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EFFECT OF ADDING OF PROPOLIS AND VITAMIN B12 TO TRIS EXTENDER AND COOLING PERIOD ON THE SEMEN VIABILITY FOR SHAMI BUCKS

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Abstract

This study provides an indication of the effect of adding alcohol extract to propolis and vitamin B12 to Tris Extender on the quality of the semen cooled in Levantine. This study was carried out in the Department of Animal Production, College of Agriculture - Diyala University, 5 male goats were used for 1.5-2 years of age with an average weight of 40 kg. The semen was collected by an artificial vagina. The semen of all male goats (semen) was collected and divided into four factors. An Extender was used in the first group (T1) control group. Places of the second group (T2) Vitamin B12 was added to the Tris Extender (3 mg / 50 mm Extender) and the third group (T3) the alcoholic extract was added to the propolis (3 mg / 50ml Extender) and in the fourth group (T4) the alcoholic extract was added to the propolis (3 mg / 50 ml Extender) and vitamin B12 (3 mg / 50 ml Extender). Group (T4), (T3) and (T2) significantly outperformed (P≤0.05) for the percentage of individual movement over group (T1) (76.42, 75.58, 74.67 and 72.08), respectively. The study also showed a significant difference in the percentage of live sperm, as group (T4), (T3) and (T2) significantly outperformed (P≤0.05) over group (T1) (76.17, 76.75, 75.00 and 72.42), respectively. The study also showed a significant difference in the percentage of plasma membrane safety, as group (T4), (T3) and (T2) significantly outperformed (P≤0.05) over group (T1) (73.08, 72.42, 71.25, 67.75), respectively. Also a significant effect on Extender PH. It also showed a significant percentage of total abnormal, as the group (T4) thinks (T3), (T2) and (T1) (9.88, 10.67, 11.63, and 13.71), respectively. It can produce the addition of the alcoholic extract of propolis (3 mg / 50 ml Extender). Vitamin B12 (3 mg / 50 mL Extender) in different cooling periods has a pronounced effect in improving semen characteristics of male goats compared to control group (T1)
Keywords: Semen, Propolis, Vitamin B12, Goat characteristics

Introduction

Artificial insemination was used to spread the good genetics of farm animals. By using semen cooling and freezing techniques (Olivera *et al*., 2013). As metabolism of dead or distorted sperms (Amirat –Briand, 2009) is a source of production of free radicals (ROS) with its various types and reactive oxygen species (Bucak *et al*., 2010; Bansal and Bilaspuri, 2011). As the free radicals resulting from the oxidation of fats and the breakdown of fructose sugar lead to decreased semen vitality (Sahnun *et al*. 2017; Gandhi *et al*. 2017; Bucak *et al*. 2017) as the accumulation of these roots increases the pH due to the formation of lactic acid (Menchaca *et al*. 2005; al-Khashab 2012). To address this problem, scientific studies have indicated the possibility of raising antioxidant concentrations by adding some antioxidants Like vitamins such as A, E, C (Mohamed *et al*., 2018) or some enzymes like clotathione and carnitine (Abdul Karim *et al*., 2017). Propolis can also be used to raise the concentration of antioxidants, as propolis acts as a protective material to protect cells and membranes against oxidative reactions and prevents the accumulation of substances and molecules that cause oxidative damage (Ahn *et al*., 2007; Atta *et al*., 2014). And it consists of infection from the strongest antioxidants that work to fight free radicals (reactive oxygen types ROS) because it contains polyphenols (polyphenols), vitamins, minerals and antioxidants, as the flavonoids present in the propolis are considered to be powerful antioxidants, which leads to the formation of Free roots have the preservation of the cell membrane from these roots (Youssef and Salama, 2009). Propolis also activates enzymatic antioxidants such as Superoxide dismutase (Jasprica *et al*. 2007). The propolis also contains approximately 300 chemical compounds, including polyphenols, phenolic, (aldehyde), (amino acids) and other organic compounds (De souse *et al*., 2001). Selem (2012) also indicated that the Egyptian propolis contains flavonoids such as 3-hydroxy methyl-1-phenyl-1-heptadecyh h-3-o1 at (13.7%) and contains flavones at (10.15%). These substances are antioxidants. Adding propolis to semen Extender to present it improves sperm motility and maintains the integrity of the plasma membrane (Al-Batawy and Brannas, 2015). And Al-Shishtawy *et al*. (2016) reported that the propolis improves the storage time and sperm viability of the rats fed to the propolis. Mohamed (2017) bear that propolis has the ability to improve sperm vitality, as it improves sperm motility and reduces harmful enzymes such as AST and ALT. El-Harairy *et al*. (2018) confirmed that adding the alcoholic extract of propolis to the semen of the Rahmani rams in a soy lecithin Extender reduces liver enzymes in the seminal plasma which leads to increased progressive sperm motility.

