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POSSIBILITY OF USING PROPOLIS AS NATURAL ANTIBIOTIC INSTEAD OF SYNTHETIC ANTIBIOTICS IN RAM SEMEN EXTENDERS

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SUMMARY

The objective of this study was to compare between propolis (as natural antibiotic) and synthetic antibiotics on sperm survivability and protection against bacterial contamination of extended ram semen. Ethanolic extract of propolis was prepared by dissolving either powder or glue (10 grams/100 ml ethanol) for 24 hrs. then stored in dark at 4ºC. In this study, seven mature rams were used for semen collection using artificial vagina. Tris-glucose-egg yolk (TGE) extender was prepared. Samples of pooled semen were diluted at extension rate 1: 10 (semen: extender). Then, extended semen was divided into 6 portions, served as T1, T2, T3, T4, T5 and T6 each contained 0.2 ml of Pen-Strep (T1), Alamycin LA (T2), Vetrocin (T3) and propolis ethanolic extract as powder (T4) or glue (T5), respectively. The 6th portion (T6) was served as control without any antibiotic addition. The characteristics of spermatozoa such as motility, livability and normality and bacteriological procedure for T1, T2, T3, T4, T5 and T6 were assayed during incubation at 37°C from 0 to 3 hours. The results indicated that extended ram semen in groups T1, T3, T4 and T5 exhibited higher ($P < 0.05$) percentages of motility, livability and normality than T2 and T6 during incubation. However, T1, T4 and T5 appeared not successful ($P>0.05$) to suppress bacterial contamination. However, T4 and T5 showed more effectiveness in controlling bacterial growth after 3 hrs of incubation. Moreover, maximum ($P<0.05$) inhibitory bacterial zones were observed in T4 and T5 than other experimental treatments.

It could be conclude that either propolis powder or glue supplementation at 0.2% of extender had a potential effect among the experimental antibiotics tested. In addition, propolis can be used to restrain the bacterial infection and improve semen quality. However, testing more different levels of propolis in a fertility trial would be applied in future studies.

Key words: *rams, propolis, antibiotics, bacterial growth, semen extender.*

INTRODUCTION

Semen preservation is an effective tool to enhance the reproductive performance and maintain the genetic of the superior mammalian males. Slow progress in semen preservation might be due to contamination, which may occur during semen handling procedures and extension, which usually takes place without access to an airflow. Addition of antibiotics to semen extender was one of the first major advances that significantly improve the fertility potential of artificial insemination (AI). Nicholson *et al.* (2014) stated that addition of antibiotics to semen extenders to control the growth of these contaminants has stipulated by national and international guidelines. On other hand, Jane and Wallgren (2014) reported that bacterial infections had a negative effect on sperm quality, either by direct competing with spermatozoa on nutrients supplied to extender or by the production of toxic metabolic by-products and endotoxins. Since antibiotics may be toxic to spermatozoa, a cocktail of natural agents used in semen extenders to reduce the effect of each individual component to limit inflammation or disease in inseminated females

(Guimaraes *et al.,* 2015). Actually, finding alternative antibiotics able to control microorganisms in semen extender for AI would be beneficial to improve sperm quality, survival and to slow the development of antibiotic resistance. However, no data have been available regarding the effect of propolis on bacterial resistance of preserved mammalian semen. Hence, propolis (bee glue) is a natural product of honeybees (*Apis mellifera*) collected from various plants, which used as building material (filling cracks and gaps) or for protection against intruders (embalms killed invader insects) in bee hive (Bonvehí and Gutiérrez, 2012). Additionally, propolis used as a folk medicine from ancient times and it is an adhesive material, dark yellow to brown balsam. It has a wide range of biological activities including antibacterial (stops the multiplication of the bacterium by damaging its cytoplasm, causing bacteriolysis), antiviral, anti-inflammatory and antioxidative properties (Petruska *et al.,* 2014). On the other hand, propolis has antibacterial activity against Gram positive strain bacteria (*S. aureus, S. pyogenes, S. viridens, D. pneumoniae,* and *C. diphtheria*) and Gram-negative strain bacteria (*E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhi, S. paratyphi* A*, S. paratyphi*-B*,* and *S. flexneri*) rods and cocci, *Helicobacter pylori*, *Tryponosoma cruzi*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Candida*, *Saccharomyces*, *Cryptococcus*, *Mycobacteria* (Haddadin *et al.,* 2008). Furthermore, propolis contains bioflavonoids (natural pigments) that play very important role in the scavenging of free radicals that damage sperm cell membrane (Gromenko *et al.,* 2008). Also, propolis contain a large amount of amino-acids such as the mono-amino-monocarbonic group, up to 41.45% (alanine, valine, glycine, leucine, isoleucine, serine, threonine), 25.53% mono-amino-dicarbonic group (aspartic acid and glutamic acid), 11.39% heterociclc (histidine, proline), 7.47% di-amino-monocarbonic (argine, lysine), 5.92% cycle-aromatic (fenylanina , tyrosine), 6.90% cystine and 1.19% methionine (Eremia and Dabija, 2007). Moreover, micro and macro elements such as Ca, Mg, K, Fe, Na, Se, P, Mn, Cu, Co and Zn found abundant in propolis (Miguel et al., 2011). Further studies revealed that propolis contained more than 200 constitutes including flavonoids as a major compounds, amino acids, fatty acids and vitamins like B1, B2, B6, C, E and enzymes like adenosine triphosphatase, succinic dehydrogenase, glucose 6-phosphatase (Zeighampour *et al.,* 2014).

