—Original Article—

Effect of Resveratrol on the Development of Porcine Embryos Produced *In Vitro*

Kiho LEE¹⁾, Chunmin WANG¹⁾, John M CHAILLE¹⁾ and Zoltan MACHATY¹⁾

¹⁾Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA

Abstract. The effects of resveratrol (a phytoalexin with a wide variety of pharmacological activities) on pig embryos produced by parthenogenesis and/or *in vitro* fertilization have been investigated. First, parthenogenetic embryos were generated and cultured in PZM-3 medium supplemented with various amounts of resveratrol (0, 0.05, 0.1, 0.5, 1.0 and 25 μ M final concentrations). In the presence of 0.5 μ M resveratrol a significantly higher percentage of parthenogenetic embryos reached the blastocyst stage by day 7 compared to non-treated control ($43.5 \pm 6.3\%$ vs. $33.0 \pm 5.4\%$; $P < 0.05$). The total cell number of blastocysts also increased as a result of incubation with 0.5 μ M resveratrol; the difference was statistically significant between treated and non-treated embryos on day 5 of culture (35.8 ± 0.9 vs. 32.1 ± 1.1 ; $P < 0.05$). Resveratrol incubation affected the expression levels of apoptosis-related genes in parthenogenetic blastocysts: the level of Bax transcripts was similar but lower expression of Bcl-2 and Caspase-3 was observed in embryos treated with 0.5 μ M resveratrol when compared to control blastocysts ($P < 0.05$). The results of the TUNEL assay were similar in blastocysts developing with or without resveratrol supplementation. In addition, when embryos produced by *in vitro* fertilization were incubated with 0.5 μ M resveratrol, the treatment led to higher frequencies of blastocyst formation (8.6% vs. 13.3%) and elevated total cell numbers (37.1 ± 2.4 vs. 43.2 ± 1.7) by the end of the 7-day culture period ($P < 0.05$). The results indicate that 0.5 μ M resveratrol during culture has a positive effect on early embryonic development of porcine embryos.

Key words: Development, Embryo, Pig, Resveratrol

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Embryo culture is valuable in the study of preimplantation embryonic development and is indispensable for the *in vitro* production of transferable embryos. In spite of significant improvements in the culture conditions, the development of *in vitro*-produced embryos is still suboptimal [1]. Because the preimplantation embryo is extremely sensitive to environmental factors, deficiencies in culture conditions often lead to aberrant embryo development that manifest in lower frequency of blastocyst formation and lower cell numbers and can affect fetal as well as postnatal life [2]. Modifications in the culture system can potentially improve the development of the cultured embryos.

Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin identified in more than 70 plant species including grapes, plums, and peanuts [3]. Its biological function is to protect the plant in case of a parasitic attack or environmental stress [4]. Originally, the search for plant-derived compounds with pharmacological properties identified resveratrol as a blocker of enzymes involved in arachidonate metabolism [5] and later, an inhibitor of partially purified kinases [6]. Based on its ability to block the cyclooxygenase COX-1 resveratrol was suggested to have anticancer properties and it was demonstrated that topical application of resveratrol decreased tumorigenesis in a mouse skin cancer model [7].

Resveratrol was later shown to exert a wide variety of pharmacological activities ranging from anti-inflammatory effects to

immunomodulation and cardioprotection [for recent reviews see 8, 9]. It is able to influence the expression of a wide range of genes that are related to DNA synthesis, cell cycle, proliferation, stress responses, and apoptosis. In addition, via the activation of sirtuin proteins, a class of histone deacetylases with a role in lifespan determination, resveratrol was shown to increase lifespan in yeast [10] as well as *C. elegans*, *Drosophila* [11] and mice [12]. These various effects of resveratrol are explained by the fact that resveratrol targets many components of intracellular signaling pathways by modulating a great number of enzymes including kinases, lipooxygenases, sirtuins, and transcription factors [8].

When cultured cells were exposed to resveratrol diverse results were obtained depending on the concentration it was applied. In human tumor cells resveratrol at high concentrations (10.0–100.0 μ g/ml) induced apoptosis and decreased mitotic activity [13] while low doses of resveratrol (0.1–1.0 μ g/ml) enhanced cell proliferation [14]. Similarly, in osteoblastic MC3T3-E1 cells resveratrol dose-dependently stimulated proliferation and differentiation [15]. Because of its ability to positively influence a number of cellular processes in cultured cells we hypothesized that resveratrol may also be beneficial for the development of preimplantation embryos under *in vitro* conditions. The aim of this study was to investigate the effect of resveratrol on the early development of parthenogenetic and IVF embryos using the pig as a model.

