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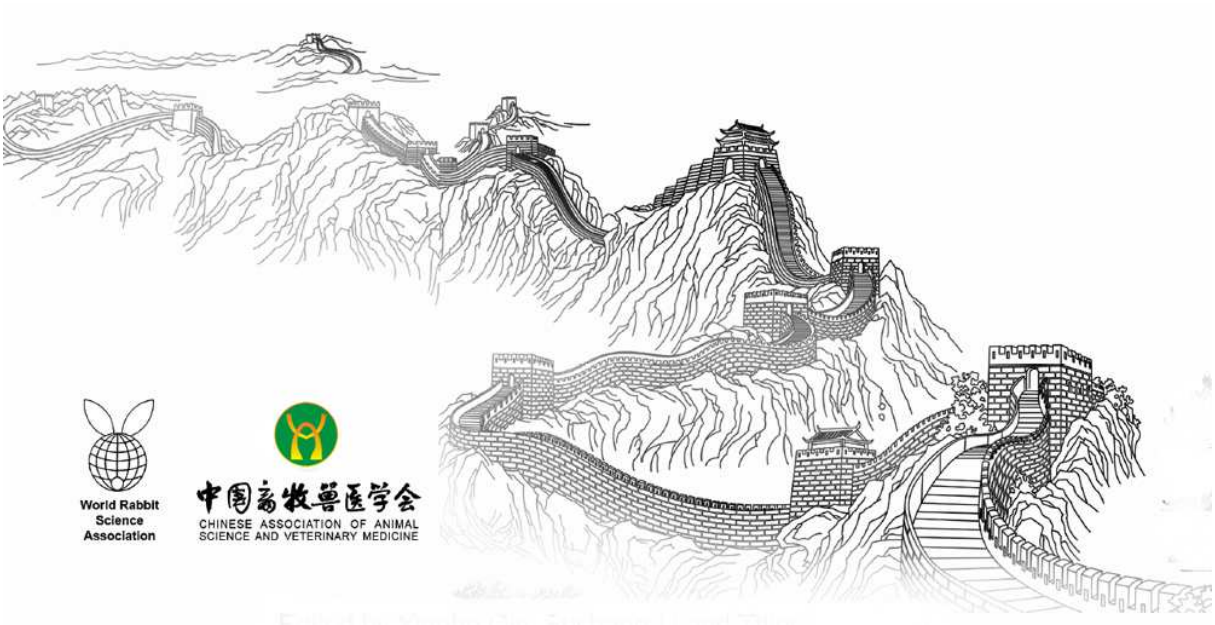

A POTENTIAL ROLE FOR COENZYME Q10 AS ENERGY AND ANTIOXIDANT AGENT IN EMBRYO PRODUCTION FROM FOLLICULAR OOCYTES IN RABBITS

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ABSTRACT

This study aimed to evaluate the effect of adding 0 (T1), 0.2 (T2) and 0.4 (T3) mg Coenzyme Q10 (CoQ10)/ml maturation medium (TCM-199) on *in vitro* maturation (IVM), fertilization (IVF) and embryo culture (IVEC) of NZW rabbit oocytes. Immediately after slaughtering 30 sexually mature does, ovaries were removed and oocytes were collected by slicing. Only compact-cumulus oocytes (COCs) were incubated in CO_2 incubator (5% CO_2, at 38°C with high humidity, HH) for 20 h. Percentage of oocytes at metaphase II (MII) was determined as maturation rate (MR). IVF was performed of COCs matured with different levels of CoQ10 with spermatozoa capacitated by heparin at 38°C for 24 h in 5% Co_2 with HH. Cleavage rate (CR) and fertilized ova were cultured for 5 days at 38°C with 5 % CO_2 with HH for determining morula (MPR) and blastocyst (BPR) production rates. Results showed that T2 and T3 increased ($P<0.05$) the percentage of oocytes at MII (87.17 and 88.99%) and decreased percentage of degenerated oocytes (2.35 and 2.44%) as compared to T1 (75.02 and 7.52%), respectively. CR (70.00 and 72.89 vs. 55.48%), MPR (36.49 and 37.21 vs. 26.35%) and BPR (15.89 and 19.53 vs. 6.45%) were higher ($P<0.05$) for T2 and T3 versus T1, respectively. In conclusion, present study cleared that supplementation of maturation medium with CoQ10 at a level of 0.4 mg/ml has improved *in vitro* maturation, cleavage and developmental potential of rabbit oocytes.

Key words: Rabbit, oocytes, CoenzymeQ10, maturation, blastocyst.

