LITTER SIZE, OVARIAN CHARACTERISTICS, AND OOCYTE *IN VITRO* MATURATION AND FERTILIZATION OF RABBITS ADMINISTRATED WITH COENZYME Q10 AND L-CARNITINE

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SUMMARY

The objective of this study was to evaluate the effect of coenzyme Q10 (CoQ10) and L-carnitine (LC) administration on litter size, ovarian characteristics and *in vitro* production of rabbit embryos. This study was carried out at the International Livestock Management Training Center (ILMTC), belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. Total of 36 mature NZW rabbit does (5-6 months of age, 3-3.5 kg LBW as well as 6 NZW bucks (7.5-8 months of age and 3.5-4.0 kg LBW) were used in this study. Does were divided into 3 similar groups, (n=12). The 1 group was control (G1), while does in the 2 and 3 groups were given daily oral dose of 10 mg CoQ10/kg LBW (G2) and 40 mg LC/kg LBW (G3) for 21 days prior to natural mating, respectively. Five does from each group were slaughtered post-mating as oocyte donors for studying the effect of treatments on ovarian characteristics and *in vitro* maturation and fertilization of oocytes. Immediately after slaughtering, ovaries were removed and oocytes were collected by slicing technique and evaluated, then only compact-cumulus oocytes (COCs) were matured and fertilized *in vitro*. For the rest number of does in each group (n=7), pregnancy was handy diagnosed by palpation 10-12 days post-mating. Also, litter size and weight at birth up to weaning were recorded. The obtained results showed that, ovarian weight and number of follicles increased ($P<0.05$) in G2 and G3. Number of bleeding follicles and recovered oocytes increased ($P<0.05$) only in G2 as compared to G1, however, oocyte recovery rate was not affected by treatment. Frequency distribution of compact and partial denuded oocytes was higher ($P<0.05$), while that of expanded and denuded oocytes was lower in G2 and G3 as compared to G1. Percentage of oocytes with full expansion (maturation rate) was 80.3 and 79.3% vs. 73.8% and fertilization rate was 67.4 and 66.7% vs. 64.5% in G2 and G3 vs. G (($P\geq0.05$). Percentage of embryos at morula stage was higher ($P<0.05$) in G2 and G3 than in G1 (30.3 and 3.1% vs. 20%), respectively. Percentage of embryos at blastocyst stage was 21.2 and 21.4% vs. 15% in G2 and G3 vs. G1, respectively. Litter size of rabbit does was higher ($P<0.05$) in G2 and G3 than in G1 at birth (7.29 and 6.71 vs. 5.43/doe), at 21 days of age (6.71 and 5.86 vs. 4.43/doe) and at weaning (6.43 and 5.43 vs. 4.14/doe). Mortality rate of kits at birth or weaning was not affected by treatment. Average kit weight at birth was higher ($P<0.05$) in G2 and G1. However, average kit weight at weaning, average litter weight from birth up to weaning and average litter daily gain from birth up to weaning were higher ($P<0.05$) in G2 and G3 than in G1.

In conclusion, treatment of rabbit does 21 days prior to insemination with CoQ10 at a level of 10 mg/kg LBW or L-carnitine at level 40 mg/kg LBW as daily oral dose is recommended to improve in vitro embryo production and also to increase litter characteristics (size and weight from birth up to weaning) of New Zealand White rabbit does.

Keywords: *Rabbit, coenzyme Q10, L-carnitine, oocyte, in vitro maturation and fertilization.*

INTRODUCTION

Mammalian ovaries contain a large number of oocytes enclosed in primordial follicles. Ovarian cyclic activity induces some of these follicles to initiate growth towards ovulation. However, in growing follicles, only subsets of oocytes are capable to support maturation, fertilization and early embryo development (Mermillod *et al.,* 2008). Metabolism and adenosine tri-phosphate (ATP) levels within the oocyte and adjacent cumulus cells are associated with oocyte quality and optimal development of a healthy embryo. Mitochondria play a key role in the physiology of eukaryotic cells during all stage of life including the pre-implantation period and their main function is to provide cells with ATP through oxidative phosphorylation (Cummins, 2004; Smith *et al.,* 2005). Mitochondrial dysfunction may lead to incomplete detoxification of the free radicals, which may lead to oxidative damage to macromolecules such as lipids, proteins, and DNA (Abdelrazik *et al.,* 2009). In vivo oocytes rely on mitochondrial

oxidative phosphorylation for energy, a process which is subsequently accompanied by reactive oxygen species (ROS) generation (du Plessis *et al.,* 2008). Thus, poor oocyte quality would lead to compromised embryo development. In the oocyte, ROS levels when present in excess, can disrupt the oocyte cytoskeleton (du Plessis *et al.,* 2008).

