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EFFECT OF HONEY BEES SUPPLEMENTATION FOR SEMEN EXTENDER ON CRYOPRESERVATION, BACTERIAL ACTIVITY AND FERTILITY TRAITS OF RABBITS

M.E.El-Speiy[1]; El-Sawy[1], F.B.A., Badri[2] and T.A. Sadaka[1]
[1] Anim. Prod. Res. Inst., Agric. Res. Center, Ministry of Agric., Egypt.
[2] Poultry Production Department, Faculty Agric., Ain-Shams Univ., Cairo, Egypt. ***E-mail: Elsawy1966@Gmail.com***

Twenty V-line male rabbits, ten months age and 80 multiparous does aged 12 months were used for the present study. Three experiments were carried out. First experiment was planned to use honey bee as a component in sperm preservation extender at incubation (37°C) for up to 6 hours or refrigeration condition (4-6°C) for up to 72 hours. Second experiment was done to study effect of different levels of honey bee on total bacterial count, enterobacteriaceas count, Staph. aureus. Third experiment was designed for studying fertility traits for does artificially inseminated by diluted pooled semen with antibiotics (control) and the best level of honey bees.

Results showed that *diluted V-line rabbits semen with 1, 3, or 5 ml honey bee/100ml extender improved significantly (P≤0.05) semen quality increased in percentages of advanced sperm motility and decreased percentages of dead spermatozoa, sperm abnormalities and acrosomal damages during cryopreservation at (4-6°C) for up to 72 hours or incubation at 37°C for up to 6 hours.*

Addition of different levels of honey bee to rabbits semen extender resulted in a gradually and significantly decrease (P≤0.05) in overall mean of MAD concentration. Total anti-oxidant enzymes activity of rabbit semen extender was increased by increasing honey bee level and this effect was significant (P≤0.05), regardless the incubation or storage time. Moreover, the results revealed that the highest honey bee levels had the highest total anti-oxidant capacity value, which was significant (P≤0.05) compared to the other levels. Honey bee at different concentrations, generally, had a broad action against total bacterial count of V-line rabbit semen, during chilled storage at 4-6°C or incubation at 37°C.

V-line rabbit does artificially inseminated using semen supplemented with a level of honey bee 5ml/100 ml extender showed the best results as compared to the control group. Also, fertility traits were significantly (P≤0.05) higher than those inseminated artificially by using semen with antibiotics.

Conclusively, *it is concluded that rabbit's semen quality, bacterial activity and fertility traits may be improved with honey bee supplementation to semen extender in V-line rabbits.*

Key words: Rabbit semen extender, honey bee, semen quality, fertility traits.

Artificial insemination (AI) a useful assisted reproductive biotechnology has been applied to achieve rapid livestock genetic improvement production (Yimer *et al.,* 2015). 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 super genetic origins of the males, technologies such as artificial insemination (AI) and *in vitro* fertilization (IVF) (Medeiros *et al*., 2002). Efforts to improve the preservation of animal semen are focused on the modification of extenders as well as on the addition of various components to maintain motility, fertilizing capacity and sperm membrane integrity (Marti *et al*., 2003 and Riha *et al*., 2006). Nowadays, semen cryopreservation has many biotechnological applications. It can be used to solve problems of infertility, life threatening diseases, preservation of semen and DNA from endangered species and conservation of biodiversity (El-Sheshtawy *et al.,* 2014). Semen extender is added to maintain spermatozoa metabolic demands, control pH changes in the extracellular environment of the spermatozoa, minimize cryogenic damage, and also control bacterial contamination.

The presence of variety microorganisms in semen leads reduction of survival rate and fertility of cells and as a consequence, results in non-viable offspring. Therefore, the suppression of undesirable contaminant microbial activity in breeder semen is a mandatory condition for artificial insemination of the pedigree stock (Amangeldy *et al.*, 2013).

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 contains high level of metabolizable energy in form of glucose and fructose and antibacterial that inhibits a broad spectrum of around 60 species of bacteria

including aerobes and anaerobes, Gram positives, and negatives activity against microorganisms (Hannan *et al.,* 2004 and AL-Waili, 2004). Also contains minor quantities of amino-acids and vitamins, antioxidant properties, phenolic acids and flavonoid (Andrade *et al.,* 1997) and certain enzymes, ascorbic acid, mineral substances (White, 1975). Molan and Russell (1988) found that honey bee has antibacterial against some microorganisms which are resistant to the common antibiotics used in extenders.

