J.Animal and Poultry Prod., Mansoura Univ., Vol.8(1): 1- 5,2017

Relevance of Honey Bee in Semen Extender on the Quality of Chilled-Stored Ram Semen

Zaghloul, A. A.

Department of Animal and Poultry Physiology, Desert Research Center , Matariya, Cairo, Egypt

ABSTRACT

The effect of Honey bee supplementation into Tris extender on semen quality and bacterial activity of chilled- stored Barky ram' semen was studied. Semen samples were processed in Barky ram's semen extender with or without honey bee. Seven adult Barky rams aged 24-36 month and weighed 43.0± 1.5 kg were used. A total of 210 ejaculates were collected from the seven rams using an artificial vagina, 30 ejaculates each, twice weekly throughout the period of the study (July- October, 2016). After collection, each ejaculate was diluted (1:10) with Tris–based extender at 37˚C immediately after dilution, the specimens were split into 4 aliquots; the first aliquot served as control (C), whereas the other 3 aliquots were supplemented with 1.5 (HB 1.5), 3 (HB 3) and 4.5 (HB 4.5) ml honey bee/100 ml extender. Both control and treated specimens were subjected to chilled preservation at 5˚C for a period of 48 h, during which semen physical characteristics were evaluated, immediately after dilution time and every 24 h thereafter until 48 h storage at 5˚C. Microbial contamination in all specimens was also determined at 0, 24 and 48 h. The results showed that supplementing semen extender with honey bee improved ($P<0.05$) sperm motility and reduced ($P < 0.05$) percent of dead and abnormal spermatozoa and acrosomal damage. In conclusion, supplementing semen extender with honey bee for ram semen preservation at 5°C has reduced the deleterious effects due to chilling condition. This would facilitate the application of assisted reproductive technologies; i.e. artificial insemination and in vitro fertilization in sheep by extending the storagability and survival of sheep sperm.

Keywords: Ram semen, chilled storage, honey bee, bacterial contamination.

INTRODUCTION

Artificial insemination (AI), a useful assisted reproductive biotechnology tool, has been applied to achieve rapid livestock genetic improvement, production (Yimer *et al.,* 2015). Nowadays, semen cryopreservation has many biotechnological applications since it can be used to solve problems of infertility, life threatening diseases, preservation of semen and sperm DNA from endangered species and conservation of biodiversity. Semen extender is added in order to maintain spermatozoa metabolic demands, control *p*H changes in the extracellular environment of the spermatozoa, minimize cryogenic damage, and also control bacterial contamination.

Commonly added nutrients in semen extender are simple sugars such as glucose and fructose (Bearden *et al.,* 2004). Egg yolk-based extender has been the common and most extensively used extender but it is a good medium for the growth of microorganisms (Geoffrey *et al.,* 1992). Honey bee, however contains high level of metabolizable energy in from of glucose and fructose and antibacterial (inhibit a broad spectrum of around 60 species of bacteria including aerobes and anaerobes, Gram positives, and Gram negatives) (Hannan *et al.,* 2004) activity against microorganisms (AL-Waili, 2004). Moreover, honey bee contains minor quantities of amino acids, vitamins, antioxidant properties, phenolic acids and flavonoids (Andrade *et al.,* 1997), certain enzymes, ascorbic acid, minerals (White, 1975) and also has antibacterial effects against some microorganisms which are resistant to the common antibiotics used in extenders (Molan and Russell.,1988). Therefore, the aim of the current study was to elucidate the effects of supplementing various levels of honey bee in the Tris-based extender on the semen quality of Barky ram's semen during preservation at 5˚C.

MATERIALS AND METHODS

Animals and Management

The study was carried out at the Artificial Insemination Lab., Mariout Research Station, Desert Research Center, Egypt. Seven sexually mature Barky rams, aged from 24 to 36 months and weighed 43.0 ± 1.5 kg were used during July to October, 2016. Throughout the experimental the rams where housed in fenced open yard they were allowed to feed daily on Egyptian clover hay (*Trifolium alexandrinum*) *ad lib.*, and a concentrate mixture was offered to meet their protein and energy requirements (NRC, 1985). Fresh water was supplied once daily after returning from the pasture. Prior to executing the experiment, all rams were clinically examined and were found free of diseases and reproductive disorders.