Based on the antibacterial activity of propolis, the present study designed to investigate the possibility of using propolis (as powder or glue) as natural antibiotic to inhibit bacterial infection in extended ram semen in comparison with some other synthetic antibiotics.

MATERIALS AND METHODS

This study was conducted at two Research Stations: Sids and El-Serw, both belong to Animal Production Research Institute (*APRI*), Ministry of Agriculture, Egypt. The experimental period carried out from September 2015 to June 2016.

Ethanolic extract of propolis preparation

Ten grams powder of propolis was dissolved and mixed at room temperature up to100 ml in ethanol (70% concentration) for 24 hours, then filtered. After 24 hrs, the filtrate completed up to 100 ml with ethanol and kept in dark bottle. The same method used to preparer 100 ml of glue propolis extract (using 10 gram of glue) in another dark bottle. Then, both bottles were stored at 4ºC until used in semen extenders.

Animals and semen collection

Seven sexually mature rams at age 24 to 30 months, maintained under the same feeding, housing and lighting conditions. They were used to collect semen by an artificial vagina. Rams were kept in pens, fed individually daily with concentrate mixture at 60% and 40% roughage/kg body weight, fresh water and minerals blocks were available *ad-libitum*. A total of 18 pooled semen ejaculates were evaluated and accepted to be used in this work, if the following criteria were met (volume ≥ 0.50 ml, sperm concentration ≥ 2×10^9/ml, the motility sperm percentage > 70%, total abnormal sperm ≤ 15% and spermatozoa livability≤ 15%).

Semen dilution and addition of antibiotics

Pooled semen diluted by Tris-glucose-egg yolk (TGE) at extension rate 1:10 (semen: extender). Then, extended semen divided into 6 portions poured in 6 sterilized tubes. Three portions contained synthetic antibiotics as: Pen-Strep, Alamycin and Vertrocin at level 0.2ml (T1, T2 and T3, respectively). The forth and fifth portions contained 0.2 ml of either propolis ethanolic extract powder (T4) or glue (T5). The last portion (T6) considered as a control extended without any antibiotic. The chemical ingredients of semen extender with different types of antibiotic are presented in Table (1). All diluted semen in T1, T2, T3, T4, T5 and T6 were incubated at 37°C for 3 hours and progressive motility, live sperm and normal sperm were investigated.

Table 1. Chemical ingredients of semen extender with different types of antibiotic.

Ingredients	Different types of antibiotic					
	T1	T2	T3	T4	T5	T6
***Tris, (g)**	3.634	3.634	3.634	3.634	3.634	3.634
glucose, (g)	0.500	0.500	0.500	0.500	0.500	0.500
Citric acid, (g)	1.990	1.990	1.990	1.990	1.990	1.990
Antibiotic, (ml)	0.200	0.200	0.200	0.200	0.200	-
Egg yolk, (ml)	20	20	20	20	20	20
Distilled water up to, (ml)	100	100	100	100	100	100

***Tris:** hydroxymethl amino methane. **T1:** containing Pen-Strep20/20 antibiotic genesis of procaine penciling 200mg, Dihydrostreptomycin sluphate250mg. **T2:** containing Alamycin AL antibiotic genesis of Oxytetracycline dehydrate equivalent 200mg base as the magnesium complex in an aqueous solution. **T3:** containing Vertrocin antibiotic genesis of Penicillin G (as procaine) 1.200.000IU, Penicillin G (as sodium) 400.000 IU, Streptomycin (as sulphate) 2.0gm. **T4:** containing natural antibiotic genesis of powder propolis ethanolic extract. **T5:** containing natural antibiotic genesis of glue propolis ethanolic extract. **T6:** control extender without any type of antibiotic.

Bacteriological procedure

Preparation of medium

The medium was prepared by dissolving 40 g of Tryptone Soya Agar (Oxoid) in 1 litter distilled water and heating to boiling point. The medium was autoclaved at 121°C under 15 pound/inch pressure for 20 minutes. Then cooled down to 50-55°C, 10% fresh de-fibrinated sheep blood was added and subsequently poured in sterilized Petri dishes. In order to check the sterility of media, Petri dishes kept in incubator at 37°C for 24 hours and the viable bacterial counts were determined in all samples using the spread plate method (Harry and Paul, 1981).

