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Antimicrobial Effects of Propolis on Preservation of Ram's Semen Extender and Its Fertility Rate

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ABSTARCT

The present study aimed to define the effect of different concentrations of propolis powder or glue extract compared with synthetic antibiotic on ram's semen quality, resistance to bacterial contamination and fertility rate. Semen samples of six mature rams aging 2.2 years old with an average 62.0 kg live body weight were collected twice a week for three weeks using artificial vagina. The semen ejaculates were pooled, extended and divided into seven portions. The final extension rate was 1 semen: 10 extender. The seven portions were (CTL, without any antibiotic type and kept as control group), 400µl pen-step ($SA_{400µl}$) and 600µl pen-step ($SA_{600µl}$) as synthetic antibiotic; 400µl and 600µl propolis powder ($PP_{400µl}$ & $PP_{600µl}$); 400µl and 600µl propolis glue ($PG_{400µl}$ & $PG_{600µl}$) as natural antibiotic, respectively. Extended semen samples were stored at 5 °C for 6 days. The obtained results showed that, spermatozoa quality was significantly ($P<0.05$) decreased with the advancement of storage period in all extended samples. In addition, sperm parameters (sperm motility, acrosomal status and normal spermatozoa), enzymatic activities (ALT, AST, ALP and LDH), bacterial count and fertility rate were improved ($P<0.05$) in extended samples treated with synthetic and natural antibiotics either powder or glue than the control. A higher antimicrobial activity after 24 hrs incubation for $PP_{600µl}$ and $PG_{600µl}$ extenders were significantly ($P<0.05$) decreased compared with other extended samples. Moreover, the $PG_{600µl}$ antibiotic was insignificantly greater litter size (1.27%) compared with (1.07%) for $SA_{600µl}$ one. Regarding to the results of this study, it could be concluded that antimicrobial effect of natural propolis either powder or glue in extended ram semen was capable to improve ram semen quality and inhibitory bacterial zones, as well as, improvement fertility rate.

Keywords: Ram semen, propolis, incubation, storage, fertility rate.

INTRODUCTION

Bacterial contamination is a dangerous and very actual preventive to beneficial semen production in all livestock. It is routinely attained in raw semen produced for artificial insemination (AI) especially when semen is collected by either electrico-ejaculate or artificial vagina method. In addition, bacterial contamination of the extended semen has a negative effect on sperm quality which occurred directly by vie with spermatozoa for nutrient sources or indirectly by production of toxic metabolic by-products. Hence, Pasing *et al.* (2013) revealed that presence of bacterial contaminant in extended semen decreased acrosome reaction, increased sperm agglutination, and may cause infection, inflammation, endotoxins and reproductive diseases with AI program. Furthermore, the insemination of contaminated extended semen may be associated with endometritis, embryonic death and infection in recipient females' genital tract, so reducing litter size of the herd (Bresciani *et al.,* 2014). Despite, the female reproductive tract may expose to bacterial contamination from the male during natural mating, however female tract has natural defense mechanisms to prevent bacteria development in genital tract (Gloria *et al.,* 2014).

Meanwhile, addition of antibiotics to semen extenders is required to prevent development and growth of bacterial contaminants (Morrell, 2016). But unfortunately, some synthetic antibiotics may be toxic to spermatozoa and were shown to have an adversely effect on sperm motility, structure of the sperm, shortening viability and caused the production of antibodies directed against the sperm glycocalyx complex (Guimaraes *et al.,* 2015). Therefore, finding alternatives of antioxidants and antibacterial to resist microorganisms in semen extender for AI programs would be profitable for improving sperm survival, consequently fertility rate. Hence, antioxidant activity of propolis is mainly attributed to its flavonoid contents, such as quercetin, flavones, isoflavones, flavonones, anthocyanins, catechins and isocatechins (Alves and Kubota, 2013). Furthermore, flavonoid contents are capable of scavenging free radicals and thereby provide protection against lipid peroxidation (Moraes *et al.,* 2014). On the other hand, El-Battawy and Brannas (2015) indicated that semen extenders containing propolis achieved the best motility, cryoprotection and maintained sperm cells integrity. In addition, El-Sheshtawy *et al.* (2016) mentioned that addition of bee products (as honey and propolis solution) in stallion semen extender improved storing, frozen sperm activity and also attended superior conception rate. Similarly, Khalifa *et al.* (2016) reported that propolis extract (as powder or glue) in ram semen extenders had greater elimination of bacterial contamination than synthetic antibiotics such as Pen-Strep, Alamycin and Vetrocin. Therefore, the present study aimed to evaluate the efficiency of antibiotics addition (natural or synthetic) on the extended ram semen quality under refrigeration condition. Enzymatic activities of the extended ram semen during storage at 5 °C for up to 6 days were also recorded. Moreover, the possibilities to point out the importance of ram semen extender with antibiotic types to evaluate reproductive traits (as fertility rate) after being stored at 5 °C for up to one day.

MATERIALS AND METHODS

All semen samples were collected from Sids and El-Serw Research Stations belonging to Animal Production Research Institute (*APRI*), Egypt.

Experimental design

Six mature rams (2.2 years old with an average live body weight 62.0 kg) were used in the present study. Pooled semen samples were extended in Tris extender at 1 part of raw semen: 10 parts of Tris extenders. Each sample of the extended semen was split into seven extender parts in sterilized test tubes. The extended semen samples were included CTL (without

any antibiotic types) served as control. While, the other six extended semen samples included $SA_{400\mu}$, $SA_{600\mu l}$ contained 400 and 600µl of synthetic antibiotic as pen-step /100ml extender, $PP_{400\mu l}$, $PP_{600\mu l}$ included 400 and 600 µl of propolis powder /100 ml extender and $PG_{400\mu l}$, $PG_{600\mu l}$ supplied with propolis glue at 400 and 600 µl /100 ml extender, respectively. The extender ingredients of Tris diluent and antibiotic levels are presented in Table 1.

Table 1. Composition of the extender and level of antibiotics used for the processing of ram semen.

Extender ingredients	Extender ingredients /100 ml of distilled water						
	CTL	$SA_{400\mu l}$	$SA_{600\mu l}$	$PP_{400\mu l}$	$PP_{600\mu l}$	$PG_{400\mu l}$	$PG_{600\mu l}$
*Tris (g)	3.634	3.634	3.634	3.634	3.634	3.634	3.634
Glucose (g)	0.800	0.800	0.800	0.800	0.800	0.800	0.800
Citric acid (g)	1.990	1.990	1.990	1.990	1.990	1.990	1.990
Synthetic antibiotic (µl)	-	400	600	-	-	-	-
Natural antibiotic as powder (µl)	-	-	-	400	600	-	-
Natural antibiotic as glue (µl)	-	-	-	-	-	400	600
Egg yolk (ml)	20	20	20	20	20	20	20

***Tris: Hydroxymethel amino methane. CTL: Free from any type of antibiotics, $SA_{400\mu l}$ & $SA_{600\mu l}$: Including Pen-Strep 20/20 antibiotic genesis of procaine penciling 200mg, Dihydrostreptomycin sulphate 250mg, $PP_{400\mu l}$ & $PP_{600\mu l}$: Containing natural antibiotic genesis of powder propolis ethanolic extract, and $PG_{400\mu l}$ & $PG_{600\mu l}$: consists of natural antibiotic genesis of glue propolis ethanolic extract.**

Extended semen samples (as CTL, $SA_{400\mu l}$, $SA_{600\mu l}$, $PP_{400\mu l}$, $PP_{600\mu l}$, $PG_{400\mu l}$ and $PG_{600\mu l}$) were placed in water bath of a 500 ml beaker containing water at room temperature up the level of extended semen. Then, the beaker was placed in refrigerator and gradually cooled until reached to 5°C within a period of 1.5-2.0 hours. Extended semen with different levels of propolis was stored at 5°C for 0, 1, 2, 4 and 6 days. All test tubes containing extended medium were covered with dark plastic sheath to avoid to light and any contamination.

