Research Article/Artículo de investigación

EFFECT OF ROYAL JELLY ON QUALITY OF CHILLED SEMEN FROM DAMASCUS BUCK

EFECTO DE LA JALEA REAL SOBRE LA CALIDAD DEL SEMEN REFRIGERADO DE MACHOS CABRIOS DAMASCO

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ABSTRACT

The objective of this study was to evaluate the effect of supplementation of different concentrations of royal jelly (RJ) in Tris-egg yolk (TEY) and Tris-soybean (TSL) extenders on sperm quality of Damascus buck chilled semen. The ejaculates were collected from five bucks using an electroejaculator twice a week during the reproductive season. Semen samples were pooled and diluted in extenders supplemented with RJ (1%, 0,75%, 0,50%, 0,25%) with a final concentration of 150x10^6 spermatozoa/mL and stored for 72 hours. The samples were evaluated for sperm quality parameters, including motility, viability, abnormality, membrane integrity, pH, osmolarity. When the RJ doses were compared among each other, 1% RJ concentration caused an increase in the percentage of abnormal and dead spermatozoa in the TSL extender. In the TEY extender, the control and 0.25% RJ concentration groups had the lowest motility and viability values. Generally, the addition of medium and high doses of RJ in the TEY extender group was found to be beneficial to sperm motility and plasma membrane integrity. Although motility was terminated at the 48th hour in the TEY control and 0.25% RJ groups, other trial groups continued. In conclusion, it was determined that the TSL extender was superior to the short-time storage of the goat spermatozoa in the between-groups evaluation, and the RJ supplementation did not achieve any success during the liquid storage at 4°C in the TSL extender.

Keywords: Royal jelly, tris, soybean, short-time storage, semen, goat.
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RESUMEN

El objetivo de este estudio fue evaluar el efecto de la suplementación de diferentes concentraciones de jalea real (RJ) en los diluyentes Tris-yema de huevo (TEY) y Tris-soja (TSL) sobre la calidad del semen refrigerado de machos cabríos Damasco. Los eyaculados se recogieron dos veces por semana durante la temporada reproductiva de cinco machos utilizando un electroeyaculador. Las muestras de semen se agruparon y diluyeron en TEY y TSJ suplementados con RJ (1%, 0,75%, 0,50%, 0,25%), a una concentración final de 150x10^6 espermatozoides/ml y se almacenaron durante 72 horas. Las muestras fueron evaluadas para parámetros de calidad como motilidad, viabilidad, anormalidad, integridad de la membrana, pH y osmolaridad. Cuando las dosis de RJ se compararon entre sí, la concentración de RJ al 1% causó un aumento en el porcentaje de espermatozoides anormales y muertos en el extensor de TSL. En el diluyente TEY, el control y los grupos de concentración de 0,25% RJ tuvieron los valores más bajos de movilidad y viabilidad. En general, se encontró que la adición de dosis medias y altas de RJ en el grupo TEY fue beneficiosa para la motilidad de los espermatozoides y la integridad de la membrana plasmática. Aunque la motilidad se terminó a las 48 horas en el control TEY y en los grupos con 0,25% de RJ, en los otros grupos de prueba la motilidad continuó. En conclusión, se determinó que el diluyente TSL fue superior para el almacenamiento a corto plazo de los espermatozoides de macho cabrío en la evaluación entre grupos, y que la suplementación con RJ no logró ningún éxito durante el almacenamiento de líquido a 4°C en el diluyente TSL .

Palabras clave: Royal jelly, tris, soybean, short-time storage, semen, goat.
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INTRODUCTION

Unlike with cattle, artificial insemination of goats is generally limited, owing to the high cost of labor, the difficulty of identifying superior sires accurately, low conception rates, especially with frozen semen, and the use of non-frozen semen. Efforts to improve the preservation of goat semen are focused on the modification of extenders (Alcay et al., 2017). The utilization of chilled semen in a breeding program is an important alternative to artificial insemination with frozen semen when insemination is performed within a short-time-interval after collection. Moreover, fertility using semen stored at 5°C is higher than that obtained with frozen semen, which decreases motility, morphological integrity, increases embryonic loss (Leboeuf et al., 2000; Paulenz et al., 2002). The extenders of semen are responsible for energy metabolism, the metabolic activity of sperms, and the protection of sperm cells from damage during the preservation process (Salamon and Maxwell, 2000; Barbas and Mascarenhas, 2009; Pamungkas et al., 2014). Due to the plasma membrane structure, which includes a high concentration of polyunsaturated fatty acids, mammalian spermatozoa are highly sensitive to oxidative stress (Motlang et al., 2014). Over a few decades, researchers have studied the potential of antioxidant supplementations to the semen extender to overcome this problem (Hu et al., 2010; Salmani et al., 2013; Shikh Maidin et al., 2014; Syarifuddin et al., 2017; Eriani et al., 2018). Antioxidant compounds increase sperm function and can improve fertility (Hamza and Amin, 2007).