Semen bacterial counting

Ten µl of fresh semen diluted in 990 µl of extender contained different types of antibiotics (T1, T2, T3, T4, T5 and T6). These samples were transferred into 99 ml dilution blanks (44-

45°C) containing 2% sodium citrate solution. Shaking carried out several times until emulsification (first dilution). Also, samples transferred 1 ml from first dilution into 99 ml dilution blanks (44-45°C) containing 2% sodium citrate solution (second). This was repeated until reaching to sixth dilution. The 5th and 6th dilutions were poured into each well of inoculated plates aseptically. The Petri dishes were then incubated at 37°C for 48 hrs and a colony counter counted the number of colonies that arose. The number of bacteria present in each Petri dish was calculated by multiplying the number of colonies with the dilution rate at which the colonies developed. The final results were expressed as CFU/ml according to Qureshi et al. (1993).

Antibiotic sensitivity for the suspected colonies (Mari, 2005)

1. Select colonies (Gram-positive and Gram-negative bacteria)
2. Prepare inoculum suspension
3. Standardize inoculum suspension
4. Inoculate plate (Tryptone Soya Agar)
5. Add antimicrobial disks
6. Incubate plate at 37 °C for 20-24 hours
7. Measure inhibition zones

After the plates were incubated at 37ºC for 24 h, they inspected for the zone of inhibition of bacterial growth. The zones of inhibition recorded as circumference (π× circle diameter) and area (π× Radius2) the value of π=3.14.

Statistical analysis

Data were expressed as means (±S.E.) and statistical analyses were performed with SPSS Version 22.0 for Windows (SPSS, 2013, Inc., Chicago, IL, USA). Duncan test of the same SPSS program was applied to determine significant differences among all parameters. Differences with probability value of P<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of antibiotics on diluted ram semen characteristics

Incubation measurements (%) of motility, live and normal spermatozoa at zero time (initial hours) at 37°C with T1, T2, T3, T4, T5 and T6 demonstrated non- significant negative effect (P>0.05) on sperm characteristics as presented in Figure (1). However, at 3hrs of incubate the effect was significant (P<0.05) on sperm morphology in relation to T1, T2, T3, T4, T5 and T6 as shown in Table (2). T1, T4 and T5 preserved sperm cells better than those other synthetic antibiotics in T2, T3 and T6. Contrariwise, T4 and T5 improved the percentages of motility, live and normal spermatozoa due to inhibitory effect on bacterial infection. Similarly, Petruska *et al.,* (2014) reported that propolis prevented the growth of bacteria and had a free radical scavenging activity. On the other hand, Miguel *et al.* (2011) found that propolis rich in polyphenols, flavonoids and other essential vitamins and minerals that thought to contribute in survivability of sperm during storage. Moreover, T4 and T5 could provide zinc in the extender, which may improve semen quality compared to the synthetic antibiotics. Finding of Dorostkar *et al.* (2014) showed positive significant correlations between zinc and sperm parameters beside that zinc is an antioxidant agent. A co-factor of copper/zinc superoxide dismutase (Cu/Zn), plays a major role in the protection of spermatozoa against peroxidative damages and reactive oxygen species (ROS).

The observed decreased reduction in sperm characteristics with the presence of synthetic antibiotics during progressive incubation time (3hrs) may be attributed to the lowering mitochondrial activity in spermatozoa, since antibiotics inhibit DNA. Madeira *et al.* (2014) revealed that this particular mechanism of action allows the elimination of strains resistant to antibiotics, which act on the cell wall, the cytoplasmic membrane or on protein

