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In Vitro Embryo Production of Doe Rabbits Administrated with Aqueous Garlic Extract

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Evaporative cooling; Passive cooling; Cooling effectiveness; Cooling pads.

ABSTRACT

The aim of the present work was evaluating the effect of daily oral administration of aqueous garlic extract, AGE (5 and 10 mg/kg live body weight (LBW) for 30-day pre-mating on ovarian activity and *in vitro* maturation and fertilization of rabbit does. Rabbit does (n=30) were allocated into three experimental groups (10/group), does in the 1st group (G1) were orally treated with distilled water (2 ml), while those in the 2nd and 3rd groups were orally treated with 5 mg AGE/kg LBW (G2) or 10 mg AGE/kg LBW (G3) dissolved in 2 ml distilled water. Does were daily administrated before morning feeding for 30 days as a treatment period. Results indicated insignificant effect of AGE on LBW, while absolute or relative ovarian weights were higher (P<0.05) by 31.6 and 36.7% in G2 and G3 compared with G1. AGE administration induced an increase (P<0.05) in large follicles number, yield, recovery rate, and quality of oocytes, the percentage of embryos at morula stages. Daily oral administration of 10 mg AGE/kg LBW pre-mating improved ovarian activity and both *in vitro* maturation and fertilization of rabbit does. This can be used as a tool for *in vitro* embryo production in rabbits with different breeds or lines.

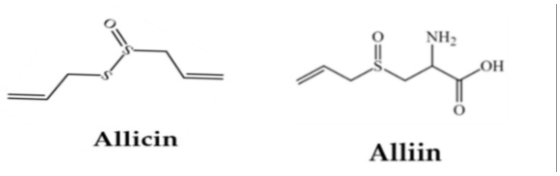
1. INTRODUCTION

Herbs are used worldwide as a therapeutic tool in case of infertility because the herbs are cheaper and more available than other chemical treatments (**Rezaeian, *et al.*, 2016**). Medicinal herbs are characterized by high antioxidant compounds and used for treating some reproductive disorders in males (**Mohammadi *et al.*, 2013; Aliakbari *et al.*, 2016; Amidi *et al.*, 2014**). Some plants have compounds affecting sex hormones while others have androgenic effects on the hypothalamus-pituitary

axis to stimulate sex hormones (**Bastampoor *et al*., 2014).**

Today, there is great attention towards the usage of phytobiotics to improve the production of animals due to their antioxidant and antimicrobial properties **(VamsiDuvvu *et al.*, 2018)**. They also have immunomodulatory and anti-inflammatory activities **(Hanieh *et al.*, 2010)** as well as action against hyperlipidemic **(Al-Shuwaili *et al.*, 2015; Muthamilselvan et al, 2016)**, toxicity, and hypercholesterolemic properties **(Lanzotti, 2008)**. Moreover, phytobiotics have ability to inhibit lipid peroxidation and promote antioxidant enzyme activities **(Marsoul *et al.*, 2016).**

Garlic (*Allium sativum*), as one of the phytobiotics, is an important and essential herb for several purposes of medicinal treatment **(Adulugba *et al.*, 2017)**. Garlic belongs to alliaceae and has a great history as a food flavor and a medicinal herb **(Rahman, 2007)** for its medicinal potential prominent **(Bahrami et al., 2014)**. Very important compounds such as: sulfurs, allicin, vitamins (B_2, B_6, B_1, A, C), flavonoids, several antioxidants (**Bahrami *et al*., 2014**). Garlic has the ability for hepatic protection, and is characterized by antithrombotic, antihypertensive and antimicrobial activities (**Rahman, 2007**), and acts like insulin receptor to decrease level of glucose **(Roozbeh *et al*., 2016**). Allicin is the main biological compound in aqueous garlic extract **(Vaidya *et al.*, 2009)** having the ability to linking the free-radicals for protection against stress **(El-katcha, *et al.*, 2016).** The key role of the garlic extract as a medicinal herb is containing allicin (dialklythiosulfinate) forming by action of allinase on alliin (*S*-alkyl-l-cysteine sulfoxide) according to **Rahman (2007**) as the following:



