Effect of Kaempferol on In Vitro Maturation of Porcine Oocytes

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Abstract

We investigated the effects of kaempferol on porcine oocytes in vitro maturation. Kaempferol is one the most studied flavonoids and is in research attention on animal cells until 1979. Flavonoids are known as polyphenolic compounds synthesized by the plants.

Cumulus-oocyte complexes aspirated from the ovaries were maturated in vitro, fertilized and embryos were cultured in a defined conditioned medium with 5, 15, 25, 35 µg/ml or without kaempferol supplementation. During in vitro maturation with highest kaempferol concentration (35 µg/ml) distinct significantly increase the rate of cumulus cell expansion in grad 4 (42.74 vs. 50.96%, $p<0.01$). The same, addition of 5 µg/ml kaempferol to the in vitro maturation medium increase significantly the rate of expansion compared to 25 µg/ml (42.20 vs. 48.67%, $p<0.05$) and increase distinct significantly the rate of expansion compared to 35 µg/ml (42.20 vs. 50.96%, $p<0.01$).

Kaempferol supplementation (15 µg/ml vs. 35 µg/ml) of the in vitro fertilization medium led to a significant increase in the rate of 4-8 cells formation (0.69 vs. 4.96%, $p<0.05$).

In conclusion, these results demonstrate that supplementation with kaempferol during in vitro maturation improved the developmental competence of porcine oocytes.

Keywords: kaempferol, in vitro maturation, oocytes, porcine

1. Introduction

The objective of present study was to examine the effect of kaempferol supplementation during in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) on the developmental competence of porcine oocytes.

Pigs are more studying for its biological similarity with human physiology.

Kaempferol (3,4',5,7-tetrahydroxyflavone) is a plant derived flavonoid from fruits and vegetables that has antioxidant action as a free radical scavenger [7]. Its chemical structure is almost identical with quercetin (3,3',4',5,7 – pentahydroxyflavone), a flavonoid antioxidant tested for in vitro pig embryos production by Kang et al. [3]. The difference between these two is the hydroxyl (OH) group at the 3-position in ring B, kaempferol has not.

It was been reported that flavonoids efficiently protect against oxidative stress [4]. Heijen et al. [2] shown that kaempferol is potent scavengers against peroxynitrite toxicity. Kaempferol it has been studied by researchers since 1979 on animal cells as Chinese hamster V79 cells [6, 8], human nervous tissue [10], human diabetics [9], monkey sperm [5], fish sperm [1].

2. Materials and methods

The solutions and chemicals used in this study were purchased from Sigma-Aldrich (USA).

Cumulus-oocyte complexes (COCs) were aspirated from 3 to 6 mm follicles of porcine ovaries obtained from a slaughterhouse. Oocytes with intact cumulus cells and evenly granulated cytoplasm were selected

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and randomly assigned to each treatment. The COCs were incubated in 45 µl droplets cover with mineral oil of IVM medium composed from TCM-199 supplemented with sodium pyruvate, L-glutamine, cysteine, fetal bovine serum 10 % and antibiotics (penicillin, streptomycin, and gentamicin). Variations of kaempferol concentrations used are 5, 15, 25, 35 µg/ml.

For the first 24 hours only, IVM medium contained Folligon (10 IU/ml), Chorulon (10 IU/ml), and COCs are incubated at 37°C in 5% CO2 in air at saturated humidity.

The matured COCs were used for IVF with refrigerated boar semen in TALP medium. After 24 hours, the fertilized oocytes are cultivated in IVC medium for development of embryos. Evaluation of embryos is done in the 7^{th} day

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. Cumulus cell expansion of COCs after 44 hours cultured in maturation media supplemented with different levels of kaempferol are shown in Table 1.