Vitamin B12 also plays a role in fighting free radicals, so Chen *et al.* (2001). Vitamin B12 also plays a major role in biochemical reactions such as amino acid metabolism and methionine synthesis (Juanchi *et al*., 2000) and Watanabe *et al.* (2003) suggest that the presence of B12 to produce reduces the amount of free radicals resulting from oxidative reactions in human semen. Ha and Zhao, (2003) and Dalvit *et al*. (2005) confirmed that we add vitamin B12 until it reaches the level of abnormal sperm preparation in male rats and several studies have shown that adding B12 to semen Extender during freezing improves the quality of sperm Cai *et al* (2004) confirmed that adding vitamin B12 to semen Extender and progressing to a significant improvement in the quality of frozen semen of Friesian oxen. Foot *et al*. (2002) indicated

that adding vitamin B12 sends to reduce the damage caused by reactive oxygen species. as the semen cooling and freezing process damages the membranes fat, leading to death due to free radicals formed during freezing (Rober, 2005). The addition of vitamin B12 to the frozen semen diluents also reduces AST in the semen in rams (Hu *et al*., 2011). It was also confirmed by Saieed *et al*. (2018) that adding vitamin B12 to Tris diluent upon cooling resulted in improved semen characteristics of Awassi rams.

Materials and Methods

This study was conducted in the field of the College of Agriculture/Diyala University. Use in this experiment (5) male sexually mature shami goats ranged between (1.5-2) years old and weights ranged between (30-40) kg. And the collection of semen from male goats by using the artificial vagina of sheep and goats in the presence of a female in the case of estrus injected with estradiol by (2.5) mg 36 hours before the collection process.

First treatment T1: control treatment

Second treatment T2: add 3 mg of vitamin B12 / 50 ml Extender Tris.

Third treatment T3: Add 3 mg of Alcoholic propolis extract to Propolis / 50 mL Tris Extender

T4 treatment: add 3 mg of vitamin B12 with the addition of 3 mg of alcohol extract to Propolis / 50 ml Tris Extender.

Sperm plasma washing was performed by mixing semen with Tris solution without adding egg yolk according to Ritar *et al*. (1990). Washing was done at a ratio of 1:10 of the solution at a temperature of (30 ° C) according to the method (Hubei *et al*., 2006) and performing an operation Centrifuge to dispose of seminal plasma using a Centrifuge at 2500 rpm for 15 minutes. Sperm plasma was eliminated, the washing process was repeated, and the filtrate was disposed of. The groups were divided into four trial groups using the Tris Extender. After that, the samples were transferred to the refrigerator for cooling at a temperature of (5°C), as the samples were placed in a tightly closed test tubes and then the tubes inserted were placed in plastic boxes containing water at a temperature of (32 °C). Then they were placed in the refrigerator where the readings were taken after In a period of 1 day, 3 days, and 5 days, individual sperm motility was estimated by Chemineau *et al*. (1991) and the live sperm ratio was calculated according to the Swasnon and Bearden method (1951) and the percentage of plasma membrane integrity was calculated according to the Jeyendran *et al*. (1984) The semen pH was estimated by the PH meter. The total abnormal sperm percentage was also calculated based on the Hancock method (1951) and. Statistical analysis of the data was performed using SPSS (spss 2011) according to the complete random design CRD and the differences between the mean of the coefficients were compared according to Duncan polynomial test (Duncan, 1955).