There are several factors affecting animal physiology and pathology that lead to a reduction in the reproduction of intensive rabbit production **(Castellini, 2007)**.Oxidative stress induced by stressors of metabolism, environment, or nutrition leads biologically to change in animal cells **(Ibrahim *et al.*, 2012)**. These changes include kidney and liver dysfunctions **(Akidwu *et al.*, 2013)**, disorders in LH and FSH hormones, incidence of lipid peroxidation and reducing activity of antioxidant enzymes **(Al-Masri, 2015)**. Under heat stress condition, resulted in decreasing the productivity of rabbit does and leading to substantial economic loss **(Attia *et al.*, 2009)** and dietary antioxidant supplementation prevents the damage in antioxidant defense system by scavenging free radical during lipid per-oxidation **(Mittler *et al.*, 2004).** Several types of natural antioxidants were used to improve the reproductive performance of doe rabbits **(El-Ratel *et al.*, 2017)** including allicin, as a strong natural antioxidant, to enhance the activities of endogenous antioxidant enzymes and decrease the deleterious effects of oxidative stress on doe rabbits **(Alam *et al.*, 2018).** Also, *in-vivo* ovarian activity, health and immune response of doe rabbit were improved by allicin **(El-Ratel et al. 2020)**. The effect of antioxidant like allicin or garlic extracts on *in-vitro* maturation and fertilization of rabbit oocytes is rarely studied as a tool for understanding the role of antioxidant in reproduction of females**.** (**Baniaghil *et al*., 2016)**

Therefore, the current experiment aimed to study the pre-treatment impact of AGE on ovulatory response, in-vitro maturation and fertilization and embryo production in rabbit doe as a laboratory model animal.

2. MATERIALS AND METHODS

This study was conducted according to the scientific co-operation between Department of Animal Production, Faculty of Agriculture, Tanta University and Animal Production Research Institute (APRI) belonging to Agricultural Research Center, Ministry of Agriculture, Egypt.

2.1 Animals:

In our experiment, the experimental animals included thirty sexually mature Niliparous New Zealand White (NZW) rabbit does. They 3.1 - 3.5 kg LBW and about 6 mos of age. They were housed individually in wire cages supplied with drinking nipples and feeders. They were kept in a private rabbit farm in Mansoura City, Dakahlia Governorate, Egypt under similar management, environment and hygienic conditions.

2.2Feeding system:

Feeding all experimental animals was ad libitum on complete feed diet (CFD) as commercial diet to cover the requirements of doe rabbits as recommended nutrient and physiology by **NRC (1994).** Ingredients and chemical composition of CFD are shown in Table 1. The standard methods of **AOAC (2012)** were performed for the chemical analyses of CFD.

Table 1. Ingredients and chemical analysis of the control diet used for feeding rabbits in the experimental treatments

Ingredient	%	Ingredient	%	Chemical analysis	%
Clover hay	30.0	DL-Methionine	0.20	Organic matter	93.15
Soybean meal (44%)	18.0	Common salt	0.50	Crude protein	18.15
Wheat bran	24.6	Minerals[1]	0.15	Crude fiber	10.19
Barley grain	21.0	Vitamins[2]	0.15	Ether extract	2.600
Molasses	3.00	Di-Calcium phosphate	1.40	Nitrogen free extract	62.21
Limestone	1.00	Total	100	Ash	6.850

[1] Each 1kg contains on Vitamin A (150, 000 UI), Vitamin E (100 mg), Vitamin B1(10 mg), Vitamin K3 (21mg), Vitamin B2 (40mg), Vitamin B6 (15mg), Vitamin B12 (0.1mg), Pantothenic acid (100 mg) Niacin (200 mg), Biotin (0.5mg), Folic acid (10mg) and Choline chloride (5000 mg). [2] Each 1kg contains on manganese (800 mg), zinc (600mg), iron (300 mg), copper (40m g), iodine (500 mg), selenium (100 mg), and cobalt (100 mg).

2.3 Experimental design:

Total of thirty rabbit does were divided based on LBW into 3 groups, 10 animals in each). Animals (n=10) were orally received 2 ml distilled water served as control group. Animals were orally administrated with 5 mg AGE/kg LBW in the 2nd group or 10 mg AGE/kg LBW in the 3rd group. Each treatment of AGE (Anhui Ruisen Biological Technology Co., China) in the 2nd and 3rd group (5 and 10 mg/kg LBW) was dissolved in 2 ml distilled water. Does were treated morning pre daily feeding for a treatment period of one month.

2.4 Collection, recovery rate, and evaluation of oocytes:

At the termination of the treatment period, total of 15 doe rabbits were slaughtered, as oocyte donors. Pre slaughter weight of does was recorded and ovaries were immediately removed after slaughter, collected, and washed by excised submerged in saline solution (0.9 % NaCl) in flacon plastic tissue culture dishes (60 x 15 mm) at 38.5°C, then dried by cleaning paper.