Enzymatic Activities

Extended semen samples were centrifuged at 600 g for 20 minutes and the supernatant was removed and used for enzymatic assay. Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH) were determined according to Graham and Pace (1967).

Semen evaluation

Percentages of sperm motility and sperm normality were estimated according to Salisbury *et al.* (1978), acrosomal status were examined by the dual stain procedure described by Didion *et al.* (1989). The following categories were detected: a- live spermatozoa with intact acrosome (LIA), b- live spermatozoa with detached acrosome (LDA), c- dead spermatozoa with intact acrosome (DIA), and d- dead spermatozoa with detached acrosome (DDA), as shown in Figure 1

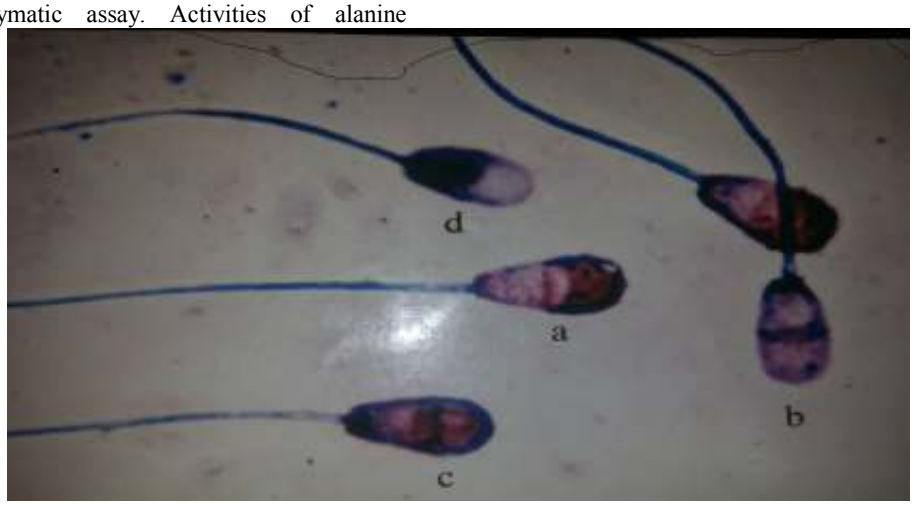

Figure 1. The four categories of spermatozoa generated by the dual stain procedure. a- live spermatozoa with intact acrosome (LIA), b- live spermatozoa with detached acrosome (LDA), c- dead spermatozoa with intact acrosome (DIA), and d- dead spermatozoa with detached acrosome (DDA).

Microbiological evaluation

Tryptone Soya Agar (Oxoid) medium was prepared by using the spread plate method (Harry and Paul, 1981). Semen bacterial counting was expressed as CFU/ml according to Qureshi *et al.* (1993). Antibiotic sensitivity of the suspected colonies for Gram-positive and Gram-negative bacteria was determined according to (Mari, 2005). The inhibition zones were recorded as circumference (π× circle diameter) and area (π× Radius2) the value of π=3.14.

Fertility trial

During the breeding season, thirty mature and healthy ewes with good reproductive and productive performance were divided into two insemination groups (n=15 each). The AI was conducted with $PG_{600\mu l}$ (as a natural antibiotic that had the best characteristics) compared to $SA_{600\mu l}$ (as a control synthetic antibiotic) post- stored at 5°C for up to one day. The ewes that came in heat, she would be inseminated with 0.5 ml of ($SA_{600\mu l}$ or $PG_{600\mu l}$) according the best results included >

400×10^6 fertile sperm. Conception rate and lambing rate of $SA_{600\mu l}$ or $PG_{600\mu l}$ were calculated as following: conception rate (number of ewes conceived / number of ewes inseminated), Lambing rate as single birth (number of ewes lambed single/ number of ewes lambed), twins birth (number of ewes lambed twins/ number of ewes lambed) and litter size (number of total lambs born / number of ewes lambed) were recorded.

Statistical analysis

Data were expressed as means (±S.E.) and statistical analyses were performed with SPSS Version 22.0 for Windows (SPSS, 2013). Duncan's New Multiple Range Test (Duncan, 1955) of the same SPSS program was applied to determine significant differences among all tested treatments and parameters. Fertility rate results were analyzed by the Chi-square test.

RESULTS

Sperm motility (%):

The effects of synthetic and natural antibiotics on ram sperm motility (%) stored at 5 °C for up to 6 days are shown in Table 2. Sperm motility (%) was decreased with the advancement of storage period in all extended semen samples. Meanwhile, the synthetic and natural antibiotics in extended samples had significantly ($P<0.05$) higher of sperm motility (%) than CTL extended samples. It was evident from Table 2 that sperm motility (%) in the natural antibiotic had the highest values followed by the synthetic antibiotic and the least one was CTL. Concerning the natural antibiotic, the values of the sperm motility (%) for the propolis contained glue were better than those contained powder. These results are in agreement with those of Khalifa *et al*. (2016).

Table 2. Effect of synthetic and natural antibiotics on sperm motility (%) and normality (%) of the extended ram semen during storage at 5 °C for 6 days.