INTRODUCTION

One of the primary goals of an embryologist is to improve the quality of embryos developing in the laboratory. As a fundamental technical bases, a stable, reliable and effective *in vitro* mammalian embryo culture system has been one of the major concerns in the of embryo engineering. In many mammalian species, the critical factors of utmost importance in the development of *in vitro* produced embryos are the culture conditions for the maturation of immature cumulus-oocyte complexes (COCs) and early-stage embryos (Manzano *et al.*, 2015). Oocytes are renowned as large cells containing lipid stores (Dunning *et al.*, 2014) and during the oocyte *in vitro* maturation (*OIVM*) the intracellular lipid stores undergo dramatic changes. Lipid droplets exhibit a pronounced peripheral distribution pattern following maturation pig *OIVM* (Sturmey *et al.* 2006) or undergo structural reorganization, aggregating centrally in mouse oocytes (Yang *et al.* 2010). Oocyte development is complex and energy-intensive, requiring high levels of functional mitochondria to produce sufficient adenosine triphosphate (ATP) for the meiotic process (May-Panloup *et al.*, 2007). Insufficient or poorly functioning mitochondria lead to reduced energy levels, which in turn can lead to arrested development of the oocyte (May-Panloup *et al*., 2005), increased aneuploidy rates (Bartmann *et al*., 2004) and decreased fertilization rates (Reynier *et al*., 2001). Mitochondria are particularly susceptible to damage, in part due to their proximity to the source of reactive oxygen species (ROS) generation, which are a natural and important part of many physiological processes (Pritchard *et al*., 2015).

In bovine, oocyte lipid content was reduced by supplementation of *IVM* medium with Coenzyme Q10 (CoQ10), a component of lipid metabolism (Abdulhasan *et al.*, 2015). CoQ10 had a beneficial role in the cellular metabolism, cellular antioxidant and prevention of lipid peroxidation in mice (Hosseinzadeh *et al*., 2015).

Therefore, this paper aimed to evaluate the effect of maturation medium supplemented with CoQ10 at two levels (0.2 and 0.4 mg/ml) on *in vitro* maturation, fertilization and embryo development of rabbit.

MATERIALS AND METHODS

Animals

Total of 30 mature rabbit does of New Zealand white (NZW, 5-6 mo of age, 3-3.5 kg LBW) as oocyte donors and 6 NZW bucks (7.5-8 mo of age and 3.5-4.0 kg LBW) for semen collection were taken from Sakha Experimental Station, Agricultural Research Center, Egypt. Immediately after slaughtering, ovaries were removed, washed by 0.9% NaCl and dried. Oocytes were collected by slicing technique into tissue culture dishes containing 4 ml of harvesting medium (Dulbecco's Phosphate buffer saline, PBS ,Sigma. Co.), supplemented with 3 mg bovine serum albumin (BSA)/ml, 0.03 % sodium pyruvate and 50 µg/ml gentamycin sulfates. Oocytes were examined and evaluated. Only compact-cumulus oocytes (COCs) were used in this study.

In vitro Maturation and fertilization

Tissue Culture Medium (TCM-199) supplemented with 10% BSA (w/v), 10 IU/ml PMSG, 10 IU/ml hCG, 1 µg/ml estradiol, 0.03 % sodium pyruvate and 50 µg/ml gentamycin sulfates was used as *IVM* medium. Three types of *IVM* medium were supplemented with 0.0, 0.2 or 0.4 mg/ml of CoQ10 (Sigma). The pH value and osmolarity of the medium were adjusted to 7.2-7.4 and 280-300 mOsmol/kg, respectively. Filtered *IVM* medium (100 µl) was placed into well dishes and covered by sterile mineral oil and incubated in 5%CO_2, at 38°C with saturated humidity for at least 60 min. COCs were washed three times with *IVM* medium, cultured and incubated under the same conditions for 20 h. After *IVM* period, COCs were washed using DPBS containing 1 mg /ml hyaluronidase, washed two times with PBS plus 2% BSA, and loaded into fixation solution (3 ethanol: 1 glacial acetic acid) overnight and stained with 1 % orcein in 45% acetic acid to determine the proportion of oocytes at metaphase II (MII).

Semen from 15 ejaculates was capacitated in DPBS medium supplemented with 3 mg/ml BSA 50 µg/ml gentamycin sulfates and 35 µg/ml heparin. Maturated COCs by types of CoQ10 were *in vitro* fertilized by pipetting 50 µl of fertilization medium under sterile liquid paraffin oil and incubated at 38°C for 2 h in 5% Co_2 and high humidity. About 50 µl washing media was added to each droplet with 10-15 oocytes following by adding 2 µl of prepared semen and then incubated together at 38°C for 24 h in 5% Co_2. After IVF, cumulus cells were separated from presumptive zygotes by pipetting and were washed twice in culture medium comprising SOF medium supplemented 10% FCS, 0.03 % sodium pyruvate and 50 µg/ml gentamycin sulfates. To determining the rate of cleavage, 10 zygotes were cultured in each four-well Petri dish containing 100 µl of culture medium under mineral oil for 5 days at 38 °C with 5 % CO_2 in air.

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) using computer program of SAS (1998). ANOVA was performed after arcsine transformation of original data expressed as proportions. Significant differences were determined by Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Proportion of oocytes at M II was higher (87.17 and 88.99%, $P<0.05$) and proportion of degenerated oocytes was lower (2.35 and 2.44%, $P<0.05$) for 0.2 and 0.4 CoQ10 levels than those in free medium (75.02 and 7.52%), respectively (Table 1).