Weight of the right and left ovaries of each doe rabbit was recorded, and follicular number

(more than 1 mm in diameter) on the surface of the ovaries was counted on the right and left side of each doe. Oocyte collection was achieved by slicing technique into glass Petri dishes with 4 ml harvesting medium (Gordon, 1994). The harvesting medium was phosphate buffer saline (PBS) supplemented with bovine serum albumin (2 mg/ml), sodium penicillin G 100 IU/ml and streptomycin 100 µg/ml (Misr Co. for Pharm., Egypt). The harvesting medium (Table 1) was adjusted for pH value (7.2-7.4) and osmolality level (280-300 m Osmol/kg), then filtered (0.22-µm millipore filter, Milieux GV, milpore, Cooperation Bedford MOA).

Table 2. Composition of harvesting medium.

Item	g/L	Item	Level
$CaCl_2$. $2H_2O$	0.133	Glucose	1.0 (g/L)
$MgSO_4 \cdot 7H_2O$	0.120	Sodium pyruvate	0.036 (g/L)
NaCl	8.000	Streptomycin	100 (mg)
KCl	0.200	Sodium Penicillin G	100,000 (IU)
$NaHPO_4$	2.170	-	-

The recovered oocytes by slicing were exposed to washing for 3 times in Petri dishes by the harvesting medium for determination the number of oocytes/doe, the recovery rate was calculated using the following formula: ***Oocyte recovery rate = (Oocyte number /follicular number) x 100.***

Stereomicroscopy was used for oocyte examining, then oocytes were classified into five categories. Compaction of the cumulus layer, number of cumulus cell layers, and homogeneity of ooplasm were the basis of classification according to **Ravindranatha *et al.*, (2003)**. Cumulus oocytes–complexes COCs: with ≥3 layers' compact cumulus cells and homogenous ooplasm; denuded oocytes: completely devoid cumulus cells with heterogeneity ooplasm; expanded oocytes: with expanded cumulus cells; Partial denuded oocytes: with incompletely surrounding cumulus cells. Only, COCs were under-went *in vitro* maturation in this study, according to **Nadri *et al.*, (2009)**.

2.5 In vitro maturation (IVM):

Tissue Culture Medium (TCM-199) supplemented with 0.3% bovine serum albumin (w/v) , sodium penicillin G (100 IU), streptomycin (100 µg) and Na pyruvate (11 mg)/dl of TCM-199 was used as a maturation medium.

TCM-199 was adjusted for the pH value to 7.2-7.4 and osmolality level of 280-300 mOsmol/kg, then filtered (0.22-µm Millipore filter). In four well dishes, the prepared TCM-199 (500 µl) was placed, then dishes were incubated in CO_2 incubator at 38 °C in condition of 5% CO_2 with saturated humidity for 60 min as an equilibration period before placing the oocytes in TCM-199. Thereafter, these dishes were covered by sterile mineral oil.

Only compact oocytes (COCs) were used for *in-vitro* maturation after washing 3 times by TCM-199. The culture dishes with oocytes and TCM-199 were incubated at 38 °C in condition of 5% CO_2 with saturated humidity for twenty hours.

At the termination of the culture period for 20 hours, cytoplasmic maturation in terms of degree of expansion of the matured oocytes was examined and oocytes were classified into oocytes with full expansion: all cumulus cells were loosened, partial expansion: only the outer cumulus layer of the oocytes was loosened, and no expansion: oocytes without any changes in the cumulus layer.

2.6 In vitro fertilization:

In-vitro matured oocytes were in-vitro fertilized in this study. About 50 µl of fertilization medium (Table 3) was pipetted under sterile liquid paraffin oil, and then the droplets of fertilization were incubated for two hours at 38°C in 5% Co_2 in air and high humidity. The prepared semen (2 µl) was incubated with washing medium (5 µl) containing oocytes (n=7-10) for 24 hours at 38°C in 5% Co_2 in air, then rate of in-vitro fertilization and embryonic stages after 72 h was investigated.

Table 3. Composition of fertilization medium.

