

# Quercetin Efficacy on in vitro Maturation of Porcine Oocytes

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## Abstract

The present study proposed to examine the effects of a polyphenol (quercetin) on in vitro matured parameters. Quercetin it has been extensively studied by researchers on animals over the 35 years. It is a plant derived flavonoid from fruits and vegetables that has antioxidant action as a free radical scavenger.

Immature porcine oocytes were untreated and treated with 5, 15, 25, 35 µg/ml quercetin during in vitro maturation. After then the mature oocytes were fertilized.

It was observed that cumulus cell expansion of COCs cultured in maturation media supplemented with 5 µg/ml quercetin in grad 3 could be very significantly increased ( $p < 0.001$ ). In grad 4 could be significantly between different levels of quercetin (5 vs. 25, 5 vs. 35,  $p < 0.001$ ).

The rates of embryos cultured in medium supplemented with different levels of quercetin did not presented significantly statistically different. The presence of 25 µg/ml quercetin in the maturation medium increased the percentage of embryos in the morula stage compared with the control. In the morula stage all the concentrations of quercetin resulted percentages increased to control. This results shows that quercetin added during in vitro maturation has a positive effect on future embryos development.

**Keywords:** in vitro maturation, oocytes, porcine, quercetin

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## 1. Introduction

Pigs are the most useful animal models for studying organs because of their physiological similarities to humans.

One of the factors that impair in vitro produced porcine embryos is the oxidative stress that is mainly caused by the imbalance reactive oxygen species generation and antioxidants activity. Oocyte quality is critically important for optimal in vitro oocyte maturation, in vitro fertilization and in vitro embryo development.

The present study was conducted to examine the effects of a polyphenol (quercetin) on in vitro matured parameters. Quercetin (3,3',4',5,7 –

pentahydroxyflavone) is a plant derived flavonoid from fruits and vegetables that has antioxidant action as a free radical scavenger [1-3]. Quercetin it has been extensively studied by researchers over the 35 years on hamster embryo cells [4], Chinese hamster V79 cells [5], human sperm [6], human HepG2 hepatocytes [7], porcine oocytes [8].

Antioxidant properties have been attributed to the ability of scavenging reactive oxygen species [9]. The relation between the chemical nature of flavonoids and their antioxidant activity has been established, in particular the number and the position of hydroxyl groups [10].

## 2. Materials and methods

All the chemicals used in this study were purchased from Sigma-Aldrich (USA). To identify the optimal quercetin concentration for improving

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oocyte maturation, in vitro maturation (IVM) medium was supplemented with four concentrations 5, 15, 25, 35  $\mu\text{g/ml}$  quercetin during the 44 hours maturation period. Then we evaluated the effects of the same four concentrations of quercetin in IVM medium on the future embryos development.

Pig ovaries were collected from sows slaughtered and transported to the laboratory in a 0.9% NaCl solution at 37°C within 2 hours. Follicular contents from the antral follicles with size between 3-6 mm diameter were aspirated using a syringe. Cumulus-oocyte complexes (COCs) which has compact, multilayered cumulus cell and homogeneous cytoplasm were selected and transferred in IVM medium containing TCM-199 supplemented with sodium pyruvate, L-glutamine, cysteine, fetal bovine serum 10 % and antibiotics (penicillin, streptomycin, gentamicin). For the first 24 hours only, IVM medium contained Folligon (10 IU/ml), Chorulon (10 IU/ml). The COCs were cultured in Petri dishes with 45 $\mu\text{l}$  droplets covered by mineral oil at 37°C in 5% CO<sub>2</sub> in air. After 44 hours of maturation, the oocytes were denuded of cumulus cells by pipetting with phosphate buffer

saline (PBS) and bovine serum albumin (BSA) at 1:1 ratio [11]. The collected COCs were classified into 4 grades on the basis of cumulus cells [12]. The COCs classified for 3 and 4 grades are considered to be mature [13] and were used for in vitro fertilization (IVF).

For embryo culture medium NCSU-23 was supplemented with sodium lactate, sodium pyruvate,  $\beta$ - mercaptoethanol, cysteine, bovine serum albumin and antibiotics (pen-strep-gent). Embryo development was evaluated after 72 hours. The number of embryos was compared to the control and the differences analyzed using the analysis of variance and interpreted using the Tukey test and the GraphPad InStat program.