The cytoplasm of ruminant oocyte and pre-implantation embryos are rich in lipid droplets, which, in compact cumulus oocytes (COCs) are associated with the endoplasmic reticulum and mitochondria and are degraded to some extent during maturation (Kruip *et al.,* 1983; McEvoy *et al.,* 2000). The ß-oxidation pathway that metabolizes lipids/fatty acids within the mitochondria to generate cellular ATP has an essential role in determining oocyte quality and its ability to support embryo development (Van Blerkom *et al.,* 1995; Stojkovic *et al.,* 2001; Fergusson and Leese, 2006).

In the animal body, carnitine can be synthesized from protein-bound lysine and methionine in two forms (L- and D-carnitine) and L-carnitine (LC) is biologically the active form (Vaz and Wanders, 2002). It is vitamin-like amino-acid as a polar natural compound (Groff and Gropper, 2000), playing an important role in the cellular detoxification (Arrigoni-Martelli and Caso, 2001) and in lipid metabolism by carrying long-chain fatty acids to the mitochondria for β-oxidation to produce ATP required for cell function (Hoppel, 2003). It is also important as antioxidant for protection of the cell membranes against oxidative damages (Kalaiselvi and Panneerselvam, 1998).

Recently, Manzano *et al.* (2015) showed that nuclear maturation and cleavage rate of COCs were not influenced by the presence of LC during the initial maturation period, but LC supplementation was effective at supporting blastocyst formation and improving its quality as evidenced by having a higher total cell count. In this respect, You *et al.* (2012) mentioned that LC plays an important role in the lipids/fatty acid metabolism as an endogenous energy source for the oocytes and embryos. There is multiple reports demonstrating that LC treatment in vitro can improve embryo development, however little is known about whether circulating carnitine or in vivo supplementation with LC can improve embryo quality (Eder, 2009). The unique dual effects of LC in terms of reducing cellular lipid content and providing antioxidative protection make it a novel candidate reagent for the non-invasive improvement of cryo-tolerance and developmental competence in embryos of farm animals (Takahashi *et al.,* 2013). There has been very little investigation of whether LC supplementations in vivo can impudence oocyte quality or embryo growth, however, Miyamoto *et al.* (2010) reported that LC in the drinking water (5 mg/ml) improved ovulation rates in the 'aged' ovaries, normalized the alterations in oocyte mitochondria and markers of oxidative stress and improved blastocyst development.

Coenzyme Q10 (CoQ10) is a fat soluble vitamin-like substance present in every cell of the body and serves as a coenzyme for several of the key enzymatic steps of in the production of energy within the cell (Kapoor and Kapoor, 2013). CoQ10 plays a key role in the mitochondrial electron transport chain, and it is a critical coenzyme in the synthesis of ATP (Abdulhasan *et al.,* 2015). Also, CoQ10 is an antioxidant that has great importance against free radicals (Bentinger *et al.,* 2007), protects the stability of the cell membrane, DNA from free radicals induced oxidative damage and helps recycling of vitamin E and maintain healthy energy levels (El-Tohamy *et al.,* 2012). Moreover, dietary supplementation with CoQ10 may increase mitochondrial activity within the oocyte and developing embryo (Bentov et al., 2010). Since tissue levels of CoQ10 decrease with age, supplementation of this agent may improve mitochondrial function in the ovary and may improve oocyte and embryo quality, especially in women of advanced reproductive age (Scott, 2013).

Therefore, aim of the present work was to study the effect of treatment of rabbit does for 21 d pre-mating with coenzyme Q10 (10 mg/kg LBW) and L-Carnitine (40 mg/kg LBW) on follicular number, and recovery rate, in vitro maturation, fertilization and development of follicular oocytes.