Table 1: Effect of adding maturation medium with CoQ10 on *in vitro* maturation of doe rabbit oocytes.

Treatment group	Total COCs	Frequency distribution (%)				
		GV	GVBD	MI	MII	Degenerated oocytes
T1 (Control)	80	3.76±0.12[a]	8.75±1.21[a]	4.95±1.10	75.02±0.56[b]	7.52±0.25[a]
T2 (CoQ10, 0.2 mg/ml)	85	2.30±1.15[ab]	1.11±1.11[b]	7.07±0.22	87.17±1.92[a]	2.35±1.18[b]
T3 (CoQ10 0.4 mg/ml)	81	1.28±1.28[b]	2.44±1.24[b]	4.84±0.92	88.99±1.70[a]	2.44±1.24[b]

Means with different letters on the same column differ significantly at P<0.05.
GV: Germinal vesicle. GVBD: Germinal vesicle breakdown. MI: Metaphase I. MII: Metaphase II.

Supplementing maturation medium with 0.2 and 0.4 mg/ml CoQ10 to *IVM* medium improved cleavage rate (70.00 and 72.89 vs. 55.48%, P<0.05), morula rate (36.49 and 37.21 vs. 26.35%, P<0.05) and blastocyst rate (15.89 and 19.53 vs. 6.45%, P<0.05) as compared to free medium, respectively (Table 2). Generally, the differences between both CoQ10 levels were not significant.

Table 2: Cleavage rate and developmental competence of rabbit oocytes *in vitro* matured in medium supplemented with CoQ10.

Treatment group	Mature oocytes	Cleavage rate (%)	Morula (%)	Blastocyst (%)
T1 (Control)	110	55.48±0.86[b]	26.35±2.09[b]	06.45±1.32[b]
T2 (CoQ10, 0.2 mg/ml)	90	70.00±1.92[a]	36.49±0.89[a]	15.89±1.62[a]
T3 (CoQ10 0.4 mg/ml)	85	72.89±0.44[a]	37.21±0.84[a]	19.53±2.95[a]

Means with different letters on the same column differ significantly at P<0.05.

The main finding of this study was that we looked for a relation between the observed improvement in the developmental potential of immature rabbit COCs and IVM, IVF and embryos at morula/blastocyst stages resulting after culture *in vitro* as affected by CoQ10 supplementation in maturation medium (TCM-199). In a recent study of Abdulhasan *et al*. (2015) with bovine oocytes, the percentage of IVM was 51.77 vs. 39.06% for maturation medium supplemented with 40 vs. 20 μM of CoQ10, but oocytes maturated *in vitro* with both levels had failed to reach the fertilization.

The observed improvement in developmental competence of oocytes in our study may be partly due to decrease of ROS concentration of matured oocytes and improvement of the quality of morula/blastocyst stage. In this respect, CoQ10 has been claimed to have a beneficial role in the cellular metabolism, cellular antioxidant and prevention of lipid peroxidation for the oocytes and embryos (Hosseinzadeh *et al*., 2015). Mitochondria are critically important for oocyte maturation and they are reliable indicators for oocyte quality achieved during the maturation process (Schatten *et al*., 2014). CoQ10 plays an important role in the mitochondrial electron transport chain; it is a critical coenzyme in the synthesis of adenosine triphosphate (ATP).The redistribution of active mitochondria is correlated with elevated ATP production during the MI–MII transition (Yu *et al*., 2010). ATP is a major product of mitochondrial respiratory oxidative phosphorylation and known to affect the MII-spindle assembly and embryonic development (Wilding *et al*., 2009). The previous results showed that CoQ10 caused progress of oocyte maturation through changing in pattern of mitochondrial distribution and polarization, the polarity of the membrane and expression of genes that involved in the electron transport chain (Stojkovic *et al*., 1999).

A superior effect of CoQ10 on rate of cleavage, morula/blastocyst formation rate, percentage of expanding blastocysts, and a larger inner cell mass was reported in bovine oocytes by Marriage *et al*. (2004) and these changes were associated with an increased ATP content in the group of embryos cultured with CoQ10. ROS may originate in the embryo or from extraneous factors. An increase in ROS production leads to arrest of embryo development (Mansour *et al*., 2009). In the *in vitro* fertilization setting, strategies to reduce ROS production, such as addition of free radical scavengers and lowering the oxygen tension, are important for improving the fertility potential in assisted reproduction (Esfandiari *et al*., 2005). Supplementation of CoQ10 prevented the depletion of free radical scavengers such as reduced glutathione levels and caused a statistically significant increase in superoxide dismutase levels (Schatten*et al*., 2014).

CONCLUSIONS

In conclusion, present study demonstrates that supplementation of maturation medium with CoQ10 has improved the developmental potential of rabbit oocytes and production embryos *in vitro* required for gen and gamete banks. Also, effective *in vitro* mammalian embryo culture system is considered as one of the major concerns in the of embryo engineering.

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