pISSN 1225-4991, eISSN 2288-0178
J. Emb. Trans. (2016) Vol. 31, No. 3, pp. 153~159
http://dx.doi.org/10.12750/JET.2016.31.3.153

Effects of (-)-Epicatechin Gallate on porcine oocyte *in vitro* maturation and subsequent embryonic development after parthenogenetic activation and *in vitro* fertilization

Min-Su Seo[1,2], Kyoung-Ha So[1,2,†], Sang-Hwan Hyun[1,2,†]

[1]Laboratory of Veterinary Embryology and Biotechnology, Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea;
[2]Institute of Stem Cell & Regenerative Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea

ABSTRACT

(-)-Epicatechin gallate (ECG) is a polyphenol compound of green tea exhibiting biological activities, such as antioxidant and anticancer effects. To examine the effect of ECG on porcine oocytes during *in vitro* maturation (IVM), oocytes were treated with 0-, 5-, 15-, and 25 μM ECG. After maturation, we investigated nuclear maturation, intracellular glutathione (GSH) and reactive oxygen species (ROS) levels and subsequent embryonic development after parthenogenetic activation (PA) and *in vitro* fertilization (IVF).

After 42 hours of IVM, the 5 μM group exhibited significantly increased ($p< 0.05$) nuclear maturation (89.8%) compared with the control group (86.1%). However, the 25 μM group observed significantly decreased ($p< 0.05$) nuclear maturation (83.5%). In intracellular maturation assessment the 5-, 15-, and 25 μM groups had significantly increased ($p< 0.05$) GSH levels and decreased ROS levels compared with the controls. The 5- and 15 μM group showed significantly increased ($p< 0.05$) embryo formation rates and total cell number of blastocysts after PA (18% and 68.9, 15% and 85.1 vs. 12% and 59.5, respectively) compared with controls. Although the 25 μM group observed significantly lower blastocyst formation rates after PA (27.6% vs. 23.2%) than control group, the 5 μM group showed significantly increased blastocyst formation rates after PA (37.2% vs. 23.2%) compared to the control group. Furthermore, the 5 μM group measured significantly increased blastocyst formation rates (20.7% vs. 8.6%) and total cell number after IVF (88.3±1.5 vs. 58.0±3.6) compared to the control group.

The treatment of 5 μM ECG during IVM affectively improved the porcine embryonic developmental competence by regulating intracellular oxidative stress during IVM.

(Key word: (-)-epicatechin gallate, porcine oocyte, *in vitro* maturation, embryonic development)

INTRODUCTION

The *in vitro* production of embryos has been widely used in reproduction technology (Prather et al., 2003, Meurens et al., 2012). However, the potential of porcine embryos to develop *in vitro* is still inferior to that of embryos obtained *in vivo*. One of the reasons of this inferiority is thought to be improper IVM systems. Several researchers have made tremendous efforts to improve IVM conditions by the addition of compounds (Marco-Jimenez et al., 2010, Avery et al., 1999).

Green tea (Camellia sinensis) is gaining attention due to its healthful properties, including chemo-preventive efficacy (Siddiqui et al., 2011) and anti-oxidant efficacy. Chemo-preventive effects of green tea are mediated by its polyphenols. The major of catechins in green tea consist of epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epicatechin (EC). In particular, the antioxidant effects of EGCG are supported by results from suppressing the inflammatory

† Corresponding author:
Kyoung-Ha So, Ph.D.
Tel: +82-43-249-1746
E-mail: khso@cbu.ac.kr
Sang-Hwan Hyun, DVM, Ph.D.
Tel:+82-43-261-3393
E-mail: shhyun@cbu.ac.kr

processes that lead to transformation, hyperproliferation, and initiation of carcinogenesis (Thawonsuwan et al., 2010). EGCG has also demonstrated other beneficial effects in studies of diabetes, Parkinson's disease, Alzheimer's disease, stroke, and obesity (Khan et al., 2006, Higdon and Frei 2003, Shankar et al., 2008). However, despite many potential benefits of green tea and EGCG consumption, it is also important to evaluate negative health-related consequences that may arise from EGCG-induced reductions in the levels of sex steroids hormones with possible negative effects on reproductive efficiency (Kao et al., 2000, Basini et al., 2005).

