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EFFECT OF NIGELLA SATIVA (AQUAS EXTRACT) ON THE VIABILITY OF RABBIT SPERMATOZOA, FERTILITY TRAITS AND BACTERIAL CONTAMINATION

(With 6 Tables)

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تأثير المستخلص المائي لحبة البركة على حيوية حيامن الأرانب والخصوبة وكذلك التلوث الميكروبي للقذفة المنوية

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أستخدم في هذه الدراسة عدد (٣٦) ذكر أرانب كاليفورنيا ناضجا جنسيا، على عمر ١٢ شهرا. اشتملت الدراسة على تجربتين أساسيتين: في التجربة الأولى تم جمع السائل المنوي اصطناعيا وتخفيفه بمخفف اللاكتوزسترات الصوديوم ثم أضيف إليه المستخلص المائي لحبة البركة على مستويات (٠، ٥٠، ١٠٠، ١٥٠، ٢٠٠ أو ٢٥٠ ميكرو مل/ مل) وتم حفظه على درجتي حرارة الثلاجة (٤ـ٦ °م) لمدة ثلاثة أيام، أو التحضين على ٣٧ °م لمدة أربعة ساعات وكانت الخصائص الطبيعية للسائل المنوي تقدر خلال فترات الحفظ المختلفة. صممت التجربة الثانية لدراسة تأثير إضافة المستخلص المائي لحبة البركة على المحتوى الميكروبي الموجود في السائل المنوي المخفف وكذلك تم تقدير معدلات خصوبة الإناث الملقحة اصطناعيا باستخدام السائل المنوي المخفف والمضاف إليه المستخلص المائي لحبة البركة. ويمكن تلخيص النتائج المتحصل عليها كما يلي:- إضافة المستخلص المائي لحبة البركة إلى سائل منوي الأرانب المخفف حسن معنويا (على مستوى ٥%) في خصائصه الطبيعية خلال فترات الحفظ على درجتي حرارة الثلاثة (٤ـ٦ °م) لمدة ثلاثة أيام، أو التحضين على ٣٧ °م لمدة أربع ساعات، أظهرت النتائج أن قيم كل من المقدرة التخزينية للسائل المنوي ومقاييس جودته كانت مرتبة ترتيبا تنازليا كنتيجة لإضافة المستخلص المائي لحبة البركة على مستويات (٢٥٠ و ٢٠٠)، ١٥٠، ١٠٠، ٥٠ ثم صفر ميكرو مل/ مل، على الترتيب. لوحظ انخفاض معنوي (على مستوى ٥%) في جودة السائل المنوي بتقدم زمن الحفظ على درجتي حرارة الثلاجة والتحضين. أدى استخدام المستخلص المائي لحبة البركة مع المضاد الحيوي (البنسلين والإستربتوميسين) إلى تأثير مثبط (على مستوى ٥%)

على المحتوى الميكروبى فى السائل المنوى المخفف. كانت قيم كل من معدل الولادات وعدد ووزن الخلفـات الملقحـة اصطناعيا باستخدام السائل المنوي المخفف والمضاف إليه ٢٠٠ مـيكرومل مسـتخلص حـبة البركة / مل معنوي (علي مستوى ٥%) أفضل من مجموعة المقارنة.