Ingredient	mM	Concentration mg/100ml
NaCl	114	666
KCl	3.2	23.5
NaHCO3	25.0	210.4
NaH2PO4.2H2O	0.4	5.52
Lactate acid	12.4	60% syrup 186 µl
$MgCl_2$-$2H_2O$	0.5	10
$CaCl_2.6H_2O$	2.1	30
Penicillin	-	6.5
Streptomycin sulfate	-	0.071
Bovine serum albumin	3.0	-
Pyruvic acid	1.25	0.110
Phenol Red	--	1
Distilled water to 1000 ml		

2.7 Criteria of fertilized oocytes:

Inverted microscope was used to evaluate the fertilized oocytes, which were classified into normal: with pronounced pro-nuclei of male and female as well as presence of the 2nd polar body in the cytoplasm and abnormal: with chromatin fragmentation (scattered chromatin) the cytoplasm and these oocytes were considered as degenerated or unfertilized oocytes. Also, the normal fertilized oocytes were examined by inverted microscope to classify embryonic stages including 2-cell, 4-cell, 8-16-cell, morula, and blastocyst after 72 h of culture.

2.8 Statistical analysis:

One-way ANOVA by a software package (SAS, 2000) was used to the statistical analysis of the obtained data according to the following model:

$$Yij = \mu + Gi + eij,$$

Where; µ = Overall mean, Gi = Treatment (1-3), and eij = Random error. Duncan's multiple range test (Duncan 1955) was used

to separate significant effect of treatment. Data were tabulated as mean standard error and the differences were set at $P<0.05$.

Chi-Square test was used to test the recovery rate, and proportion of oocytes at different maturation and embryonic stages.

3. RESULTS AND DISCUSSION

Effect of treatment of doe rabbits with aqueous garlic extract (AGE) on:

3.1Ovarian characteristics

Effect of AGE treatment on ovarian characteristics of NZW doe rabbits is presented in Table 4. There were non-significant differences in LBW of does in the experimental groups. The statistical analysis revealed that ovarian characteristics including

ovarian weights and ovarian weight relative to LBW of doe rabbits were affected significantly (P<0.001) by AGE treatment, being significantly (P<0.05) higher G2 and G3 as compared to G1 (control). These results indicated that ovarian weight or relative ovarian weight significantly increased by 31.6 and 36.7% when doe rabbits were treated with 5 and 10 mg/kg LBW as compared to control, respectively.

Table 4. Effect of AGE administration on ovarian weight of NZW does

Item	Experimental group			
	G1 (control)	G2 (5 mg/kg)	G3 (10 mg/kg)	P-value
Average weight				
Doe rabbit (g)	3010±6.27	3005±6.63	3003±9.21	0.795^{NS}
Ovaries/doe (g)	0.49±0.01^{b}	0.64±0.02^{a}	0.67±0.01^{a}	0.000 ***
ROW (g)/kg BW	0.16±0.005^{b}	0.21±0.007^{a}	0.22±0.005^{a}	0.000***

$^{a \text{ and } b}$: Means within the same row with different superscripts are significantly different at P<0.05. ^{NS}: Not significant. *** Significant at P<0.001. ROW: relative ovarian weight (g)/kg BW.

3.2Number of ovarian follicles:

Effect of AGE treatment on follicular number per ovary of does is presented in Table 5. The statistical analysis revealed that only the large follicles number was affected significantly (P<0.05) by AGE treatment, while small and total follicles numbers were not affected by AGE treatment. Treatment of AGE at the highest dose in G3 (10 mg/kg LBW) showed significance (P<0.05) a great number of the large follicle than the lower AGE dose (G2) and the control (G1). However, the lower AGE dose in G2 (5 mg/kg LBW) showed increased number of the large follicles, but did not differ significantly from G3 and G1. On the other hand, number of small and total follicles tended to be slightly lower in G2 and G3 than in G1, without significant group differences.

Table 5. Effect of AGE administration on number of follicles/doe of NZW does

Item	Experimental group			
	G1 (control)	G2 (5 mg/kg)	G3 (10 mg/kg)	P-Value
Follicles number/doe				
Large follicles	35.29±1.87^{b}	41.43±1.34^{ab}	43.57±2.03^{a}	0.010**
Small follicles	14.57±0.97	12.28±1.95	14.14±2.14	0.630^{NS}
Total follicles	49.86±2.21	53.71±2.58	57.71±2.26	0.089^{NS}

$^{a \text{ and } b}$: Means within the same row with different superscripts are significantly different at P<0.05. ^{NS}: Not significant. ** Significant at P<0.05.