MATERIALS AND METHODS

This study was carried out at the International Livestock Management Training Center (ILMTC), belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Animals:

Total of 36 New Zealand white (NZW) mature rabbit does (5-6 mo of age and 3.0-3.5 kg live body weight) as embryo donors as well as 6 NZW bucks aging 7.5-8.0 mo and weighing 3.5-4.0 kg for natural mating were used in this study during the experimental period.

Experimental groups:

Rabbit does were divided randomly into 3 groups, (n=12) per group. The 1st group (G1) was considered as a control group without treatment (G1), while does in the 2nd and 3rd groups were given 10 mg Coenzyme Q10 (CoQ10)/kg LBW and 40 mg L-Carnitine (LC)/kg LBW as daily oral doses for 21 days as a treatment period prior to mating. Natural mating was carried with fertile bucks and pregnancy was handy diagnosed by palpation ten to twelve days post mating to detect the pregnancy.

Experimental procedures:

Post-mating, five rabbit does were taken from each group, slaughtered, and immediately after slaughtering ovaries of each doe were removed, washed by distilled water and dried by cleaning paper. Ovaries were excised, submerged in a flacon plastic tissue culture dishes (60 x 15 mm) containing saline solution at 38.5°C for in vitro study. On the other hand, the remainder number of rabbit does in each group (n=7) were kept under the same feeding and managerial conditions up to kindling for in vivo study.

In vitro study:

Oocyte recovery:

Follicular oocytes were collected using slicing technique into tissue culture dishes containing 4 ml of phosphate buffer saline (PBS) as a harvesting medium, supplemented with 4 mg bovine serum albumin (BSA)/ml, 100 IU sodium penicillin G/ml, (Misr Co. for Pharm., Egypt) and 100 µg streptomycin/ml. Oocytes were examined under stereomicroscopy and classified according to their compaction, number of cumulus cell layers and homogeneity of ooplasm as described by Ravindranatha et al. (2003) to compact cumulus oocytes (COCs), expanded cumulus oocytes, partial denuded cumulus oocytes and denuded cumulus oocytes. Oocytes were recovered from only compact-cumulus oocytes (COCs) were used in this study.

In vitro maturation (IVM):

Maturation in vitro rate of oocytes was evaluated using tissue culture medium (TCM-199) containing 10% (v./v.) from doe rabbit serum (DRS) supplemented with 100 IU sodium penicillin G and 100 µg streptomycin per ml. The pH value of the medium was adjusted to 7.2-7.4 and osmolarity of 280-300 mOsmol/kg. The medium was filtered by 0.22-µm millipore filter (Milieux GV, milpore, Cooperation Bedford MOA). Each of 500 µl from prepared maturation medium was placed into four well dishes and covered by sterile mineral oil. Before placing the oocytes in culture dishes, the medium was incubated in CO2 incubator (5% CO2, at 38 °C with saturated humidity) for at least 60 minutes to attain equilibrium between the temperature and gases. Compact cumulus oocytes (COCs) were washed three times with maturation medium, then cultured in the medium and incubated under the same conditions for 20 h.

The morphology of matured oocytes was evaluated for cumulus cell expansion as full, partial and no expanded oocytes. In vitro maturation rate was expressed in term of percentage of oocytes with full expansion.

Sperm capacitation and in vitro fertilization:

Semen was collected from rabbit bucks and the jelly mass of each ejaculate was discarded and net volume of 10 ejaculates was used for sperm capacitation. PBS supplemented with 100 IU sodium penicillin g and 100 µg streptomycin and 4 mg/ml BSA was used. Medium was adjusted to pH 7.2-7.4 and osmolarity of 280-300 mOsmol/kg and filtered by 0.22-µm millipore filter. Sperm capacitation was performed by 35 µg/ml of heparin. Fertilization droplets were prepared by pipetting 50 µl of fertilization medium under sterile liquid paraffin oil and incubated at 38°C for 2 h in 5% CO2 in air and high humidity. About 5 µl of washing media was added to each droplet with 7-10 oocytes followed by adding 2 µl of prepared semen and then incubated together at 38°C for 24 h in 5% CO2 in air. Fertilizing rate after 24 h and developmental competence of embryos at different embryonic stages after 72 h were investigated.