Results and Discussion

Effect of addition of alcohol extract to propolis and vitamin B12 on percentage of individual movement, percentage of live sperm, percentage of plasma membrane integrity, Extender PH and percentage of sperm abnormal

The results of the statistical analysis Table (1) showed that there was a significant effect (P≤0.05) with respect to the individual movement of the group T4, T3, and T2 on the control group T1 and the results were (76.42, 75.58, 74.67, 72.08) respectively. This superiority in the percentage of individual sperm motility in T4 over other experimental groups within different cryopreservation periods may be due to propolis containing active substances such as polyphenolic, vitamins, minerals and flavonoids that reduce free radical damage (Yousef and Salama, 2009).

The results showed Table (1), a significant difference between the experimental groups for the percentage of live sperm, whereas there was a mathematical superiority for the group T4, T3, and T2 over T1 (76.75, 76.17, 75.00, 73.42), respectively.

The superiority of the percentage of live sperms in T4 over the rest of the experimental groups for propolis containing antioxidants that protect cell membranes and prevent the accumulation of oxidizing damaging molecules (Atta, 2014). This finding was consistent with El-Seadawy *et al*. (2017). In addition, the addition of vitamin B12 works to fight free radicals through its use as an antioxidant Foot (*et al*. 2002).

Also, the results of the statistical analysis, Table (1) showed a significant difference (P≤0.05) between the experimental groups of the percentage of plasma membrane safety as T4, T3, T2 group outperformed the T1 group (73.08, 72.42, 71.25, 67.75), respectively. This superiority in the percentage of the integrity of the plasma membrane of the sperm in the T4 group indicates the rest of the experimental groups within different cryopreservation periods because of propolis containing flaviodones in addition to other antioxidants that reduce free radical damage and thus reduce the accumulation of fatty acids and thus preserve them from Fat peroxide (Yousef and Salama, 2009).

The results of the statistical analysis, Table (1) showed a significant difference (P≤0.05) for the PH between the experimental groups, as the group T4 and T3 were superior to T2 (6.58, 6.55, 6.48, 6.46), respectively. The reason may be to maintain the pH (PH) in group T4 by maintaining sperm vigor due to propolis containing phenolic compounds and antioxidants that prevent the accumulation of free radicals which prevents the formation of lactic acid produced from metabolic processes (Olezyk *et al*., 2017).

The results of the statistical analysis, Table (1) also showed a significant decrease (P≤0.05) for the percentage of sperm distortions, as the T4 group on the groups T2, T1 and T3 decreased (9.88, 10.67, 11.63 and 13.71) respectively.

Table 1 : Effect of addition of alcoholic extract to propolis with vitamin B12 in percentage of individual movement, percentage of live sperm, percentage of plasma membrane integrity, Extender PH and percentage of abnormal sperms. (Average ± standard error)

Factors	Individual movement (%)	Alive sperms (%)	Safety of plasma membrane (%)	PH	Abnormal sperms (%)
control T1	72.08 ± 0.996 b	73.42 ± 1.264 b	67.75 ± 1.207 b	6.46 ± 0.033 b	13.71 ± 0.664 c
Vit B12 mg3 T2	74.67 0.980± a	75.00 ± 1.059 ab	71.25 ± 1.008 a	6.48 ± 0.022 b	11.63 ± 0.338 bc
3 mg alcoholic extract of propolis T3	75.58 ± 0.793 a	76.17 ± 0.757 ab	72.42 ± 1.221 a	6.55 ± 0.021 a	10.67 ± 0.437 b
3 mg Alcoholic Extract of Propolis Propolis +3 mg Vit B12 T4	76.42 ± 0.484 a	76.75 ± 1.067 a	73.08 ± 1.485 a	6.58 ± 0.020 a	9.88 ± 0.262 a
	*	*	*	*	*

The storage times affect the percentage of individual movement, the percentage of live sperm, the percentage of plasma membrane integrity, diluted PH and the percentage of sperm deformities.