3.3 Yield and recovery rate of oocytes

Effect of AGE treatment on oocyte yield and oocyte recovery rate of NZW doe rabbits is presented in Table 6. Results showed that AGE treatment significantly (P<0.05)

increased yield and recovery rate of oocytes. Oocyte yield and recovery rate were significantly (P<0.05) higher in G2 and G3 than in G1.

Table 6. Effect of AGE administration on number and recovery rate of oocytes in NZW does

Item	Experimental group			
	G1 (control)	G2 (5 mg/kg)	G3 (10 mg/kg)	P-Value
Oocyte yield				
Oocytes (n)/doe	40.29±1.42[b]	47.43±1.52[a]	50.57±1.51[a]	0.045[*]
Recovery rate (%)	80.04±3.26[b]	88.28±1.80[a]	87.49±1.98[a]	0.042[*]

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

3.4Category of the recovered oocytes:

Effect of AGE treatment on categories of the recovered oocytes in NZW doe rabbits is shown in Table 7. Analysis of variance revealed that proportions of compact oocytes (P<0.001) and denuded (P<0.001) oocytes were affected significantly by AGE treatment. However, proportion of partial denuded oocytes was not affected significantly by AGE treatment. Results showed that proportion of compact oocytes significantly (P<0.05) increased, while that of denuded oocytes significantly (P<0.05) decreased by both AGE doses as compared to control. Proportion of compact oocytes was the most frequent as compared to that of denuded or partial denuded oocytes in all groups.

Table 7. Effect of aqueous garlic extract administration on oocytes category of NZW does

Oocytes category	Experimental group					
	G1 (control)		G2 (5 mg/kg)		G3 (10 mg/kg)	
	n	%	N	%	n	%
Compact	27/41	65.85[b]	38/48	79.17[a]	42/52	80.76[a]
P. denuded	4/41	9.76	7/48	14.58	6/52	11.53
Denuded	10/41	24.39[a]	3/48	6.25[b]	4/52	7.69[b]

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

3.5 Oocyte maturation rate:

Effect of AGE treatment on *in vitro* cytoplasmic maturation of NZW doe rabbits is shown in Fig. 1. Analysis of variance revealed that proportions of full-expanded, partial expanded, and no expanded oocytes were affected significantly (P<0.001) by AGE treatment. After *in vitro* maturation of the recovered oocytes, percentage of full-expanded oocytes significantly (P<0.05) increased, while percentage of oocytes of partial expanded and without expansion significantly (P<0.05) decreased by both AGE doses, being insignificantly better with the high than low AGE doses.

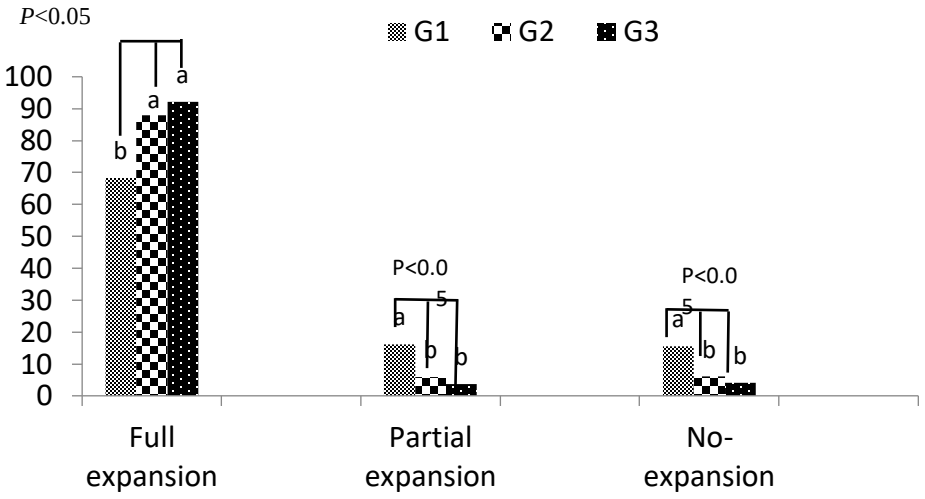


Fig. 1. Oocyte stages of *in vitro* cytoplasmic maturation of doe rabbits treated with different AGE levels. (P<0.05: significant group differences)

3.6 In vitro fertilization:

Effect of AGE treatment on *in-vitro* fertilization of *in-vitro* mature oocytes of NZW doe rabbits is shown in Table 8. Analysis of variance of AGE revealed that fertilization rate and the percentage of embryos at 8-16 cell, and degenerated oocytes were affected significantly (*P*<0.001) by AGE treatment. However, embryos at 2-cell, 4-cell, morula, and blastocyst stages were not affected significantly by AGE treatment.