Semen extenders	Sperm motility (%) Storage time (day)					Means
	Zero	1st	2nd	4th	6th	
CTL	79.2±2.0^{b}	67.5±2.81^{c}	55.0±2.9^{b}	41.7±3.1^{d}	25.0±2.9^{b}	53.5±3.7^{c}
$SA_{400\mu l}$	81.7±1.7^{ab}	69.2±3.3^{bc}	60.0±3.2^{ab}	48.3±3.3^{cd}	37.5±2.8^{ab}	59.2±3.1^{bc}
$SA_{600\mu l}$	82.5±1.7^{ab}	70.8±2.4^{abc}	64.2±2.4^{ab}	54.2±2.4^{bc}	43.3±2.5^{ab}	62.8±2.6^{ab}
$PP_{400\mu l}$	83.3±1.1^{a}	72.5±1.7^{abc}	64.2±2.4^{ab}	54.2±3.0^{bc}	45.8±4.5^{ab}	63.8±2.7^{ab}
$PP_{600\mu l}$	84.2±0.8^{a}	73.3±1.7^{abc}	65.8±2.4^{ab}	56.7±2.8^{ab}	46.7±4.4^{ab}	65.2±2.6^{ab}
$PG_{400\mu l}$	85.0±0.8^{a}	75.8±0.8^{ab}	70.0±1.3^{a}	62.5±1.1^{a}	50.8±2.0^{a}	68.7±2.2^{a}
$PG_{600\mu l}$	85.8±0.8^{a}	76.7±1.05^{a}	70.0±1.3^{a}	63.3±1.7^{a}	52.5±2.1^{a}	69.5±2.1^{a}
Means	83.1±0.6^{A}	72.3±0.9^{B}	64.2±1.1^{C}	54.4±1.4^{D}	43.1±1.8^{E}	63.2
	Sperm normality (%)					Means
	Zero	1st	2nd	4th	6th	
CTL	86.5±1.5^{e}	68.3±2.6^{e}	57.5±2.1^{c}	49.7±2.1^{d}	36.3±2.2^{e}	59.7±3.3^{c}
$SA_{400\mu l}$	89.2±1.0^{d}	73.7±0.9^{d}	64.2±1.6^{b}	57.3±1.6^{c}	47.3±2.6^{d}	66.3±2.7^{bc}
$SA_{600\mu l}$	90.8±0.9^{cd}	76.0±1.6^{cd}	64.3±2.4^{b}	57.8±2.4^{c}	50.2±2.4^{cd}	67.8±2.8^{b}
$PP_{400\mu l}$	91.8±0.8^{bcd}	77.0±0.9^{bcd}	67.0±0.9^{b}	61.2±1.14^{bc}	54.8±1.2^{bc}	70.4±2.4^{ab}
$PP_{600\mu l}$	93.2±0.9^{abc}	78.5±1.5^{bc}	66.7±1.8^{b}	60.7±0.9^{bc}	55.0±1.1^{bc}	70.8±2.6^{ab}
$PG_{400\mu l}$	94.5±0.4^{ab}	81.0±0.6^{ab}	71.8±0.6^{a}	64.8±0.6^{ab}	59.2±1.1^{ab}	74.3±2.3^{ab}
$PG_{600\mu l}$	95.2±0.3^{a}	83.3±0.4^{a}	73.0±0.5^{a}	67.5±1.0^{a}	61.8±1.0^{a}	76.2±2.2^{a}
Means	91.6±0.5^{A}	76.8±0.9^{B}	66.4±0.9^{C}	59.9±1.0^{D}	52.1±1.4^{E}	69.3

$^{a:e}$, Means with different superscripts, within each column, are significantly different (P<0.05).
$^{A:E}$, Means with different superscripts, within each row, are significantly different (P<0.05).
CTL: Control, $SA_{400\mu l}$: 400µl pen-step, $SA_{600\mu l}$: 600µl pen-step, $PP_{400\mu l}$: 400µl propolis powder, $PP_{600\mu l}$: 600µl propolis powder, $PG_{400\mu l}$: 400µl propolis glue and $PG_{600\mu l}$: 600µl propolis glue.

Sperm normality (%):

The obtained data in Table 2 indicated that the effect of synthetic and natural antibiotic on normal spermatozoa (%) during storage at 5 °C for 6 days. Percentage of normal sperm morphology may be an indicator of the fertility potential of given male. Normality (%) gradually decreased significantly ($P<0.05$) with the advancement of storage period among antibiotic types. Normality (%) in natural and synthetic antibiotics contained extender were significantly ($P<0.05$) higher than in CTL extended semen samples. Concerning natural antibiotics both $PG_{400\mu l}$ and $PG_{600\mu l}$ were better than $PP_{400\mu l}$ and $PP_{600\mu l}$.

Acrosome status:

The effect of synthetic and natural antibiotic on the live and dead spermatozoa with intact and detached acrosome (%) which were stored at 5 °C for 6 days are presented in Tables 3&4.

The percentages of live spermatozoa with intact and detached acrosome were significantly ($P<0.05$) decreased and the percentages of dead spermatozoa with intact and detached acrosome were significantly ($P<0.05$) increased with successive storage time in all samples. Intact acrosome was significantly ($P<0.05$) declined with longer storage time in all extended semen samples. Both natural and synthetic antibiotic had higher values in the percentage of live spermatozoa with intact acrosome than CTL one. Among natural antibiotic samples, the percentage of live spermatozoa with intact acrosome were better in $PG_{400\mu l}$ and $PG_{600\mu l}$ than $PP_{400\mu l}$ and $PP_{600\mu l}$ ones during storage at 5 °C up to 6 days. Similar trend was reported by El-Gaafary (1987) who clarified that the percentage of spermatozoa with damaged acrosomes showed significantly increased following incubation of ram semen at 37 °C for 6 hours. From another point of view, Jones and Stewart (1979) indicated that extension and cooling of bull semen to 5 °C caused acrosomal swelling in about 50% of the spermatozoa.

Table 3. Effect of synthetic and natural antibiotics on live spermatozoa with intact and detached acrosomes (%) of the extended ram semen during storage at 5 °C for 6 days.

Semen extenders	Live spermatozoa with intact acrosomes (%)					Means
	Storage time (day)					
	Zero	1st	2nd	4th	6th	
CTL	69.4±1.9^{e}	47.9±1.5^{e}	28.4±1.3^{f}	17.7±1.2^{g}	8.4±0.7^{g}	34.4±4.1^{d}
$SA_{400µl}$	75.1±0.9^{d}	55.8±1.0^{d}	41.6±0.6^{e}	30.2±0.9^{f}	17.8±0.7^{f}	44.1±3.7^{c}
$SA_{600µl}$	80.9±2.0^{c}	62.0±1.3^{c}	47.8±1.4^{d}	38.0±1.1^{e}	24.3±1.0^{e}	50.6±3.7^{bc}
$PP_{400µl}$	82.0±1.0^{bc}	64.1±1.1^{bc}	51.3±1.4^{c}	41.7±1.3^{d}	29.0±1.1^{d}	53.6±3.4^{abc}
$PP_{600µl}$	84.2±1.5^{abc}	66.0±1.2^{ab}	54.7±0.3^{b}	45.4±0.8^{c}	33.4±1.2^{c}	56.8±3.3^{ab}
$PG_{400µl}$	85.8±0.8^{ab}	66.8±1.0^{ab}	58.5±1.1^{a}	49.3±0.7^{b}	36.8±0.8^{b}	59.5±3.1^{ab}
$PG_{600µl}$	88.2±0.8^{a}	68.9±0.4^{a}	61.2±0.8^{a}	52.5±1.2^{a}	41.5±0.8^{a}	62.5±2.9^{a}
Means	80.8±1.1^{A}	61.7±1.1^{B}	49.1±1.7^{C}	39.3±1.8^{D}	27.3±1.7^{E}	51.6
	Live spermatozoa with detached acrosomes (%)					Means
	Zero	1st	2nd	4th	6th	
CTL	11.1±0.7^{c}	20.1±1.1^{c}	18.1±0.8	16.0±0.9^{b}	10.5±0.9^{d}	15.1±0.8
$SA_{400µl}$	8.5±0.7^{c}	17.0±0.4^{c}	18.5±0.2	19.0±0.8^{a}	16.7±0.8^{c}	15.9±0.8
$SA_{600µl}$	4.7±0.5^{c}	14.0±0.6^{c}	18.9±0.7	19.9±1.0^{a}	18.8±0.7^{bc}	15.2±1.1
$PP_{400µl}$	4.5±0.4^{c}	13.6±0.6^{c}	18.0±0.7	21.6±1.1^{a}	19.8±0.8^{ab}	15.5±1.2
$PP_{600µl}$	3.9±0.6^{c}	13.7±0.4^{c}	17.8±0.5	21.6±1.3^{a}	20.8±1.0^{ab}	15.5±1.2
$PG_{400µl}$	3.4±0.2^{b}	13.5±0.5^{b}	17.8±0.9	21.2±0.7^{a}	21.7±1.1^{a}	15.5±1.3
$PG_{600µl}$	3.8±0.3^{a}	13.8±0.6^{a}	17.4±0.6	21.5±0.9^{a}	22.3±0.8^{a}	15.8±1.3
Means	5.7±0.5^{D}	15.1±0.4^{C}	18.1±0.2^{B}	20.1±0.5^{A}	18.7±0.7^{B}	15.5