Therefore, the aim of the present study was evaluating the effect of different levels of honey bee addition to tris-based diluent of rabbit's semen during preservation at refrigeration or incubation condition, on fertility traits of rabbit does and semen bacterial counts.

MATERIALS AND METHODS

Twenty V-line male rabbits, ten months age weighting about 3.5 kg and 80 multiparous does aged 12 month were used for the present study. They were housed in clean, separate and wire-floor metal cages and maintained under standard laboratory conditions. The ambient temperature was 25±2°C with 55-64% relative humidity and a 16:8 hrs light: dark daily. Bucks and does were allowed to a standard pellet diet (17% crude protein, 2.56% crude fat and 2500 Kcal/kg-ration digested energy and 12.5% crude fiber). Food and water were available *ad. libitum.* All rabbits were kept under the same managerial condition, were healthy and clinically free of external and internal parasites.

Three consecutive semen samples were collected from each male and the interval time between samples was 10 days. Semen was collected artificially by an artificial vagina as described by Lòpez and Alvarino (2000). Semen ejaculates were individually evaluated microscopically and only ejaculates showing advanced sperm motility more than 70% were pooled and used. Semen was then divided into four equal comparable experimental portions and diluted with tris–honey extender supplemented with 0, 1, 3 and 5% honey bee. The final dilution rate was 1 part semen: 4 parts diluent. The diluted semen was preserved either incubated (37 °C) for up to 6 hours or refrigerated (4-6°C) for up to 72 hours.

Percentages of advanced motility, dead, abnormality sperms and acrosomal damages were estimated according to Boiti *et al.,* (2005). Samples of diluted semen were taken at 24, 48 and 72 hours of preservation at 4-6°C and after 0, 2 and 6 hours of incubation at 37°C for evaluating each of physical semen quality and enzymatic activity. Preserved semen was centrifuged at 3000 r.p.m. for 15 minutes and the supernatant was removed and stored at -20°C until enzymatic assay, malonyaldehyde (MAD) and total antioxidant capacity (TAC) according to Koracevic *et al.*, (2001). All

Table 1: Composition of Tris- extender with different levels of honey bees.

Composition	0.0 ml 1.0 honey	1ml honey	3ml honey	5ml Honey
Tris* (gm)	3.028	3.028	3.028	3.028
Citric acid(gm)	1.5	1.5	1.5	1.5
Egg yolk (ml)	5	5	5	5
Sodium penicillin (IU).	50000	-	-	-
Streptomycin sulphate (mg)	50	-	-	-
D-glucose (gm)	1.25	-	-	-
Double distillated water up to	100	100	100	100
pH	7.2	7.4	7.4	7.5

* Tris (Hydroxymethyl) aminomethane, Aldrich Chemical Co.Ltd., Gillingham, Doreset England.

biochemical parameters were analyzed by commercially available kit methods. GNW-Model: SM-721Spectrophotometers, Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis. Moreover, each parameter was done according to the instructions of its kit.

Semen from the second sample was divided into 4 portions for bacteriological examination. Different levels of honey bee (0, 1, 3, or 5 ml) were added to the 1st, 2nd, 3rd and 4th fractions, respectively. Total bacterial count; *Enterobacteriaceas* count, *Staph. aureus* count was carried out according to Cruickshank *et al.*, (1975) and the extended semen sample was examined bacteriologically by culturing on nutrient agar (Buxton and Fraser, 1977).

The third samples, forty V-line multiparous rabbit does were intramuscularly injected with 20 µg gonadotrophin-releasing hormone analogue (GnRH, Recptal, Intervet Co. Lab) to induce ovulation immediately after artificially insemination. Rabbit does were divided into two comparable experimental groups. The 1st and 2nd groups were artificially inseminated by diluted pooled semen with antibiotics (control) and the best level of honey bee showed good semen quality, respectively. The artificial insemination was carried out as described by Boiti *et al.,* (2005). Kindling rate and litter size and weight at birth were recorded.