synthesis. Current knowledge about propolis is well known as a highly valuable biological, pharmacological properties, antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, immunomodulatory and wound healing (Pascoal *et al.,* 2014). The best sperm parameters with both T4 and T5 extenders during incubation might attributed to enhancing the activity of mitochondrial respiration. This agree with that carried out by Toreti *et al.* (2013) who suggested that propolis might have positive influence on mitochondrial membrane potential via increased permeability of the inner mitochondrial membrane. The present results are consistent also with those reported by Cedikova *et al.* (2014) who revealed that propolis affects either mitochondrial respiration which enhanced oxygen consumption by~50% or the activity of mitochondrial enzymes involved in the electron transport system. In view of this, the last authors stated that propolis increased dehydrogenase thus rising motility sperm activity and affected the coupling of the electron transport to adenosine triphosphate (ATP) synthesis. It is expected that the adequate amount of energy components of propolis required by sperm during storage to maintain movement and other physiological functions (Akandi *et al.,* 2015). Furthermore, ferulic acid in propolis was also reported to elevate sperm motility and viability (Niculae *et al.,* 2015). Actually, a high concentration of free radical may be obtained by advancing incubation time of semen at 37°C (produced by cell metabolism, cellular respiration and consequence of pathological events) which could damage all biomolecules like lipids, carbohydrates, proteins and both mitochondrial and nuclear DNA (Madeira *et al.,* 2014). In addition, ROS can generated from exogenous and endogenous sources and they may cause damage to different molecules and parts of spermatozoa (Shamsi *et al.,* 2011). Apparently, the ROS affect not only the acrosome and flagellum of spermatozoa but also intervene into the mitochondrial system with a subsequent influence on respiration (Rozkot *et al.,* 2013). Thus, too much production of ROS might be cytotoxic through the production of free radicals that negatively affect spermatozoa function. Budai *et al.* (2014) observed that ram sperm cells are vulnerable to free radical attack, since they are rich in polyunsaturated fatty acids (PUFA). In this case, ROS can readily combine with PUFA, that performed directly lipid peroxidation (LPO) which causes tissue injury. For this reason, propolis may played a major role to obliteration of free radicals. This corresponds to the opinion of Çetin *et al.* (2011) who stated that propolis can reduce the levels of ROS; such as hydrogen peroxide (H_2O_2) and nitric oxide (NO) that might be responsible for its anti-inflammatory effects, and also caffeic acid phenethyl ester (CAPE) as another compound in propolis that can be provided stumbling block to generate ROS. In previous researches, Ates *et al.* (2006) showed that CAPE in propolis could regulate antioxidant enzymes, inhibit lipid peroxidation and reduce cell injury. In addition, Ozyurt *et al.* (2007) reported the antioxidant properties of CAPE could be similar to those of vitamin E. On the other hand, nonylphenol (NP) and octylphenol (OP) may cause tissue injury by enhancing ROS generation and induce cell death. This result is in agreement with Shalaby and Eman (2011) who explained the ability of propolis to ameliorate the oxidative stress toxicity which induced by NP and OP. Otherwise, propolis may have an important role in balancing antioxidant systems and has an antiperoxidant effect on tissues, thus propolis provides protection against infertility by improving sperm quality (Moraes *et al.,* 2014). Generally, El-Battawy and Brannas (2015) concluded that extenders containing propolis showed higher motility percentage, an appropriate cryoprotective agent and maintained the integrity of the fish spermatozoa cells and this effect may be due to the phenolic components of propolis and their antioxidant activity.

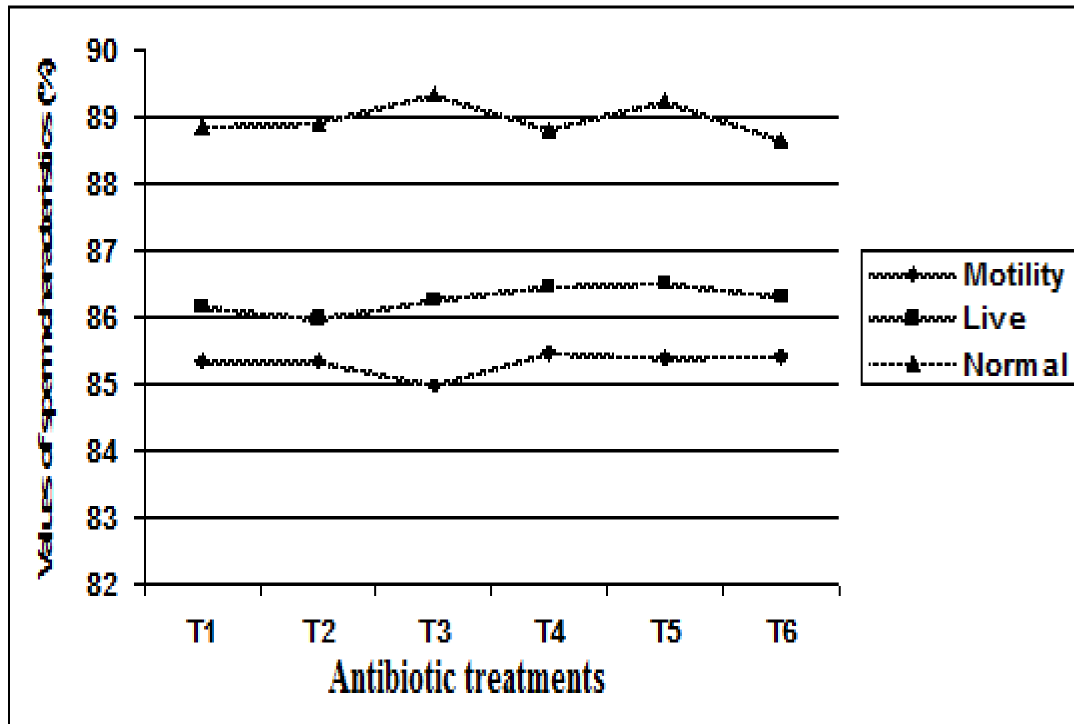

Figure 1. Effect of antibiotic types on diluted ram semen characteristics at zero time of incubation at 37°C.