$^{a:g}$, Means with different superscripts, within each column, are significantly different (P<0.05).
$^{A:E}$, Means with different superscripts, within each row, are significantly different (P<0.05).
CTL: Control, $SA_{400µl}$: 400µl pen-step, $SA_{600µl}$: 600µl pen-step, $PP_{400µl}$: 400µl propolis powder, $PP_{600µl}$: 600µl propolis powder, $PG_{400µl}$: 400µl propolis glue and $PG_{600µl}$: 600µl propolis glue.

Table 4. Effect of synthetic and natural antibiotics on dead spermatozoa with intact and detached acrosomes (%) of the extended ram semen during storage at 5 °C for 6 days.

Semen extenders	Dead spermatozoa with intact acrosomes (%)					Means
	Storage time (day)					
	Zero	1st	2nd	4th	6th	
CTL	16.7±1.0^{a}	22.6±1.0^{a}	32.6±0.9^{a}	34.7±1.2^{a}	36.1±1.6^{a}	28.5±1.5^{a}
$SA_{400µl}$	14.7±0.9^{ab}	20.8±0.9^{ab}	27.5±0.4^{b}	31.2±0.7^{b}	33.9±1.3^{ab}	25.6±1.4^{ab}
$SA_{600µl}$	12.3±1.7^{bc}	19.6±1.5^{abc}	23.9±1.0^{c}	27.7±0.9^{c}	32.0±1.0^{bc}	23.1±1.4^{bc}
$PP_{400µl}$	11.0±0.6^{cd}	18.4±0.9^{bcd}	22.7±0.5^{cd}	24.1±0.8^{d}	30.3±1.0^{cd}	21.3±1.2^{c}
$PP_{600µl}$	9.5±0.7^{d}	16.8±1.0^{cde}	20.8±0.6^{d}	22.5±1.3^{de}	28.3±1.1^{de}	19.6±1.2^{c}
$PG_{400µl}$	8.3±0.4^{d}	16.4±0.8^{de}	18.2±1.1^{e}	20.7±0.4^{ef}	26.2±1.3^{ef}	17.9±1.1^{c}
$PG_{600µl}$	5.5±0.2^{e}	14.5±0.7^{e}	16.6±0.9^{e}	18.5±0.7^{f}	23.5±0.7^{f}	15.7±1.1^{c}
Means	11.2±0.6^{E}	18.4±0.5^{D}	23.2±0.8^{C}	25.6±0.9^{B}	30.0±0.8^{A}	21.7
	Dead spermatozoa with detached acrosomes (%)					Means
	Zero	1st	2nd	4th	6th	
CTL	2.8±0.4	9.4±0.6^{a}	20.9±1.1^{a}	31.7±1.6^{a}	45.0±1.7^{a}	22.0±2.9^{a}
$SA_{400µl}$	1.6±0.1	6.3±0.3^{b}	12.3±0.3^{b}	19.7±0.9^{b}	31.6±1.2^{b}	14.3±2.0b
$SA_{600µl}$	2.1±0.3	4.4±0.3^{c}	9.5±0.6^{c}	14.5±0.7^{c}	24.8±0.8^{c}	11.0±1.5^{bc}
$PP_{400µl}$	2.5±0.4	3.9±0.3^{cd}	8.0±0.6^{cd}	12.5±0.8^{cd}	20.8±1.0^{d}	9.6±1.3^{c}
$PP_{600µl}$	2.3±0.4	3.5±0.3^{cd}	6.7±0.1^{de}	10.6±0.3^{de}	17.6±0.8^{e}	8.1±1.1^{c}
$PG_{400µl}$	2.5±0.4	3.3±0.2^{d}	5.5±0.4^{ef}	8.8±0.3^{ef}	15.3±0.5^{ef}	7.1±0.9^{c}
$PG_{600µl}$	2.6±0.8	2.9±0.1^{d}	4.7±0.2^{f}	7.5±0.4^{f}	12.7±0.4^{f}	6.1±0.7^{c}
Means	2.3±0.2^{D}	4.8±0.4^{D}	9.7±0.8^{C}	15.0±1.2^{B}	24.0±1.7^{A}	11.2

$^{a:f}$, Means with different superscripts, within each column, are significantly different (P<0.05).
$^{A:E}$, Means with different superscripts, within each row, are significantly different (P<0.05).
CTL: Control, $SA_{400µl}$: 400µl pen-step, $SA_{600µl}$: 600µl pen-step, $PP_{400µl}$: 400µl propolis powder, $PP_{600µl}$: 600µl propolis powder, $PG_{400µl}$: 400µl propolis glue and $PG_{600µl}$: 600µl propolis glue.

Enzymatic activities (U/10^9 spermatozoa):

Tables 5 and 6 show that, the extended ram semen with natural antibiotics especially $PP_{600µl}$ showed significantly (P<0.05) lower the release of ALT, AST, ALP and LDH enzymes into the extracellular medium than synthetic antibiotics and control during storage period.

The storage at 5 °C for up to 6 days increased the amount of ALT, AST, ALP and LDH enzymes released into the extracellular medium. These results are in agreement with those of Zeidan *et al.* (2004).

Bacterial count and bacterial inhibition zone diameters

Bacterial count and bacterial inhibition zone diameters are shown in Table 7 and Figure 2, respectively. After 24hrs of incubation at 37°C, bacterial count (CFU/ml) was 610.67, 108.33, 87.67, 66.00, 51.67, 20.00 and 12.33 CFU/ml in CTL, $SA_{400µl}$, $SA_{600µl}$, $PP_{400µl}$, $PG_{400µl}$, $PP_{600µl}$ and $PG_{600µl}$ extenders, respectively. From the results, the $PG_{600µl}$ semen treatment showed the best protective film against bacterial contamination, followed by $PP_{600µl}$ and the least one was CTL.