Statistical analysis:

Data were analyzed using tow-way analysis of variance for honey bee level and preservation period and their interaction using the General Linear Model (GLM) procedure of SAS (2002) as following model:

$$Y_{ijk}=\mu + T_i + P_j + (TP)_{ij} + e_{ijk},$$

Where: Y_{ijk} = An observation; μ = Overall mean; T_i = Honey bee level; P_j = Preservation temperature and period; TP_{ij} = Interaction between honey bee level

and preservation period; and e_{ijk} = Experimental error. When significant differences among means were tested by using Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Data presented in Tables 2 and 3 showed that addition of diluted V-line rabbit semen with 1, 3, or 5 ml honey bee/100 ml extender improved significantly (P≤0.05) semen quality (represented by increase in percentages of advanced sperm motility and decrease in percentages of dead spermatozoa, sperm abnormalities and acrosomal damages) during cryopreservation at (4-6°C) for up to 72 hours or incubation at 37°C for up to 6 hours compared with non-supplemented semen. These results confirm that honey bee in the reactivated medium seemed to have beneficial effect on increasing percentage of motile spermatozoa and consequently increasing semen viability and decreasing the percentages of dead and abnormal spermatozoa and acrosomal damages. Deleterious effects on semen quality were associated with an increase in preservation period. Diluted semen preserved either at (4-6°C) or at 37°C/6 hrs decreased significantly (P≤0.05) the percentages of sperm motility and increased significantly (P≤0.05) the percentages of dead spermatozoa, sperm abnormalities and acrosomal damages (Table 2 and 3). These observations were in agreement with the results obtained by Seleem *et al.*, (2007). The observed reduction in semen quality with progression of conservation period may contribute to the increase in metabolic agent accumulation as a result of sperm anaerobic metabolism leading to changes in both the osmotic pressure and pH of the media, which might exert a toxic effect on the sperm cell (Riad, 2003). According to Januskauskas *et al*., (2002) sperm motility induced by cryopreservation is believed to be mainly associated with mitochondrial damage in human spermatozoa; mitochondrial enzymatic activities were shown to be correlated with spermatozoa motility. Furthermore, results from this study revealed that highest semen quality during preservation under refrigeration or incubation conditions was recorded in semen samples supplemented with 3 or 5 ml honey bee/l00 ml extender. No significant effects were recorded between supplementing 3 and 5 ml honey bee diluted semen on percentage of sperm motility at 5 °C/6 hrs. These results supported the improvement of semen quality with honey bee, which may act as antibacterial agent (Kacániová *et al.,* 2012). Kacániová *et al.*, (2012) reported that the component (antioxidant activity, phenolic content, antibacterial agent) play a major factor in sperm protecting sperm against harmful effects of reactive oxygen species and improve sperm motility and membrane integrity, during sperm liquid storage.

Thus, the improvements in semen may be attributed to increase 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) and amino acids, proteins improved rabbits' semen quality during conservation and in the same time increases fertilizing ability (Elspeiy *et al*., 2014). Muhammad *et al*., (2014) reported that honey bee has been used with cryoprotectant medium. However, normally bee honey exists below its melting point, and it is a super cooled liquid. At very low temperatures, honey will not be freeze solid. Instead, as the temperatures become colder, the viscosity of honey increases. Like most viscous liquids, the honey will become thick and condense with decreasing temperature. While appearing or even feeling solid, it will continue to flow at very slow rates. Olayemi *et al.* (2011) stated that addition of small proportion of honey bee 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 bee and this illustrated the good semen quality of preserved semen with extenders containing honey bee additive. In another study, Akandi *et al*. (2015) reported that the addition of 1-2% honey bee to a boar semen extender (based on glucose, sodium bicarbonate, sodium citrate and EDTA) proved its effectiveness in preserving boar semen .Honey bee is also a highly concentrated product and has the 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 bee the property of a non-permeable cryoprotectant.

More specifically, *in vivo* supplementation of honey bee to food has been also reported to increase significant concentrations of different antioxidants and a decrease in oxidative stress biomarker present in seminal plasma of humans exposed to a stress factor compared to those who did not take honey bee (Tartibian *et al.,* 2011). Therefore, this could be additional mechanism by which the *in vitro* addition of honey bee at 2.5% into tris extender improved quality by reducing percentage of sperm cells with abnormal morphology compared to other tris based extenders tested. The decrease in post-cryopreservation semen quality with an increase in honey bee supplement of more than 2.5% might be associated with excess hyperosmotic extracellular environment created due to high concentration of honey bee that can lead to excessive intracellular dehydration similar to effect of high concentration of non-penetrating cryoprotectants (Lemma, 2011).