T1: Pen- Strep, T2: Alamycin, T3: Vertrocin, T4: propolis powder, T5: propolis glue and T6: control without antibiotic type.

Table 2: Effects of antibiotics (Mean ± SE) on diluted ram semen characteristics after 3hrs of incubation at 37°C.

Item	Antibiotic types	Sperm characteristics		
		Motility (%)	Live (%)	Normal (%)
Synthetic antibiotic	T1	78.75±1.64[a]	77.83±1.68[ab]	80.42±1.66[ab]
	T2	74.17±1.21[bc]	72.50±1.06[bc]	75.50±1.90[bc]
	T3	76.25±0.90[ab]	75.58±1.98[b]	78.85±1.66[ab]
Natural antibiotic	T4	80.42±1.56[a]	81.25±1.40[a]	82.50±1.09[a]
	T5	81.67±0.94[a]	82.75±1.84[a]	82.92±1.83[a]
Control	T6	71.25±1.96[c]	69.75±1.21[c]	72.33±1.29[c]

Means under each parameter in the same column, with different superscript differ significantly (P < 0.05). T1: Pen-Strep, T2: Alamycin, T3: Vertrocin, T4: propolis powder, T5: propolis glue and T6: control without antibiotic type.

Bacteriological parameters

The results displayed increase in viable bacterial counts in all antibiotic types of diluted semen, stored from 0 to 3 hrs at 37°C as presented in Table (3). Moreover, inhibition zones in Petri dishes are shown in Fig. (2). Also, bacteriological picture fields of antibiotic types in Petri dishes presented as Gram-positive bacteria Fig (3) and Gram-negative bacteria Fig (4) for T1, T2, T3, T4, T5 and T6. Comparing with the control (T6), all antibiotic types recoded higher bacterial inhibition (P<0.05) during incubation time from 0 to 3hrs at 37°C. The different antibiotics supplementation in T1, T3, T4 and T5 had observed higher effect on controlling bacterial contamination at final incubation than T2. Moreover, statistical analysis between T1, T3, T4 and T5 tend to be non-significant. Interestingly, the bacterial count did not differ

significantly between T4 and T5 but, it was lower in T5 (197.00) than T4 (204.00). Souza *et al.* (2014) verified that ethanol extract of propolis interfered on the bacterial growth by inhibiting protein synthesis that may be conflicted with the enzymatic activity of some bacteria. Obviously, the lowest bacterial count in T5 may be related to volatile oils in propolis glue, which caused several behavioral biological activities. These results are in accordance with those noted by Probst *et al.* (2011) that volatile oils of propolis glue contribute to fight the bacterial infections and also it has different mechanisms of action that are important for the antimicrobial activity. In addition, activity mechanism of volatile oils of propolis is complex and may attributed to the cooperation among some of its components. Recently, Niculae *et al.,* (2015) established that the chemical constitution of volatile oils in propolis have antimicrobial action against microorganisms and positively correlated efficacy towards *E. coli* strains.

In the current study, bacterial concentration varied (P<0.05) according to incubation time (0hr, and 3hrs of ram semen). In zero time (0hr) of incubation, bacterial growth varied respectively for T1, T2, T3, T4, T5 and T6 at 189.33, 207.00, 196.33, 192.00, 180.00 and 308.33 CFU/ml. However, at 3hrs of incubation, higher number of bacteria concentration found in T6 compare to T1, T2, T3, T4 and T5 concentration, being 471.33, 195.33, 232.33, 201.33, 237.00 and 197.00 CFU/ml, respectively. Akandi *et al.* (2015) found that propolis added basically to semen could reduce lipid peroxidation; an antimicrobial protective role and provide vitamins and minerals. The phenolic compounds of various structures (as tannin, flavonoids, anthocyanins, simple phenolic glycosides, etc.) and some phenolic acids (as caffeic, chlorogenic, Gallic and quinic), are primarily responsible for the biological activity of propolis and are more specific to the microbial agents (Chirumbolo, 2011). The action against bacterial infections is an essential characteristic of propolis and this fact has been recognized. The present results revealed the means of inhibition zone diameters (P<0.05) among T1, T2, T3, T4, T5 and T6 for both Gram-positive and negative bacteria. The greatest circumference zone diameters were 4.20, 3.20 and 2.83mm and 2.20, 1.70 and 2.17 with T5, T4 and T1 for both Gram-positive and negative bacteria, respectively compared to 2.83, 2.53 and 2.37 Gram-positive and 1.67, 1.93 and 1.47 Gram-negative bacteria with T3, T2 and T6, respectively. Accordingly, Bankova *et al.* (2014) reported efficacy of propolis ethanolic extracts with inhibition zone diameters ranging between 7-12 mm and minimum inhibitory (MIC) at 0.625% (v/v). According to Hegazi *et al.* (2014) who stated the efficacy of Egyptian propolis on several Gram-positive bacteria (*Staphylococcus aureus*, coagulase negative staphylococci, *Streptococcus agalactiae,* *Streptococcus dysgalactiae*) and balancing of activity against Gram-negative bacteria (*E. coli* and *Pasteurella spp.*) In general, analysis of propolis showed that presence of derivatives of the following acids; caffeic, cinnamic, *P* –coumaric, ferulic and 3, 4 dihydroxy benzoic had positive resistance to microorganisms (Silva *et al.,* 2015).