Table 5. Effect of synthetic and natural antibiotics on alanine aminotransferase and aspartate aminotransferase enzymes (U/10^9 spermatozoa) activity of the extended ram semen during storage at 5 °C for 6 days.

Semen extenders	Alanine aminotransferase (U/10^9 spermatozoa)					Means
	Storage time (day)					
	Zero	1st	2nd	4th	6th	
CTL	17.0±0.1[a]	22.8±0.1[a]	25.5±0.2[a]	38.6±0.4[a]	55.1±0.1[a]	31.8±3.1
$SA_{400µl}$	16.6±0.2[b]	22.7±0.1[a]	25.8±0.2[a]	36.9±0.3[b]	50.4±0.3[b]	30.5±2.7
$SA_{600µl}$	15.6±0.2[c]	21.7±0.1[b]	24.7±0.2[b]	36.4±0.2[b]	49.8±0.1[b]	29.6±2.8
$PP_{400µl}$	14.2±0.2[d]	19.7±0.1[c]	22.1±0.1[c]	34.7±0.2[c]	46.8±0.3[c]	27.5±2.7
$PP_{600µl}$	14.1±0.1[d]	19.4±0.1[d]	21.6±0.1[c]	33.4±0.2[d]	45.6±0.2[d]	26.8±2.6
$PG_{400µl}$	12.0±0.1[e]	17.9±0.1[e]	20.7±0.1[d]	33.0±0.1[de]	44.9±0.1[de]	25.7±2.7
$PG_{600µl}$	11.4±0.1[f]	17.1±0.1[f]	19.8±0.1[e]	32.3±0.1[e]	43.6±0.2[e]	24.8±2.7
Means	14.4±0.4[E]	20.2±0.4[D]	22.9±0.4[C]	35.0±0.4[B]	48.0±0.7[A]	28.1
	Aspartate aminotransferase (U/10^9 spermatozoa)					Means
	Zero	1st	2nd	4th	6th	
CTL	35.0±0.4[a]	41.7±0.4[a]	48.2±0.3[a]	65.5±0.6[a]	79.0±0.6[a]	53.9±3.8[a]
$SA_{400µl}$	33.8±0.4[b]	38.8±0.5[b]	43.4±0.6[b]	61.6±0.6[b]	76.5±0.5[b]	50.8±3.6[ab]
$SA_{600µl}$	31.3±0.5[c]	36.1±0.4[c]	40.5±0.4[bc]	59.4±0.6[c]	70.6±0.6[c]	47.6±3.4[ab]
$PP_{400µl}$	29.2±0.3[d]	33.9±0.5[d]	38.3±0.7[cd]	56.8±0.6[d]	67.0±0.5[d]	45.0±3.3[ab]
$PP_{600µl}$	29.0±0.4[d]	33.6±0.5[de]	37.9±0.5[cd]	56.4±0.5[d]	67.4±0.7[d]	44.9±3.4[ab]
$PG_{400µl}$	28.3±0.2[de]	32.5±0.2[de]	36.3±0.3[d]	55.3±0.5[de]	65.8±0.5[de]	43.6±3.3[ab]
$PG_{600µl}$	27.7±0.2[e]	31.7±0.2[e]	35.3±0.1[d]	54.3±0.4[e]	64.8±0.4[e]	42.8±3.3[b]
Means	30.6±0.5[E]	35.5±0.7[D]	40.0±0.9[C]	58.5±0.7[B]	70.2±1.0[A]	46.9

[a:f], Means with different superscripts, within each column, are significantly different (P<0.05).
[A:E], Means with different superscripts, within each row, are significantly different (P<0.05).
CTL: Control, $SA_{400µl}$: 400µl pen-step, $SA_{600µl}$: 600µl pen-step, $PP_{400µl}$: 400µl propolis powder, $PP_{600µl}$: 600µl propolis powder, $PG_{400µl}$: 400µl propolis glue and $PG_{600µl}$: 600µl propolis glue.

Table 6. Effect of synthetic and natural antibiotics on alkaline phosphatase and lactic dehydrogenase enzymes (U/10^9 spermatozoa) activity of the extended ram semen during storage at 5 °C for 6 days.

Semen extenders	Alkaline phosphatase (U/10^9 spermatozoa)					Means
	Storage time (day)					
	Zero	1st	2nd	4th	6th	
CTL	81.6±0.5[a]	85.1±0.5[a]	98.5±0.7[a]	104.3±0.6[a]	144.7±0.8[a]	102.8±5.2[a]
$SA_{400µl}$	77.1±0.2[b]	84.9±0.6[a]	94.5±0.9[b]	104.6±0.6[a]	133.7±0.5[b]	99.0±4.5[ab]
$SA_{600µl}$	76.6±0.3[b]	84.4±0.3[ab]	92.7±0.9[b]	102.7±0.3[a]	128.2±0.6[c]	96.9±4.1[ab]
$PP_{400µl}$	74.7±0.2[c]	82.6±0.2[bc]	90.0±0.2[c]	94.0±0.2[b]	126.5±0.3[cd]	93.6±4.1[ab]
$PP_{600µl}$	73.8±0.1[c]	80.5±0.2[cd]	87.3±0.1[d]	92.9±0.2[b]	124.6±0.2[d]	91.8±4.0[ab]
$PG_{400µl}$	73.5±0.1[c]	80.3±0.1[d]	86.9±0.1[d]	92.3±0.2[bc]	114.5±0.5[e]	89.5±3.2[ab]
$PG_{600µl}$	70.9±0.1[d]	79.1±0.3[d]	85.8±0.2[d]	90.7±0.2[c]	109.6±0.2[f]	87.2±3.0[b]
Means	75.5±0.6[E]	82.4±0.5[D]	90.8±0.9[C]	97.4±1.1[B]	126.0±2.1[A]	94.4
	Lactic dehydrogenase (U/10^9 spermatozoa)					Means
	Zero	1st	2nd	4th	6th	
CTL	193.2±1.1[a]	210.1±1.6[a]	243.5±1.7[a]	309.3±1.7[a]	404.7±2.0[a]	272.1±17.7
$SA_{400µl}$	188.6±1.2[b]	209.9±1.1[a]	239.5±1.9[b]	309.6±1.6[a]	393.7±1.7[b]	268.3±17.2
$SA_{600µl}$	188.1±1.3[b]	209.4±1.3[ab]	237.7±1.9[b]	307.7±1.4[a]	388.2±1.9[c]	266.2±16.8
$PP_{400µl}$	186.3±1.2[c]	207.6±1.2[bc]	235.0±1.2[c]	299.0±1.2[b]	386.5±1.2[cd]	262.9±16.6
$PP_{600µl}$	185.4±1.1[c]	205.5±1.2[cd]	232.3±1.1[d]	297.9±1.3[b]	384.6±1.4[d]	261.1±16.6
$PG_{400µl}$	185.1±1.1[c]	205.3±1.1[d]	231.9±1.1[d]	297.3±1.2[bc]	374.5±1.1[e]	258.8±15.9
$PG_{600µl}$	182.5±1.1[d]	204.1±1.4[d]	230.8±1.2[d]	295.7±1.2[c]	369.6±1.2[f]	256.5±15.6
Means	187.0±0.6[E]	207.4±0.5[D]	235.8±0.9[C]	302.4±1.1[B]	386.0±2.1[A]	263.7

[a:f], Means with different superscripts, within each column, are significantly different (P<0.05).
[A:E], Means with different superscripts, within each row, are significantly different (P<0.05).
CTL: Control, $SA_{400µl}$: 400µl pen-step, $SA_{600µl}$: 600µl pen-step, $PP_{400µl}$: 400µl propolis powder, $PP_{600µl}$: 600µl propolis powder, $PG_{400µl}$: 400µl propolis glue and $PG_{600µl}$: 600µl propolis glue.