Materials and Methods

Unless stated otherwise, all chemicals were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA).

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Correspondence: Z Machaty (e-mail: zmachaty@purdue.edu)

Table 1. Primer sequences used for real-time RT-PCR

Gene	Primer sequences (5'–3')	Product size (bp)	Annealing temperature (C)	GenBank accession number
<i>Bax</i>	F: AAGCGCATTGGAGATGAACT R: CGATCTCGAAGGAAGTCCAG	251	60	AJ606301
<i>Bcl-2</i>	F: ACTGAATGCCCTCCGGTACC R: ATCCCATGGCTGCAGTGAA	80	60	NM214285
<i>Caspase-3</i>	F: ACACGCCATGTCATCTTCAGTCC R: TTCATAATTCAGGCCTGCCGAG	81	60	NM214131
<i>GAPDH</i>	F: ACATCAAGAAGGTGGTGAAG R: ATTGTCGTACCAGGAAATGAG	151	52	AF017079

Oocytes

Ovaries of prepubertal Large White gilts were collected at an abattoir and transported to the laboratory in physiological saline maintained at ~30 C. The follicular fluid was aspirated from medium size (3 to 6-mm) follicles using a syringe attached to a 20-gauge hypodermic needle and collected in a 50-ml centrifuge tube. Oocytes with intact cumulus investment and evenly dark cytoplasm were selected and washed in Hepes-buffered Tyrode's lactate (TL-Hepes) medium. They were then matured in TCM-199 medium (50 oocytes per 500 μ l of medium) supplemented with 0.1 mg/ml cysteine, 0.5 IU/ml LH, 0.5 IU/ml FSH and 10 ng/ml epidermal growth factor at 39 C in 5% CO₂ in air. After 42 h, the oocytes were collected and the surrounding cumulus cells were removed by vortexing in 0.1% hyaluronidase.

Oocyte activation

In order to generate embryos for the studies porcine oocytes were parthenogenetically activated by electroporation. For this purpose mature oocytes were transferred into electroporation medium (300 mM mannitol, 0.1 mM CaCl₂, 0.1 mM MgSO₄, 0.5 mM Hepes, 0.01 mg/ml BSA) between two stainless steel electrodes of the electroporation chamber. The oocytes were then activated by applying two direct current electric pulses (120 V/mm, 60 μ sec each, 1 sec apart) followed by an incubation with 10 μ g/ml cycloheximide and 7.5 μ g/ml cytochalasin B, for 5 h. Activated oocytes (in groups of 10) were then placed in 20 μ l drops of Porcine Zygote Medium 3 (PZM-3) containing various concentrations of resveratrol (0, 0.1, 0.5, 1.0, 25 μ M; Sigma-Aldrich catalog # R5010) covered with mineral oil. Resveratrol was dissolved in dimethyl sulfoxide (DMSO) and kept frozen as a stock (84 mM) until it was added to the medium. The dishes were incubated in a humidified incubator at 39 C under 5% CO₂ in air.

In vitro fertilization

Mature oocytes were rinsed in a modified Tris-buffered medium consisting of 113.1 mM NaCl, 3 mM KCl, 7.5 mM CaCl₂ $\times$ 2H₂O, 20 mM Tris (crystallized free base; Fisher Scientific, Fair Lawn, NJ, U.S.A.), 11 mM glucose, 5 mM sodium pyruvate, 0.1% BSA, and 1 mM caffeine [16]. Groups of 30 to 35 oocytes were placed into 50 μ l droplets of the same medium covered with warm mineral oil. Fresh semen was collected from a Large White boar, diluted in Modena extender and stored at 17 C for up to three days. Prior to

use, the extended semen was washed three times by centrifugation at 1,900 g for 4 min in Dulbecco's phosphate buffered saline (DPBS; without calcium and magnesium) containing 0.1% BSA, 75 μ g/ml potassium penicillin G, and 50 μ g/ml streptomycin sulfate (pH 7.2, 39 C). The final sperm pellet was diluted with the modified Tris-buffered medium described above. The sperm suspension at a final concentration of 5×10^5 cells/ml was added to each 50 μ l droplet containing the oocytes. The gametes were co-incubated for 5 h at 39 C in an atmosphere of 5% CO₂ in air; potential zygotes were then transferred to PZM-3 medium for culture.