The results of the statistical analysis, Table (2), showed that the first period (1 day) was the best as it significantly outperformed (P≤0.05) over both the second period 3 days and the third period 5 days (77.06, 74.75, 72.25) respectively. Individual sperm is due to the storage process in which the metabolism process and fat oxidation are not stopped, as the accumulation of toxic substances, especially free radicals resulting from metabolism, and the effect of these compounds in inhibiting the degradation of fructose sugar and the air breathing process led to a decrease in individual movement during the periods of the cooling process (Sahnoun *et al*., 2017).

The results of the statistical analysis, Table (2), found that the percentage of live sperm decreased, and there was a significant significance (P≤0.01) for the first 1 day of cryopreservation over the second period 3 days and the third period 5 days (77.75, 75.31 and 72.94), respectively. The reason for the low percentage of live sperm is due to the storage process in which the metabolism process and fat oxidation are not stopped, as the accumulation of toxic substances, especially the free radicals resulting from the metabolism process and the effect of these compounds in inhibiting the degradation of fructose sugar and the air breathing process led to a lower percentage For live sperm during periods of the cooling process (Gandhi *et al*., 2017).

The results of the statistical analysis, Table (2) showed a highly significant superiority (P≤0.01) in the percentage of plasma membrane integrity for the first (1 day) period of cryopreservation over the second period (3 days) and the third period (5 days) (73.88, 70.94, 68.56), respectively. The reason for the low percentage of plasma membrane integrity is due to the increased accumulation of toxic substances especially free radicals resulting from the metabolism during the storage process in which the metabolism process is not stopped and the oxidation of the cooling periods (Bucak *et al*., 2017).

The results of the statistical analysis showed Table (2) there was significant superiority (P≤0.05) in the pH for the first (1 day) period of cryopreservation over the second period (3 days) and the third period (5 days) (6.58, 6.52, 6.44) respectively. The reason for the decrease in pH is due to the increase in lactic acid due to the increase in the accumulation of toxic substances, especially the free radicals resulting from the metabolism during the storage process, which increases the acidity of the dilute during the periods of refrigerated storage (Al-Khashab, 2012).

The results of the statistical analysis, Table (2) showed a significant decrease (P≤0.05) in the percentage of abnormal sperms for the first (1 day) period of cryopreservation over the second period (3 days) and the third period 5 days (10.53, 11.34, 12.53) respectively. The high percentage of sperm deformities is due to increased.

Table 2 : Effect of storage periods on percentage of individual movement, percentage of live sperm, percentage of plasma membrane integrity, diluted PH and percentage of abnormal sperms. (Average ± standard error)

	Individual movement (%)	Alive sperms (%)	Safety of plasma membrane (%)	pH	Abnormal sperms (%)
The first period is 1 day	77.06 ± 0.528 a	77.75 ± 0.849 a	73.88 ± 0.926 a	6.58 ± 0.013 a	10.53 ± 0.327 a
The second period is 3 day	74.75 ± 0.680 b	75.31 ± 0.830 b	70.94 ± 1.070 b	6.52 ± 0.024 b	11.34 ± 0.480 a
The third period is 5 day	72.25 ± 0.750 a	72.94 ± 0.761 c	68.56 ± 1.136 b	6.44 ± 0.021 c	12.53 ± 0.622 b
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Effect of interference between addition of alcoholic extract to propolis with vitamin B12 and cryopreservation times on percentage of individual movement, percentage of live sperm, percentage of plasma membrane integrity, diluted PH and percentage of sperm deformities