After *in-vitro* fertilization of *in vitro* mature oocytes, both AGE levels in G2 and G3 significantly (P<0.05) increased fertilization rate, while significantly (P<0.05) reduced the degenerated oocytes as compared to control. However, the highest AGE level in G3 increased the percentage of embryos at morula and blastocyst stage, but the differences were not significant. On the other hand, percentage of embryos at 8-16 cell stage significantly (P<0.05) increased only in G2 (Table 8).

Table 8. Effect of AGE administration on embryonic stages after fertilization of *in vitro* mature in NZW does.

Embryo stage	Control		Aqueous garlic extract			
	G1		G2		G3	
	N	%	N	%	N	%
Fertilized	11/18	61.11[b]	25/32	78.13[a]	31/39	79.49[a]
2-cell	1/11	9.09	2/25	8.00	2/31	6.45
4-cell	1/11	9.09	2/25	8.00	4/31	12.90
8-16 cell	1/11	9.09[b]	6/25	24.00[a]	3/31	9.68[b]
Morula	4/11	36.36	8/25	36.00	13/31	41.93
Blastocyst	2/11	18.18	5/25	20.00	7/31	22.58
Degenerated	2/11	18.18[a]	2/25	8.00[b]	2/31	6.45[b]

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

4. DISCUSSION

The phytobiotics have attracted major attention in animal production due to their different biological activities, including antioxidant, antimicrobial **(VamsiDuvvu, *et al.*, 2018)**, immune modulatory, anti-inflammatory **(Hanieh *et al.*, 2010)**, hypo lipidemic **(Al-Shuwaili *et al.*, 2015; Muthamilselvan et al., 2016)**, anti-toxicity, hypo cholesterolemic properties **(Lanzotti, 2008)**, inhibition of lipid peroxidation and promotion of the activities of antioxidant enzymes **(Marsoul *et al.*, 2016).** Among these phytobiotics, garlic (*Allium sativum*) is one of the most essential and useful herbs for various medicinal purposes **(Adulugba *et al.*, 2017).** The objective of this study was the impact of daily oral administration of two levels of AGE (5 and 10 mg/kg LBW) for 30-day pre-mating on ovarian activity, and *in vitro* maturation and fertilization of oocytes recovered from rabbits. The obtained results indicated that ovarian weight or relative ovarian weight significantly increased by
31.6 and 36.7% when doe rabbits were treated with 5 and 10 mg/kg LBW as compared to control, respectively. Increasing ovarian eight and relative ovarian weight was attributed to the greatest number of the large follicles and the lowest number of small follicles in in treatment groups than in control one (P<0.05). The obtained results also indicated increasing oocyte yield and recovery rate in treatment groups in association with the observed greater number of large follicles in each group. IN this respect, number of recovered oocytes and recovery rate was reported to be extremely affected by the follicular size or/and to small number of oocyte donors. Further, our study indicated positive impact of both AGE levels on improving quality of the recovered oocytes by increasing proportion of compact oocytes and decreasing the proportion of other fair or undesired oocytes. In accordance with the positive impact of AGE in our study on ovarian characteristics, **Younan *et al*. (2015)** found that treatment of doe rabbits, 21 days prior to insemination, with coenzyme Q10 at a level of 10 mg/kg LBW or L-carnitine at level 40 mg/kg LBW as daily oral dose, increased ovarian weight, number of large follicles, number of bleeding follicles and recovered oocytes as compared to control. Also, injection of hydro-alcoholic green tea extract as antioxidants in 10 days increased the

number of follicles at various stages. It also increased the number of corpus luteum and reduced the number of cystic follicles (**Ghafurniyan *et al.*, 2015).** Improving oocyte quality as affected by AGE was reported on embryo quality as reported by **El-Ratel *et al.*, (2020)**, who found increase in yield and recovery rate of embryo in rabbits as affected by allicin treatment. They added that this may be due to a direct effect of allicin aa a main compound in AGE on ovarian tissues and/or indirect effect on healthy status and immunity of rabbit does. Also, **Abdel-Khalek *et al.*, (2016)** showed that daily oral administration of doe rabbits with Coenzyme Q10 or L-Carnitine, as antioxidants, can improve quality of recovered rabbit embryos.