Quantitative real-time PCR

Parthenogenetic blastocysts were collected on day 7 from the control as well as the 0.5 μ M resveratrol-treated groups (40 blastocysts from each group generated by a single round of oocyte activation). They were rinsed three times in PBS containing 0.1 mg/ml polyvinyl alcohol (PVA) and 0.1 mg/ml diethylpyrocarbonate (DEPC) and then placed into 100 μ l lysis/binding buffer consisting of 100 mM Tris-HCl, 500 mM LiCl, 10 mM EDTA, 1% LiDS and 5 mM dithiothreitol (DTT). Messenger RNA was then extracted from the lysed embryos by the Dynabeads mRNA direct kit (Invitrogen Corporation; Carlsbad, CA, USA) according to the manufacturer's protocol. Magnetic beads with mRNA attached were re-suspended in 20 μ l of reverse transcription (RT) reaction mix consisting of 4 μ l of 5X iScript Reaction Mix, 1 μ l of iScript Reverse Transcriptase and 15 μ l of nuclease free water (iScriptTM cDNA Synthesis Kit, Bio-Rad; Hercules, CA, USA). Reverse transcription reactions were carried out in the presence of the Dynabeads under conditions of 25 C for 5 min and 42 C for 30 min followed by 85 C for 5 min. The cDNA was then used for real-time PCR. Primers for real-time PCR were designed (in the case of *Bcl-2* and *Caspase-3*) or obtained from previous reports (*Bax*, [17]); the *GAPDH* primers were received as a generous gift from Dr. Rebecca Krisher of University of Illinois at Urbana-Champaign. The primer sequences are shown in Table 1. Each reaction mixture consisted of 2.5 μ l of cDNA, 5 μ l each of forward (1 μ M) and reverse (1 μ M) primers, and 12.5 μ l SybrGreen PCR Master Mix (Bio-Rad) in a total reaction volume of 25 μ l in 96-well plates. PCR reactions were performed in three replicates; for each replicate cDNA from a single pool of embryos was sub-divided to amplify all genes during the same PCR assay. As a negative control, an RT reaction in which the reverse transcriptase enzyme was

replaced with nuclease-free water was used as a template. PCR was performed on a MyiQ single color real-time thermal cycler (Bio-Rad). The program used for amplification included an initial temperature of 94 C for 5 min followed by 40 cycles of 5 sec at 94 C, 30 sec at 60 C (52 C for GAPDH), and 30 sec at 72 C. Real time fluorescence data were collected during the extension time. A melting curve was produced to verify the identity of each PCR product; successful amplification was further confirmed by separating the amplicons by agarose gel electrophoresis. In the first replicate transcripts were also cloned and sequenced to confirm product identity.

Transcript levels of Bax, Bcl-2, and Caspase-3 were quantified using the relative quantification method based on comparative threshold cycles (C_t) values. The transcript abundance of each gene was determined relative to that of the internal control gene GAPDH. The C_t value, i.e. the cycle number during the log-linear phase when the amount of amplified product rose above background level was determined for each gene and subtracted from that of the GAPDH gene to obtain ΔC_t . Control group C_t values served as calibrators and were used subsequently to obtain $\Delta\Delta C_t$ values. Fold differences in transcript abundance were calculated for Bax, Bcl-2, and Caspase-3 assuming an amplification efficiency of 100% and using the equation $2^{-\Delta\Delta C_t}$. To verify that amplification efficiencies were similar for each amplicon a validation test was run prior to the experiment.

TUNEL assay

At the end of the 7-day culture period, parthenogenetic blastocysts were fixed in 4% paraformaldehyde overnight at 4 C and permeabilized in 0.1% Triton X-100 in 0.1% sodium citrate for 2 min. The activations were performed in three replications to produce the blastocysts for the assay. The embryos were then incubated for 1 h at 39 C in a TUNEL reaction medium (In Situ Cell Death Detection Kit, Roche Applied Science; Indianapolis, IN, USA) that specifically labels the exposed 3'-OH ends of single-

strand DNA breaks (a process associated with late stage apoptosis and necrosis) with the fluorochrome FITC. After rinsing in PBS, nuclei of the labeled embryos were stained with 5 μ g/ml Hoechst 33342 for 10 min. The embryos were then mounted on microscope slides and examined under UV light using an epifluorescence microscope. Nuclei showing green fluorescence were counted as apoptotic/necrotic; the total number of nuclei per blastocysts was determined based on Hoechst 33342 staining. Two-cell embryos collected after 1 day of culture were stained as negative controls; for positive control blastocysts treated with DNase I were used.

