The Effects of Resveratrol on Oocyte Maturation and Preimplantation Embryo Development

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ABSTRACT

Biotechnologies for cloning animals and *in vitro* embryo production have the potential to produce biomedical models for various researches. Especially, pigs are a suitable model for xenotransplantation, transgenic production and various areas of reproductive research due to its physiological similarities to human. However, utilization of *in vitro*-produced embryos for transfer remains limited. Despite improvement over past few decades, obstacles associated with the production of good quality embryos *in vitro* still exist which limit the efficiency of cloning. One of major problems includes improper *in vitro* maturation (IVM) and culture (IVC). Oxidative stress caused from *in vitro* culture conditions contributes to inadequate IVM and IVC which leads to poor developmental competence of oocytes, failure of fertilization and embryo development. To reduce the oxidative stress, various antioxidants have been used to IVM and IVC system. However, limited information is available on the effects of resveratrol on livestock reproductions. Resveratrol is a polyphenolic natural product and well known as an antioxidant in foods and beverages (e.g. in grapes and red wine). Resveratrol is known to be cardioprotective, anticarcinogenic, anti-inflammatory, antioxidant and antiapoptotic. This paper will review the effects of resveratrol on *in vitro* maturation of oocytes and embryo development.

(Key words : resveratrol, oocyte maturation, embryo development, antioxidants, pig)

INTRODUCTION

Biotechnologies for producing transgenic animals and *in vitro* embryo production have the potential to produce biomedical animal models for various researches. Especially, pigs can be a suitable model for xenotransplantation, transgenic production and various areas of reproductive research due to its physiological similarities to human (Abeydeera, 2002). In order for the animals to remain a suitable model for research purposes, embryos which are viable and have good quality need to be produced efficiently. Recently, the *in vitro* culture environment is well studied and becoming more defined (Nagai, 2001; Gil *et al.,* 2010); however, mammalian embryos produced *in vitro* are known to have reduced developmental competence as compared with embryos produced *in vivo* (Wang *et al.,* 1997; Wang *et al.,* 1999). Because oocytes and embryos in the *in vitro* culture system are vulnerable to oxidative stress. Therefore, many researches are aimed at reducing oxidative stress of this system through antioxidant treatment and elucidating the reproductive and cellular mechanisms associated with the oocytes maturation and embryos development.

Several criteria for example the intracellular glutathione (GSH) and reactive oxygen species (ROS) levels, the maturation promoting factor activity and cortical granule exocytosis have been used as indicators to examine the cytoplasmic maturity of matured oocytes. Among them, the intracellular levels of GSH and ROS are well known to be critical factors that influence the oocyte *in vitro* maturation (IVM) and embryo development (Abeydeera *et al.,* 1998; De Matos and Furnus, 2000; You *et al.,* 2010). The ROS such as hydrogen peroxide, superoxide anions and hydroxyl radicals, are generated during intermediate steps of oxygen reduction and when ROS is overproduced and the cell cannot adapt, oxidative stress occurs. High level of ROS can damage cell membranes (Nasr-Esfahani *et al.,* 1990), DNA (Halliwell and Aruoma, 1991), RNA transcription and protein synthesis (Takahashi *et al.,* 2000), and might play a role in apoptosis (Yang *et al.,* 1998). These detrimental effects of high ROS level in matured oocytes and embryos are supposed

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to cause detrimental effects on embryonic development and subsequently lead to early embryonic death (Guerin *et al.,* 2001; You *et al.,* 2010; Kwak *et al.,* 2012).

The intracellular GSH, a ubiquitous intracellular sulfhydryl compound, in matured oocyte is a molecular marker to predict cytoplasmic maturation in porcine oocytes (Wang *et al.,* 1997; Abeydeera *et al.,* 1998), and involved in various cellular processes including the synthesis of DNA and proteins, the metabolism of chemicals, cellular protection and amino acid transport (Meister and Anderson, 1983). Also, it has been found that intracellular GSH plays an important role in protecting the cell from oxidative damage (Meister, 1983) and regulates the intracellular redox metabolism (Luberda, 2005). Intracellular GSH has also been linked to increased thermo-tolerance in early bovine embryos (Edwards *et al.,* 2001). On the other hand, low intracellular GSH concentration is responsible for lower developmental competence of porcine oocytes (Brad *et al.,* 2003). Thus, having a high concentration of intracellular GSH and low ROS level of *in vitro*-matured oocytes and embryos may improve the oocytes and embryos viability and contribute to the development of assisted reproduction technologies in livestock species.