In the tropic, heat stress impairs animal performance, causing a substantial loss to animal production elsewhere; thus, agents that improve the outstanding of animals to heat stress are of great interest **(Marai *et al.*, 2003).** The superiority of rabbits in treatment groups may be due to a variety of physiological and pathological factors causing a decrease in reproductive activity of intensively farmed rabbits **(Castellini, 2007).** Exposing to metabolic, environmental or nutritional stressors, induces oxidative stress may alter several biological activities, cellular and intracellular levels **(Ibrahim *et al.*, 2012)**, increases damage of kidney and liver functions **(Akidwu *et al.*, 2013),** gonado-pituitary hormone (LH and FSH) disturbance, increases lipid peroxides and decrease enzymatic antioxidant activity **(Al-Masri, 2015)**. These conditions leads to reduced reproductive and productive performance of doe rabbits, and consequently results in substantial economic loss **(Attia *et al.*, 2009).** Based on the present results, AGE administration may suggest beneficial effects on oogenesis and ovarian activity of doe rabbits**.** Antioxidants have the ability to prevent cellular damage in antioxidant defense system by counteracting the oxidants and other cellular protection **(Mittler *et al.*, 2004).** There are many natural antioxidants, which play a vital role in enhancing reproduction and health status of doe rabbits **(El-Ratel *et al.*, 2017).**

The medical properties of garlic are due to contain garlic allicin (dially thiosulfinate) as one of various biological organosulfur. Garlic also contains several enzymes (allinase, per oxidases and myrosinase), essential amino acids, volatile oils, steroidal glycosides, anthocyanins, lectins, prostaglandins, fructan, pectin, adenosine, vitamins (B1, B2, B6, C and E), biotin, nicotinic acid, fatty acids, glycolipids and phospholipids **(Calvo-Gomez *et al.*, 2004; Bozin *et al.*, 2008**). Garlic contains a lot of antioxidants, flavonoids, sulfur compounds and allicin (**Bahrami *et al.*, 2014**). Allicin (dialklythiosulfinate) plays a key role in the garlic medicinal properties, however, this compound is not found in fresh garlic, and made by action of allinase on alliin **(El-Ratel *et al.*, 2020)**. Garlic protects the liver and has anti cholesterolemic, antithrombotic, antihypertensive and antimicrobial activity (**Rahman, 2007**), and consumption of garlic acts like insulin receptor and reduces glucose levels in diabetic patients (**Roozbeh *et al.*, 2016**). Allicin is the main biologically active component of aqueous garlic extract **(Vaidya *et al.*, 2009)** decomposes to form 2-propene sulfenic acid, and this compound is capable of binding the free radicals as an agent against stress **(El-katcha *et al.*, 2016).** In this respect, the essential components of garlic, like allicin, is considered as a strong natural antioxidant to increase the endogenous antioxidant enzyme activity and reduce inflammation and oxidative stress of male rabbits **(Alam *et al.*, 2018).** The hypoglycemic and hypo-lipidemic effects of allicin were proved in broilers **(Singh *et al.*, 2017)** and pigs **(Omojola *et al.*, 2009).** Allicin also reduces enzyme activity of kidney and liver and improves immune response of broiler chicken **(El-katcha *et al.*,**

2016). Several authors indicated the save use of allicin as a natural antioxidant, having properties for scavenging ROS (**El Katcha *et al.*, 2016**), anti-microbial, anti-inflammation, immunomodulatory and hypoglycemic (**Viswanathan *et al.*, 2014; Indrasanti *et al.*, 2017**). These properties characterized allicin as a garlic extract with many biological activities for good health status (**Alam *et al.*, 2018**). **Barakat *et al.* (2014**) showed that antioxidant acts as a direct scavenger of toxic oxygen derivatives and has the ability to reduce the formation of ROS.