SUMMARY

Thirty-six sexually mature bucks of Californian rabbits (12 months age) were used in three experiments. In experiment 1, semen was collected artificially, pooled and extended with lactose-yolk citrate extender. Nigella sativa extract was added to the extended semen at levels 0, 50, 100, 150, 200 or 250 µl / ml, then the extended semen was stored at refrigeration temperature (4-6 ^{0}C) for up to three days or incubated at 37 ^{0}C for up to four hours. Percentages of sperm motility, dead spermatozoa and acrosme damages were recorded at the different stages of the preservation. Experiment 2 was planned to evaluate the effect of Nigella sativa extract on some bacteria present in extended rabbit semen .In experiment 3, the does were inseminated artificially by using either the extended semen free or supplemented with 200 µl Nigella sativa extract (the lowest dose which showed the best effects on semen quality) to estimate the fertility traits. The results obtained showed that, supplementing Nigella sativa extract to the extended rabbit semen improved significantly ($P \leq 0.05$) the sperm motility and viability while the percentages of dead spermatozoa and acrosome damages were decreased ($P \leq 0.05$) during chilled storage at (4-6 ^{0}C) for up to three days or incubation at 37 ^{0}C for up to four hours. Semen quality and storagability were arranged discendingly as obtained by supplemented extended semen with 250, 200, 150, 100, 50 µl Nigella sativa extract /ml than control. Semen quality decreased significantly ($P \leq 0.05$) with progression of time at different preservation temperatures. Nigella sativa extract at level 200 µl combined with antibiotics (500 IU sodium penicillin + 500 µg streptomycin sulphate/ 1 ml extended semen showed better inhibitor effect on the microbial count compared with Nigella sativa or antibiotics treatment alone. Kindling rate and litter size and weight at birth values were significantly ($P \leq 0.05$) better in rabbit does inseminated artificially by using extended semen and supplemented with 200 µl/ ml Nigella sativa extract than those inseminated by using extended semen free from Nigella sativa (control group).

Key words: *rabbits, Nigella sativa, semen characteristics, preservation, microbial count*

INTRODUCTION

Rabbits own a number of characteristics that make them suitable as meat-producing small live stock in developing countries including Egypt. Recently, artificial insemination (A.I) is favorable and most suitable for the large commercial Rabbitries (Rashwan and El-Gaafary, 1992; Daader and Seleem, 1999 and Lavara *et al.*, 2000).

Many attempts have been applied to use some stimulators additives in the extenders in order to prolong survivability of spermatozoa and fertilizing ability during storage at different temperatures. The additives included hormones (Daader and El-Keraby, 1982), like hormones (Zeidan, 1994 and Abd-El-Kariem *et al.*, 1998), stimulating hormones (Seleem, 1996 and Daader *et al.*, 2002) and chemical substances (Marwa, 2002). The frequent use of antibiotics randomly in Rabbitries either to animals or to semen may cause antibiotics-resistance strains of bacteria (Rowida 2000). So great attention must be given to use some natural materials as semen additives. Nagwa, 2000 showed that Nigella sativa have promising effect upon isolates resistance to antibiotic. Also Zaki *et al.*, 2000 and Daader *et al.*, 2002 proved that Nigella sativa improved the reproductive performance of rabbit. The present work was carried out to investigate the effects of supplementation of the extended rabbit semen with different levels of Nigella sativa extract on semen quality during storage at different temperatures and to choose the best concentration that provided the lowest bacterial count in coordination with high semen quality and on fertility traits of does inseminated artificially.

MATERIALS and METHODS

The present study was carried out in an Industrial Rabbitry, near El-Aiyat city, Giza Province, Egypt. A number of 36 sexually mature Californian rabbit bucks (12 months age) were used in two experiments. Experiment 1 was designed to study the effect of supplementation of Nigella sativa aquas extract to extended semen on its quality during preservation at different temperatures. Semen was collected artificially by using an artificial vagina. The ejaculates of each buck were evaluated microscopically and only ejaculates that showed advanced motility more than 70% were pooled and extended with lactose-yolk citrate extender (2.90 gm sodium citrate dehydrate + 1.25 gm lactose + 0.04 gm citric acid anhydrous + 10.00 ml egg yolk/100 ml extender) at 1: 4 extension rate. The extended semen was divided into two parts; the first part is

subdivided into several portions, supplemented with different concentrations of Nigella sativa extract (50, 100, 150, 200 or 250 µl/ml). Antibiotics 50000 IU sodium penicillin 50000 µg streptomycin sulphate/100 ml extender was added to control sample (o). Each sample of treated semen was divided into 2 portions, the first one was kept at refrigeration condition (4-6 ^{0}C) for up to 3 days and the second was incubated at 37 ^{0}C for up to 4 hours. Percentages of advanced sperm motility, abnormal and dead spermatozoa and acrosomal damages were recorded at different stages of preservation according to Watson (1975) and Salisbury *et al.* (1978). In experiment two, the second part of extended semen was subdivided into 4 portions for bacteriological examination. Antibiotics, 200 µl/ ml Nigella sativa extract and antibiotics with Nigella sativa (200 µl/ ml) were added to the 1st, 2nd and 3rd fractions respectively. The fourth was left without any additives as a control. The total bacterial count, Enterobacteriaceae count and, *Staph. aureus* count was carried out according to Cruickshank *et al.* (1975). These bacteriological examinations were carried out in the bacteriology lab of ARRI for presence of bacteria. Nigella sativa extract was prepared as described by Hanafy (1991).