Previous studies have been demonstrated the potential effects of EGCG; however, few studies have investigated the effect of ECG on livestock oocyte maturation and embryonic development. In this study, we investigated the antioxidant effect of ECG on porcine IVM and subsequent embryonic development after PA and IVF. We observed oocyte nuclear maturation, intracellular levels of GSH and ROS, and embryonic cleavage.

MATERIALS AND METHODS

1. Chemicals

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

2. Oocyte collection and *in vitro* maturation

Ovaries were collected from prepubertal gilts at a local abattoir and transported to the laboratory in physiological saline supplemented with 100 IU/L penicillin G and 100 mg/ml streptomycin sulfate maintained at 32 to 35°C within 2 h of collection. The cumulus oocyte complexes (COCs) were aspirated from 3- to 6 mm in diameter superficial follicles and washed with HEPES-buffered Tyrode's medium (TLH) containing 0.05% (wt/vol) polyvinyl alcohol (TLH-PVA). Next, only COCs having $\geq$ 3 uniform layers of compact cumulus cells and homogenous cytoplasm were selected, and 60 COCs were placed into each well of a four-well Nunc dish (Nunc, Roskilde, Denmark) containing 500 μl of culture medium (TCM199; Invitrogen Corporation, Carlsbad, CA, USA), which was supplemented with 0.6 mM of cysteine, 0.91 mM of sodium pyruvate, 10 ng/ml of epidermal growth factor, 75 μg/ml of kanamycin, 1 μg/ml of insulin, 10% (vol/vol) of porcine follicular fluid (pFF), 10 IU/ml of equine chronic gonadotropin (eCG), and 10 IU/ml of hCG (Intervet, Boxmeer, Netherland). The selected COCs were maturated with hormones from 22 to 23 h and without hormones from 18-19 h at 39°C in 5% CO_2 in 95% humidified air. The COCs during IVM were treated with or without low concentration (0-, 5-, 15- and 25 μM) of ECG according to the experimental design.

3. Evaluation of nuclear maturation

After IVM (41 to 42 h), the metaphase II (MII) stage of oocytes were analyze to nuclear maturation. The oocytes (total 968 oocytes used) were denuded by gently pipetting with 0.1% of hyaluronidase in IVM medium and washed in TLH-PVA. The denuded oocytes were fixed with 2% formaldehyde and 0.25% glutaraldehyde and later stained with 5 μg/ml Hoechst 33342 in TLH-PVA for 5 min. The stained oocytes were evaluated by fluorescence microscopy (Nikon Corp., Tokyo, Japan) and classified as germinal vesicle (GV), metaphase I (MI), anaphase-telophase I (AT-I), or MII according to meiotic maturation stage. The oocytes at MII were considered to have matured.

4. Measurement of intracellular GSH and ROS levels

The oocytes after IVM were sampled to determine intracellular GSH and ROS levels that were carried out according to methods described in detail elsewhere (You et al., 2010, Nasr-Esfahani et al., 1990). Briefly, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA; Invitrogen Corporation, Paris, France) was used to detect intracellular ROS as green fluorescence, and 4-chloromethyl-6, 8-difluoro-7-hydroxycoumarin (CellTracker Blue, CMF2HC; Invitrogen Corporation, Paris, France) was used to detect intracellular GSH levels as blue fluorescence, respectively. Ten oocytes of each treatment groups were incubated with 10 μM H_2DCFDA and 10 μM CellTracker Blue in TLH-PVA for 30 min in the dark. After incubation, oocytes were washed with Dulbecco's phosphate buffered saline (DPBS) (Invitrogen Corporation, Paris, France) containing 0.1% (wt/vol) polyvinyl alcohol (PVA), were aliquoted into 10 μl droplets, and fluorescence was evaluated under an epifluorescence microscope (TE300; Nikon, Tokyo, Japan) with UV filters (460 nm for ROS and 370 nm for GSH). The experiment was replicated three times (total of examined oocytes: GSH samples, N = 21, ROS samples, N = 26).