To improve the developmental competence of oocytes *in vitro* matured and preimplantation embryonic development cultured *in vitro*, various antioxidants such as β-mercaptoethanol (β-ME), cysteine (De Matos and Furnus, 2000), cysteamine (Kobayashi *et al.,* 2006), anthocyanin (You *et al.,* 2010) and resveratrol (Lee *et al.,* 2010; Kwak *et al.,* 2012) that have been added to porcine IVM and IVC system are summarized in Table 1 and Fig. 1. Recently, it was reported that treatment of resveratrol during IVM and IVC improves developmental potential of porcine oocytes and porcine embryo development (Lee *et al.,* 2010; Kwak *et al.,* 2012). Resveratrol (3,4',5-trihydroxystilbene) is a naturally occurring polyphenolic product (Gusman *et al.,* 2001; Pervaiz and Holme, 2009) phytoalexin, secondary plant metabolite, produced by the interaction of plants with microorganism found in the root of *Polygonum cuspidatum*, *Vitis vinifera*, red wines, mulberries, etc., and has the ability to protect plants against fungal and bacterial infections (Langcake *et al.,* 1979). Resveratrol exerts various biological activities including chemopreventive, anti-inflammatory, antioxidant, antiproliferative, proapoptotic, cardioprotective and anticancer (Gusman *et al.,* 2001; Pervaiz and Holme, 2009). More biological characteristics of resveratrol are described below.

Many studies have been performed to examine the physiological functions and the biological activities of resveratrol for therapeutic purposes in humans; however, there is limited information available in livestock species oocytes and embryos on the effects of resveratrol on oocytes maturation and embryo development. In this review, therefore, we discuss the effects of resveratrol on *in vitro* maturation of oocytes and embryo development focused in porcine model.

1. Biology of Resveratrol

Resveratrol is a polyphenolic natural product and has a stilbene structure (Fig. 1). Its biological function is to protect the plant under attack by pathogens such as fungi or stress conditions (Soleas *et al.,* 1997). It exists in foods and beverages for example in grapes and red wines and is widely consumed. The effects of resveratrol are recently a hot topic of various animal and human studies as well as in embryos. Resveratrol exerts various biological activities ranging from anticancer effects to life extension. By the activation of sirtuins, a class Ⅲ of histone deacetylase with a role in lifespan determination, resveratrol increased lifespan in yeast (*Saccharomyces cerevisiae*) (Howitz *et al.,* 2003), *Caenorhabditis elegans*, *Drosophila melanogaster* (Wood *et al.,* 2004) and even in mice (Baur *et al.,* 2006). Resveratrol is also known as SIRT1 activator (Howitz *et al.,* 2003; Baur *et al.,* 2006; Lagouge *et al.,* 2006; Bai *et al.,* 2008). However, its effects on the lifespan extension of various model organisms and its effects on SIRT1 activation remain controversial (Bass *et al.,* 2007; Beher *et al.,* 2009; Pacholec *et al.,* 2010).

"The French paradox" is known as moderate drinking of red wine reduces the risk of heart disease (Szmitko and Verma,

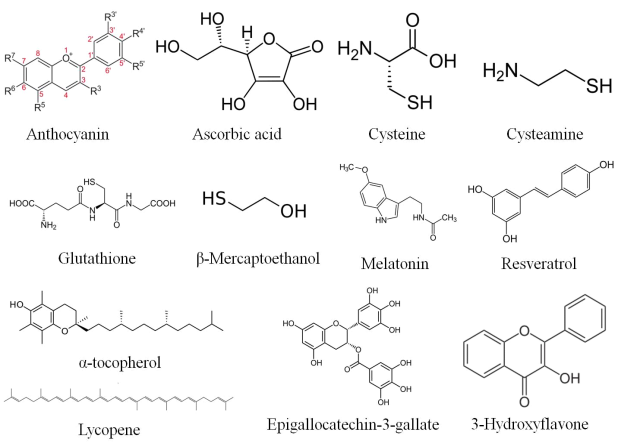

Fig. 1. Various antioxidants structures. All structure pictures from the Wikipedia.

Table 1. Various antioxidants on the porcine *in vitro* maturation (IVM) and *in vitro* culture (IVC)