In fertility traits, Californian rabbit does (174) were divided into two comparable experimental groups. The 1st & 2nd groups were artificially inseminated by extended pooled semen supplemented with, 200 µl Nigella sativa extract/ 1 ml extended semen with antibiotic (the dose showed the best quality) and antibiotic (control) respectively. The artificial insemination was carried out as described by Adams (1981). Kindling rate and litter size and weight at birth were recorded.

Animals were fed ad libitum a commercial diet according to NRC (1977) recommendations. All animals were kept under the same managerial and hygienic conditions.

Data were subjected to analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Program of SAS (1990).

RESULTS and DISCUSSION

Data presented in Tables 1&2 showed that, supplementation of extended Californian rabbit semen with 50, 100, 150, 200, or 250 µl Nigella sativa extract/ ml improved significantly ($P \leq 0.05$) semen quality (represented by increase in advanced sperm motility percentage and decrease in percentages of each of abnormal, dead spermatozoa and acrosomal damages), during chilled storage at (4 - 6 ^{0}C) for 3 days or

incubation at 37 °C for 4 hours. These results emphasize that, Nigella sativa in the reactivated media seemed to have beneficial effect on increasing percentage of motile spermatozoa and consequently increasing semen storagability and decreasing percentages of dead and abnormal spermatozoa and acrosomal damages.

Advancement of conservation time of extended semen at refrigeration temperature at (4 - 6 °C) for 3 days or at incubation condition for 4 hours decreased ($P \leq 0.05$) percentage of sperm motility and increased ($P \leq 0.05$) the percentages of dead and abnormal spermatozoa and acrosomal damages. These findings are in agreement with the results of Seleem (1996); Daader *et al.* (1999 & 2000) and Rowida (2003). The observed reduction in semen quality with progression of conservation period may contribute to the increase in lactic acid accumulation as a result of sperm anaerobic metabolism leading to changes in both the osmotic pressure and PH of the media, which might exert a toxic effect on the sperm cell (Zeidan, 1994; Seleem, 1996 and Rowida, 2003).

It seemed that the beneficial effects of Nigella sativa extract on semen quality, during preservation at refrigeration or incubation conditions were most noticeable in semen samples supplemented with 200 or 250 µl Nigella sativa/ ml extended semen. No significant effects was recorded between supplementing 200 and 250 µl Nigella sativa/ ml extended semen on percentage of perm motility and dead spermatozoa while it increased significantly ($P \leq 0.05$) in acrosme damages in 250 than 200 µl Nigella sativa and on storagability. These results showed that the improvement of semen quality supplemented with Nigella sativa which may act as anti – oxidant (Meral *et al.*, 2001) where it is a source of calcium, iron, sodium and potassium that main function is to act as essential cofactors in various enzyme functions or act as antibacterial agent Nagwa (2000). Daader *et al.* (2002) revealed that, feeding pellets contained 5% Nigella sativa seeds improved ($P \leq 0.05$) semen quality of rabbit bucks.

As shown in Tables 3, 4 and 5, the bacteriological examination of the prepared semen samples included determination of the effect of Nigella sativa extract on total bacterial count, total entrobateriaceae count and *Staph aureus* count. The obtained results showed that the best concentration of Nigella sativa extract (200 µl) causes considerable decrease of total bacterial count, this could be attributed to the antibacterial properties of Nigella sativa which was used for clinical application (Toppozada *et al.*, 1965 and Ali and Bblunden, 2003). On