Table 3: Effects of antibiotics on Bacterial count (Mean ±SE) of diluted ram semen incubated for 3 hours at 37°C.

Item	Antibiotic types	Bacterial count CFU/ml	
		At zero time (0hr)	After 3hrs
Synthetic antibiotic	T1	189.33±4.09[c]	195.33±4.70[c]
	T2	207.00±7.55[b]	232.33±13.28[b]
	T3	196.33±2.03[bc]	201.33±5.21[c]
Natural antibiotic	T4	192.00±3.00[bc]	237.00±9.61[b]
	T5	180.33±2.03[c]	197.00±2.52[c]
Control	T6	308.33±9.40[a]	471.33±12.41[a]

Means under each parameter in the same column, with different superscript differ significantly ($P < 0.05$). T1: Pen-Strep, T2: Alamycin, T3: Vertrocin, T4: propolis powder, T5: propolis glue and T6: control without antibiotic type.

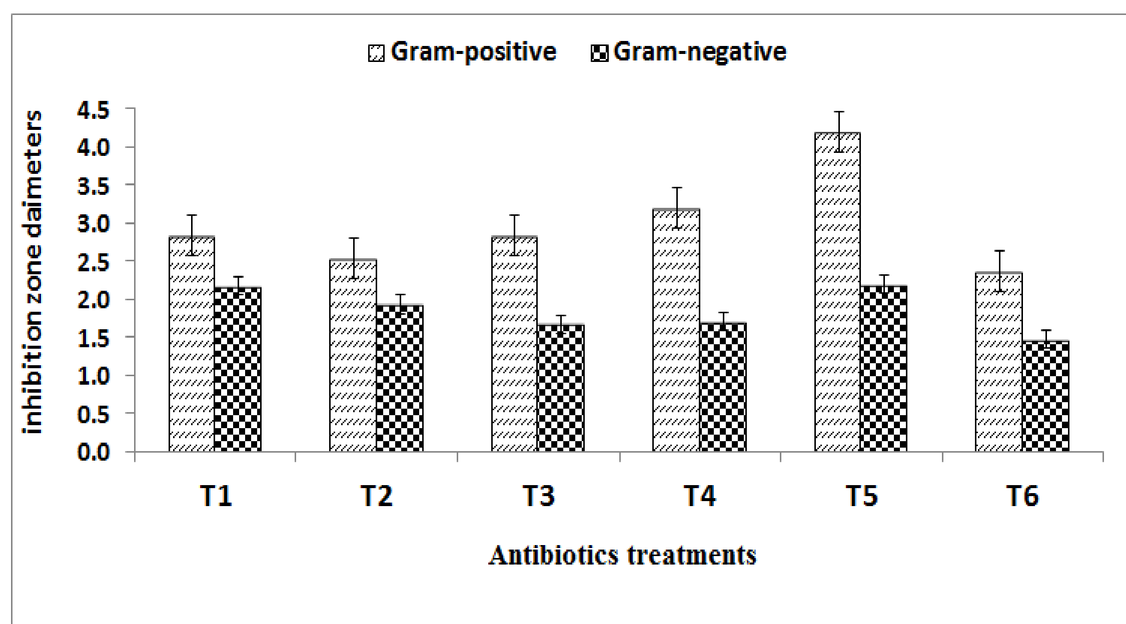

Figure 2. Effect of antibiotics on Gram-positive and negative bacteria inhibition zone diameters in petri dishes of diluted ram semen incubated for 3hrs at 37°C.

T1: Pen- Strep, T2: Alamycin, T3: Ver+trocin, T4: propolis powder, T5: propolis glue and T6: control without antibiotic type.