Semen extender

Ram semen was extended by a Tris - citrate-egg yolk extender was prepared as described by El-Bahrawy *et al.* (2004). Briefly, the extender was composed of Tris buffer (0.25 M, 3.025 %), citric acid (1.67 %), and egg - yolk (5 %). The extender was freshly prepared 24 h prior to the collection sessions.

Semen collection

The ejaculates (210) were collected from the rams, 30 ejaculates each, throughout the period of study (July-October, 2016). Collection of semen was performed at 07:00 a.m. twice weekly using an artificial vagina as previously described (El-Bahrawy *et al.,* 2004).

Experimental design

Raw semen samples were transported to the laboratory immediately after collection. Ejaculate volume (ml), hydrogen-ion concentration (*p*H), mass motility, and sperm concentration were recorded for each raw ejaculate. The mean values of mentioned criteria throughout the experimental period were 0.99 ±0.04 ml, 6.85 ±0.08, 4.47 ±0.07 and 234.021 ±15.29 $\times 10^9$sperm/ml, respectively. Thereafter, each ejaculate

was diluted (1:10) with Tris - citrate-egg yolk extender, and was split into 4 aliquots using a split-sample technique.

The four designed treatments were; control (C) without honey bee, HB1.5 which contained 1.5 ml of honey bee per 100 ml of extender, HB3 which contained 3 ml honey bee in 100 ml extender and HB4.5 which contained 4.5 ml honey bee per 100 ml extender. All diluted semen specimens were evaluated for sperm physical characteristics immediately at zero time (T0) and thereafter at 24 and 48 h of storage at 5˚C.

Semen evaluation

A phase - contrast microscope (Leica) at X 400 magnification was used to evaluate the sperm forward motility in the diluted semen, primary and secondary sperm abnormalities and sperm viability (live and dead sperm, %) were examined. The percentages of sperm primary and secondary abnormalities as well as acrosome damage were evaluated using Romanowski's triple-stain technique (DIFF-QUICK III, Vertex, Egypt). Smears preparation and staining procedure were conducted following instructions provided by the manufacturer, and the stained smears were evaluated using a phase-contrast microscope at X 1000 magnification. Sperm plasma membrane integrity was determined using hypo-osmotic swelling test (HOST) according to the modified method of Mosaferi *et al.* (2005) at X 400 magnification.

Determination of microbial load

Within 15 min. after the periodical evaluation of semen characteristics (T0, T24, T48), a sample of each chilled specimen was transported at cool temperature to the lab. The samples were exposed to automated determination of colony forming unit (CFU) and microbial identification using the VITEK® 2 system (VITEK® 2, Bio Mérieux, USA). The determination procedure was conducted according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using GLM procedure of SPSS (1999) to evaluate the differences in sperm criteria among different treatments (0, 1.5, 3 and 4.5 ml honey bee/100ml) and observation time (0, 24 and 48 h). Significant differences were detected using Duncan Multiple Range test (Duncan, 1955) and was considered at $P<0.05$. The following statistical model was applied;

$$\mathbf{Y_{ijk} = \mu + C_i + T_j + CT_{ij} + e_{ijk}}$$

Where:

Y_{ijk} = an observation on item K_j,

μ = overall mean,

C_i = a fixed effect of the i^{th} treatment,

T_j = a fixed effect of the j^{th} time of preservation,

CT_{ij}= interaction between treatment and preservation time,

e_{ijk} = random error assumed to be normally distributed with mean = 0 and variance = $\sigma^2 e$.

RESULTS AND DISCUSSION

Effect of honey bee and storage time on semen quality

Sperm cells are the endpoint of male spermatogenesis and have particular anatomic and metabolic features. Nowadays, sperm cryopreservation and storage are great demand for conserving the supergenetic origins of the males for the use in artificial insemination (AI) and in vitro fertilization (IVF) (Medeiros *et al.*, 2002).