5. Parthenogenetic activation of oocytes

For PA, the COCs after IVM were denuded by gently pipetting with 0.1% hyaluronidase, washed three times with TLH-PVA, and then rinsed twice in activation medium consisting of 280 mM mannitol solution, 0.01 mM CaCl2, and 0.05 mM MgCl2. For activation, the matured oocytes (at MII stage) were placed between electrodes covered with activation medium in a chamber connected to an electrical pulsing machine (LF101; NepaGene, Chiba, Japan). Oocytes were activated with two direct-current (DC) pulses of 120 V/mm for 60 μsec. After electrical activation, oocytes were immediately placed into IVC medium supplemented with 5 μg/ml cytochalasin B for 6 h. The PA embryos were washed three times in fresh IVC medium, aliquoted into 30 μl IVC droplets (10 gametes per drop), covered with pre-warmed mineral oil, and then cultured at 39°C under 5% O_2, 5% CO_2, and 90% N2 humidified atmosphere for 7 days.

6. *In vitro* fertilization and culture

Liquid semen obtained from Veterinary Service Laboratory (Department of Livestock Research, Yong-in, Korea) was diluted according to Kwak *et al.* (Kwak et al., 2012). After being sperm was washed in DPBS contained with 0.1% BSA at 2000 g for 2 min each, the sperm pellet was suspended in modified Tris-buffered medium (mTBM). The denuded oocytes were co-incubated with fresh sperm of 1×10^6 sperm/ml for 20 min at 39°C in a humidified atmosphere of 5% CO_2 and 95% O_2. Loosely attached sperm cells were removed from the zona pellucid by gentle pipetting, and then the oocytes were washed and incubated in mTBM without sperm for 5-6 h at 39°C in a humidified atmosphere of 5% CO_2 and 95% O_2. Thereafter, gametes were washed three times with embryo culture medium and cultured in 30 μl droplets of porcine zygote medium 3 (PZM3) (Yoshioka et al., 2002) with pre-warmed mineral oil (10 gametes/drop). The embryos were incubated at 39°C for 168 h under a humidified atmosphere of 5% O_2, 5% CO_2, and 90% N_2. In all experiments, the culture media were replaced at Day 2 (48 h) and were treated with 3 μl of fetal bovine serum (FBS) at Day 4 (96 h) after IVF.

7. Embryo evaluation and total cell count of blastocysts

The day of PA or IVF was considered Day 0. The embryos were evaluated under a stereomicroscope for cleavage on Day 2 (48 h). Evenly cleaved embryos were classified into three groups (2-3, 4-5, and 6-8 cells). At Day 7, blastocysts were collected for counting the total cell number which was washed with 1% (wt/vol) PBS-BSA and stained with 5 μg/ml Hoechst-33342 for 5 min. Then, embryos were fixed in 4% PBS-paraformaldehyde and mounted with 100% glycerol on glass slides. Embryos were observed under a fluorescence microscope (Nickon Corp., Tokyo, Japan) at 400X magnification.

8. Statistical analysis

The statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Percentage of data (e.g., rates of maturation, cleavage, and number of nuclei) was compared by one-way ANOVA, followed by Duncan's multiple range test. All results are expressed as means ± SEM. *p-values* < 0.05 were considered to be statistically significant.

RESULTS

1. Effect of ECG on nuclear maturation during IVM

We evaluated the effect of various concentrations (0-, 5-, 15- and 25 μM) of ECG on oocyte nuclear maturation. There was no significant difference in maturation (Metaphase II stage) between the control group (86.1%) and the 15 μM ECG group (88.4%) (Table 1). However, the 5 μM ECG group (89.8%) showed a significantly increased ($p< 0.05$) number of Metaphase II stage oocytes compared with the control group. In addition, the 25 μM group (83.5%) had significantly decreased ($p< 0.05$) Metaphase II stage oocytes compared with the control group.