Antioxidants	Biology	References	
		IVM	IVC
Anthocyanin	Flavonoids	You *et al.,* 2010	You *et al.,* 2010
Ascorbic acid (Vitamin C)	Vitamin C	Tatemoto *et al.,* 2001a Tatemoto *et al.,* 2001b Tao *et al.,* 2004 Tao *et al.,* 2010	Jeong *et al.,* 2006 Hossein *et al.,* 2007 Huang *et al.,* 2011b Hu *et al.,* 2012 Chawalit *et al.,* 2012
Cysteine	Amino acid	Abeydeera *et al.,* 1999 Jeong and Yang, 2001 Viet Linh *et al.,* 2009 Choe *et al.,* 2010 Whitaker and Knight, 2010	
Cysteamine	Chemical compound	Grupen *et al.,* 1995 Kobayashi *et al.,* 2006	
Epigallocatechin-3-gallate	Type of catechin	Spinaci *et al.,* 2008	
Glutathione	Tripeptide		Ozawa *et al.,* 2006 Wang and Day, 2002 Choe *et al.,* 2010
3-Hydroxyflavone	Flavonoid		Uhm *et al.,* 2010
Lycopene	Red carotenoid pigment	Watanabe *et al.,* 2010	
β-Mercaptoethanol	Chemical compound	Abeydeera *et al.,* 1998	Kitagawa *et al.,* 2004 Choe *et al.,* 2010 Yuh *et al.,* 2010
Melatonin	N-acetyl-5-methoxytryptamine hormone	Kang *et al.,* 2009 Shi *et al.,* 2009	Rodriguez-Osorio *et al.,* 2007 Choi *et al.,* 2008 Shi *et al.,* 2009
Resveratrol	Stilbenoid Phytoalexin	Kwak *et al.,* 2012	Lee *et al.,* 2010
Selenium	Chemical element (No. 34)	Jeong *et al.,* 2008	Uhm *et al.,* 2007
Superoxide dismutase	Enzyme	Park *et al.,* 1997	
α-Tocopherol (Vitamin E)	Vitamin E	Tao *et al.,* 2004 Miclea *et al.,* 2009 Tao *et al.,* 2010	Kitagawa *et al.,* 2004 Jeong *et al.,* 2006 Hossein *et al.,* 2007 Gajda *et al.,* 2008 Yuh *et al.,* 2010

2005). Cardioprotective effects of resveratrol are related to its abilities of inhibition of vascular smooth muscle cell proliferation (Haider *et al.,* 2005), stimulation of endolethelial nitric oxide synthase activity (Wallerath *et al.,* 2002) and inhibition of platelet aggregation (Stef *et al.,* 2006). In addition, it also exerts the anticancer effects that resveratrol applications prevented skin cancer development in mice (Jang *et al.,* 1997). There have been many studies of the anticancer activity of resveratrol in cell and animal models (Jang and Surh, 2001; Baur and Sinclair, 2006). It was reported the proapoptotic activity of resveratrol in platelets (Lin *et al.,* 2009) and smooth muscle (Zou *et al.,* 1999). To date, however, there are no results of human clinical trials for cancer (Athar *et al.,* 2007). Not only the above effects of resveratrol, but also it have va-

rious effects: Antidiabetic (Su *et al.,* 2006), antiapoptotic (Jang and Surh, 2001), neuroprotective (Karuppagounder *et al.,* 2009), anti-inflammatory (Gentilli *et al.,* 2001) and antiviral effects (Docherty *et al.,* 2006).

In reproduction field, resveratrol is known to be an estrogen receptor modulator (Gehm *et al.,* 1997; Bhat *et al.,* 2001) and an aromatase inhibitor (Wang *et al.,* 2006). So, resveratrol supplementation increased testosterone levels in mice (Shin *et al.,* 2008) and it has been reported to increase sperm output in rats (Juan *et al.,* 2005). It was reported that resveratrol, known as aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo (a) pyrene (Revel *et al.,* 2001).

2. Resveratrol in *In Vitro* Maturation and Fertilization

It was demonstrated that 2 μM resveratrol during IVM of porcine oocytes showed beneficial effects on oocyte maturation and subsequent embryonic development of porcine embryos derived by parthenogenetic activation (PA) and *in vitro* fertilization (IVF). Treatment of porcine oocytes with 2 μM resveratrol during IVM effectively reduced the intracellular level of ROS and increasing GSH concentration in mature oocytes, and subsequently reduced apoptosis-related gene expressions in matured oocytes and cumulus cells. Consequently, 2 μM resveratrol treated oocytes enhanced subsequent *in vitro* development of PA and IVF embryos and reduced apoptosis-related gene expression in blastocysts (Kwak *et al.,* 2012). From this finding, resveratrol may have a role in reducing intracellular ROS in mature oocyte, and this is consistent with previous study that resveratrol is a good scavenger of ROS (Mahal and Mukherjee, 2006).