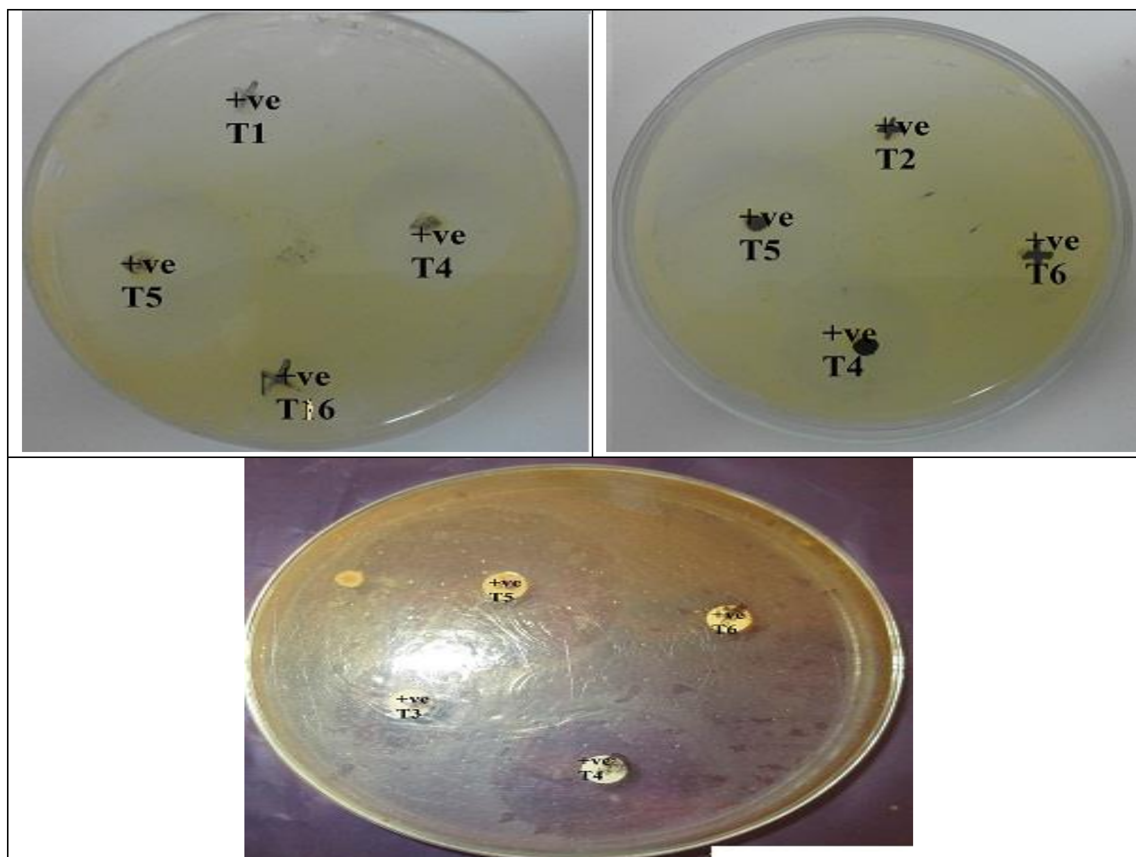

Figure 3: Gram-positive bacteria

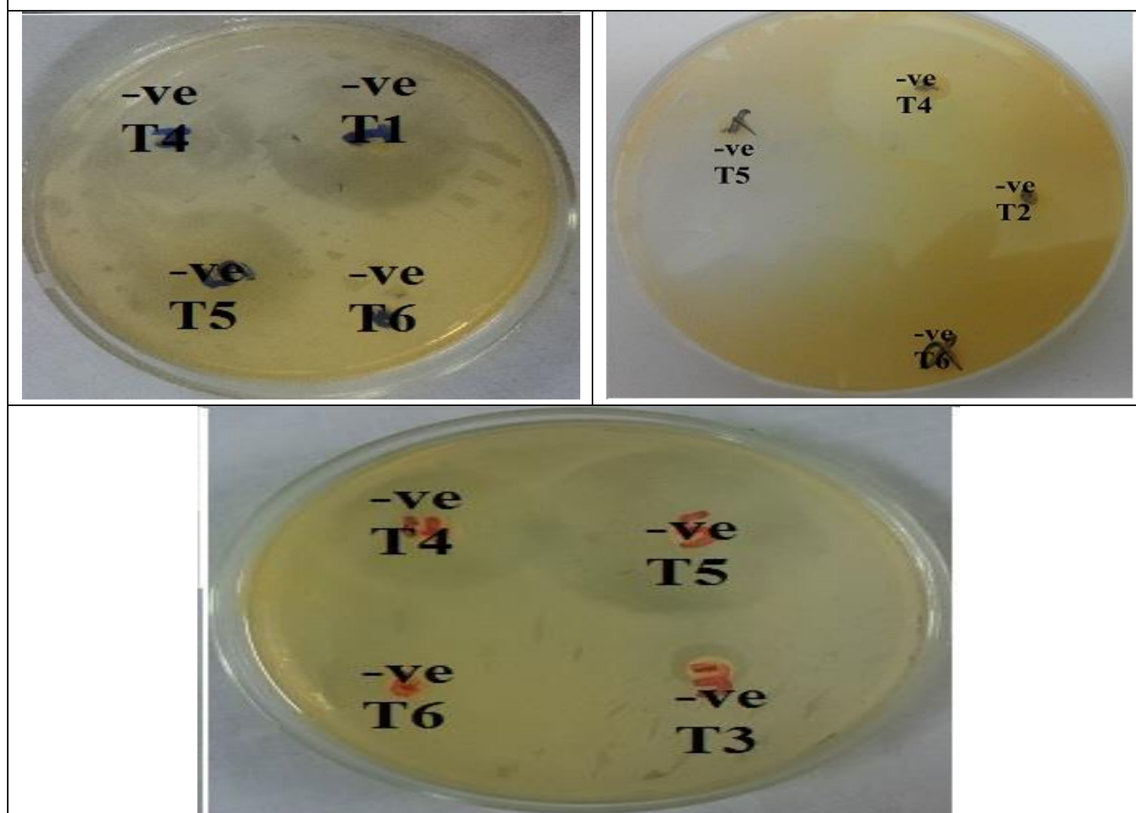

Figure 4: Gram-negative bacteria

CONCLUSION

Based on our findings, survivability of diluted ram spermatozoa stored and incubated at 37ºC can maintain longer livability in the presence of propolis either as powder or as glue compared with synthetic antibiotics. Furthermore, semen extended with either propolis powder or glue at the dose of 0.2 % (v/v) had the best effect on resistance of bacterial contamination to semen than some synthetic antibiotics. Further studies are needed in the future to determine the main active level of different levels of propolis that having the beneficial effect on fertility rate.

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الملخص العربى

إمكانية استخدام البروبوليس كمضاد حيوى طبيعى بدلا من المضادات الحيوية المصنعة فى مخففات السائل المنوى للكباش

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الغرض من هذه الدراسة مقارنة تأثير البروبوليس كمضاد حيوى طبيعى بالمضادات الحيوية المصنعة على حيوية الحيوانات المنوية المخففة للكباش وحمايتها من التلوث البكتيرى. جهز مستخلص من البروبوليس بإذابة كلا من البروبوليس كمسحوق أو صمغ (10جرام/100 مل كحول اثيلى) لمدة 24 ساعة ثم حفظت المستخلاصات فى الظلام على درجة 4م5. واستخدم في هذه الدراسة 