Criteria of fertilized oocytes:

After fertilization, oocytes were examined using inverted microscope into normal fertilized oocytes containing both male and female pronucli in their cytoplasm plus second polar body, unfertilized oocytes with unknown criteria and degenerated oocytes exhibiting fragment or scattered chromatin complement in their cytoplasm. Also, the stage of cleavage was classified into embryos at 2-4 cell, 8-16 cell, morula and blastocyst stages.

In vivo study:

In this study, the rest number of does in each group (seven does) was allowed to continue to pregnancy and kindling. After the positive mating the nest boxer were supplied with sawdust in the 25

days of pregnancy to provide a comfortable and warm nest for bunnies. Litter size at birth, 21 d of age and weaning (28 d of age), mortality rate at birth and weaning as well as litter weight at birth, 1st, 2nd, 3rd and 4th wk of age were determined.

Statistical analysis:

Data were analyzed by analysis of variance using computer program of SAS (1998). The significant differences among group means were performed using Duncan Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

In vitro study:

Follicular number and oocyte recovery:

Data in Table (1) revealed that Ovarian weight and number of follicles increased (P<0.05) in G2 and G3. Number of bleeding follicles and recovered oocytes increased (P<0.05) only in G2 as compared to G1. However, oocyte recovery rate was not affected by treatment.

It is of interest to note that increasing ovarian weight of does in both treatment groups was associated with increasing number of bleeding and large follicles in G2 and only increasing number of large follicles in G3. In addition, number of recovered oocytes increased significantly (P<0.05) by CoQ10 treatment and insignificantly by LC treatment. No information are available on the effect of LC or CoQ10 on the ovarian activity of animals, but in a single study of El-Shahat and Abo-Elmaaty (2010) in ewes, dietary supplementation of 250 ppm LC for 56 days increased the number of medium and large ovarian follicles. Also, LC supplementation in the drinking water (5 mg/ml) improved ovulation rates in the 'aged' ovaries (Miyamoto *et al.*, 2010). The addition of LC to cultured mouse follicles for 12 days, from the pre-antral to large-antral stage, increased β-oxidation (Dunning *et al.*, 2011).

Table (1). Ovarian characteristics and ovulatory response of rabbit does as affected by CoQ10 and L-Carnitine (LC) treatments.

Characteristics	Experimental group		
	G1 (control)	G2 (CoQ10)	G3 (LC)
Ovarian weight, g	0.37±0.002b	0.48±0.042a	0.51±0.024a
Number of bleeding follicles, n	1.00±0.5b	2.40±0.2a	1.60±0.2ab
Number of visible follicles, n	20.00±0.5c	24.40±0.4a	21.80±0.5b
Number of recovered oocytes, n	16.00±1.0b	19.20±0.4a	17.40±0.2ab
Oocyte recovery rate, %	84.0±1.0	87.3±0.7	87.9±1.2

a, b and c: Means within the same row with different superscripts are significantly different at P<0.05.

Categories of recovered follicular oocytes:

Data in Table (3) clearly revealed that frequency distribution of compact and partial denuded oocytes was significantly (P<0.05) higher, while that of expanded and denuded oocytes was significantly (P<0.05) lower in G2 and G3 as compared to G1. This means that both treatments either CoQ10 or LC had impact on quality of recovered oocytes in term of frequency distribution of compact oocytes as compared to controls, being insignificantly better with CoQ10 than LC.

Table (2). Frequency distribution of rabbit oocyte categories as affected by CoQ10 and L-Carnitine treatment.

Oocyte category (%)	Experimental group		
	G1 (control	G2 (CoQ10)	G3 (LC)
Compact	52.4±0.6b	63.7±2.2a	60.9±1.1a
Expanded	6.4±0.51a	5.2±0.13b	5.8±0.12ab
Partial denuded	11.1±0.7c	16.5±1.7b	21.8±1.2a
Denuded	30.1±0.7a	14.6±0.8b	11.5±0.2c

a, b and c: Means within the same row with different superscripts are significantly different at P<0.05.

Oocyte quality is important for fertilization and development into viable offspring. Quality compromised oocytes are correlated with infertility, developmental disorders, reduced blastocyst cell number and embryo loss, but the

mechanisms underlying these effects are not well understood. Oocyte quality is achieved during the maturation process. Maturation defects can have several causes and have been associated with mitochondrial dysfunction (Schatten and Sun, 2011a, b) due to insufficient ATP, calcium homeostasis, hormonal effects, and several others. The ß-oxidation pathway that metabolizes lipids/fatty acids within the mitochondria to generate cellular ATP has an essential role in determining oocyte quality and its ability to support embryo development (Fergusson and Leese, 2006).