2. Effect of EGC on intracellular GSH and ROS levels during IVM

Intracellular GSH levels were significantly increased in the matured oocytes of the 5- and 15 μM ECG groups ($p< 0.05$) (Figure 1). Intracellular ROS levels were decreased significantly in MII oocytes derived from the groups with maturation medium supplemented with 5-, 15-, and 25 μM ECG ($p< 0.05$).

3. Effect of ECG added to IVM media on subsequent embryonic development after PA and IVF

Mature oocytes from each IVM group were subjected to PA and IVF. The cleavage patterns showed significantly more 4-5 cell PA embryos in the 5- and 15 μM ECG groups compared with the control groups, whereas there were significantly fewer

Table 1. Effect of ECG treatment during IVM on nuclear maturation

ECG concentration (μM)	Oocytes cultured for maturation,N*	Number of oocytes at the stage of							
		Germinal vesicle (%)		Metaphase I (%)		Anaphase and Telophase I (%)		Metaphase II (%)	
0 (Control)	238	6	(2.5±2.3)	18	(7.6±4.2)^{a}	9	(3.8±2.0)	205	(86.1±2.7)$^{a, b}$
5	245	3	(1.2±1.2)	14	(5.7±2.5)$^{a, b}$	8	(3.3±1.2)	220	(89.8±1.3)^{b}
15	242	5	(2.1±2.5)	18	(7.4±4.2)$^{a, b}$	5	(2.1±0)	214	(88.4±3.1)$^{a, b}$
25	243	6	(2.5±1.6)	27	(11.1±4.5)^{b}	7	(2.9±2.4)	203	(83.5±5.6)^{a}

Values with different superscript letters within the same column are significantly different (p< 0.05).
The data represent the means ± SEM.
* Three times replicated
ECG, (-)-epicatechin gallate; IVM, *in vitro* maturation

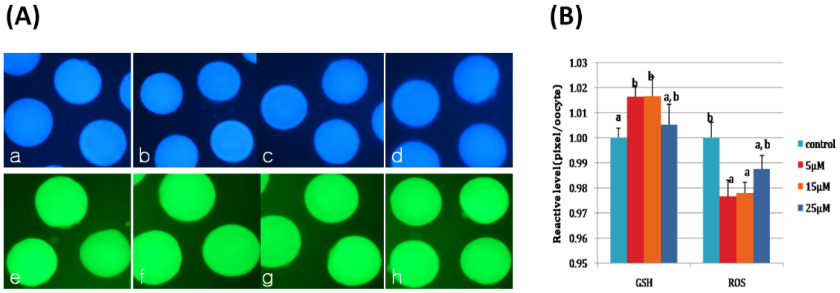

Figure 1. Epifluorescent photomicrographic images of *in vitro* matured porcine oocytes. (A) Oocytes were stained with CellTracker Blue (a – d) and H_2DCFDA (e – h) to detect intracellular levels of glutathione (GSH) and reactive oxygen species (ROS), respectively. (B) Effect of ECG in maturation medium on intracellular GSH and ROS levels in *in vitro* matured porcine oocytes. Within each group (GSH and ROS), bars with different letters (a – b) are significantly different (p< 0.05). GSH samples, N = 21; ROS samples, N = 26. Experiment was replicated three times.

Table 2. Effect of ECG treatment during IVM on embryonic development after PA.

ECG concentration (μM)	No. of embryos cultured*	No. of embryos developed into (%)				Cell number in blastocyst (N)
		≥ 2-cell embryos		Blastocysts		
0 (Control)	105	66	(61.1±2.0)	25	(23.2±3.6)$^{a, b}$	59.5±4.9 (15)^{a}
5	112	78	(67.2±1.7)	43	(37.2±4.6)^{b}	68.9±2.8 (15)$^{a, b}$
15	120	77	(70.6±3.9)	30	(27.6±7.1)$^{a, b}$	85.1±5.8 (15)^{b}
25	103	63	(60.2±6.9)	16	(15.2±3.8)^{a}	52.8±13.3 (15)^{a}

Values with different superscript letters within same column are significantly different (p< 0.05). The data represent the means ± SEM.
* Three times replicated
ECG, (-)-epicatechin gallate; IVM, *in vitro* maturation; PA, parthenogenetic activation.