Data presented in Tables 1, 2,3, and 4 show that supplementing ram's semen with 1.5,3, and 4.5 ml honey bee/100 ml extender significantly ($P<0.05$) improved semen quality (represented by increase in percentage of advanced sperm motility and decreases in percentages of dead spermatozoa, sperm abnormalities and acrosome damages during chilling storage for up to 48 hours.. Time progression of diluted semen stored at 5˚C for up to 48 hours significantly ($P<0.05$) decreased the percentages of sperm motility and increased the percentages of dead and abnormal spermatozoa, and acrosome damage (Tables 2,3 and 4) these observations were in agreement with the results obtained by Seleem *et al.* (2007). According to Januskauskas and Zillinskas (2002), sperm motility reduction resulted from cryopreservation is believed to be mainly associated with mitochondrial damages and lack of its enzymes in human spermatozoa. Furthermore, results from current study revealed that highest semen quality during cold storage were shown in semen samples supplemented with 3 or 4.5ml honey bee /ml00 diluted semen. No significant effects were recorded between supplementing 3 or 4.5 ml honey bee diluted semen on percentage of motility. These results supported the idea that honey bee acts as an antibacterial agent (Kacániová et al., 2012). Moreover, other researchers suggested an antioxidant activity (Ferreres *et al.*, 1992), phenolic content activity (Andrade *et al.*, 1997) and antibacterial activity (Mullai and Menon, 2007). reported that honey bee components (antioxidant activity, phenolic content, antibacterial agent) play major roles to protect sperm against harmful effects of reactive oxygen species (ROS) and to improve sperm motility and membrane integrity during cold storage. Thus, these improvements in semen due to the existence of honey bee in the dilution media might be attributed to increasing oxidative stability. On the other hand, honey bee is a supersaturated solution of sugars, of which fructose (38%), glucose (31%) are the main contributors as additive energy source of sperm cells (White, 1975). Additionally, amino acids and proteins in honey bee improved rabbits' semen quality during chilling condition which increased fertilizing ability (Elspeiy *et al.*, 2014). Muhammad *et al.* (2014) reported that honey bee has been used in cryoprotectant medium. The clear explanation of its cryoprotective effects is its low melting point. Olayemi *et al.* (2011) stated that addition of small proportion of honey in egg yolk-extender (5 ml honey + 15 ml egg yolk + 80 ml sodium citrate) gave the highest percent of sperm motility and live/dead ratio of liquid goat cooled semen. Also, Aljady *et al.* (2000) recorded an antioxidant and antibacterial effects of honey and this illustrated the good semen quality of preserved semen with extenders containing honey. In a recent study, Akandi *et al.* (2015) reported that the addition of 1-2% honey to a boar semen extender (based

on glucose, sodium bicarbonate, sodium citrate and EDTA) proved its effectiveness in preserving boar semen. One extra theory of the physical effect of honey is its high viscosity creates potential hyperosmotic extracellular environment around sperm cells that enhances efflux of intracellular fluid thereby minimizing formation of ice crystals inside the sperm cytoplasm which has been linked to sperm damage during cryopreservation (Fakhrildin *et al.*, 2014). This mechanism of protection gives honey the property of a non-permeable cryoprotectant. More specifically, *in vivo* supplementation of honey has been also reported to increase different antioxidants and a decreases oxidative stress biomarkers present in seminal plasma of humans (Tartibian *et al.*, 2011).

Table 1. Mean percentage of sperm motility of the extended Barky ram's semen supplemented with honey bee during storage at 5ºC

Storage time / Treatment	0 h	24 h	48 h	Mean	SEM
C	82.2^{c}	67.6^{g}	61.60^{h}	70.47^{A}	2.43
HB1.5	82.2^{c}	78.33^{de}	72.20^{f}	77.58^{B}	1.24
HB 3	86.2^{b}	81.20^{cd}	78.2^{de}	81.87^{C}	1.05
HB 4.5	90.2^{a}	79.2^{cde}	77.2^{e}	82.20^{C}	1.63
Mean	85.20^{A}	76.08^{B}	72.80^{C}		
SEM	0.9	1.3	1.7		

A,B,C Means within column (treatment), or row (time) with different superscript significantly differ at P<0.05; a‹b‹c‹ d‹e‹f‹ g‹h Means in the same column with different superscripts significantly differ at P<0.05.