The present study indicated also that treatment of doe rabbits with both AGE doses showed acceptable *in-vitro* cytoplasmic maturation rate (88-92%) in comparison with control (68%). Also, the highest AGE level had positive impact on embryo production of doe rabbits in terms of increasing fertilization rate, and the percentage of embryos at morula and blastocyst stages, while decreased the percentage of degenerated ova. *In-vivo* embryonic development may be influenced by the ROS produced in the female genital system (**Bedaiwy *et al.*, 2002**). According to **Du Plessis *et al.* (2008)**, *in-vivo* embryos rely on mitochondrial oxidative phosphorylation for energy, a process which is subsequently accompanied by ROS generation. The antioxidant capacity of the embryos against the harmful assault of oxidation, because the fast-developing embryo produces energy via ATP generation through mitochondrial oxidative phosphorylation and glycolysis (**Agarwal *et al.*, 2014**). As it develops, the embryo is capable of producing ROS through several pathways, namely oxidative phosphorylation, NADPH and xanthine oxidase systems (**Guerin *et al.*, 2001**). The pronounced improvement in oocyte quality of does treated with AGE is associated with improved embryo quality by dietary supplementation with antioxidant such as CoQ10 in human (**Scott, 2013**) or by in vivo administration of the antioxidant epigallocatechin gallate (EGCG) in mouse (**Roth *et al.*, 2008**). This finding concurs with the results observed in the current study may be justified the establishment of supplementation antioxidants allicin promoted equilibrium between oxidative agents and the anti-oxidative system, which may have improved embryo quality. In accordance with the present results, **Roth *et al.*, (2008)** reported that *in-vivo* administration of EGCG as antioxidant improved developmental competence of mouse embryos. Also, **Cerri *et al.* (2009)** found that increased selenium of the re-productive tract in dairy cows may improve competence of embryo development, pregnancy and fetal development. This was indicated in the current study in doe rabbits treated with allicin. Furthermore, impact of different types of antioxidant in *in-vitro* culture medium, on developmental competence of embryos was proved by several workers. In this line, addition of melatonin at a level of 10−6 M for rabbit embryos (**Mehaisen and Saeed, 2015**), GT polyphenols for bovine embryos (**Wang *et al.*, 2013**), and LCarnitine (**Abdelrazik *et al.*, 2009**) and vitamin C at a level of 50 µmol/ L for mouse embryos (**Wang *et al.*, 2002**). markedly improved embryo development rate in vitro. Improving the developmental competence of embryos produced from doe rabbits treated with AGE may be attributed to those exogenous antioxidants activities of phenolic compounds in garlic due to their structure and particularly ability to donate a hydrogen ion to the proxy radical generated as a result of lipid peroxidation **(El-Ratel *et al.*, 2020)**. Also, **Agarwal *et al.*, (2003)** mentioned that *in-vitro* blastocyst formation is suboptimal, and supplementation with

antioxidants may improve blastocyst development. According to the present results and the previous findings, antioxidants are likely to play a significant role in preventing subsequent loss or damage to the embryo (**Abdelrazik *et al*., 2009**).

5. CONCLUSION

Daily oral administration of 10 mg aqueous garlic extract/kg LBW pre-mating improved ovarian activity, and both in-vitro maturation and fertilization of rabbit does. This can be used as a tool for in-vitro embryo production in rabbits with different breeds or lines.

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إنتاج أجنة الأرانب معمليا باستخدام المستخلص المائي للثوم

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الملخص

كان الهدف من الدراسة هو تقييم تأثيرالمعاملة بالمستخلص المائي للثوم (AGE) لمدة 30 يوم بمعدل (5 ، 10 مجم/ كجم وزن حي) وذلك على نشاط المبيض ، والإنضاج والإخصاب المعملي لأمهات الأرانب. تم تقسيم الأرانب (ن = 30) إلى ثلاث مجموعات تجريبية (10 / مجموعة) ، تم معاملة المجموعة الأولى (G1) عن طريق الفم بالماء المقطر (2 مل) ، بينما تم معاملة المجموعة الثانية والثالثة عن طريق الفم مع 5 مجم AGE / kg LBW (G2)أو 10 مجم AGE / kg LBW (G3) مذاب في 2 مل من الماء المقطر.أشارت النتائج إلى عدم وجود تأثير معنوي (AGE)على LBW لكن الاوزان المطلقة أو النسبية للمبيض كانت أعلى($P<0.05$)بنسبة 31.6 و 36.7٪ في G2 و G3 مقارنة معG1 ،أدت المعاملة بالمستخلص المائي للثوم إلى زيادة عدد الحويصلات المبيضية الكبيرة ، إنتاج البويضات ، معدل الاسترداد ، نوعية البويضات ونسبة الأجنة في مراحل الموريولاوالبلاستوسيست

نستخلص من هذه الدراسة ان معاملة الأرانب بمستوى 10 ملجم من المستخلص المائي للثوم يوميًا عن طريق الفم / كجم LBW قبل التزاوج إلى تحسين كل من النشاط المبيضي والإنضاج والإخصاب المعملي لأمهات الأرانب وان استخدام المستخلص المائي للثوم كأداة لإنتاج الأجنة معمليا في مختلف السلالات.