The current results showed that inhibition zone diameters were significantly (P<0.05) among extender antibiotic types for both gram positive and negative bacteria (Fig. 2). The highest values for circumference zone diameters were recorded with $PG_{600µl}$ (4.27 and 3.43mm) followed by $PG_{400µl}$ (4.00 and 2.97mm); $PP_{600µl}$ (3.80 and 2.83mm) and $PP_{400µl}$ extender (2.47 and 2.90mm) for both gram positive and negative bacteria, respectively. On the other hand, the synthetic antibiotic extenders ($SA_{600µl}$ & $SA_{400µl}$) and CTL had the lowest (P<0.05) values (2.17 and 2.57); (1.90 and 2.40) and (1.87 and 1.40) for both gram positive and negative bacteria, respectively.

Table 7. Effect of different antibiotics on bacterial count (CFU/ml) of diluted ram semen during 24 hrs of incubation at 37°C

Semen samples	Incubation at 37°C (hour)			Means
	Zero hr.	After 3 hrs.	After 24 hrs.	
CTL	309.33±6.25[a]	474.67±6.86[a]	610.67±13.99[a]	464.89±37.49[a]
$SA_{400µl}$	170.67±2.09[b]	183.00±3.24[b]	108.33±3.12[b]	154.00±9.97[b]
$SA_{600µl}$	153.33±3.92[c]	156.67±3.12[d]	87.67±5.17[c]	132.56±9.82[bc]
$PP_{400µl}$	173.67±2.90[b]	186.00±3.67[b]	66.00±4.14[d]	141.89±16.36[bc]
$PP_{600µl}$	118.33±3.12[d]	122.67±2.25[e]	20.00±2.04[e]	87.00±14.35[bc]
$PG_{400µl}$	166.33±3.30[b]	169.33±3.06[c]	51.67±4.25[d]	129.11±16.62[cd]
$PG_{600µl}$	105.00±5.40[e]	94.67±3.66[f]	12.33±1.03[e]	70.67±12.66[d]
Means	170.95±11.94	198.14±22.55	136.67±37.80	168.59

[a:f], Means within each column with different superscripts are significantly different (P<0.05).

CTL: Control, $SA_{400µl}$: 400µl pen-step, $SA_{600µl}$: 600µl pen-step, $PP_{400µl}$: 400µl propolis powder, $PP_{600µl}$: 600µl propolis powder, $PG_{400µl}$: 400µl propolis glue and $PG_{600µl}$: 600µl propolis glue.

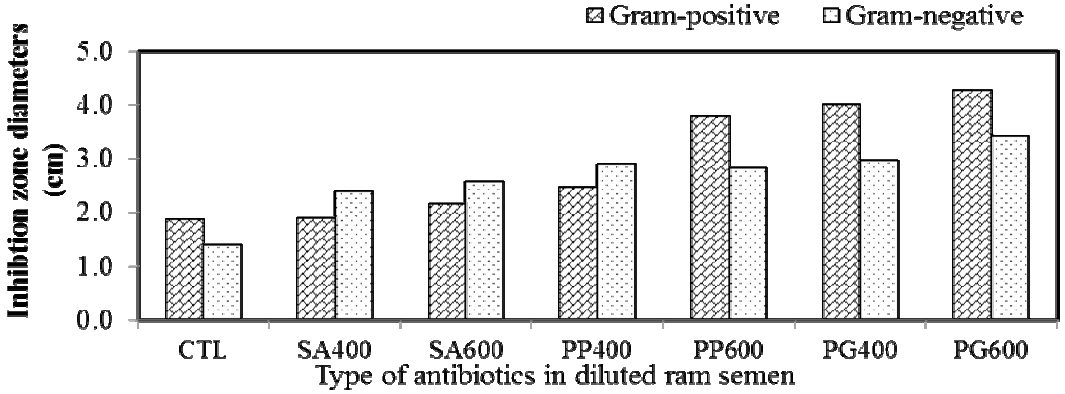

Figure 2. Effect of different antibiotics on bacterial inhibition zone diameters in petri dishes of diluted ram's semen during 24hrs of incubation at 37 °C.

CTL: Control, SA_{400}: 400µl pen-step, SA_{600}: 600µl pen-step, PP_{400}: 400µl propolis powder, PP_{600}: 600µl propolis powder, PG_{400}: 400µl propolis glue and PG_{600}: 600µl propolis glue.

Fertility rate (%):

Fertility rates results of ewes inseminated with diluted ram semen containing $SA_{600µl}$ or $PG_{600µl}$ antibiotics stored at 5 °C for up to one day are presented in Table (8). Fertility rates as conception rate after 1st and 2nd insemination , single born rate %, twins born rate % and litter size were (80.00 *vs* 73.33); (20.00 *vs* 26.67); (73.33 *vs* 93.33); (26.67 *vs* 6.67) ; (1.27 *vs* 1.07) for both $PG_{600µl}$ and $SA_{600µl}$ antibiotics, respectively. Generally, the fertility parameters of the ewes artificially inseminated after one day of storage at 5 °C of the extended ram semen with $PG_{600µl}$ were insignificantly higher than $SA_{600µl}$ one.

Table 8. Fertility traits of ewes artificially inseminated with diluted ram's semen with $SA_{600µl}$ or $PG_{600µl}$ and stored at 5 °C for one day

Items	Antibiotic types	
	$SA_{600µl}$	$PG_{600µl}$
No. of ewes inseminated at 1st service	15	15
No. of ewes conceived at 1st service	11	12
Conception rate after 1st service (%)	73.33	80.00
No. of ewes reiterated insemination at 2nd services	4	3
Conception rate after 2nd service (%)	26.67	20.00
Conception rate after 1st and 2nd services (%)	100.00	100.00
Total number of ewes lambing after 1st and 2nd services	15	15
No. of ewes lambing single	14	11
Single rate (%)	93.33	73.33
No. of ewes lambing twins	1	4
Twins rate (%)	6.67	26.67
Litter size	1.07	1.27

$SA_{600µl}$: 600µl pen-step and $PG_{600µl}$: 600µl propolis glue.