Results (Table 3) showed the effect of interference between treatment and times of cryopreservation at a temperature of 5 °C to the presence of mathematical superiority between addition and period after one day of cryopreservation, as they did not differ significantly between them and were in charge. As for cryopreservation after 3 days, the two groups excelled T4 and T3 over groups T2 and T1 (76.5, 75.75, 75.00, 71.75) respectively. As for cryopreservation after 5 days, group T4 outperformed T3, T2, T1 (74.75, 73.25, 72.25, 68.75), respectively. The superiority of the percentage of individual sperm motility in T4 over the rest of the experimental groups within different cryopreservation times is due to the containment of propolis It contains active substances such as polyphenolic polyphenols, vitamins, minerals and flavonoids that reduce free radical damage (Yousef and Salama, 2009). It also contains antioxidants that protect the cell membranes and prevent the accumulation of molecules causing oxidative damage (Atta *et al*., 2014) and this result was consistent with what El-Seadawy *et al.* (2017) also stated. Vitamin B12 plays a major role in biochemical reactions such as amino acid metabolism and the synthesis process Methionine (Juanchi *et al*., 2000) and this finding was consistent with Saieed *et al* (2018).

The results of the statistical analysis, Table (3), showed the overlap between addition and duration, there was no significant difference in the percentage of live sperm in relation to the first (1 day) period of cryopreservation, and the results took the same curve with respect to the second period of cryopreservation (3 days). 5 days) from cryopreservation there was significant superiority (P≤0.05) for group T4, T3 over group T2, T1 (74.75, 73.75, 72.75, 70.5) respectively. The reason for the superiority in the percentage of live sperms and the decrease in the percentage of dead sperms in T4 over the rest of the experimental groups within different cryopreservation periods is because the propolis contains flaviodones (Atta, 2014) on the rest of the experimental groups within different cryopreservation periods. It also contains antioxidants that protect cell membranes and prevent the accumulation of oxidizing damaging molecules (Atta *et al*., 2014). This finding was consistent with El-Seadawy *et al*. (2017). In addition, the addition of vitamin B12 works to fight free radicals by using it as an antioxidant Foot (*et al*. 2002) and this finding was agreed by Saieed *et al* (2018).

The results of the statistical analysis, Table (3), indicated the interference between addition and duration, there was a significant difference (P≤0.05) in the percentage of plasma membrane integrity totals T4, T3, T2, over group T1 (72.75, 72.5, 71.25, 67.25) respectively for the second period. (3 days) from cryosurgery. The results took the same curve for the third (5 day) period of cryopreservation. There was significant (P≤0.05) for group T4, T3, T2 over group. T1 (71.25, 70.5, 68.75, 63.75) respectively. This superiority in the percentage of the integrity of the plasma membrane of the sperm in the T4 group may indicate the rest of the experimental groups within different cryopreservation periods due to propolis containing flaviodones in addition to other antioxidants that reduce free radical damage and thus reduce the accumulation of fatty acids and thus preserve them From lipid peroxide (Yousef and Salama, 2009). The reason is due to the action of flavonoids in fighting free radicals and thus an improvement in the percentage of safety of the plasma membrane (Mohamed, 2017). Additionally, the addition of vitamin B12 works to fight free radicals through its use as an antioxidant (Saieed *et al*., 2018).

The results of the statistical analysis, Table (3), showed the effect of interference between addition and cryopreservation times. There was significant superiority (P≤0.05) in the extender PH of group T4, T3 over group T1, T2 (6.59, 6.58, 6.49, 6.43), respectively, with respect to For the second (3 day) period of cryopreservation. As for the third (5 days) of cryopreservation, there was significant (P≤0.05) for group T4 superiority over T3, T2, T1 (6.54, 6.47, 6.39, 6.38), respectively. The reason for maintaining the pH in group T4 by keeping sperm alive may be due to propolis containing phenolic compounds and antioxidants that prevent the accumulation of free radicals which prevents the formation of lactic acid resulting from metabolic processes (Miguel *et al*., 2014; Olezyk *et al*., 2017). This finding was consistent with Menchaca *et al.* (2005); Al-Khashab (2012). Vitamin B12 also helps in that preventing the formation of lactic acid, a decrease in the amount of pH and an increase in acidity, which leads to a decrease in sperm vitality for the rest of the experimental groups.