### 3. Results and discussion

Seven replicate trials were done for each type of experiment. The results of the cumulus cell expansion of COCs after 44 hours cultured in TCM-199 supplemented with different levels of quercetin are presented in Table 1.

**Table 1.** Development in culture of pig oocytes treated with different concentrations of quercetin during in vitro maturation (original)

Concentration ( $\mu\text{g/ml}$ )	Number oocytes	COCs in the grads of expansion % ( <sup>a</sup> )				
		0	1	2	3	4
0 (control)	269	10.87 $\pm$ 1.2	14.53 $\pm$ 2.1	20.80 $\pm$ 3.4	17.37 $\pm$ 3.2	35.76 $\pm$ 2.2
5	280	9.46 $\pm$ 1.8	11.47 $\pm$ 1.0	24.46 $\pm$ 2.0	33.80 $\pm$ 2.8	20.77 $\pm$ 1.9
15	273	11.12 $\pm$ 1.8	14.35 $\pm$ 1.3	22.40 $\pm$ 2.4	23.96 $\pm$ 1.7	28.13 $\pm$ 1.8
25	267	8.28 $\pm$ 1.4	12.11 $\pm$ 1.1	16.23 $\pm$ 1.0	21.16 $\pm$ 2.3	42.17 $\pm$ 2.2
35	258	10.26 $\pm$ 1.5	15.76 $\pm$ 1.3	18.21 $\pm$ 1.7	19.20 $\pm$ 1.0	36.41 $\pm$ 2.8

(<sup>a</sup>) mean $\pm$ SEM

Gr 3: 0 vs. 5 \*\*\* p<0.001;

Gr 4: 5 vs. 25 \*\*\* p<0.001; 5 vs 35 \*\*\* p<0.001; 15 vs 25 \*\* p<0.01

The results showed that the rate of cumulus cell expansion in grad 3 could be very significantly (p<0.001) increased from 17.37% to 33.80% by supplementing 5  $\mu\text{g/ml}$  of quercetin to control media. At the same time, the expansion rate in grad 4 could be very significantly (p<0.001) increased from 20.77% to 42.17% supplementing from 5  $\mu\text{g/ml}$  of quercetin to 25  $\mu\text{g/ml}$  of quercetin media, and very significantly (p<0.001) increased from 20.77% to 36.41% supplementing from 5  $\mu\text{g/ml}$  of quercetin to 35  $\mu\text{g/ml}$  of quercetin media, and distinct significantly (p<0.01) increased from 28.13% to 42.17%

supplementing from 15  $\mu\text{g/ml}$  of quercetin to 25  $\mu\text{g/ml}$  of quercetin media.

The results of the development of embryos from 2 cell to 16-32 cell up to 7 days of culture are summarized in Table 2.

The presence of 25  $\mu\text{g/ml}$  quercetin in the maturation medium increased the percentage of embryos in the morula cell stage (48.86%) compared with the control (26.92%). In the morula cell stage all the concentrations of quercetin resulted percentages increased compared to the control.

**Table 2.** In vitro culture of oocytes matured in media supplemented with different levels of quercetin (original)

Concentration ( $\mu\text{g/ml}$ )	Number oocytes cultured	Embryos developed % (*)			
		2 cell	4-8 cell	8-16 cell	morula
0 (control)	146	3.01 $\pm$ 1.9	5.75 $\pm$ 2.9	6.53 $\pm$ 5.0	26.92 $\pm$ 6.6
5	148	7.05 $\pm$ 3.2	6.70 $\pm$ 3.2	6.62 $\pm$ 2.6	36.15 $\pm$ 3.3
15	154	2.23 $\pm$ 2.2	4.76 $\pm$ 1.6	7.66 $\pm$ 2.7	36.82 $\pm$ 6.3
25	160	1.53 $\pm$ 1.5	1.85 $\pm$ 1.5	2.30 $\pm$ 1.2	48.86 $\pm$ 8.3
35	145	1.61 $\pm$ 1.1	2.15 $\pm$ 1.6	4.82 $\pm$ 2.4	37.77 $\pm$ 8.4

(\*) mean $\pm$ SEM

Quercetin supplementation had effect though morula cell stage were developed in 25  $\mu\text{g/ml}$  media (48.86%) to control media (26.96%).