7كباش لتجميع السائل المنوى بالمهبل الصناعى. وخفف السائل المنوى بمخفف الترس بنسبة 1 سائل منوى : 10 مخفف وقسم السائل المنوى المخفف الى 6 اجزاء لتكوين ت1، ت2، ت3، ت4، ت5، ت6 حيث يحتوى كل جزء على 2و0مل من المضادات الحيوية. وكانت انواع المضادات الحيوية المضافة كالتالى بنسترب، الميسين، فيتروسين، مسحوق البروبوليس وصمغ البروبوليس لكلا من ت1، ت2، ت3، ت4، ت5 على التوالى. والجزء السادس (ت6) بدون اضافة أى مضاد حيوى للمقارنة. وتم تقييم حيوية الحيوانات المنوية كالحركة، ونسبة الحى ، والطبيعى لكلا من ت1، ت2، ت3، ت4، ت5،ت6 اثناء التحضين على 37م5 من صفرالى 3 ساعات. اظهرت النتائج فروق معنوية عالية بين المعاملات فى كل من ت1،ت3، ت4، ت5 مقارنة مع ت2،ت6 خلال التحضين لمد 3ساعات. ولاتوجد فروق معنوية فى قيم التلوث البكتيرى بين ت1، ت4، ت5 للسائل المنوى المخفف. واظهرت كلا من ت4، ت5 فروق غير معنوية فى التحكم فى النمو البكتيرى للسائل المنوى المخفف بعد التحضين لمدة 3ساعات. وكانت هناك فروق معنوية عالية لكلا من ت4، ت5 للحماية من التلوث البكتيرى مقارنة مع ت1 ت2، ت3، ت6 . اوضحت النتائج من خلال الدراسة أن كلا من مسحوق وصمغ البروبوليس عند 0.2 % (جم/جم) فى مخفف السائل المنوى من الممكن استخدامه كمضاد حيوى طبيعي مقارنة بالمضادات الحيوية المصنعة. البروبوليس يمكنه مقاومة التلوث البكتيرى وتحسين جودة السائل المنوى فى المخففات.ولكن استخدام مستويات مختلفة من البروبوليس وتحديد قدرته الإخصابية تتطلب مزيد من الدراسات المستقبلية.