Also, the results of the statistical analysis, Table (3) showed the effect of interference between addition and times of cryopreservation. There was a significant superiority (P≤0.05) in the percentages of sperm abnormal of groups with respect to the first (1 day) period of cryopreservation, as the group T4, T3, T2 overtook the group T1. (9.38, 10.25, 10.75, 11.75) respectively. There was significant superiority (P≤0.05) for group T4, T3, T2 over group T1 (9.75, 10.63, 11.38, 13.63) respectively for the second period of cryopreservation (3 days). As for the third (5 days) period of cryopreservation was There was a significant superiority (P) 0.05) for the group T4 and T3 over the groups T2, T1 (10.5, 11.13,12.75, 15.75), respectively. Others work to reduce free radical damage and thus reduce the accumulation of fatty acids and thus preserve them from fat peroxide (Yousef and Salama, 2009). Likewise, the antioxidants that protect cell membranes and prevent the accumulation of oxidizing damage molecules (Mohamed), 2017). Vitamin B12 also play a major role in biochemical reactions such as amino acid metabolism and methionine synthesis (Juanchi *et al*., 2000).

Table 3 : Effect of interference between addition of alcoholic extract to propolis with vitamin B12 and cryopreservation times in percentage of individual movement, percentage of live sperm, percentage of plasma membrane integrity, diluted PH and percentage of abnormal sperms.

(Average ± standard error).

Factor	Period	Individual movement (%)	Alive sperms (%)	Safety of plasma membrane (%)	PH	Abnormal sperms (%)
control T1	First period I day	75.75 ± 0.854 ab	76.5 ± 1.848 ab	72.25 ± 0.854 ab	6.56 ± 0.034 ab	11.75 ± 0.520 bcd
	Second period 3 days	71.75 ± 1.109 de	73.25 ± 2.097 abc	67.25 ± 1.250 bc	6.43 ± 0.057 de	13.63 ± 0.898 d
	Third period 5 days	68.75 ± 0.854 e	1.936±70.5 c	63.75 ± 1.250 c	6.38 ± 0.040 e	15.75 ± 1.051 e
3mgVit B12 T2	First period I day	76.75 ± 1.436 ab	77.5 ± 1.555 ab	73.75 ± 1.652 ab	6.56 ± 0.018 abc	10.75 ± 0.323 abc
	Second period 3 days	75.00 ± 1.581 abcd	74.75 ± 1.702 abc	71.25 ± 1.652 ab	6.49 ± 0.020 bcd	11.38 ± 0.427 abc
	Third period 5 days	72.25 ± 1.601 cd	72.75 ± 1.797 bc	68.75 ± 1.250 abc	6.39 ± 0.040 e	12.75 ± 0.520 cd
3 mg alcoholic extract of propolis T3	First period I day	77.75 ± 1.250 a	78.25 ± 0.854 ab	74.25 ± 2.287 a	6.60 ± 0.014 a	10.25 ± 0.777 ab
	Second period 3 days	75.75 ± 0.854 ab	76.5 ± 0.957 ab	72.5 ± 2.102 ab	6.58 ± 0.021 ab	10.63 ± 0.826 abc
	Third period 5 days	73.250 ± 1.19 bcd	73.75 ± 1.109 abc	70.5 ± 2.102 ab	6.47 ± 0.027 cde	11.13 ± 0.826 abc
3 mg Alcoholic Extract of Propolis Propolis +3 mg Vit B12 T4	First period I day	78.00 ± 0.408 a	78.75 ± 2.016 a	75.25 ± 2.626 a	6.62 ± 0.028 a	9.38 ± 0.427 a
	Second period 3 days	76.5 ± 0.645 ab	76.75 ± 1.702 ab	72.75 ± 2.78 ab	6.59 ± 0.040 a	9.75 ± 0.433 ab
	Third period 5 days	74.75 ± 0.479 abcd	74.75 ± 1.702 abc	71.25 ± 2.626 ab	6.54 ± 0.029 abc	10.5 ± 0.408 ab
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