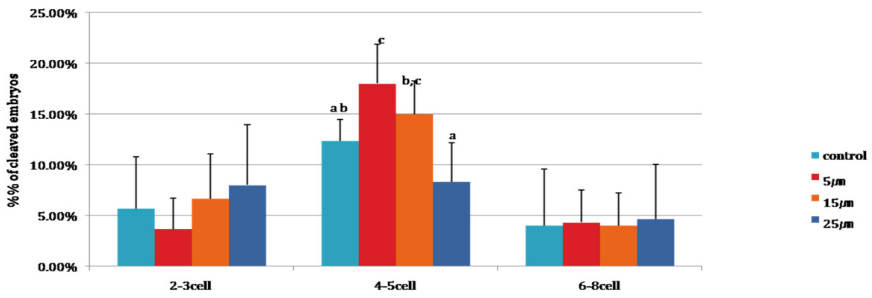

Figure 2. Effect of different concentrations of ECG treatment during IVM on the cleavage patterns of parthenogenetic activation (PA) embryos at day 2. Bars with different letters (a, b and c) are significantly different (p< 0.05) for different concentrations of ECG treatment. Experiment was repeated three times.

4-5 cell PA embryos in the 25 μM ECG group compared with the control group (Figure 2). However, no significant differences were observed in the cleavage pattern of 2-3 cell PA embryos and 6-8 cell PA embryos. The 5 μM ECG group had increased blastocyst formation rates (37.2%) compared with control groups (23.2%) (Table 2). Furthermore the 5- and 15 μM ECG groups had a significantly increased total cell number (68.92 and 85.08, respectively) than the control group (59.46) ($p< 0.05$).

As shown in Table 3, IVF embryos from the 5 μM ECG group displayed the highest ($p< 0.05$) blastocyst formation rates and total cell numbers (20.7%, and 88.3, respectively) compared with the other groups, whereas the cleavage pattern of each stage was not significant (Figure 3).

DISCUSSION

Assisted reproduction technology strategies, including *in vitro* production of embryos, has been-developed and used over many decades, and application in porcine reproduction is well-established worldwide (Hall et al., 2013). However, in particular, there are still many problems in current *in vitro* maturation systems that are inefficient compared with *in vivo* systems.

The pig has been an especially difficult species in which to obtain high rates of fertilization and subsequent blastocyst development *in vitro* (Gil et al., 2010). The main obstacle is environmental differences between *in vivo* and *in vitro* conditions, and one of the biggest problems is the accumulation of ROS (Funahashi and Day 1997, Coy and Romar 2002). Therefore, many antioxidants have been used to prevent the accumulation of ROS. EGCG and ECG are antioxidants that contain most of the material in the polyphenol family, green tea extract. In the anti-oxidation process, EGCG releases superoxide, but ECG does not release superoxide (Kondo et al., 1999). However, ECG has not been studied extensively. We demonstrated that treatment with 5-, 15-, and 25 μM ECG during IVM had detrimental effects on oocyte maturation and subsequent embryonic development of PA and IVF embryos.

Oocyte maturation includes both nuclear and cytoplasmic maturation. First, in terms of nuclear maturation, the maturation rate of the 5-μM ECG IVM group was significantly increased, suggesting that treatment with 5 μM ECG leads to

Table 3. Effect of ECG treatment during IVM on embryonic development after IVF.

ECG concentration (μM)	No. of embryos cultured*	No. of embryos developed into (%)		Cell number in blastocyst (N)
		≥ 2-cell embryos	Blastocysts	
0 (Control)	105	55 (52.6±4.0)	9 (8.6±0.3)[a]	58.0± 3.6 (6)[a]
5	112	66 (59.2±4.7)	23 (20.75±2.9)[b]	88.3± 1.5 (6)[b]
15	120	75 (62.6±4.6)	11 (9.2±0.8)[a]	53.0± 8.7 (4)[a]
25	103	55 (54.1±7.1)	6 (5.9±0.3)[a]	47.7±16.3 (6)[a]

Values with different superscript letters within the same column are significantly different ($p< 0.05$). The data represent the means ± SEM.
* Three times replicated
ECG, (-)-epicatechin gallate; IVM, *in vitro* maturation; IVF, *in vitro* fertilization.