Statistical analysis

The frequencies of blastocyst formation in the treatment groups were compared by the Chi-square test. Total cell numbers and results of the TUNEL assay were compared by Student's *t*-test. Differences in gene expression were analyzed by ANOVA; differences at $P < 0.05$ were considered significant.

Results

In preliminary experiments we determined the most effective dose of resveratrol. These experiments (a total of 8 replications) demonstrated that resveratrol had a distinct dose-dependent effect on the development of parthenogenetic porcine embryos (Table 2). Blastocyst formation in the control group was 33.0% while in the presence of 25 μ M resveratrol only 4.5% of the embryos formed blastocysts. Incubation with 0.5 μ M resveratrol had no effect and blastocyst development in the presence of 0.1 μ M resveratrol tended to be higher compared to the control group. Based on these data we then selected the concentration of 0.5 μ M as our resveratrol treatment and investigated its effect on embryos compared to those that developed in the absence of resveratrol. When parthenogenetic embryos were cultured in the presence of 0.5 μ M resveratrol, embryonic development improved significantly compared to the control group (four replications; Table 3). As a result of resveratrol

Table 2. Effect of resveratrol on parthenogenetic blastocyst formation by the end of the 7 day culture period

Resveratrol concentration	No. of parthenotes	No. of blastocysts (% $\pm$ S.E.M.)
0 μ M (control)	312	103 (33.0 $\pm$ 5.4)
0.05 μ M	252	83 (32.9 $\pm$ 6.7)
0.1 μ M	203	81 (39.9 $\pm$ 3.8)
0.5 μ M	301	131 (43.5 $\pm$ 6.3)
1.0 μ M	195	61 (31.3 $\pm$ 4.9)
25 μ M	89	4 (4.5 $\pm$ 1.2)

Table 3. Cleavage, blastocyst formation and total cell number (mean $\pm$ S.E.M.) in embryos developed with or without resveratrol supplementation

Group	Cleaved embryos (% $\pm$ S.E.M.)	Day 5 Blastocysts*		Day 7 Blastocysts	
		Formed (% $\pm$ S.E.M.)	Total cell number	Formed (% $\pm$ S.E.M.)	Total cell number
Control	121/181 (66.8 $\pm$ 5.2) ^A	83/306 (27.1 $\pm$ 9.2) ^A	32.1 $\pm$ 1.1 ^A (n=73)	55/181 (30.4 $\pm$ 0.7) ^A	41.5 $\pm$ 1.6 ^A (n=32)
0.5 μ M Res.	139/171 (81.3 $\pm$ 2.7) ^B	102/294 (34.7 $\pm$ 9.8) ^B	35.8 $\pm$ 0.9 ^B (n=85)	77/171 (45.0 $\pm$ 2.7) ^B	45.4 $\pm$ 1.4 ^A (n=40)

^{A, B} Different superscripts indicate significant differences ($P < 0.05$). *For the analysis, Day 5 Blastocysts were collected from separate experiments

Fig. 1. Expression of apoptosis-related genes in parthenogenetic blastocysts developed in the presence of resveratrol. Different superscript letters indicate statistically significant differences between treatment groups ($P < 0.05$).

supplementation, the percentage of cleaved embryos increased from $66.8 \pm 5.2\%$ to 81.3 ± 2.7 and blastocyst formation at the end of the 7-day culture period improved from 30.4 ± 0.7 to 45.0 ± 2.7 ($P < 0.05$). The average cell numbers of the developing parthenogenetic blastocysts are also shown in Table 3. Control and 0.5μ M resveratrol-treated day 7 blastocysts had a total of 42.4 ± 2.1 and 44.0 ± 1.9 cells, respectively; the difference was not statistically significant. However, when in separate experiments development was evaluated after only 5 days of culture, the average cell number in the resveratrol-treated blastocysts was significantly higher than that in the control group (35.8 ± 1.0 vs. 32.1 ± 1.1 ; $P < 0.05$).

Results from real-time RT-PCR indicated significant changes in the expression levels of apoptosis-related genes as a result of incubation with resveratrol; the data are shown in Fig. 1. Transcript levels of the Bcl-2 and Caspase-3 genes were lower in parthenogenetic embryos cultured with 0.5μ M resveratrol compared to control embryos ($P < 0.05$). The expression level of the Bax gene

was not different between control and resveratrol-treated (0.5μ M) embryos. The results of the TUNEL assay demonstrated that there was no difference in DNA fragmentation after resveratrol treatment (Table 4); the average number of nuclei showing DNA fragmentation was similar in the control and resveratrol-treated groups.