other hand the use of combination of Nigella sativa extract and antibiotics yielded the lowest bacterial count, this could be explained by synergistic action between Nigella sativa and antibiotics as described by Hanafy (1991). The bacterial contamination causing reproductive problems in inseminated females which complicated if these bacteria acquired antibiotic purposes. For more follow up the effect of Nigella sativa on bacterial content of the treated semen resistance to the antibiotic used in semen diluent or from uncontrolled use of antibiotic for treatment and prophylactic. The prepared semen was examined for total Enterobacteriaceae and *Staph aureus* (table 4 and 5). The tables revealed that the incidence of Enterobacteriaceae in semen was lower than *Staph aureus*, although the extract of Nigella sativa was found to be more effective on Staph *aureus* than Enterobacteriaceae, these result could be explained by the anti-microbial activities of Nigella sativa extract (Agarwal *et al.* 1979 and Nagwa 2000) and due to the presence of many compounds of which thynohydroquinone was to have high anti-microbial effect against gram-positive microorganism (El-Fatatry, 1975). These results indicated that, Nigella sativa extract was successful in preventing the growing and increasing the microbial content in the extended rabbit semen.

Concerning reproductive performance of rabbit does, data presented in Table 6 indicated that, fertility traits (represented by kindling rate and litter size and weight at birth) of Californian rabbit does inseminated artificially using extended semen and supplemented with 200 µl Nigella sativa extract / ml was significantly ($P \leq 0.05$) higher than control. Daader *et al.*, (2002) found that Nigella sativa supplemented to diet of rabbit bucks increased the kindling rate and litter size at birth.

In conclusion, supplementation of 200 µl Nigella sativa aques extract/ml combined with antibiotics improved semen quality and viability during conservation at refrigeration and incubation temperatures, as well as decreased the microbial count presented in the extended rabbit semen.