The saline-containing solutions, sodium citrate-egg yolk, sodium citrate-sugar-egg yolk, saccharose-EDTA, CaNa2-egg yolk, whole-fat, semi-skimmed and skim milk components, and various commercial diluents are used for short-time storage of goat sperm (Arı and Yıldız, 2018). The egg yolk contains low-density lipoproteins (LDLs) and protects the sperm against damage during storage, cooling, and freezing and protects the sperm membrane from cold shock (Jerez-Ebensperger et al., 2015). Even if, egg yolk is a predisposing factor for microbial contamination which cause a reduction of the fertilization capacity of sperm, represent a sanitary risk of disease transmission, and it also interacts with egg yolk coagulating enzyme that hydrolyzes egg yolk lecithin into fatty acids and lysolecithin which is toxic to buck spermatozoa (Hafez and Hafez, 2000; Beccaglia et al., 2009; Yodmingkwan et al., 2016). Therefore, extenders free of animal protein have been tested in recent years, and several commercial vegetable origin extenders have been reported to show promising results (Leboeuf et al., 2000; Marco-Jimenez et al., 2004; Bittencourt et al., 2008; Yodmingkwan et al., 2016; Kakati et al., 2017). An alternative to egg yolk in semen extenders is the soybean lecithin, a natural mixture of phosphatidylcholine and several fatty acids that confer structural stability to cells (Oke et al., 2010).

Royal jelly (RJ) is a secretion from the hypopharyngeal and submandibular glands of young worker bees of the genus *Apis mellifera* primarily for feeding young larvae and maintaining the adult queen bee (Nakajima et al., 2009; Zahmatkesh et al., 2014). It contains water (60–70%), proteins, hormone-rich substances including testosterone and insülin-like growth factor-1, lipids including sterols and fatty acids, sugars, small amounts of mineral salts and essential amino acids particularly cystine, lysine and arginine which have high antioxidant potency (Robak and Marcinkiewicz, 1995; Hattori et al., 2007; Kodai et al., 2007; Abd-Allah, 2010; Amini et al., 2019;). It has been reported that the protective effects of RJ on the sperm membrane during the cooling process might be associated with the physiologic effects of amino acids, especially cysteine and proline, which work as potent antioxidants. Royal jelly also contains enzymes, antibacterial properties, vitamins A, B, C, D, and E and possesses many pharmacological functions in experimental animals such as antioxidant, antibacterial, anti-allergic, antitumor, anti-inflammatory, antihypertensive and anti-aging (Hashimoto et al., 2005; Abd-Allah, 2010; Abd-Allah, 2012;). The beneficial role of RJ supplementation on male fertility has been reported in lab model animals (Elnagar, 2010; Zahmatkesh et al., 2014; Ghanbari et al., 2015) and a few studies have been conducted about the effect of RJ on sperm quality during incubation at 37°C or cold storage in domestic animals (Abd-Allah, 2012; Moradi et al., 2013). Additionally, there are no studies about the effect of RJ on sperm quality during the liquid storage of Damascus buck semen. Therefore, the objective of this study was to determine the effects of different concentrations of royal jelly on sperm parameters during the short-time storage of buck semen diluted with Tris-egg yolk and Tris-soybean lecithin extenders at 4ºC for 72 hours.

MATERIALS AND METHODS

The study was carried out during the breeding season (August- October) at the Pan Livestock Breeding Goat Farm in Nurdağı/Gaziantep, Turkey. Semen samples were obtained from 5 mature Damascus bucks, which were 3-5 years old and without reproductive problems. Ejaculates were collected twice a week using the electroejaculation method (Ruakura Ram Probe, Manufactured for Shoof International Ltd, New Zealand), according to Demirci (2002) and Tekin (1994) and processes were repeated ten times. Immediately after collection, the ejaculates were transferred to a water bath (37ºC) and then evaluated. Fresh semen samples were examined for volume, mass activity, and motility. Only the ejaculates with a volume >0.5 ml; mass activity >3; and motility >70% were used. Semen was pooled to reduce individual

differences and was evaluated for volume, motility, mass activity, concentration, dead spermatozoa, abnormal spermatozoa, membrane integrity, and pH values. After dilution and storage at 4ºC, motility was evaluated every 12 hours, plasma membrane integrity, while percentages of dead and abnormal spermatozoa, osmolarity, and pH values were determined every 24 hours.