In accordance with the present study Miyamoto *et al.* (2010) has shown that LC can improve oocyte quality in mice by normalizing the alterations in oocyte mitochondria and markers of oxidative stress. In the same line, dietary supplementation with CoQ10 have also been considered to improve mitochondria functions in quality-compromised oocytes (Bentov *et al.*, 2010) and may increase mitochondrial activity and function within the oocyte (Bentov et al., 2010; Scott, 2013), especially with decreasing levels of CoQ10 with age in women (Scott, 2013). CoQ10 is a coenzyme that aids in the transport of electrons along the mitochondrial respiratory chain.

Maturation, fertilization and embryonic stages of follicular oocytes:

Data in Table (3) showed that percentage of oocytes with full expansion (Maturation rate) was 80.3 and 79.3% vs. 73.8% and fertilization rate was 67.4 and 66.7% vs. 64.5% in G2 and G3 vs. G1 without significant differences among groups ($P \geq 0.05$). However, percentage of embryos at morula stage was significantly ($P<0.05$) higher in G2 and G3 than in G1 (30.3 and 3.1% vs. 20%), respectively. Yet, the differences in percentage of embryos at blastocyst stage among groups were not significant, being 21.2 and 21.4% vs. 15% in G2 and G3 vs. G1), respectively. Such findings may indicate positive effect of both treatments only on morula production rate.

Table (3). Oocyte expansion (maturation rate), fertilization rate and embryonic stages of oocytes in vitro matured as affected by CoQ10 and L-Carnitine treatments.

Experimental group	N	Oocyte expansion		Fertilized oocytes		Embryonic stage							
						2- 4cell		8-16 cell		Morula		Blastocyst	
		n	%	n	%	n	%	n	%	n	%	n	%
G1 (control)	42	31	73.8	20	64.5	6	30.0	7	35.0a	4	20.0b	3	15.0
G2 (CoQ10)	61	49	80.3	33	67.4	7	21.2	9	27.3ab	10	30.3a	7	21.2
G3 (LC)	53	42	79.3	28	66.7	6	21.4	7	25.0b	9	32.1a	6	21.4

a and b: Means within the same column with different superscripts are significantly different at P<0.05.
N: Total number of oocytes.

Mitochondria are critically important for oocyte maturation and they are reliable indicators for oocyte quality achieved during the maturation process (Schatten *et al.*, 2014).The number of mitochondria varies in different species but in most mammalian species mitochondria are closely located around the MII spindle (Katayama *et al.*, 2006). During oocyte maturation, the oocyte grows and undergoes remodeling on cellular and molecular levels. This remodeling requires ATP likely supplied by mitochondria; thereby allowing timely and accurate cytoplasmic and nuclear maturation (Sirard *et al.*, 2006). Mitochondria supplied ATP is also important for protein phosphorylation and dephosphorylation which are among the regulatory events that play key roles in oocyte maturation, and include centrosome and microtubule dynamics for the formation of the meiotic spindles during meiosis I (MI) and II (MII) (Ai *et al.*, 2009; Swain and Pool, 2008; Gosden and Lee, 2010; Schatten and Sun, 2011a,b). In the mouse oxidation is significantly up-regulated during both in vivo and in vitro oocyte maturation (Dunning *et al.*, 2010).

LC is an enhancer of lipid metabolism in animal cells including oocytes; it has a role in the transport of fatty acids from the cytosol to the mitochondria to fuel beta-oxidation (Kerner and Hoppel, 2000). Supplementation of culture medium with LC reduced lipid content in bovine oocytes (Chankitisakul et al., 2013). The observed tendency of in vitro maturation in term of full expanded oocytes when recovered from rabbit does treated with LC is in agreement with the results of You *et al.* (2012), who found that immature mammalian COCs matured with LC had improved glutathione level during maturation, indicating that its influence is more prominent on the cytoplasmic maturation rather than nuclear maturation. LC improved the meiotic competence of mammalian oocytes by holding back the apoptosis of granulosa cells and enhancing its mitochondrial activity (Hashimoto, 2009; Somfai *et al.*, 2011).

