocytes cultured in vitro. Apoptosis. 10(4), 863-874. doi:10.1007/s10495-005-0367-8.

Shi S, Grothe S, Zhang Y, O'Connor-McCourt MD, Poole AR, Roughley PJ and Mort JS. (2004). Link protein has greater affinity for versican than aggrecan. Journal of Biological Chemistry. 279(13). 12060–12066.

Slimen IB, Najar T, Ghram A, Dabbebi H, Ben Mrad M and Abdrabbah M. (2014). Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. International Journal of Hyperthermia. 30(7). 513–523.

Son YJ, Lee SE, Hyun H, Shin MY, Park YG, Jeong SG, Kim EY and Park SP. (2017). Fibroblast growth factor 10 markedly improves in vitro maturation of porcine cumulus-oocyte complexes. Molecular Reproductionand Development. 84(1). 67–75.

Sun GW, Kobayashi H and Terao T. (1999). Expression of link protein during mouse follicular development. Journal of Histochemistry & Cytochemistry. 47(11). 1433–1442.

Tsang CK, Liu Y, Thomas J, Zhang Y and Zheng XF. (2014). Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance. Nature Communications. 5. 3446.

Trumbo P, Yates AA, Schlicker S and Poos M. (2001). Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Journal of the American Dietetic Association. 101(3). 294–301.

Vancompernolle K, Herreweghe FV, Pynaert G, Craen MVD, Vos KD, Totty N, Sterling A, Fiers W, Vandenabeele P and Grooten J. (1998). Atractyloside-induced release of cathepsin B, a protease with caspase-processing activity. FEBS Letters. 438(3). 150–158.

Verdoucq L, Viqnols F, Jacquot JP, Chartier Y and Meyer Y. (1999). In vivo characterization of a thioredoxin h target protein defines a new peroxiredoxin family. Journal of Biological Chemistry. 274(28). 19714–19722.

Wood Z, Schröder E, Robin Harris J and Poole LB. (2003). Structure, mechanism and regulation of peroxiredoxins. Trends Biochemical Sciences. 28(1). 32–40.

Yamanaka Y, Ralston A, Stephenson RO and Rossant J. (2006). Cell and molecular regulation of mouse blastocyst. Developmental Dynamics. 235(9). 2301–2314.

Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi KW and Oh KS. (1998). Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Human Reproduction. 13(4). 998–1002.

You J, Kim J, Lim J and Lee E. (2010). Anthocyanin stimulates in vitro development of cloned pig embryos by increasing the intracellular glutathione level and inhibiting reactive oxygen species. Theriogenology. 74(5). 777–785.

Zhang DX, Park WJ, Sun SC, Xu YN, Li YH, Cui XS and Kim NH. (2011). Regulation of maternal gene expression by MEK/MAPK and MPF signaling in porcine oocytes during in vitro meiotic maturation. Journal of Reproduction and Development. 57(1). 49–56.

Zhou P, Wu YG, Li Q, Lan GC, Wang G, Gao D and Tan JH. (2008). The interactions between cysteamine, cysteine and cumulus cells increase the intracellular glutathione level and developmental capacity of goat cumulus-denuded oocytes. Reproduction. 135(5). 605– 611.

Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H and Jin DY. (2000). Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. Biochemical and Biophysical Research Communications. 268(3). 921–927.

Zuccotti M, Merico V, Sacchi L, Bellone M, Brink TC, Bellazzi R, Stefanelli M, Redi CA, Garagna S and Adjaye J. (2008). Maternal Oct-4 is a potential key regulator of the developmental competence of mouse oocytes. BMC Developmental Biology. 8. 97.

Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H and Jin DY. (2000). Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. Biochemical and Biophysical Research Communications. 268(3). 921–927.

Khazaei M. and Aghaz F. (2017). Reactive oxygen species generation and use of antioxidants during in vitro maturation of oocytes. Int J Fertil Steril, 11(2), 63-70. doi:10.22074/ijfs.2017.4995.

Kim W. J., Lee S. E., Park Y. G., Jeong S. G., Kim E. Y. and Park S. P. (2019). Antioxidant hesperetin improves the quality of porcine oocytes during aging in vitro. Mol Reprod Dev, 86(1), 32-41. doi:10.1002/mrd.23079.

Sun QY, Lai L, Bonk A, Prather RS and Schatten H. (2001). Cytoplasmic changes in relation to nuclear maturation and early embryo deveopmental potential of porcine oocytes: effects of gonadotropins, cumulus cells, follicular size, and protein synthesis inhibition. Mol Reprod Develop 59:192-198.