Table 2. Mean percentage of dead sperm of the extended Barky ram's semen supplemented with honey bee during storage at 5ºC

Storage time / Treatment	0 h	24 h	48 h	Mean	SEM
C	15.80^{cd}	21.06^{b}	25.92^{a}	20.93^{A}	1.24
HB1.5	14.80^{cd}	16.80^{c}	22.80^{ab}	18.13^{A}	1.07
HB 3	12.80^{def}	14.80^{cde}	22.80^{ab}	16.80^{B}	1.29
HB 4.5	10.80^{f}	11.80^{ef}	16.80^{c}	13.13^{C}	0.90
Mean	13.55^{A}	16.12^{B}	22.08^{C}		
SEM	0.66	0.91	0.90		

A,B,C Means in the same column (treatment) and row (time) with different superscript significantly differ at P<0.05; a‹b‹c‹ d‹e‹ f‹h Means in the same column with different superscripts significantly differ at P<0.05.

Table 3. Mean percentage of sperm abnormalities of the extended Barky ram's semen supplemented with honey bee during storage at 5ºC

Storage time / Treatment	0 h	24 h	48 h	Mean	SEM
C	11.80^{ab}	15.80^{ab}	22.80^{a}	16.80^{A}	1.34
HB 1.5	11.80^{ab}	15.80^{ab}	21.80^{a}	16.47^{A}	1.24
HB 3	9.80^{b}	12.80^{c}	16.80^{b}	13.13^{B}	0.96
HB 4.5	9.80^{b}	13.80^{c}	15.80^{b}	13.13^{B}	0.88
Mean	10.80^{A}	14.55^{B}	19.30^{C}		
SEM	0.54	0.57	0.85		

A,B,C Means within the same column (treatment) and row (time) with different superscript significantly differ at P<0.05; a‹b‹c Means in the same column with different superscripts significantly differ at P<0.05.

Table 4. Mean percentage of acrosome damage of the extended Barky ram's semen supplemented with honey bee during storage at 5ºC

Storage time / Treatment	0 h	24 h	48 h	Mean	SEM
C	13.80^{ab}	15.80^{a}	19.80^{a}	16.47	0.88
HB 1.5	11.80^{b}	13.80^{b}	18.80^{ab}	14.80	0.97
HB 3	11.80^{b}	14.80^{ab}	17.80^{abc}	14.80	0.87
HB 4.5	11.80^{b}	15.80^{a}	17.80^{abc}	15.13	0.88
Mean	12.30^{A}	15.05^{B}	18.55^{C}		
SEM	0.53	0.53	0.53		

A,B,C Means within row with different superscript significantly differ at P<0.05.

a‹b‹c‹ d‹e Means with different superscripts, differ significantly (p<0.05).

Total bacterial count in semen as a consequence of honey bee supplementation

Data in Table (5) clearly illustrate that, honey bee at the different concentrations, generally, had a broad action against total bacterial count in diluted semen stored at 5°C.

Results revealed a linear relationship between honey level and bacterial count. Moreover, the highest honey bee level (4.5 ml /100 ml extender) had the lowest (P<0.05) total bacterial count. Time wise, the total bacterial count significantly (P<0.05) increased with the advancement of storage time. It is important to efficiently control the population of micro-organisms in the semen when using artificial insemination. Sone (1982) showed low effectiveness of streptomycin and penicillin against six species of semen microflora. In a comparative study by Sevinc *et. al.* (1984), they found that semen supplemented with chloramphenicol has the highest fertility (93.1%), followed by the semen containing penicillin and streptomycin (30-80%) and the worst semen resulting the poorest fertility was that supplemented with ampicillin (29%). The antimicrobial activity in most honeys is probably attributed to the enzymatic production of hydrogen peroxide (Mandal *et al.*, 2010). Also, its mechanism might be related to the low *p*H of honey and its high sugar content (high osmolality) that is enough to hinder the growth of microbes, the acidic properties of gluconic acid and the antiseptic properties of its H_2O_2 (O'Grady *et al.*, 1997). It is interesting to notice that antimicrobial properties of honey *in vitro* include H_2O_2, methylglyoxal, antimicrobial peptide and bee defensin-I, are distinct mechanisms involved in the bactericidal activity of honey (Khan *et al.*, 2007). These inherent properties of honey are responsible for its high antimicrobial properties. In the same trend, Raju and Goli, (2013) documented that the antimicrobial properties of honey might be ascribed to its contents of both hydrogen peroxide as well as non-peroxide components (i.e. lysozyme, phenolic acids and flavonoids). Mundo *et al.* (2004) investigated effects of honey on various bacteria and found its inhibitory effects on 60 species of bacteria, including aerobes and anaerobes, gram-positives and gram-negatives. Honey was found to have inhibitory effects on bacterial growth (*Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus cereus*) comparable to the antibiotic

penicillin, kanamycin and streptomycin (Mierzejewski, 2014). Recently, Abubaker *et al.* (2015) indicated that honey bee inhibited the growth of some pathogenic bacteria as *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. These findings agree with the results reported by Oyeleke *et al.* (2010) who showed that undiluted honey was also able to inhibit the growth of *Proteus mirabilis*, *P. aerussginosa*, *E. coli*, *Streptococcus faecalis*, *Clostridium perfingens* and *S. aureus*.