DISCUSSION

1- Effect of synthetic and natural antibiotics in the extender on ram semen characteristics:

Using of natural origin antibiotics (as propolis) instead of synthetic antibiotic in ram semen preservation is interesting in the recent years. Petruska *et al.* (2014) suggested that propolis had an extensive span of biological activities including antibacterial, antiviral, anti-inflammatory and antioxidant properties. Moreover, Akandi *et al.* (2015) proved that propolis in semen medium could protect some semen characteristics as motility, viability and DNA integrity. In addition,

propolis reduces lipid peroxidation concentration, antimicrobial resistancy and supplied vitamins and minerals to semen diluent which keep high quality of viable sperm. The results of the current research are in agreement with those reported by Khalifa *et al*. (2016). They noticed that extenders containing 0.2% of propolis powder or glue showed higher motility, live and normal spermatozoa percentages compared with other experimental antibiotics tested. Furthermore, the values of sperm characteristics of propolis glue extender were better than those of propolis powder. Also, Khalifa *et al*. (2017) reported that traditional extenders as Tris (E1) and sodium citrate (E2) and non-traditional extender as lecithin plus propolis extract (E3) were insignificantly affected semen characteristics during incubation at 37°C for up to 4 hours or storage at 5°C for up to 4 days. The same later author found that semen characteristics had better values in E3 followed by E1 than E2 extenders during preservation condition. On the other hand, Moraes *et al.* (2014) stated that possible better semen quality was observed with inclusion of 1.25 g powder propolis/kg in the rabbit's diet.

From the present results, longer storage period decreased sperm characteristics among extender types. Olurode and Ajala (2016) stated that the drastic decline in sperm characteristics could be attributed to the gradual decrease of nutrients such as potassium, sodium and plasma protein required for high metabolic demands of sperm. Moreover, the effect of peroxidation that comes from polyunsaturated fatty acids in ram sperm cytoplasm membrane lead to loss of cytoplasm membrane, decreased sperm motility and inhibits fructolysis and respiration (Albiaty *et al.,* 2016). In addition, Acharya *et al.* (2016) noticed that the reduce of sperm characteristics be associated with waste products of reactive oxygen species (ROS) and free radical (as superoxide anion O^{-}_{2}, hydrogen peroxide H_2O_2 and hydroxyl radical OH^{-}) might be involved in damaged sperm membrane. Storage of semen for prolongation periods causes ultra-structural, biochemical and functional damage of spermatozoa resulting in a reduction of sperm motility, live sperm impaired transport reduced fertility.

The present study demonstrated that both antibiotics in the extender improved sperm characteristics compared with the control. The best value was recorded with $PG_{600\mu l}$ and the least one was CTL, other treatments came in between. From the obtained results, natural antibiotics in the extenders exhibited the highest ability to sustain the viability of sperm motility. This could be attributed to capability of these antibiotics to supply protection and nutrients afford to sperm cells plus they inhibit microbial growth (by propolis extract) and improve physiologic condition. Hence, the reduction in sperm parameters with longer storage time could be attributed to the gradual consumption of nutrients required for sperm metabolic through preservation.

Similarly, Ciftci *et al*. (2012) stated that using propolis in semen extender resulted in an increase in sperm count and motility, plasma testosterone levels, and a decrease in dead and abnormal sperm count.

Furthermore, the possible physiological reasons for the decline in motility might be due to extracellular oxidative stress, effects of seminal plasma volume-constituents and endogenous free radical production. Substances from seminal plasma protect spermatozoa from premature aging during storage (Kasimanickam *et al*., 2006).

Mammalian tissues contain several enzymes scavenging reactive oxygen species (ROS) such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione-S-transferase (GST) and reduced glutathione (GSH) as controlling system of ROS and protecting cells under stress condition. Also, there are some natural compounds contribute to the detoxification process from ROS such as propolis (Jasprica *et al*., 2007 and Yousef and Salama, 2009).

Thus, using propolis in semen extender as antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes leading to cell damage. Many studies showed that propolis possesses antioxidant activity. This may be due to the free radical scavenging activity of propolis that protects sperm membrane from the deleterious action of oxidative attacks and reduces their barbituric acid reactive substances formation (Russo *et al*., 2006).

The inclusion of propolis powder in the diet increased normal spermatozoa percentage and reduced spermatozoa abnormalities. The propolis powder did not affect the progressive spermatic motility, spermatic vigor or spermatic concentration. However, a small reduction in semen volume was observed, without any negative effect on the other semen characteristics evaluated. Thus, it is possible to observe better semen quality with inclusion of 1.25 g propolis powder /kg in the diet for reproducer rabbits (Moraes *et al.*, 2014).

2- Effect of synthetic and natural antibiotics in the extender on enzymatic activities:

The continuous increase in leakage of ALT, AST, ALP and LDH enzymes into the extracellular medium during incubation or storage may reflect the breakdown of the cellular sperm membrane during incubation and storage (Zeidan *et al.*, 2004). The alkaline phosphatase enzyme leakage from the spermatozoal cells due to cold shock or incubation at 37°C into the seminal plasma was significantly ($P<0.05$) increased (White *et al.,* 1954). Such increase in the enzyme activity after incubation and preservation, however, may be a sign for increasing the cell damage which occurred during storage process (Zeidan, 1994).

In eutherian mammals, the acrosome contains digestive enzymes (including hyaluronidase and acrosin). These enzymes break down the outer membrane of the ovum (zona pellucida), allowing the haploid nucleus in the sperm cell to join with the haploid nucleus in the ovum.

3- Effect of synthetic and natural antibiotics in the extender on bacterial count and inhibition zone:

In this study, propolis contained extenders was significantly ($P<0.05$) higher inhibition for Gram-positive and negative bacteria, bacterial zone diameters and lower bacterial count than either synthetic antibiotic or CTL in extended ram semen during storage condition. These results are in accordance with those noted by Takaisi-Kikumi and Schilder (1994) who observed that the antibacterial action against *Streptococococcus agalactiae* was complex, propolis involving several mechanisms such as the formation of pseudo-multicellular streptococci; disorganization of the cytoplasm, the cytoplasmatic membrane and the cell wall; partial bacteriolysis and inhibitation of protein synthesis. In addition, Mirzoeva *et al.* (1997) reported that propolis had bacterioststic activity against gram-positive and some gram-negative bacteria. The mechanism action of propolis is likely to be related to the change in the bioenergetic status of the bacterial membrane, which inhibits bacterial motility.

Moreover, Khalifa *et al*. (2016) reported that semen extender with propolis showed more effectiveness in inhibitory bacterial zone and reduced bacterial count compared with other extenders and propolis can be used to restrain the bacterial infection and improved semen quality. Khalifa *et al*. (2017) reported also that propolis addition to the extender instead of synthetic antibiotics could resist bacterial contamination which improved sperm viability during incubation or storage.