Oxidative stress markers status:

Data of Tables 4 and 5 showed the effect addition of different levels of honey bee to rabbit semen extender and the effect of incubation and conservation periods on the lipid peroxidation malonyaldehyde concentration (MAD) and total anti-oxidant capacity (TAC). Results showed that increasing honey bee level supplementation to rabbits semen extender resulted in a gradually and significantly decrease ($P \leq 0.05$) in the overall mean of MAD concentration and this effect was honey bee levels-dependent manner, regardless the incubation or conservation time. On the other hand, the MAD concentration in rabbits semen extender was increased significantly ($P \leq 0.05$) with increasing the incubation or storage period. Total anti-oxidant enzymes activity of rabbits semen extender was increased by increasing honey bee level and this effect was significant ($P \leq 0.05$), regardless the incubation or storage time. Moreover, the results revealed that the highest honey bee levels (5ml/100 ml extender) had the highest total anti-oxidant capacity value, which was significant ($P \leq 0.05$) compared to the other levels.

It could explain the role of total anti-oxidants enzymes on sperm functions by understanding the job carried out by these enzymes. From the previous studies, the mammalian sperm membrane is particularly rich in unsaturated fatty acids (PUFA). This renders the sperm very susceptible to lipid peroxidation (LPO), which occurs as a result of the oxidation of the membrane lipids through auto-oxidative reactions that result in the formation of lipid hydro-peroxides (superoxide, hydrogen peroxide and hydroxyl) and malonyaldehyde (MAD) (Bernhard and Phyllis, 1998). Peroxidation can change membrane fluidity and affect several cell functions such as ion permeability and ATPase activity (Tretter and Adam, 1996). Lipid peroxidation of the sperm membrane ultimately leads to the impairment of sperm function due to the attacks by reactive oxygen species acrosome (ROS), which affect and reduce the sperm motility and fertility (Kankofer *et al.*, 2005).

Currently, there is overwhelming evidence that free radicals cause oxidative damage to lipids, proteins, and nucleic acids, leading to many biological complications (Halliwell and Gutteridge 1989). Honey bee can produce many powerful effects as antioxidant (Erejuwa *et al*., 2010). Completed antioxidant system is required to neutralize and minimize Reactive Oxygen Species (ROS) damage (Hassan *et al.,* 2010).

Several enzymes were detected in honey bee content such as glucose oxidase, diastase, invertase, catalase and peroxidase (Bogdanov *et al.,* 2008). In addition, they studied other active ingredients, organic acids, ascorbic acid, trace elements, vitamins, amino acids, and proteins.

The antioxidant activity of honey bee is generally attributed to its phenolic compounds and flavonoids (Kishore *et al*., 2011). The main phenolic and flavonoid compounds in honey bee include ellagic, gallic, syringic, benzoic, cinnamic, ferulic acids, myricetin, chlorogenic, coumaric and caffeic acids, hesperetin, isoramnetin, chrysin, quercetin, galangin, luteolin and kaempferol (Petrus *et al*., 2011) and L-ascorbic acid, α-tocopherol and β-carotene (Balz *et al*., 1989).

Phenolic compounds are very efficient scavengers of peroxyl radicals because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogens (Aruoma 1994). Moreover, the action of phenolic compounds can be related to their capacity to reduce or chelate divalent ions that catalyze lipid peroxidation (Gazzani *et al*., 1998).

On the other hand, phenolic antioxidants (Ar OH) may interrupt radical-initiated chain reactions by hydrogenatom transfer (Eq. 3) or by electron transfer (Eq. 4) with the formation of phenoxyl radical cation (Ar OH_), which is rapidly and reversibly deprotonated, forming phenoxyl radical(ArO) (Eq. 5) (Ladas *et al.,* 1995). Finally, Honey bee supplementation significantly increased the concentrations of seminal superoxide dismutase (SOD) and catalase (CAT). This antioxidant effect of honey bee was also associated with low elevations in the seminal reactive oxygen species (ROS) and malonyaldehyde (MAD) levels (Tartibian *et al*., 2011).

Total bacterial count of semen as affected by supplemented honey bee:

Data in Tables 6 and 7 showed that increasing additive level of honey bee to rabbits' semen extender resulted in a gradually and significantly decrease ($P \leq 0.05$) in the overall mean bacterial count and this effect was dependent manner, regardless the incubation or conservation time. Moreover, the results revealed that the highest honey bee levels (5ml/100 ml extender) had the lowest total bacterial count, which was significant ($P \leq 0.05$) compared to the other levels. Also, the total bacterial count in rabbits' semen extender was increased significantly ($P \leq 0.05$) with increase the storage period.