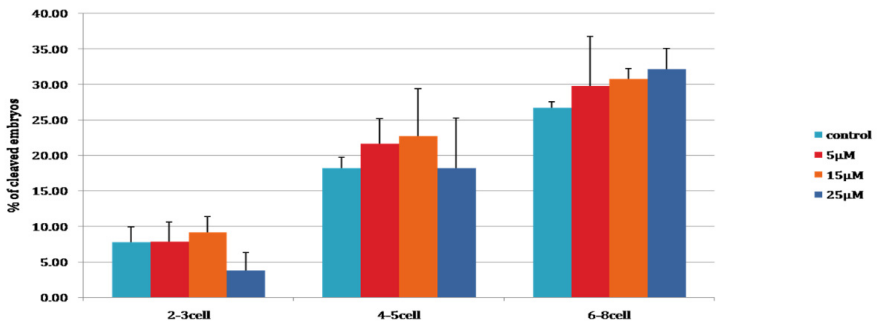

Figure 3. Effect of different concentrations of ECG treatment during IVM on the cleavage patterns of *In vitro* fertilization (IVF) embryos at day 2.

oocytes successfully progressing beyond anaphase and telophase I. We investigated intracellular GSH and ROS levels to examine cytoplasmic maturation. Intracellular levels of GSH and ROS are critical factors that influence oocyte IVM and oocyte developmental potential after parthenogenetic activation and *in vitro* fertilization (De Matos and Furnus 2000, Abeydeera et al., 1998). Intracellular GSH is a molecular marker in mature oocytes that predicts cytoplasmic maturation in porcine oocytes (Luberda 2005). Low intracellular GSH concentrations are responsible for lower developmental competence in porcine oocytes (Brad et al., 2003). Moreover, intracellular GSH plays a pivotal role protecting cells against the destructive effects of reactive oxygen intermediates and free radicals (Meister 1983). ECG (5-, 15-, and 25 μM) during IVM significantly increased intracellular GSH levels and decreased intracellular ROS levels. Here, we demonstrated that ECG treatment at levels higher than 5 μM has a beneficial effect on cytoplasmic maturation of porcine oocytes.

Embryonic development and blastocyst viability after PA and IVF were also improved by 5 μM ECG treatment, due to the beneficial effect of ECG on nuclear and cytoplasmic maturation. A previous study examined the effect of green tea polyphenols (GTP) on bovine embryonic development and showed that supplementation with 15 μM GTPs during *in vitro* maturation and *in vitro* culture improved the developmental competence of bovine oocytes (Wang et al., 2007). In comparison with bovine, our results indicate that 5 μM ECG significantly increased porcine blastocyst formation rates after PA and IVF. This difference might be due to the different species or that porcine COCs are more sensitive to ECG.

In conclusion, ECG treatment during IVM was beneficial for cytoplasmic maturation of porcine oocytes by increasing intracellular GSH levels, thereby decreasing ROS concentrations. Furthermore, porcine oocytes treated with 5 μM ECG may have increasing developmental competence, which would greatly increase blastocyst formation in PA- and IVF-derived embryos. Therefore, our results suggest that ECG improves the quality of porcine oocytes and subsequent *in vitro* development when 5 μM ECG is supplemented.

ACKNOWLEDGMENTS

This research was financially supported by the Ministry of Trade, Industry & Energy(MOTIE), Korea Institute for Advancement of Technology(KIAT) through the Leading Industry Development for Economic Region (Project No. R0004357)

REFERENCES

Prather RS, Hawley RJ, Carter DB, Lai L and Greenstein JL. 2003. Transgenic swine for biomedicine and agriculture. Theriogenology 59:115-123.

Meurens F, Summerfield A, Nauwynck H, Saif L and Gerdts V. 2012. The pig: a model for human infectious diseases. Trends Microbiol 20:50-57.

Marco-Jimenez F, Llobat L and Vicente JS. 2010. Effects of lanosterol on in vitro maturation of porcine oocytes. Anim Reprod Sci 117:288-294.