The current study indicated significant improvement in fertilization in vitro and developmental competence of embryos to reach blastocyst stage due to treatment of rabbit does with CoQ10 or LC. In this respect, Manzano et al. (2015) found that LC supplementation was effective at supporting blastocyst formation and improving its quality as evidenced by having a higher total cell count. Addition of LC to culture medium enhanced lipid metabolism and mitochondrial activity and decreased ROS levels, as well

as improves MII and cleavage rates (Somfai *et al.*, 2011), although the treatment does not impact blastocyst formation.

Inhibition of oxidation compromises meiotic resumption (Downs *et al.*, 2009) and impairs embryonic development formation following fertilization (Dunning et al., 2010), suggesting that lipid metabolism plays essential roles in both nuclear and cytoplasmic maturation in the mouse. Furthermore, enhancing oxidation by supplement LC to the culture medium during in vitro follicle culture and in vitro maturation significantly improves mouse oocyte developmental competence (Dunning *et al.*, 2010, 2011). In addition, Dunning and Robker (2012) demonstrated that LC to in vitro oocyte maturation and embryo growth media improves embryo outcomes, most likely by supplying the oocyte and embryo with an essential co-factor required to utilize fatty acids. Thus in vitro systems must be optimized to include adequate carnitine, in order to mimic in vivo physiological levels. In vivo, circulating carnitine levels are normally regulated within a narrow range and thus dietary carnitine supplementation may only affect tissue levels when carnitine levels are systemically dysregulated. Dunning *et al.* (2011) found that oocytes isolated from the follicles treated with LC had greater rates of maturity (metaphase II/MII), higher fertilization rates and improved blastocyst development. Also, Manzano *et al.* (2015) reported that LC supplementation was effective at supporting blastocyst formation and improving its quality as evidenced by having a higher total cell count.

Regard to the beneficial effects of CoQ10, Mirit Gendelman and Zvi Roth (2012) found that CoQ10 increased the expression of genes associated with the mitochondrial electron transport chain, and improved developmental competence of oocytes. Also, Kishi *et al.* (1993) found that incorporation of CoQ10 into the oocytes enhanced the proportion of embryos that developed to the blastocyst stage.

In vivo study:

Litter size of does and mortality rate of bunnies:

Data presented in Table (4) show that litter size of rabbit does was significantly (P<0.05) higher in G2 and G3 than in G1 at birth (7.29 and 6.71 vs. 5.43/doe), at 21 days of age (6.71 and 5.86 vs. 4.43/doe) and at weaning (6.43 and 5.43 vs. 4.14/doe), being significantly (P<0.05) higher in G2 than in G3 at 21 d and weaning age. Mortality rate of bunnies at birth or weaning was not affected by treatment, being insignificantly the lowest in G2, followed by G3 and the highest in G1. These results indicated beneficial effect of treatment of rabbit does with CoQ10 and LC on their litter size and mortality rate of their bunnies.

Table (4). Litter size of rabbit does and mortality rate of their bunnies as affected by CoQ10 and L-Carnitine treatments.

Item	Experimental group			SEM
	G1(Control)	G2 (CoQ10)	G3 (LC)	
Litter size/doe:				
At birth (alive)	5.43b	7.29a	6.71a	0.286
At 21 days of age	4.43c	6.71a	5.86b	0.261
At weaning (28 days of age)	4.14c	6.43a	5.43b	0.297
Mortality rate (%):				
At birth	17.14	7.40	12.42	3.787
At weaning	22.38	11.57	19.56	4.514

a and b: Means within the same row with different superscripts are significantly different at P<0.05.

Litter weight of bunnies:

Results shown in Table (5) revealed that average bunny weight at birth was significantly (P<0.05) higher in G2 than in G1 and G3. However, average bunny weight at weaning, average litter weight from birth up to weaning and average litter daily gain from birth up to weaning were significantly (P<0.05) higher in G2 and G3 than in G1, being significantly (P<0.05) higher in G2 than in G3. This means that both treatments improved litter size and litter weight of does with further superiority of those treated with CoQ10.