Microorganisms can affect the male reproductive function directly, causing the agglutination of motile sperm, reducing the ability of acrosome reaction and causing alterations in cell morphology and indirectly, through the production of reactive oxygen species generated by the inflammatory response to the infection (Moretti *et al.,* 2009). However, there is no complete agreement on the detrimental role of the presence of bacteria in the semen. Detection of bacteria in semen does not necessarily indicate infection, because sample contamination and transference of surface genital colonization can readily occur (Sanocka-Maciejewska *et al.,* 2005). In cases in which bacteria have been

detected, sperm morphology was deemed acceptable and few ejaculates contained inflammatory cells. Presence of bacteria in the ejaculates can affect fertilization directly (Morrell and Geraghty, 2006), by adhering to spermatozoa (Diemer *et al.,* 1996), impairing their motility (Kaur *et al.*, 1986), and inducing acrosome reaction (El-Mulla *et al.,* 1996). Endotoxins produced by these bacteria interfere with spermatozoa survival time in semen and cause sperm agglutination and reduced motility (Okazaki *et al*., 2010). Thus in the use of AI, it is important to control efficiently the population of micro-organisms in the semen.

Sone (1982) showed that the streptomycin and penicillin has low effectiveness against six species of semen microflora. Also, Sevinc *et al*., (1984) found that a semen with chloramphenicol has the highest fertility (93.1%), followed by the semen containing penicillin and streptomycin (30-80%); the semen with ampicillin was the worst one (29%).

The antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide (Mandal *et al.,* 2010). Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes and 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 bee *in vitro* found that H_2O_2, methylglyoxal and an antimicrobial peptide, bee defensin-1, are distinct mechanisms involved in the bactericidal activity of honey bee (Khan *et al.,* 2007). Akandi *et al*., (2015) reported that the dilution of honey bee up to 30% formed the maximum amount of hydrogen peroxide in media. These inherent properties of honey bee are responsible for its high antimicrobial properties.

In the same trend, Raju and Goli (2013) documented that the antimicrobial properties of honey bee have been attributed to both the hydrogen peroxide as well as non-peroxide components. Non-peroxide factors may also contribute to antimicrobial properties of honey bee such as lysozyme, phenolic acids and flavonoids.

Currently, the best known of the honeys, has been reported to have an inhibitory effect on around 60 species of bacteria, including aerobes and anaerobes, gram-positives and gram-negatives (Mundo *et al*., 2004).

Honey bee was found to have inhibitory effects on bacterial growth (*Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis* and *Bacillus cereus*) comparable in strength to the antibiotic Penicillin, Streptomycin and Kanamycin (Mierzejewski, 2014).

Fertility traits:

It is obvious from the data in Table 8 that V-line rabbit does artificially inseminated using semen supplemented with 5% of honey bee showed the best results as compared to the control group. The results indicated that kindling rate and litter size and weight at birth were significantly (P≤0.05) higher than those inseminated artificially by using semen with antibiotics (control group).

Table 8. Fertility traits of V-line rabbit does inseminated artificially by semen extender supplemented with honey bee (Ls means ± SE)

Items	Honey bee levels (ml/100ml extender)	
	0	5
Mated does (N)	40	40
Conceived does (N)	26	34
Kindling rate (%)	65.33^{b}±3.18	84.67^{a}±2.84
Litter size at birth	6.67^{b}±0.33	9.00^{a}±0.58
Litter weight at birth (g)	266.67^{b}±1.67	345.33^{a}±3.76
Bunny weight at birth (g)	40.33±1.86	39.00±3.08

Means within the same row (a, b) bearing different letter superscripts are significantly different (P≤0.05)

Energy is very crucial for the maintenance of sperm motility and viability (Machebe *et al*., 2012). Thus, it is expected that an adequate amount of energy is required by sperm during storage to maintain movement and other physiological functions. Hence, energy substrate like sugars in the storage media forms the source of materials for production of energy for motility.

Average motility of sperm during storage in honey extender was higher than that of other treatments. Honey bees have a multifactorial function. It contains source of energy, antioxidant, antibacterial, amino acid and protein, mineral, other phenolic compounds and flavonoid (Kwakman *et al*., 2010). These inherent properties of honey are responsible for contributed to the maintenance of high motility of sperm cells over time when stored in honey extenders. Fayemi *et al.,* (2006) found that sperm motility might partly contribute to the conception rate and litter size. On the other hand, improvement of fertility traits as a result of V-line rabbit does artificially inseminated by diluted semen containing honey bee can be attributed mainly to its role in control of bacterial growth in semen. In this respect, Poolperm (2001) showed that the bacterial contamination in semen could cause a tremendous reduction in fertility and breeding performance in several farms.