The treatment of 10 μM resveratrol during IVM, however, significantly decreased nuclear maturation. At certain concentrations, resveratrol has been shown to exert pro-apoptotic properties in somatic cells (Kim *et al.,* 2004) and to decrease nuclear maturation *in vitro* in bovine oocytes by binding to arylhydrocarbon receptors (Pocar *et al.,* 2004). In bovine, resveratrol treatment of 20 and 40 μM during IVM significantly reduced the percentage of oocytes that reached the MⅡ stage. Therefore, high concentrations ($\geq$ 10 μM) of resveratrol might have a detrimental effect on oocyte maturation. From the recent study (Park *et al.,* 2012), it might be caused by the competitively inhibiting activity of various phosphodiesterases, which result in an increase in cytosolic concentration of cAMP, which can block the maturation.

Because of the beneficial effects on cytoplasmic maturation, embryonic development and viability of blastocyst after PA and IVF were also improved. After fertilization, GSH participates in sperm decondensation in parallel to oocyte activation, and in the transformation of the fertilizing sperm head into the male pronucleus (Yoshida *et al.,* 1992). The competence of monospermic fertilization is dependent on the degree of cytoplasmic maturation in the matured oocytes (Han *et al.,* 1999) and subsequently influences early developmental potential (Koo *et al.,* 2005). Taken together, it was shown that monospermic fertilization and developmental competence were greatly improved by 2 μM resveratrol treatment during IVM (Kwak *et al.,* 2012). These results suggest that resveratrol is useful supplement on IVM medium, leading to successful cytoplasmic maturation and subsequent *in vitro* fertilization in porcine.

There are some studies of the effects of resveratrol on cryopreservation and vitrification. In human, resveratrol prevents DNA damage induced by cryopreservation in human semen (Branco *et al.,* 2010; Garcez *et al.,* 2010). In other study, the scavenger properties of resveratrol were demonstrated *in vitr*o in human sperm, thus resveratrol could be added to the media used in assisted reproduction techniques and cryopreservation when oxidative stress is exacerbated (Collodel *et al.,* 2011). In ram, resveratrol treatment increase sperm motility and reduce acrosomal defect in conservation of ram semen, so it can improve the ram semen preservation (Sarlos *et al.,* 2002). In cat, resveratrol showed reversible and beneficial effects to cat oocyte survival during vitrification (Comizzoli *et al.,* 2009).

3. Resveratrol in Embryo Development

It was reported that 0.5 μM resveratrol improved blastocyst formation, increased total cell numbers of PA and IVF blastocysts and affected events related to apoptosis by down-regulating the *Caspase-3* mRNA expression in the developing embryos (Lee *et al.,* 2010). This result is also in agreement with our unpublished data. In this study, high concentration (25 μM) of resveratrol in the culture medium had toxic side effects and was detrimental to the developing embryos. Similarly, in cell culture studies, resveratrol had a dose-dependent effect on the proliferation of cultured cells. At high concentrations of resveratrol induced apoptosis and decreased mitotic activity (Clement *et al.,* 1998) whereas at low concentrations it prompted cell division in various human cell lines (Szende *et al.,* 2000).

In previous studies, resveratrol protected against hazardous effects of 2-bromopropane on maturation of mouse oocytes, fertilization and fetal development (Huang and Chan, 2012). In addition, resveratrol protected against methylglyoxal-induced apoptosis and disruption of embryonic development in mouse blastocysts (Huang *et al.,* 2011a). It also reported that protective effects of resveratrol on ethanol-induced apoptosis in embryonic stem cells and disruption of embryonic development in mouse blastocysts (Huang *et al.,* 2007).

To date, the molecular mechanisms by which resveratrol exerts its antioxidative and antiapoptotic effects on oocyte maturation and embryonic development remains elusive. Intracellular GSH is one of the major antioxidants and plays key roles in maintaining redox homeostasis, scavenging peroxides and detoxifying xenobiotics (Meister, 1992; Hayes *et al.,* 2005). Recent studies have shown that resveratrol could increase GSH levels (Sharma and Gupta, 2002; Savaskan *et al.,* 2003; Yen *et al.,* 2003; Li *et al.,* 2006), suggesting that modulating the GSH homeostasis by resveratrol may play a significant role in the beneficial effects in oocyte maturation and embryo development.

CONCLUSION

Resveratrol supplementation in the *in vitro* maturation and culture media also in the semen cryopreservation is advantageous for oocyte cytoplasmic maturation, embryo development and semen cryopreservation by reducing the intracellular ROS level, increasing GSH concentration, and down-regulation of apoptosis-related genes. Furthermore, resveratrol treatment of porcine oocytes could have better developmental competence, so greatly improve monospermic fertilization, blastocyst formation, and blastomere viability in PA and IVF-derived embryos. These findings will be useful for improving the early development of livestock embryos by reducing oxidative stress. Therefore, resveratrol treatment during IVM and IVC has the potential to increase the efficiency of cloned animal production and establishment of embryonic stem cells.

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