Embryos produced by *in vitro* fertilization also showed better development when cultured in the presence of 0.5μ M resveratrol (Table 5). This experiment was performed in seven replications. In the control group the frequency of blastocyst formation was $8.6 \pm 2.1\%$ whereas a significantly higher percentage of resveratrol-treated embryos formed blastocysts by the end of the 7-day culture period (13.3 ± 1.1). In addition, resveratrol treatment also affected cleavage frequency. A higher percentage of 2-cell embryos were registered in the resveratrol-treated group as compared to the non-treated control (56.5 ± 5.9 vs. 67.1 ± 3.3). As shown in Table 5, by day 7 following fertilization the average total cell number was also higher in IVF blastocysts that developed in the presence of resveratrol than that in the control embryos (43.2 ± 1.7 vs. 37.1 ± 2.4 ; $P < 0.05$).

Discussion

This study demonstrated that resveratrol had a marked effect on the *in vitro* development of preimplantation pig embryos. Supplementation of the culture medium with 0.5μ M resveratrol enhanced blastocyst formation, increased total cell numbers of parthenogenetic and IVF blastocysts and affected events related to programmed cell death in the developing embryos.

No previous reports are available regarding the influence of resveratrol on preimplantation embryo development. In cell culture studies, resveratrol had a dose-dependent effect on the proliferation of cultured cells: at high concentrations resveratrol exerted an anti-proliferation effect and induced apoptosis whereas at low concentrations it promoted cell division in various human cell lines [14, 18]. In this study a similar effect of resveratrol on parthenogenetic pig embryos was observed. High levels (25μ M) of resveratrol in the culture medium had toxic side effects and were detrimental to the developing parthenogenetic embryos. However,

Table 4. DNA fragmentation in day 7 parthenogenetic embryos as determined by the TUNEL assay

Resveratrol concentration	No. of blastocysts	Percentage of apoptotic nuclei (mean $\pm$ S.E.M.)
0 μ M (control)	32	$12.6 \pm 1.6\%$
0.5 μ M	40	$11.5 \pm 1.1\%$

Table 5. Effect of resveratrol on the development of embryos produced by *in vitro* fertilization

Resveratrol concentration	No. of embryos	No. of embryos cleaved (% $\pm$ S.E.M.)	No. of blastocysts (% $\pm$ S.E.M.)	No. of cells in blastocysts (mean $\pm$ S.E.M.)
0 μ M (control)	370	209 (56.5 ± 5.9) ^A	32 (8.6 ± 2.1) ^A	37.1 ± 2.4 ^A
0.5 μ M	368	247 (67.1 ± 3.3) ^B	49 (13.3 ± 1.1) ^B	43.2 ± 1.7 ^B

^{A, B} Different superscripts indicate significant differences ($P < 0.05$). Blastocyst development was evaluated after 7 days in culture.

lower concentrations tended to be beneficial and a concentration of 0.5 μM increased blastocyst formation and supported cell proliferation in the parthenotes. These results are consistent with the pro-proliferation characteristic of resveratrol reported in the above-mentioned studies [14, 18]. Moreover, reduced blastocyst formation in the presence of elevated concentrations of resveratrol seems to support the notion that resveratrol at high levels induces apoptosis in cells [19], although this was not directly evaluated in the present study.

Apoptosis in preimplantation embryos can be induced by suboptimal culture conditions *in vitro*, and its incidence in the developing embryos can be correlated to embryo quality [20–22]. Apoptotic activity has recently been demonstrated in parthenogenetic porcine preimplantation embryos [23, 24]. Although no reports are available regarding the effects of resveratrol on embryos, the results of studies that focused on the physiological action of resveratrol in cultured cells implied a role for resveratrol in affecting early embryo development. According to published data the action of resveratrol in cell cultures depended on the cell types and the concentration of resveratrol used. In various cancer cell lines resveratrol activated p53 by phosphorylation which in turn suppressed proliferation of the cells [25–27]. Moreover, elevated transcript levels of genes related to apoptosis such as Bak, Bcl-2, and p21 were reported in those studies. On the other hand, in rheumatoid fibroblast-like synoviocytes resveratrol induced programmed cell death but the levels of Bax and Bcl-2 were not changed. Instead, resveratrol triggered the mitochondria-related apoptotic pathway by activating Bid cleavage via Caspase-8 [28]. Despite such inconsistencies most studies support the idea that above a certain threshold level resveratrol induces apoptosis and stimulates apoptosis-related genes characterizing resveratrol as an anti-cancer reagent [19, 29, 30].