#### 4. Conclusions

Several studies have shown that supplementation of porcine IVM medium with antioxidants such as  $\alpha$ -tocopherol and ascorbic acid [14]. The other one have reported toxic effects of other flavonoids on embryos from different species [15].

The results of the present study were comparable to the observation by Kang et al. [8] who reported a beneficial effect of quercetin treatment on embryonic development.

In our investigation shown that the presence of quercetin in IVM medium increased embryos development. It is not clear what level of quercetin is optimal for porcine.

#### References

- Chen, C., Zhou, J. Ji. C., Quercetin: A potential drug to reverse multidrug resistance, In Life Science, 2010, 87, 333-338
- Middleton, E. J., Effect of plants flavonoids on immune and inflammatory cell functions, In Advances in Experimental Medicine and Biology, 1998, 439, 175-182
- Noroozi, M., Wilson, J. A., Lean, M. E. J., Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes, Am. J. Clin. Nutr., 1998, 67, 1210-8
- Kazuo, U., Taijiro, M., Takashi, S., Tadashi, H., Machiko, T., Yoichi, K., Shozo, T., In vitro transformation of hamster embryo cells by quercetin, Toxicology Letters, 1977, 1, 3, 175-178
- Nakayama, T., Yamada, M., Osawa, T., Kawakishi, S., Suppression of active oxygen-induced cytotoxicity by flavonoids, Biochem. Pharmacol., 1998, 45, 1, 265-7
- Khanduja, K. I., Verma, A., Bhardwaj, A. Impairment of human sperm motility and viability by quercetin is independent of lipid peroxidation, Andrologia, 2001, 33, 277-81

7. Dong, G. Z., Kim, C. Y., Cho, W. S., Shin, Y. J., Lee, K., Cryoprotective role of the flavonoid quercetin in mammalian cells, Korean J. Genetics, 2006, 28, 3, 269-277

8. Kang, J. T., Kwon, D. K., Park, S. J., Kim, S. J., Moon, J. H., Koo, O. J., Jang, G., Lee, B. C., Quercetin improves the in vitro development of porcine oocytes by decreasing reactive oxygen species levels, Journal of Veterinary Science, 2013, 14, 1, 15-20

9. Ishige, K., Schubert, D., Sagara, Y., Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms, Free Radical Biology & Medicine, 2001, 30, 4, 433-446

10. Celik, H., Arinç, E., Evaluation of the protective effects of quercetin, rutin, naringenin, resveratrol and trolox against idarubicin-induced DNA damage, Journal of Pharmacy and Pharmaceutical Sciences 2010, 13, 231-41

11. Hoque, S. A. M., Khandoker, M. A. M. Y., Kabiraj, S. K., Asad, L. Y., Fakruzzaman, M., Tareq, K. M. A. Effect of goat follicular fluid on in vitro production of embryos in Black Bengal goats. Iranian Journal of Applied Animal Science 2001, 2, 3, 287-294

12. Khandoker, M. A. M. Y., Imai, K., Takahashi, T., Hashizume, K. Role of gelatinase on follicular atresia in the bovine ovary, Biol. Reprod., 2001, 65, 720-732

13. Tao, Y., Zhou, B., Xia, G., Wang, F., Wu, Z., Fu, M., Exposure to L-ascorbic acid or  $\alpha$ -tocopherol facilitates the development of porcine denuded oocytes from metaphase I to metaphase II and prevents cumulus cells from fragmentation, Reprod. Domest. Anim., 2004, 3, 52-57

14. Miclea, Ileana, Pacala, N., Zahan, M., Orlovski, Delia, Carlea, L., Miclea, V., Alpha-tocopherol and ascorbic acid combination have a positive effect on the maturation of pig oocytes, Bulletin UASVM Animal Science and Biotechnologies, 2012, 69, 1-2

15. Sakatani, M., Suda, I., Oki, T., Kobayashi, S., Kobayashi, S., Takahashi, M., Effects of purple sweet potato anthocyanins on development and intracellular redox status of bovine preimplantation embryos exposed to heat shock, J Reprod Dev., 2007, 53, 605-614