The antibacterial action of propolis on different bacterial strains has been observed by several authors (Bankova, 2005; Khalifa *et al*., 2016) they found that biological activity of propolis could be almost identical (i.e., antimicrobial, antitumor, antioxidant, anti-inflammatory, etc.). Also, Itavo *et al*. (2011) confirmed that propolis has bacteriostatic activity against gram-positive and some gram-negative bacteria, *via* related to changes in the bioenergetic status, which inhibits bacterial motility.

The antimicrobial activity of propolis ethanolic extract against *Candida albicans*, *Escherichia coli*, *Staphylococcus sp*, and *Streptococcus sp*, the major microorganisms (somatic cell counts) that causing mastitis was observed *in vitro* as well as *in vivo* even in ovine or bovine species (Pinto *et al*., 2001). Cattani *et al*. (2012) reported that the higher antimicrobial activity of propolis against gram-positive than gram-negative bacteria lead to improve the feed efficiency because gram-positive bacteria produce more ammonia, hydrogen and lactate than gram-negative species, also the antioxidant activity of propolis components would lessen oxidative stress, thus promoting better conditions for rumen microbial growth, consequently enhancing the fermentation process. Probst *et al.* (2011) observed that essential oils of propolis glue contribute to fights the bacteria and also it has different mechanism of action that are important for the antimicrobial activity. In addition, activity mechanism of volatile oils of propolis is complex and may be attributed to the cooperation among some of its components. Niculae *et al.* (2015) established also that the chemical constituent of volatiles oil in propolis had antimicrobial actions against microorganisms and positively correlated with efficacy towards *E. coli* strains.

4- Effect of synthetic and natural antibiotics in the extender on fertility:

The major finding of the present study demonstrated that $PG_{600\mu l}$ addition to the extended ram semen stored at 5°C improved fertility rate compared with $SA_{600\mu l}$ one.

These results are in accordance with that noticed by Yousef and Salama (2009) who reported that propolis could provide protection against infertility by improving sperm production, motility, sperm count and quality in male rats exposed to aluminum chloride toxicity because of propolis induced a significant increase in the level of antioxidant enzymes. The highest value of fertility was recorded with PG_{600ul} may be due to the free radical scavenging activity of propolis that protects sperm membrane from the deleterious action of oxidative attacks and reduces their barbituric acid reactive substances formation (Russo *et al*., 2006).

In addition, many studies referred that using of propolis increased fertility and improved the blood criteria according to Bankova (2005). Moreover, owing to the high content of propolis in flavonoids, it can affect the reproductive performance of the animals. For females, propolis flavonoids due to their non-polarity or in complex with serum albumin, can pass the plasma membrane and can attach to the cytoplasmic steroid receptor. Consequently, flavonoids are carried into the cell nucleus to the transcription complex at the genes controlling the expression of estrogen receptors and may be also of other proteins participating in the growth, reproduction and function of the mammary gland. Propolis administration was found to have a good impact on pregnant ewes' health and perhaps a promising feed additive during critical periods such as flushing (Morsy, 2013).

Schulze *et al.* (2016) demonstrated that antibiotics were of great importance in semen extenders to ensure long life of spermatozoa and to reduce transmission of pathogens into the female tract. However, the use of synthetic antibiotics carries a risk of developing resistant bacterial strains in artificial insemination laboratories and their spread via artificial insemination. The highest reproductive performance of ewes treated by PG600µl may be related to using of propolis extract compared to SA600µl. Based on the results, 600µl of propolis glue extract presented in PG600µl extender could preserve sperm plasma membrane and improve the cells normal morphology, viability and reduce of acrosomal damage by preventing free radical and ROS compared with the SA600µl extender.

Indeed, the propolis extract was found to possess the ability to protect sperm membrane from the harmful action of oxidative aggression, while reducing the thiobarbituric acid-reactive substance (TBARS) and LDH enzyme release.

In conclusion, from current results, it was evident that the natural antibiotic improved semen quality and maintenance of enzymatic activities compared to either

synthetic antibiotic or CTL extenders. Concerning the addition of natural antibiotic to extenders, semen characteristics with propolis glue extenders were better than those contained propolis powder. Furthermore, the best semen extender was PG600µl and it can be used to inhibit bacterial zone, to successfully inseminate to enhance lambing rates in ewes under Egyptian environmental condition. Particularly, in those regions of Asia and Africa, where the liquid nitrogen may not be available for freezing semen for the long time to come.

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تأثيرات البروبوليس المضادة للبكتيريا على حفظ السائل المنوى المخفف للكباش ومعدل خصوبته

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هدفت الدراسة الحالية الى تحديد تأثير استخدام تركيزات مختلفة من مستخلص البروبوليس كمسحوق أو صمغ مقارنة مع المضادات الحيوية المصنعة على خصائص السائل المنوي للكباش، ومقاومة التلوث البكتيري، ومعدل الخصوبة. تم جمع عينات السائل المنوي باستخدام المهبل الاصطناعى من ستة كباش ناضجة مرتين أسبوعيا لمدة ثلاثة أسابيع. تم خلط وتخفيف السائل المنوي للقذفات المجمعة وتقسيمها إلى سبعة أجزاء بمعدل تخفيف ١ سائل المنوي : ١٠ مخفف. واشتملت السبعة أجزاء على المعاملة الضابطة (بدون إضافة أى مضاد حيوى) والتى استخدمت كمقارنة ، المعاملات الأخرى شملت المضاد الحيوي المصنع (بنسترب ٤٠٠ و ٦٠٠ ميكرون) ، المضاد الحيوي الطبيعي وهو البروبوليس (مسحوق ٤٠٠ و ٦٠٠ ميكرون أو صمغ ٤٠٠ و ٦٠٠ ميكرون) على التوالي. تم تخزين هذه العينات على درجة ٥م° لمدة ٦ أيام. أظهرت النتائج أن خصائص الحيوانات المنوية انخفضت معنويا ($P<0.05$) مع تقدم فترة التخزين في جميع أنواع المخففات. بالإضافة إلى ذلك ، تم تحسين خصائص الحيوانات المنوية ($P<0.05$) و كذلك انخفاض النشاط النزيمي لها حيث كانت الأفضل في العينات المعاملة بالمضادات الحيوية المصنعة أو الطبيعية سواء البودر أو الصمغ مقارنة بالكنترول. أيضا لوحظ نشاط مضاد للبكتيريا أعلى ($P<0.01$) بعد ٢٤ ساعة من التحضين للعينة المعاملة بـ ٦٠٠ ميكرون مسحوق أو صمغ البروبوليس مقارنة بعينات السائل المنوي الأخرى. النعاج المعاملة بـ ٦٠٠ ميكرون من صمغ البروبوليس حصلت على أكبر حجم للخلفة (١,٢٧) مقارنة بالنعاج المعاملة بـ ٦٠٠ ميكرون بنسترب (١,٠٧).نستنتج من نتائج هذه الدراسة أن تأثير البروبوليس الطبيعي المضاد للبكتريا سواء المسحوق أو الصمغ في عينات السائل المنوي المخففة للكباش قد أدى الى تحسين نوعية السائل المنوي المخفف (وتثبيط النمو البكتيري) وكذلك تحسين معدل الخصوبه .