Avery B, Færge I, Grøndahl C and Ottesen J. 1999. Nuclear maturation and embryo development of bovine oocytes, matured in a semi-defined medium supplemented with meiosis activating sterol (MAS). Theriogenology 51:367.

Siddiqui IA, Asim M, Hafeez BB, Adhami VM, Tarapore RS and Mukhtar H. 2011. Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. FASEB J 25:1198-1207.

Thawonsuwan J, Kiron V, Satoh S, Panigrahi A and Verlhac V. 2010. Epigallocatechin-3-gallate (EGCG) affects the antioxidant and immune defense of the rainbow trout, Oncorhynchus mykiss. Fish Physiol Biochem 36:687-697.

Khan N, Afaq F, Saleem M, Ahmad N and Mukhtar H. 2006. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Res 66:2500-2505.

Higdon JV and Frei B. 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 43:89-143.

Shankar S, Ganapathy S, Hingorani SR and Srivastava RK. 2008. EGCG inhibits growth, invasion, angiogenesis and metastasis of pancreatic cancer. Front Biosci 13:440-452.

Kao YH, Hiipakka RA and Liao S. 2000. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 141:980-987.

Basini G, Bianco F and Grasselli F. 2005. Epigallocatechin-3-gallate from green tea negatively affects swine granulosa cell function. Domest Anim Endocrinol 28:243-256.

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 74:777-785.

Nasr-Esfahani MH, Aitken JR and Johnson MH. 1990. Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed in vitro or in vivo. Development 109:501-507.

Kwak SS, Jeung SH, Biswas D, Jeon YB and Hyun SH. 2012. Effects of porcine granulocyte-macrophage colony-stimulating factor on porcine in vitro-fertilized embryos. Theriogenology 77:1186-1197.

Yoshioka K, Suzuki C, Tanaka A, Anas IM and Iwamura S. 2002. Birth of piglets derived from porcine zygotes cultured in a chemically defined medium. Biol Reprod 66:112-119.

Hall V, Hinrichs K, Lazzari G, Betts DH and Hyttel P. 2013. Early embryonic development, assisted reproductive technologies, and pluripotent stem cell biology in domestic mammals. Vet J 197:128-142.

Gil MA, Cuello C, Parrilla I, Vazquez JM, Roca J and Martinez EA. 2010. Advances in swine in vitro embryo production technologies. Reprod Domest Anim 45 Suppl 2:40-48.

Funahashi H and Day BN. 1997. Advances in in vitro production of pig embryos. J Reprod Fertil Suppl 52:271-283.

Coy P and Romar R. 2002. In vitro production of pig embryos: a point of view. Reprod Fertil Dev 14:275-286.

Kondo K, Kurihara M, Miyata N, Suzuki T and Toyoda M. 1999. Scavenging mechanisms of (-)-epigallocatechin gallate and (-)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. Free Radic Biol Med 27:855-863.

De Matos D and Furnus C. 2000. The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development: effect of β-mercaptoethanol, cysteine and cystine. Theriogenology 53:761-771.

Abeydeera L, Wang W, Cantley T, Prather R and Day B. 1998. Presence of β-mercaptoethanol can increase the glutathione content of pig oocytes matured in vitro and the rate of blastocyst development after in vitro fertilization. Theriogenology 50:747-756.

Luberda Z. 2005. The role of glutathione in mammalian gametes. Reprod Biol 5:5-17.

Brad AM, Bormann CL, Swain JE, Durkin RE, Johnson AE, Clifford AL and Krisher RL. 2003. Glutathione and adenosine triphosphate content of in vivo and in vitro matured porcine oocytes. Mol Reprod Dev 64:492-498.

Meister A. 1983. Selective modification of glutathione metabolism. Science 220:472-477.

Wang ZG, Yu SD and Xu ZR. 2007. Effect of supplementation of green tea polyphenols on the developmental competence of bovine oocytes in vitro. Braz J Med Biol Res 40:1079-1085.

Received July 08, 2016, Revised September 01, 2016, Accepted September 07, 2016