Table 1:- Effects of Nigella sativa extraction supplementation on Californian rabbit semen quality, during chilled storage at (4 – 6 ^{0}C) for up to three days (Means ± SE.).

Items	Chilled periods (Days)	Nigella sativa levels (µm / ml.)						Means ± SE.
		0	50	100	150	200	250	
Advanced – sperm motility (%).	0.0	70.7 ± 2.7	71.0 ± 1.8	72.4 ± 1.7	76.9 ± 3.1	77.5 ± 2.9	77.6 ± 3.1	**74.4 ± 2.2** ^{A}
	1.0	52.3 ± 3.1	64.3 ± 2.1	67.1 ± 2.3	71.4 ± 3.2	73.9 ± 3.1	74.1 ± 2.1	**67.2 ± 2.0** ^{B}
	3.0	41.1 ± 2.8	25.1 ± 3.0	61.8 ± 2.7	66.3 ± 2.9	69.5 ± 3.2	69.6 ± 3.1	**60.1 ± 2.4** ^{C}
Means ± SE.		**54.7 ± 2.2** ^{e}	**62.5 ± 1.6** ^{d}	**67.1 ± 1.8** ^{c}	**71.5 ± 2.0** ^{b}	**73.6 ± 2.1** ^{a}	**73.8 ± 1.9** ^{a}	**67.2 ± 1.7**
Storagability (%). *		**58.1 ± 3.1** ^{d}	**73.4 ± 2.9** ^{c}	**85.4 ± 3.0** ^{b}	**86.2 ±2.2** ^{b}	**89.7 ± 3.3** ^{a}	**89.7 ± 3.5** ^{a}	**80.4 ± 1.9**
Sperm – abnormalities (%).	0.0	16.7 ± 1.6	17.0 ± 1.3	16.8 ± 1.6	16.2 ± 1.4	16.9 ± 1.6	16.5 ± 1.3	**16.7 ± 1.1** ^{C}
	1.0	21.7 ± 1.7	21.0 ± 2.0	16.9 ± 1.9	17.9 ± 1.6	17.3 ± 1.5	17.4 ± 1.7	**18.7 ± 1.4** ^{B}
	3.0	24.3 ± 1.7	23.2 ± 1.7	21.9 ± 1.7	20.9 ± 1.4	20.1 ± 1.2	20.1 ± 1.3	**21.8 ± 0.9** ^{A}
Means ± SE.		**20.9 ± 1.2** ^{a}	**20.4 ± 1.2** ^{a}	**18.5 ± 1.4** ^{b}	**18.3 ± 1.2** ^{b}	**18.1 ± 1.7** ^{b}	**18.0 ± 1.8** ^{b}	**19.0 ± 1.1**
Dead spermatozoa (%).	0.0	21.5 ± 2.1	21.6 ± 1.9	21.4 ± 2.7	21.4 ± 3.1	20.9 ± 208	21.3 ± 2.7	**21.4 ± 1.6** ^{C}
	1.0	30.4 ± 3.1	28.9 ± 2.2	27.2 ± 2.9	24.5 ± 3.0	22.7 ± 3.1	22.3 ± 2.7	**26.0 ± 1.6** ^{B}
	3.0	46.7 ± 2.9	40.9 ± 2.4	35.3 ± 2.6	30.6 ± 2.5	25.6 ± 2.7	25.5 ± 2.4	**34.1 ± 1.7** ^{A}
Means ± SE.		**32.9 ± 2.1** ^{a}	**30.5 ± 1.8** ^{b}	**28.0 ± 1.9** ^{c}	**25.5 ± 1.6** ^{d}	**23.1 ± 1.3** ^{e}	**23.0 ± 1.5** ^{e}	**27.2 ± 1.4**
Acrosomal damages (%).	0.0	14.3 ± 2.1	14.5 ± 2.3	14.3 ± 1.9	14.4 ± 1.7	14.2 ± 1.8	14.6 ± 1.9	**14.4 ± 1.0** ^{C}
	1.0	19.7 ± 1.9	18.6 ± 1.7	17.8 ± 2.2	15.7 ± 1.9	15.5 ± 1.5	15.6 ± 1.7	**17.2 ± 1.3** ^{B}
	3.0	23.7 ± 1.9	21.9 ± 2.1	20.9 ± 1.8	17.9 ± 1.6	16.7 ± 1.3	17.4 ± 1.8	**20.6 ± 1.3** ^{A}
Means ± SE.		**19.2 ± 1.4** ^{a}	**18.3 ± 1.8** ^{ab}	**17.7 ± 1.5** ^{b}	**16.0 ± 1.3** ^{c}	**15.5 ± 0.9** ^{c}	**16.5 ± 1.0** ^{b}	**18.0 ± 0.7**