***Conclusively*,** it could be concluded that rabbits semen quality during preservation at different temperatures and fertility traits of rabbit does inseminated artificially could be improved by adding 5 ml honey bee/100 ml extender diluted semen.

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تأثير إضافة عسل النحل لمخفف سائل منوى الأرانب على حفظ الحيوانات المنوية والنشاط البكتيرى وصفات الخصوبة

محمد السيد السبيعى*- محمد عبد العزيز الصاوى*- فيصل بيومى عبد السلام بدرى - طارق أمين صدقه***

*معهد بحوث الأنتاج الحيوانى – مركز البحوث الزراعية – وزارة الزراعة – مصر.

** قسم إنتاج الدواجن - كلية الزراعة – جامعة عين شمس – مصر.

إستخدم فى هذة التجربة عدد ٢٠ ذكر أرنب عمر ١٠ شهور ، ٨٠ أنثى عمر ١٢ شهر من□ط V-Line، حيث تم إجراء ٣ تجارب كالتالى :

التجربة الأولى : □ممت لدراسة تأثير عسل النحل على حفظ الحيوانات المنوية أثناء فترة التحضين على ٣٧°م لمدة ٦ ساعات أو الحفظ بالثلاجة على درجة ٤-٦°م حتى ٧٢ ساعة.

التجربة الثانية : تم إجرائها لدراسة تأثير نسب مختلفة من عسل النحل على العد البكتيرى الكلى وعدد البكتيريا المعوية وبكتيريا الإستاف.

التجربـة الثالثـة : تـم إجرائهـا لدراسـة صـفات الخصـوبة للأمهـات الملقحـة إصـطناعيا بالسـائل المنـوى المضـاف إليـه عسـل النحـل بأفضـل مسـتوى حسـب التجربـة الأولـى مقارنـة بالمضـاف إليه مضادات حيوية (كنترول).

وقد أوضحت النتائج مايلى:

- حـدث تحسـن معنـوى عنـد مسـتوى (٥%) نتيجـة إضـافة ١، ٣، ٥مـل عسـل نحل/١٠٠مل مخفف متمثلا فى زيادة نسبة كل من الحركة التقدمية للحيوانات المنوية وإنخفاض النسبة المئوية للحيوانات المنوية الميتة والمشوهة وتشوهات الأكروسوم وذلك أثناء الحفظ على ٤-٦°م لمدة تصل ٧٢ ساعة أو أثناء التحضين على ٣٧°م لمدة ٦ ساعات.
- إضافة مستويات مختلفة من عسل النحل إلى مخفف السائل المنوى للأرانب أدى لإنخفاض معنوى عند مستوى (٥%) فى مستوى الـ MAD ، بينما زاد مستوى إنزيمات الـ TAC معنويا عند مستوى (٥%) بزيادة مستوى عسل النحل وكانت أفضل نسبة لإضافة عسل النحل هى ٥مل/١٠٠مل مخفف.
- أظهر عسل النحل بكل مستوياته المدروسة (١، ٣، ٥ مل/١٠٠مل مخفف) تأثير مضاد واسع ضد العد البكتيرى الكلى فى السائل المنوى سواء عند التخزين على ٤-٦°م أو الحفظ على ٣٧°م.
- أظهرت الإناث الملقحة إصطناعيا بسائل منوى مخفف مضافا له ٥مل عسل نحل/١٠٠مل مخفف أعلى أداء إنتاجى مقارنة بالكنترول.
- صفات الخصوبة متمثلة فى كل من معدل الولادات، عدد الخلفة ووزنها عند الميلاد كانت أعلى معنويا عند مستوى (٥%) من الإناث فى مجموعة الكنترول.

التوصية: إضافة ٥مل عسل نحل/١٠٠مل مخفف للسائل المنوى للأرانب يؤدى إلى تحسن معنوى عند مستوى (٥%) صفات السائل المنوى أثناء فترة الحفظ على درجة ٤-٦°م لمدة ٧٢ ساعة أو التحضين على ٣٧°م لمدة ٦ ساعات.