In accordance with improving litter size of rabbit does in both treatment groups, LC was reported to play an important role in improvement of reproduction of buffalo cows (Noseir, 2003) and rams (Noseir and El-Amrawi, 2001). Also, LC has beneficial effects in both productive and reproductive performance of monogastric species including sows (Freemaut et al., 1993; Eder *et al.* (2003), mares (Iben and Leibertseder, 1994) and poultry (Leibertseder and Iben, 1994). In this respect, dietary LC fed during gestation and lactation increased the number of pigs born live (Musser et al., 1999) and number of pigs

weaned (Eder *et al.,* 2001). Since the amount of LC synthesized endogenously does not cover the requirement for maximum sow performance during pregnancy and lactation, dietary LC either increases the ovulation rate or reduces embryonic mortality in sows Eder *et al.* (2003).

An adequate supplementation of LC in animal nutrition enables to overcome an energy bottleneck with minimal performance losses improves energy, fatty and amino acid utilization and decreases nitrogen excretion (Baumgartner and Jacobs, 1999).

Table (5). Litter weight and litter daily gain of rabbit does and mortality rate of their bunnies as affected by CoQ10 and L-Carnitine treatments.

Item	Experimental group			SEM
	G1 (Control)	G2 (CoQ10)	G3 (LC)	
Average kit weight (g):				
At birth	52.29b	57.19a	52.65b	1.519
At weaning	341.1b	443.2a	429.3a	16.03
Average litter weight (g):				
At birth	282.9c	416.4a	353.6b	16.83
At one wk of age	554.3b	903.6a	763.6a	54.81
At two wk of age	771.4c	1382.1a	1137.1b	54.15
At three wk of age	997.1c	1800.0a	1473.6b	62.41
At weaning (4 wk of age)	1402.9c	2837.1a	2277.1b	116.5
Average litter daily gain (g/litter/d):				
Birth ~1st wk	38.78b	69.59a	58.57a	6.726
1st ~ 2nd wk	31.02c	68.37a	53.37b	4.333
2nd ~ 3rd wk	32.25c	59.69a	48.06b	2.362
3rd ~ 4th wk	57.96c	148.2a	114.8b	9.709

a and b: Means within the same row with different superscripts are significantly different at P<0.05.

Also, LC protects cell membrane and DNA against damage (Ye *et al.,* 2010) by reducing the accumulation of ROS, enhancing the activity of numerous antioxidant enzymes, eg., superoxide dismutase, catalase and glutathione peroxidase (Rizzo et al., 2010) and has a pivotal role in mitochondrial oxidation of long-chain fatty acids which increase energy supply to the cell (Zhou et al ., 2007). Moreover, Musser et al. (1999) showed that dietary LC increased the concentrations of insulin and IGF-1 in the blood of sows during pregnancy. Other researchers have observed effects of LC supplementation on IGF system by increasing plasma IGF-I and IGF-II (Doberenz et al., 2005) when LC is supplemented in the diet.

On the other hand, dietary supplementation with CoQ10 may also improve embryo quality (Scott, 2013). CoQ10 increased the ATP content in expanded blastocysts (Stojkovic et al., 1999), stimulated ATP formation in myocardial cells of mouse fetuses (Kishi et al., 1993). Also, dietary supplementation with CoQ10 may increase mitochondrial activity and function within the developing embryo (Bentov et al., 2010). Subsequently, a superior rate of early bovine embryo cleavage, blastocyst formation, percentage of expanding blastocysts, and a larger inner cell mass as affected by CoQ10 (Marriage et al., 2004). In addition, CoQ10 is antioxidant that has great importance against free radicals (Bentinger et al., 2007).

In conclusion, treatment of rabbit does 21 days prior to insemination with CoQ10 at a level of 10 mg/kg LBE or L-carnitine at level 40 mg/kg LBW as daily oral dose is recommended to improve in vitro embryo production and also to increase litter characteristics (size and weight from birth up to weaning) of NEW Zealand White rabbit does.