Means within the same row (*a,b,c,d,e*) or the same column (*A,B,C*) bearing different letter superscripts are significantly different (P ≤ 0.05)

* Storagability = $\frac{\text{Final advanced sperm motility (after 3 days)}}{\text{Initial advanced sperm motility (at 0 time)}} \times 100$ (Seleem, 1996)

Table 2: Effects of Nigella sativa extraction supplementation on Californian rabbit semen quality, during incubation at 37 °C for up to four hours (Means ± SE.).

Items	Incubation periods (Hours)	Nigella sativa levels (µm / ml.) O	50	100	150	200	250	Means ± SE.
Advanced – sperm motility (%).	0.0	74.5 ± 3.1	75.1 ± 3.0	76.2 ± 2.9	78.5 ± 2.8	79.2 ± 2.9	79.7 ± 2.7	77.2 ± 1.8 ^{A}
	2.0	63.6 ± 3.3	66.9 ± 2.8	69.3 ± 3.0	74.3 ± 2.6	77.5 ± 3.1	77.1 ± 3.0	71.5 ± 2.3 ^{B}
	4.0	52.2 ± 2.8	57.9 ± 3.3	60.2 ± 3.8	68.6 ± 3.1	73.5 ± 3.0	74.0 ± 2.7	64.4 ± 2.1 ^{C}
Means ± SE.		63.4 ± 2.5 ^{d}	66.6 ± 2.6 ^{c}	68.6 ± 1.8 ^{c}	73.8 ± 1.6 ^{b}	76.7 ± 1.1 ^{a}	76.9 ± 1.1 ^{a}	71.0 ± 0.7
Storagability (%). *		70.1 ± 2.0 ^{d}	77.1 ± 2.3 ^{c}	79.0 ± 1.9 ^{c}	87.4 ± 1.6 ^{b}	92.8 ± 2.1 ^{a}	92.8 ± 1.9 ^{a}	83.2 ± 1.3
Sperm – abnormalities (%).	0.0	14.9 ± 1.3	14.6 ± 2.1	14.8 ± 1.9	14.7 ± 0.9	14.2 ± 0.9	14.3 ± 1.3	14.6 ± 0.8 ^{C}
	2.0	18.8 ± 1.5	17.7 ± 2.1	17.4 ± 1.9	16.4 ± 1.1	15.1 ± 1.2	15.1 ± 1.7	16.8 ± 1.0 ^{B}
	4.0	22.1 ± 1.9	20.8 ± 1.9	20.2 ± 2.1	19.3 ± 1.3	17.3 ± 1.6	17.1 ± 1.6	19.5 ± 1.1 ^{A}
Means ± SE.		18.6 ± 1.7 ^{a}	17.7 ± 1.3 ^{ab}	17.5 ± 1.7 ^{ab}	16.8 ± 1.2 ^{b}	15.5 ± 1.2 ^{c}	15.5 ± 1.0 ^{c}	17.0 ± 0.6
Dead spermatozoa (%).	0.0	19.6 ± 1.9	19.6 ± 2.1	19.3 ± 1.7	19.2 ± 1.7	18.4 ± 2.1	18.1 ± 2.1	19.0 ± 1.3 ^{C}
	2.0	28.4 ± 1.9	26.4 ± 2.3	25.5 ± 2.2	22.3 ± 1.9	20.5 ± 2.1	20.2 ± 1.7	23.9 ± 1.1 ^{B}
	4.0	35.2 ± 2.1	31.6 ± 1.8	29.3 ± 2.1	27.2 ± 1.9	23.8 ± 2.4	24.0 ± 1.9	28.5 ± 1.3 ^{A}
Means ± SE.		27.7 ± 1.5 ^{a}	25.9 ± 1.8 ^{b}	24.7 ± 1.6 ^{b}	22.9 ± 1.5 ^{c}	20.9 ± 2.1 ^{d}	20.8 ± 1.3 ^{d}	23.8 ± 0.9
Acrosomal damages (%).	0.0	12.9 ± 1.3	12.7 ± 2.0	12.7 ± 1.9	12.6 ± 1.1	12.1 ± 0.9	12.3 ± 1.2	12.6 ± 1.1 ^{C}
	2.0	16.9 ± 1.8	16.0 ± 1.6	15.6 ± 2.0	14.5 ± 1.3	13.4 ± 0.9	14.6 ± 1.1	16.0 ± 0.8 ^{B}
	4.0	21.5 ± 1.6	19.5 ± 1.8	18.7 ± 1.9	17.4 ± 0.9	16.0 ± 1.0	15.7 ± 1.3	18.1 ± 0.9 ^{A}
Means ± SE.		17.1 ± 1.0 ^{a}	16.1 ± 0.9 ^{b}	15.7 ± 1.2 ^{bc}	14.8 ± 0.7 ^{c}	13.8 ± 1.0 ^{d}	14.9 ± 0.9 ^{c}	16.2 ± 0.7

Means within the same row (*a,b,c,d*) or the same column (*A,B,C*) bearing different letter superscripts are significantly different (P ≤ 0.05)

$$\text{* Storagability} = \frac{\text{Final advanced sperm motility (after4 hours)}}{\text{Initial advanced sperm motility (at 0 time)}} \times 100$$ (Seleem, 1996)

Table 3: The effect of antibiotic and Nigella sativa extract on total bacterial count of pooled buck semen

No. of pooled semen sample	Semen + antibiotic*	Semen + N. S. extract (200 µl)	Semen + antibiotic and N. S. extract (200 µl)	Control **
1	2.4×10^2	5.3×10^2	3.5×10^2	9.8×10^4
2	4×10^2	5.9×10^2	3.7×10^2	11×10^4
3	2.3×10^2	2.9×10^2	3×10^2	9.2×10^4
4	2.4×10^2	5×10^2	2.5×10^2	9.5×10^4
5	2.5×10^2	4.8×10^2	2×10^2	9×10^4
6	2.7×10^2	4.5×10^2	2.2×10^2	9.7×10^4
mean	**2.72×10^2**	**4.733×10^2**	**2.816×10^2**	**9.7×10^4**

* The used antibiotic was Sodium Penicillin and Streptomycin Sulphate
** Control = diluted semen without antibiotic and without Nigella sativa extract

Table 4: The effect of antibiotic and Nigella sativa extract on *Staphylococcus aureus* count of pooled buck semen/ml