Table 5. Effect of honey bee level in semen extender on total bacterial count of Barky ram's semen during preservation at 5ºC for 48 hours

Storage time / Honey bee	0 h	24 h	48 h	Mean	SEM
C	11980^{a}	12480^{a}	13000^{a}	12487^{A}	124
HB 1.5	7393^{b}	9480^{b}	11380^{b}	9418^{B}	481
HB 3	7147^{b}	8507^{c}	8813^{c}	8156^{B}	224
HB 4.5	6880^{b}	7347^{d}	7980^{d}	7402^{BC}	157
Mean	8350^{A}	9454^{B}	10293^{C}		
SEM	506	445	466		

A,B,C Means in the same column (treatment) and row (time) with different superscript significantly differ at P<0.05; a،b،c، d Means within a column with different superscripts significantly differ at P<0.05.

CONCLUSION

In conclusion, supplementing ram semen with honey bee in its extension media proved to be not only effective as a source of energy for the sperm during cold storage, but it also acts as natural antibiotics against pathogenic bacteria hindering the sperm survival and its subsequent fertilizing ability. Further studies are warranted to address the efficiency of this regime of semen preservation technique on sperm DNA integrity and its fertilization capacity.

REFERENCES

Abubaker, S.A.; H. Ali; A.M. Fathelrahman and M.A. Ibrahim (2015). Evaluation of antibacterial activity of Sudanese bee honey against four species of bacteria. American Journal of Research Communication, 3(4): 132-142.

Akandi, A.;S.O. Ugwu and N.S. Machebe (2015). Survivability of boar sperm stored under room temperature in extenders containing some natural products. Open Access Anim. Physiol., 7: 57-64.

Aljady, A.M.; M.Y. Kamaruddin; A.M. Jamal andM.Y. Mohd Yassin (2000). Biochemical study on theefficacy of Malaysian honey in infected wounds:An animal model. Med. J. Islam. Acad. Sci.,13(3): 125-132.

AL-Waili, N.S. (2004). Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infection of surgical wounds and conjunctiva. Medicinal Food, 7(2): 210-222.

Amangeldy, R.;. N.Z. Eleuqalieva; K.M. Lakhanova; L.M. Mambetova; I.A. khatova and G. Khalikova (2013). Development of Antibacterial complex for Sanitation of Boar and Bull Semen. Life Science Journal, 10(12s): 592-596

Andrade, P.; F. Ferreres and M.T. Amaral (1997). Analysis of honey phenolic acids by HPLC, its application to honey botanical characterization. J. Liq Chromatogr Relat Technol. 20: 2281-8.

Bearden, H.J. J.W. In Fuquay and S.T. Willard (2004). Applied Animal Reproduction, 6th Eddition. Pearson Education, Inc., Upper Saddle River, New Jersy. (eds).

Duncan, B.D.(1955). Multiple range and multiple F-test. Biometrics, 11; 1-42.

El-Bahrawy K.A., El-Hassanein E.E., Fathelbab A.Z., Zeitoun M.M., Yaseen A.M.(2004). Desert climatic effects on freezability and some biochemical constituents of Barki ram semen. Mansoura Journal of Agricultural Science, 29, 3123-3132.

El-Speiy, M.E.; A.E. Elkomy and K.I. Kamel (2014).Effect of Adding Protein High Viscosity (Gelatin) in Tris Extender on Semen Conservation Status,Fertility Rates, Antioxidant Status and Sex Ratio of Rabbits Global Veterinaria 12(6): 840-849.

Fakhrildin, M.B.;M.R. Rana and A.R. Alsaadi (2014). Honey supplementation to semen-freezing medium improves human sperm parameters post-thawing. J. Family and Reprod. Health, 8: 27-31.