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حجم البطن والخصائص المبيضية والانضاج والاخصاب المعملى لبويضات امهات الارانب المعاملة با لكوانزيم كيو10والـ كارنيتين

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تهدف هذه الدراسة الى تقييم تأثير الكوانزيم كيو10 والـ كارنيتين على عدد الخلفات والخصائص المبيضية والانتاج المعملى لأجنة الارانب. اجريت هذه الدراسة بالمركز الدولى للتدريب على رعاية الحيوان بسخا التابع لمعهد بحوث الانتاج الحيوانى بمركز البحوث الزراعية . استخدمت فى هذه الدراسة 36 من امهات الارانب النيوزيلاندى البيضاء الناضجة ذات عمر من 5-6 شهور ووزن حى3- 3.5 كجم بالاضافة الى6 ذكور ذات عمر 7.5-8 شهور ووزن حى 3.5-4 كجم. قسمت الامهات الى 3 مجاميع متماثلة (12 أم فى كل مجموعة). المجموعة الاولى كنترول بينما جرعت المجموعة الثانية يوميا بـ 10 ملجم كوانزيم كيو 10 لكل كجم وزن حى وأما المجموعة الثالثة فقد جرعت يوميا بـ40 ملجم الـ كارنيتين لكل كجم وزن حى لمدة 21 يوم لكلا المجموعتين. تم تلقيح جميع المجاميع طبيعيا وتم ذبح خمس امهات فى كل مجموعة بعد التلقيح وذلك لدراسة تأثير المعاملات على الخصائص المبيضية والانضاج والاخصاب المعملى للبويضات . بعد الذبح مباشرة تم أخذ المبايض وجمع البويضات من الحويصلات المبيضية الموجودة على سطح المبيض بطريقة التشريح وعمل تقييم للبويضات و انضاج واخصاب معملى للبويضات الجيدة منها فقط . اما باقى الامهات فى كل مجموعة والتى لم يتم ذبحها وعددها(7) تم تشخيص الحمل فيها بعد 1 -12 يوما من التلقيح كما تم تسجيل عدد الخلفات ووزن الخلفات بعد الولادة وعند الفطام .

اظهرت النتائج زيادة فى متوسط وزن المبيض وعدد الحويصلات المبيضية معنويا فى المجموعة الثانية والثالثة. وقد سجلت الحويصلات المدممة وعدد البويضات المستردة زيادة معنوية فقط فى المجموعة الثانية مقارنة بالمجموعة الاولى . لم يتأثر معدل استرداد البويضات بالمعاملات. كان معدل التوزيع النسبى للبويضات الجيدة والمعراة جزئيا اعلى معنويا بينما كانت البويضات المقبولة والمعراة كليا اقل فى المجموعة الثانية والثالثة مقارنة بالمجموعة الاولى. كان معدل الإنضاج المعلمى للبويضات هو 80.3 و79.3% مقابل 73,8% وكان معدل الاخصاب المعملى هو 67.4 و66.7% مقابل 64.5 % فى المجموعة الثانية والثالثة مقارنة بالمجموعة الاولى. كانت نسبة الأجنة فى مرحلة الموريولا اعلى معنويا فى المجموعة الثانية والثالثة عن المجموعة الاولى (30.3 و3.1 % مقابل 20.0 %) على التوالى . بينما كانت نسبة الاجنة فى مرحلة البلاستوسيست 21.2 و21.4 % مقابل 15.0 % فى المجموعة الثانية والثالثة مقابل المجموعة الاولى على التوالى). كان عدد الخلفات للأمهات فى المجموعة الثانية والثالثة اعلى معنويا عن المجموعة الاولى عند الولادة (7.29 و6.71 مقابل 5.43 لكل ام) وكانت عند عمر 21 يوم (6.71 و5.86 مقابل 4.43 لكل ام) وكانت عند الفطام (6.73 و5.43 مقابل 4.14 لكل ام) ولم يتأثر معدل النفوق للخلفات عند الولادة او الفطام معنويا بالمعاملات . كان متوسط وزن الخلفات عند الولادة اعلى معنويا فى المجموعة الثانية عن المجموعة الأولى. وكان متوسط وزن البطن عند الفطام ومتوسط وزن الخلفات من الولادة وحتى الفطام ومتوسط الزيادة اليومية لحجم البطن اعلى معنويا فى المجموعة الثانية و الثالثة عن المجموعة الاولى.

نستخلص من هذه النتائج ان تجريع امهات الأرانب النيوزيلاندى الابيض يوميا لمدة 21 يوم قبل التلقيح بمستوى 10 ملجم من الكوانزيم كيو10 لكل كجم وزن حى أو بمستوى 40 ملجم الـ كارنيتين لكل كجم وزن حى حسنت انتاج الاجنة معمليا بالاضافة الى زيادة خصائص البطن (عدد ووزن الخلفات من الولادة وحتى الفطام) .