Wang QL, Zhao MH, Jin YX, Kim NH and Cui XS. (2013). Gonadotropins improve porcine oocyte maturation and embryo development through regulation of maternal gene expression. J of Embry Transf 28:361-371.

Wang WH, Abeydeera LR, Prather RS and Day BN. (2000). Actin filament distribution in blocked and developing pig embryos. Zygote 8:353-358.

Balboula AZ, Yamanaka K, Sakatani M, Hegab AO, Zaabel SM and Takahashi M. (2013). Cathepsin B activity has a crucial role in the developmental competence of bovine cumulus-oocyte complexes exposed to heat shock during in vitro maturation. Reproduction. 146:407-417.

ABSTRACT IN KOREAN

난자의 시험관내 성숙 및 배아 발달은 체내에 비해 세포 내부에서 생성되는 활성산소 (ROS)에 의해 난모세포가 손상된다. 본 연구에서는 돼지 난모세포의 체외 성숙 동안 광어알 추출물 (paralichthys olivaceus egg extract; POEE)의 처리가 산화 스트레스에 노출 된 난자의 성숙과 발달에 미치는 영향을 조사하였다. POEE 의 성분을 분석한 결과, 비타민 B9(엽산, FA)의 함량이 다른 성분보다 높았으며, DPPH 의 라디칼 소거능을 이용하여 POEE 의 IC_{50} 을 계산하였다. 돼지 난자에 과산화 환경을 조성하기 위해 200μM 의 H_2O_2를 처리하였고 미성숙 돼지 난자에 0, 600, 650, 700, 750 ppm POEE 농도를 처리했다. 예비 시험으로 FA 를 0, 0.1, 0.2, 0.3, 0.4 mM (0 FA, 0.1 FA, 0.2 FA, 0.3 FA, 0.4 FA)으로 처리하였다. 돼지 배아의 난할 및 배반포 형성 속도는 0.3 FA 에서 유의하게 증가했다. 650 POEE 그룹에서는 생존율이, 700 POEE 그룹에서는 난할율이 유의적으로 증가했다. 또한, 650 POEE 그룹은 대조군에 비해 글루타티온(GSH) 수치를 유의하게 증가시켰고, 0.3 FA 그룹은 대조군에 비해 활성산소종 (ROS) 활성을 유의하게 감소시켰다. 650 POEE 그룹과 0.3 FA 그룹은 대조군에 비해 비정상적인 방추 조직 및 염색체 오정렬을 예방했으며, 또한 대조군에 비해 배반포기의 총 세포 수를 유의하게 증가시키고 세포 사멸 세포를 감소시켰다. 이러한 결과는 POEE 와 FA 가 돼지 난자의 산화 스트레스를 줄이고 품질과 발달 잠재력을 향상시킴을 보여준다.

ACKNOWLEDGEMENT

학부로 들어왔을 때부터 지금까지 5 년의 시간 동안 실험실 생활을 하면서 도움을 주신 모든 분들께 감사의 말씀을 드립니다. 특히 저희 학생들을 지켜봐주시고 생각해주시며 좋은 실험 환경을 제공하여 지원을 아끼지 않으시고 졸업할 수 있게 도와주신 박세필 교수님과 김은영 소장님, 바쁘신 와중에도 세심하게 실험과 논문 지도해주시며 부족함이 많은 저를 포기하지 않고 끝까지 이끌고 도움을 주신 이승은 박사님께 진심으로 감사의 말씀을 드립니다. 항상 저희들을 챙겨주시고 실험실 생활에 많은 도움을 주신 박민지 박사님께 감사의 말씀 드립니다. 그리고 이른 아침부터 오후까지 학생들의 실험을 위해 애써주시고 도와주신 박철희 부장님, 실험실 생활에 도움을 주시고 조언을 해주신 고리안 선생님과 손종헌 국장님께도 감사의 말씀 드립니다. 또한 석사과정 생활을 하는 동안 함께 해주신 문성호 교수님, 오창언 선생님, 김연옥 선생님, 오경돈 팀장님께도 감사의 말씀을 전합니다. 대학원 또는 학과 선·후배 사이로 실험실 생활을 함께 했던 지금은 졸업한 현혁 선배님, 신민영 선배님, 박윤귀 선배님, 정상기 선배님, 손여진 선배님, 김원재 선배님과 윤재욱, 박찬오, 김소희, 이도건, 오승환, 편다빈 연구원 및 학생들, 천정민 선생님과 대학원 후배가 될 한동훈, 임은서, 이한비 학생들 모두 같이 있느라 고생많았고 감사드립니다. 마지막으로 대학원 생활을 지원해주시고 도움을 주신 사랑하는 부모님께 감사의 말씀 전합니다.