No. of pooled semen sample	Semen + antibiotic*	Semen + N. S. extract (200 µl)	Semen + antibiotic and N. S. extract (200 µl)	Control **
1	34	20	-ve	2.1×10^2
2	-ve	-ve	-ve	2.0×10^2
3	-ve	10	-ve	1.8×10^2
4	15	-ve	-ve	1.9×10^2
5	-ve	11	-ve	1.2×10^2
6	30	35	-ve	1.7×10^2
mean	**1.32 x 10**	**1.27 x 10**	**-ve**	**1.78×10^2**

* The used antibiotic was Sodium Penicillin and Streptomycin Sulphate
** Control = diluted semen without antibiotic and without Nigella sativa extract

Table 5: The effect of antibiotic and Nigella sativa extract on Enterobacteriacea count of pooled buck semen/ml

No. of pooled semen sample	Semen + antibiotic*	Semen + N. S. extract (200 µl)	Semen + antibiotic and N. S. extract (200 µl)	Control **
1	40	118	-ve	138
2	-ve	-ve	-ve	-ve
3	-ve	-ve	-ve	-ve
4	-ve	-ve	-ve	-ve
5	-ve	-ve	-ve	-ve
6	9	5	-ve	72
mean	**0.82 x 10**	**2.05 x 10**	**-ve**	**3.5 x 10**

* The used antibiotic was Sodium Penicillin and Streptomycin Sulphate
** Control = diluted semen without antibiotic and without Nigella sativa extract

Table 6: Fertility traits of Californian rabbit does inseminated artificially by using extended semen supplemented with Nigella sativa extraction (Means ± SE.).

Items	*Antibiotic* *	Nigella sativa levels (µl / ml.) + antibiotics
	(control) 0	200
Mated does (No.).	84	90
Conceived rate (%).	67.86	74.44
Kindling rate (%).	67.86 ^{b}	74.40 ^{a}
Litter size at birth	6.1 ± 1.2 ^{b}	7.9 ± 1.8 ^{a}
Litter weight at birth (gm).	262.9 ± 21.5 ^{b}	302.0 ± 16.9 ^{a}

Means within the same row (*a,b*) bearing different letter superscripts are significantly different (P ≤ 0.05).
* 50000 IU sodium penicillin + 50000 µg streptomycin sulphate/100 ml extender.

REFERENCES

Abd-El-Kariem, M.A.; Gabr, H.A. and Seleem, T.S.T. (1998): Effects of oxytocin or adrenaline on reproductive performance of New-Zealand White male rabbits. 1st Inter. Conf. on Anim. Prod. and Health in Semi-Arid Arease, El-Arish, NorthSinai, 1-3 Sept., 417-422.

Adams, C.E. (1981): Artificial insemination in the rabbit. The technique and application to practice. J. Appl. Rabb. Res., 4: 10-13.

Agarwal, R.; Kharya, M.D. and Shrivastava, R. (1979): Anti-microbial and anti helmentic activities of the essential oil of Nigella sativa Linn. Indian J.Exp. Biol. Nov, 17 (11): 1264-1265.

Ali, B.H. and Blunden, G. (2003): Pharmacological and toxicological properties of Nigella sativa. Phytother Res. Apr, 17 (4): 299-305.

Cruickshank, R.; Duguid, J.T.; Marmion, B.P. and Swain, R.H.A. (1975): Medical Microbiology Churchill Livingston Edinburg and New York, 12th Ed. Vol II ,

Daader, A.H. and El-Keraby, F. (1982): Survival rate of bull spermatozoa supplemented with estrogen, progesterone and prostaglandin $F_2 \propto$. 6th International Conference on Animal and Poultry Production, Zagazig, A.R.E., 1: 192-197.

Daader, A.H. and Seleem, T.ST. (1999): Recent trends in rabbit production. 1st Inter. Conf. on Indigenous Versus Acclimatized Rabbits. 7-9 Sept., El-Arish, North-Sinai, Egypt, 23-50.