Ferreres, F.; A. Ortiz; C. Silva; C. Viguera; F.A. Barbera´n and F.T. Lorente (1992). Flavonoids of "La Alcarria" honey. Z Lebensm Unters Forsch, 194: 139-43.

Geoffrey, H.A.; E.N. David and P. Harold (1992). Artificial Insemination. In Veterinary Reproduction and Obstetrics, 6th edition, Sunders, pp:567.

Hannan, A.; M. Barkaat; S. Saleem; M. Usman and W.A. Gilani (2004). Manuka honey and its antimicrobial potential against multi drug resistant strains of Typhoidal salmonellae, Ph.D. thesis, Department of Microbiology, University of Health Science, Lahore, Pakistan.

Januskauskas, A. and H. Zillinskas (2002). Bull semen evaluation post-thaw and relation of semen characteristics to bull's fertility. Veterinarijairzootechnika, 17:1392-2130.

Kacániová1, M.; N. VUKOVI, ; R. Chlebo; P. HaŠČík; K. Rovná; J. CUBON; M. Dżugan and A. PASTERNAKIEWIC (2012). The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. Arch. Biol. Sci., Belgrade, 64: 927-934.

Khan, F.R.; Z. Ul Abadin and N. Rauf (2007). Honey: nutritional and medicinal value.Int J Clin Pract., 61: 1705-1707.

Mandal, S.; M. Deb Mandal and N.K. Pal (2010). Synergistic anti-Staphylococcusaureus activity of amoxicillin in combination with Emblica officinalis and Nymphaeodorata extracts. Asian Pac J Trop Med., 3: 711-714.

Marti, J. I.; E. Marti; J.E. cebrian-perez and T. muino-blanco (2003). Survival rate of antioxidant enzyme activity of ram spermatozoa after dilution with different extenders or selection by a dextran swim-up procedure. Theriogenologyram semen. Acta. Vet. Hung., 50(2): 235-245.

Medeiros, C.M.; F. Forell; A.T. Oliveira and J.L. Rodrigues (2002). Current status of spermcryopreservation: why isn't better. Theriogenol., 57: 327-344.

Mierzejewski, M. (2014). The antimicrobial effects of royal jelly, propolis and honey against bacteria of clinical significance in comparison to three antibiotics; College of Arts & Sciences/Biology.

Molan, P.C. and K.M. Russell (1988). Non-peroxide antibacterial activity in some New Zeland honyes, J. Apic. Res., 27(10):62-67.

Mosaferi S., Niasari N.A., Abarghani A., Gharahdaghi A.A., Gerami A., 2005. Biophysical and biochemical characteristics of bactrian camel semen collected by artificial vagina. Theriogenology, 63, 92–101.

Muhammad, M.R.; M.D. Fakhrildin; A. Rana and R. Alsaadi (2014). M.Sc.Honey Supplementation to Semen-Freezing Medium ImprovesHuman Sperm Parameters Post-Thawing.Journal of Family and Reproductive Health 27-31.

Mullai, V. and T. Menon (2007). Bactericidal activity of differenttypes of honey against clinical and environmental isolates ofPseudomonas aeruginosa. Journal of Alternative and Complementary Medicine, 13(4): 439-441.

Mundo, M.A.; O.L. Padilla-Zakour and R.W. Worobo (2004). Growth inhibition offoodborne pathogens and food spoilage organisms by select raw honeys. Int JFood Microbiol., 97: 1-8.

NRC. National Research Council (1985). Nutrition requirements of sheep. 6th Rev. Ed, National Academy Press, Washington, D.C., USA.

O'Grady, F.W.; H.P. Lambert; R.G. Finch and D. Greenwood(1997). Antibiotic andChemotherapy. (7thedn). New York: Churchill Living Stone.

Olayemi, F.; R. Adedayo; R. Muhummad andE. Bamishaiye (2011).The nutritional quality of threevarieties of zobo(Hibiscus sabadariffa) subjected to the same preparation condition. Plant Food Hum. Nutr.,6:10-15.

Oyeleke, S.B.; B.E. Dauda; T. Jimoh and S.O. Musa (2010).Nutritional analysis and antibacterial effect of honey on bacterial wound pathogen Journal of applied Sciences research, 6(11):1561-1565.