In the present study, supplementation of the culture medium with low (0.5 μM) concentrations of resveratrol resulted in decreased levels of Bcl-2 transcripts in the parthenogenetic blastocysts suggesting that resveratrol suppresses the expression of pro-survival genes of the intrinsic apoptosis pathway in embryos. However, the expression level of Bax, a pro-apoptosis gene in the intrinsic pathway was not altered. Thus, it seems that 0.5 μM resveratrol does not stimulate programmed cell death in pig embryos. In addition to its role in regulating apoptosis, Bcl-2 is also known as an antiproliferative gene that regulates the cell cycle by driving cells into the G0 phase and delaying the G0 to S phase transition [31]. Lower expression of Bcl-2 in resveratrol-treated blastocysts may reflect faster G0 to S phase transition leading to a higher proliferation rate as indicated by higher total cell numbers in these embryos. Caspase-3 expression was also downregulated in blastocysts that developed in the presence of resveratrol suggesting a decreased apoptotic activity in the embryos. Caspase-3 is an effector caspase located downstream of Bcl-2 in the apoptosis pathway. Based on these results low levels of resveratrol seem to suppress apoptotic events in preimplantation embryos at least at the transcript level. A similar anti-apoptosis effect of resveratrol, i.e. the suppression of caspase activity was reported in human keratinocyte cell lines. Treatment with resveratrol could protect the cells from cell death induced by ultraviolet B irradiation by attenuating the

caspase pathway [32].

Results from the TUNEL assay indicate that resveratrol does not induce DNA fragmentation in embryos. Fragmentation of DNA is associated with late stage apoptosis. Although the TUNEL assay also labels the fragmented DNA of necrotic cells it is generally accepted to be a good indicator of late-stage apoptosis [22]. Based on our results we conclude that 0.5 μM resveratrol in the culture system has a pro-proliferative (Bcl-2 downregulation) and anti-apoptotic (Caspase-3 downregulation) effect in parthenogenetic porcine embryos. This is consistent with results obtained during a study in which stress response of embryos after resveratrol treatment was investigated. When apoptosis was induced by ethanol in mouse blastocysts, resveratrol had a protective effect on the embryos [33]. In addition, resveratrol-treated blastocysts were resistant to ethanol-induced apoptosis and showed lower expression of Caspase-3 and Caspase-9 when compared to non-treated embryos.

Enhanced development of IVF embryos in the presence of 0.5 μM resveratrol implies that the effect of resveratrol is not limited to parthenogenetic embryos only. The frequency of cleavage, blastocyst formation and the total cell number was also higher in resveratrol-treated IVF embryos suggesting that resveratrol positively affected embryonic development after fertilization. The percentage of embryos that reached the blastocyst stage after *in vitro* fertilization was relatively low; seasonal and/or boar effects may be accountable for this limited efficiency (typically the frequency of blastocyst formation following *in vitro* fertilization is around 15% in our laboratory [our unpublished results]). Nevertheless, the data obtained from IVF embryos supports our notion that resveratrol is beneficial for embryonic development *in vitro*.

The molecular mechanism by which resveratrol exerts its effect on preimplantation embryos is not clear. One potential mechanism of action for resveratrol can be speculated from its structure. Resveratrol shows structural similarity to diethylstilbestrol (DES; 4, 4'-dihydroxy-trans- α , β -diethylstilbene), a synthetic estrogen that was shown to bind to human estrogen receptors in binding assays [34]. The same study also demonstrated that resveratrol induced proliferation of various human cell lines by stimulating the estrogen receptor in a dose dependent manner. The estrogen receptor has long been implicated in the regulation of cellular development [35] and its expression was shown in preimplantation pig embryos [36]. In addition, in another report low concentrations (5–10 μM) of resveratrol induced S-phase entry and this pro-proliferation effect was limited to cells possessing androgen receptors [18]. These results suggest a relationship between resveratrol and steroids and seem to support a hypothesis whereby low concentrations of resveratrol may act as an agonist for specific steroid hormone receptors during preimplantation embryo development *in vitro*.

In conclusion, treatment of pig embryos with 0.5 μM resveratrol has a beneficial effect on preimplantation development leading to enhanced blastocyst formation and improved embryo quality in terms of total cell number in the blastocysts. The treatment also resulted in decreased expression of the Bcl-2 and Caspase-3 genes; however, understanding the mechanisms involved needs further investigations.

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