The findings show that the rate of cumulus cell expansion in grad 1 could be significantly ($p<0.05$) increased from 6.10% to 12.50% by supplementing 5 µg/ml of kaempferol to control media. In the same time, the expansion rate in grad 4 could be significantly ($p<0.05$) increased from 42.20% to 48.67% supplementing from 5 µg/ml of kaempferol to 25 µg/ml of kaempferol media. Also on this grad 4 was find distinct significantly ($p<0.01$) increased from 42.74% to 50.96% supplementing 35 µg/ml of kaempferol to control media, and increased from 42.20% to 50.96% supplementing from 5 µg/ml of kaempferol to 35 µg/ml of kaempferol media.

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

In the stage of 4-8 cell the rate could be significantly ($p<0.05$) increased from 0.69% to 4.96% by supplementing from 15 µg/ml of kaempferol to 35 µg/ml of kaempferol media. The presence of 5 µg/ml of kaempferol in the maturation medium increased the percentage of embryos in morula stage (53.13%) compared with the control (48.63%). Starting concetration15 µg/ml of kaempferol in the morula stage all the concentrations of kaempferol resulted percentages decreased to control.

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

Concentration (µg/ml)	Number oocytes	COCs in the grads of expansion % (ª)				
		0	1	2	3	4
0 (control)	372	8.11 ± 1.0	6.10 ± 1.0	16.06 ± 1.9	26.97 ± 1.1	42.74 ± 1.7
5	357	6.82 ± 1.1	12.50 ± 1.9	14.94 ± 2.2	23.52 ± 2.5	42.20 ± 1.4
15	381	7.08 ± 1.4	9.54 ± 1.6	11.04 ± 1.3	24.03 ± 2.6	48.28 ± 1.6
25	369	5.77 ± 1.2	8.70 ± 1.3	14.82 ± 1.3	22.56 ± 1.1	48.67 ± 1.7
35	377	7.65 ± 0.9	9.85 ± 1.1	10.76 ± 1.3	20.93 ± 1.5	50.96 ± 1.2

(ª) mean±SEM

Gr 1: * 0 vs 5 $p<0.05$

Gr 4: * 5 vs 25 $p<0.05$; ** 0 vs 35 $p<0.01$; ** 5 vs 35 $p<0.01$

Results of development of embryos from 2 cells to morula up to 7 days of culture are prezented in Table 2.

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

Concentration (µg/ml)	Number oocytes cultured	Embryos developed % (ª)			
		2 cell	4-8 cell	8-16 cell	morula
0 (control)	146	2.07 ± 0.9	1.43 ± 0.9	10.12 ± 2.8	48.63 ± 7.6
5	148	2.90 ± 1.8	2.53 ± 0.8	6.38 ± 1.9	53.13 ± 3.5
15	154	3.99 ± 1.6	0.69 ± 0.4	5.78 ± 2.0	42.66 ± 5.3
25	160	2.10 ± 1.0	1.54 ± 0.8	4.81 ± 1.8	41.78 ± 7.1
35	145	1.95 ± 1.3	4.96 ± 1.5	6.52 ± 2.8	38.77 ± 3.9

(ª) mean±SEM, 4-8 cell * 15 vs 35 $p<0.05$

In the stage of 4-8 cell the rate could be significantly ($p<0.05$) increased from 0.69% to 4.96% by supplementing from 15 µg/ml of kaempferol to 35 µg/ml of kaempferol media. The presence of 5 µg/ml of kaempferol in the maturation medium increased the percentage of embryos in morula stage (53.13%) compared with the control (48.63%). Starting concetration15 µg/ml of kaempferol in the morula stage all the concentrations of kaempferol resulted percentages decreased to control.

4. Conclusions

After our study, we conclude that concentration 5 µg/ml of kaempferol has beneficial effect for IVM and then IVF obtaining morula. Oocytes maturated with concentration 35 µg/ml of kaempferol at grad 4 not further develop embryos. The effects and optimal concentrations of kaempferol for in vitro embryos develop must be better understood thru many other studies.

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