Raju, V.N and D. Goli (2013).In-vitro comparative antimicrobial activity of commercial and raw honey against various bacteria isolated from ear discharges. Acad. J. Pharm., 2(1):5-11.

Seleem, T.S.T.; K.H. El-Kholy and T.A. El-Aasar (2007). Effect of Arak (Salvadorapersica) aqueous extract on rabbit semen preservation, microbial contamination and artificial insemination. The 5th Inter. Con. on Rabbit Prod. in Hot Clim., Hurghada, Egypt, 381- 390.

Sevinc, A.; N. Yurdaydin; N. Tekin; A. Ekici and N. Aydin (1984). Different antibioticsobtained by participating frozen bull semen donor Fertility. Ankara Univ.,31(4): 491-497.

SPSS (1999). SPSS Based 10.0 for Windows Users Guide. SPSS, Inc., Editors, N.S.A.

Sone, M. (1982). Effect of various antibiotics on the control of bacteria in boar semen. Veter.Rec., 111(3): 11-14.

Tartibian, B.; M.B. Hajizadeh and A. Abbasi (2011). The effects of honey supplementation on seminal plasma cytokines, oxidative stress biomarkers and anti-oxidants during 8 weeks of intensive cycling training. J. Andrology,33(3): 449-461.

White, J.W. (1975). Composition of honey. In: Crane E, Ed. Honey, a comprehensive survey. London: Bee research Association and Chalfont St Peter, 157-206.

Yimer, N.; N. Muhammad; K. Sarsaifi; Y. Rosnina; H. Wahid; A.M. Khumran and A. Kaka (2015). Effect of honey supplementation into Tris Extender on Cryopreservation of Bull Spermatozoa.J. Anim. Sci. 18(2): 47-54.

أهميه عسل النحل في المخفف علي صفات السائل المنوي المخزن بالتبريد للكباش

عوض عبد الغنى زغلول

قسم فسيولوجيا الحيوان والدواجن – شعبة الإنتاج الحيوانى والدواجن مركز بحوث الصحراء بالمطرية – القاهره

لدراسة تأثير إضافة عسل النحل لمخفف الترس على المقدرة الحفظية والنشاط البكتيرى للسائل المنوى لكباش البرقى – تم جمع عينات السائل المنوى من سبع كباش برقى تتراوح اعمارها ما بين 24-36 شهر ومتوسط أوزانها 43 كجم ± 1.5 كجم تم جمع 210 قذفه من السائل المنوى (بمعدل 30 قذفه من كل كبش) بأستخدام المهبل الصناعى مرتين أسبوعيا على مدار فترة التجربة (يوليو – اكتوبر2016) بعد الجمع مباشرة تم تخفيف القذفات بنسبة 10:1 بمخفف الترس – سترك – صفار بيض ، قسمت العينات المخففه بالتساوى لأربع مجموعات تجريبيه ، استخدمت المجموعه الاولى كمجموعه مقارنه (غير معامله) بينما دعمت المجموعه الثانية بـ 1.5 ملى عسل نحل / 100 ملى مخفف ، دعمت المجموعه الثالثه بـ 3 ملى عسل نحل /100 ملى مخفف ودعمت المجموعه الرابعه بـ 4.5 ملى عسل نحل / 100 ملى مخفف تم تقييم الخصائص الطبيعية للحيوانات المنوية أضافة لتقدير الحمل المكروبى لجميع العينات بدأ من بعد التخفيف مباشرة وعلى مدار 48 ساعة من الحفظ بالتبريد على 5°م، وقد اشارت النتائج ان اضافة عسل النحل للمخفف مباشرة حسن الحركة التقدمية للحيوانات المنوية بنسبة ($p<0.05$) كما أنخفضت معنويا ($p<0.05$) كلا من نسبة الحيوانات المنوية الميتة والمشوهة ونسبة الاختلالات فى غشاء الأكروسوم وتشير نتائج هذه التجربة الى كفائة استخدام عسل النحل فى الحفظ بالتبريد للسائل المنوى فى الكباش بما يساهم فى تطبيق التقنيات التناسليه الحديثه المساعده وخاصة التلقيح الأصطناعى والاخصاب المعملى فى الأغنام .

الكلمات الدلالية: سائل منوي الكباش ، تخزين بالتبريد ، عسل النحل ، تلوث بكتيري