Daader, A.H.; Gabr, H.A. and Seleem, T.S.T. (1999b): Productive and reproductive performance of New-Zealand White and californian rabbit bucks as affected by supplementing natural clay to the diet, during summer and winter seasons. 7th Sci., Conf on Anim. Nutr., 19-21 Octob., El-Arish, North Sinai, Egypt, 565-579

Daader, A.H.; Gabr, H.A.; Leila B.Bahgat and Seleem, T.S.T. (2002): Reproductive capabilities of Californian rabbits as affected by Gn-RH injection or supplementation to semen. 3rd Sci. Conf on Rabb. Prod. in Hot Climates, 8 – 11 Octob; Hurghada, Egypt; 275–285.

El-Fatatry, H.M. (1975): Isolation and structure assignment of an antimicrobial principle from the volatile oil of Nigella sativa L. seeds. Pharmazie, Feb. 30 (2): 109-111.

Hanafy, M.S.M. (1991): Studies on the sensitivity of certain bacterial pathogens to some plant extracts. Ph.D. Thesis, Faculty of Med. Vet., Cairo University, Giza, Egypt.

Khan, K.I.; Khan, F.Z. and Nazar, S. (1985): The antimicrobial activity of *Allium* – sativum garlic, *Allium* cepa onion and *Raphanus* – sativus raddish. J. Farm. (Lahore)., 8, 59–72.

Lavara, R.; Moce, E.; Andreu, E.; Pascual, J.J.; Cervera, C.; Viudes-De-Castro, M.P. and Vieente, J.S. (2000): Effects of environmental temperature and vitamin supplements on seminal parameters from a rabbit line selected by high growth rate. 7th World Rabb. Cong., Valencia, Spain, 4-7 July, 167-171.

Marwa, E.H. (2002): Physiological studies on storage of rabbit semen. M. Sci. Thesis, Fac. Agric., Zagazig Univ., Zagazig, Egypt.

Meral, I.; Yener, Z.; Kahraman, T. and Mert, N. (2001): Effect of Nigella sativa on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damagein experimental-induce diabetic rabbit. J. Vet. Med. A Physiol Pathol Clin Med. Des. 48 (10): 593-9.

Nagwa, M.M. (2000): Anti-microbial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiological Polonica, 49 (1): 63–74.

NRC (1977): Nutrient Requirements of Rabbits. National Academy of Science, Washington, DC. USA.

Rashwan, A.A. and El-Gaafary, M.N. (1992): Investigations on some factors affecting reproductive traits of New-Zealand White rabbits. Egyp. J. Rabb. Sci., 2 (2): 161-170.

Rowida, M.R. (2000): Effect of seminal bacterial pathogens on rabbit bucks performance Ph. D. Thesis Fac. of Vet. Med. Sci. Cairo Univ. Egypt.

Rowida, M.R. (2003): A comparative study of different additives of extended rabbit semen. J. Egypt. Vet. Med. Assoc., 63 (4),: 351–361.

SAS (1990): SAS/ STAT User's Guide (Version 6, 4th ed.). SAS Inst. Inc., Cary, NC.

Salisbury, G.W.; Van Demark, N.L. and Lodge, J.R. (1978): Physiology of Reproduction and Artificial Insemination of Cattle. W.H. Freeman and Company, San Francisco, USA.

Seleem, T.S.T. (1996): Studies on some reproductive characteristics in rabbits. M. Sci., Thesis, Fac. of Agric., Zagazig Univ., Zagazig, Egypt.

Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods. 2nd Ed. Iowa Univ. Press. Ames, Iowa.

Toppozadz, H.H.; Mazhoum, H.A. and El-Dakhakhory, M. (1965): The antibacterial properties of the Nigella sativa I. Seeds. Active principle with some clinical applications. J. Egypt Med. Assoc., 48: Suppl: 187-202.

Watson, P.F. (1975): Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Res. 97: 12-15.

Zaki, T.G.; Zeidan, A.E.B. and Seleem, T.S.T. (2000): Some physiological and reproductive aspects of Baladi Red buck rabbits fed Fenugreek or Nigella sativa, under hot climates. Conf. of Social and Agric. Develop. of Sinai, 16-19 May, El-Arish, North. Sinai, 89-99.

Zeidan, A.E.B. (1994): New aspects in freezing cattle semen. Ph.D. Thesis, Faculty of Agric., Zagazig University, Zagazig, Egypt.