Chemicals used in this study were obtained from Sigma Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Tris-egg yolk extender was prepared according to Camara et al., 2001; containing: Tris 3,605 g, fructose 1,488 g, citric acid 2,024 g, Penicillin G (İ.E. 1,000,000 IU, Istanbul, Turkey) 100,000 IU, Streptomycin (İ.E.ULAGAY, 1 g, Istanbul, Turkey) 100 mg, egg yolk 12% (v/v) and distilled water (100 ml). Tris-soybean lecithin extender composition was prepared with 3,605 g Tris, 1,488 g fructose, 2,024 g citric acid, Penicillin G 100,000 IU, Streptomycin 100 mg, 1,5% soybean lecithin and 100 ml of distilled water. The pooled sample was split into ten equal groups in a 37ºC water bath and diluted with tris-egg yolk or tris-soybean lecithin based extenders supplemented with 0,25%, 0,50%, 0,75% or 1% concentrations of RJ (Fanus, 20,000 mg pure royal jelly, Trabzon, Turkey). Each group was diluted to a final concentration of $150x10^6$ spermatozoon/ml.

For the preparation of the RJ stock solution, pure royal jelly (2 g) was diluted with 100 ml of distilled water and vortexed for a few minutes. All concentrations of royal jelly were prepared using the stock solution. After the first dilution, semen samples were placed in 10 ml glass tubes and were diluted, and semen was gradually cooled to 4 ºC within 2-3 hours. The diluted samples were prepared by adding 5% glycerol using the two-step dilution method, according to Üstüner et al. (2014) and stored at 4ºC, and sperm parameters evaluation was conducted for 72 hours.

Semen Evaluations

The volume of each ejaculate was determined in graduated tubes. The pH value of the pooled semen samples was measured using pH meter (Hanna HI 83141). For the evaluation of mass activity, a drop of semen was examined (Tekin, 1990). Sperm motility was assessed subjectively using a phase-contrast microscope (400x) with a warm slide (37 ºC) (Tekin, 1990). Sperm viability was determined by eosin staining method (Jeyendran et al. (1984; Tekin 1990). Spermatozoon concentration was determined using Hayem solution with hemocytometric method (Hafez and Hafez, 2000; Tekin 1990; Tekin (1994). The morphological examination was carried out using Hancock solution (Tekin 1994). To evaluate membrane integrity, we used the hypoosmotic solution (1.1 g fructose, 0.55 g sodium citrate, 100 ml of distilled water) according to the protocol established by Jeyendran et al. (1984) with slight modifications. Ten µl of semen were mixed with 100 µl of 100 mOsm hypoosmotic solution at 37ºC for 60 min. After incubation, a total of 200 sperm cells were evaluated under 1000X magnification with a phase-contrast microscope, and the percentage of curled and swollen tails was determined. The osmolarity values of the semen samples were measured using an osmometer, which works on the principle of the cooling system with measuring range 0-3000 mOsmol/kg H_2O (Gonotec, OSMOMAT 3000).

Statistical analysis

The results were analyzed using SPSS 22 and presented as mean ± standard error, and differences were considered significant at $P<0.05$. One-way analysis of variance (ANOVA) was used to evaluate the data, and Duncan Test was used to determine the differences between the groups. The Scientific Ethical Committee approved the experimental setups and evaluation techniques at Hatay Mustafa Kemal University, No: 2018/5-2.

RESULTS

The average volume of the pooled ejaculate was 6.3 ± 0.63 ml. Average mass activity and motility were 3.85±0.08 and 77±0.82%, respectively. Average sperm concentration was found to be 2375±57,60 x 10^6 spermatozoa/ml. Average dead and abnormal spermatozoa percentage, pH value, and membrane integrity were 16.2±1.40%, 12.3±0.94%, 6.47±0.06 and 78.3±1.63%, respectively.

As shown in Table 1, Tris egg yolk control (TEY control) group resulted in significantly higher motility compared to TEY-1 group while similar to the other royal jelly supplemented groups at the 0 hours. At the 24 and 36 hours, the lowest motility was observed in control and TEY-0.25 groups. At the 48 and 60 hours, the highest motility was observed in TEY-1 groups ($P<0.05$). At the 24 hours in the Tris soybean control (TSL control) group was significantly higher than that of the TSL-1 group while similar to those of other royal jelly supplemented groups. In the evaluation between groups (Table 2), the motility in TEY control group was lower than that of TSL control group, and TEY-0.25 group was found to have significantly lower motility than TSL-0.25 ($P<0.05$) at 24, 36, 48 and 60 hours. At 48 hours in TEY-0.75 group was lower than TSL-0.75 group. At 36, 48, 60, and 72 hours in TSL-0.50 group superior to TEY-0.50 group ($P<0.05$).

As shown in Table 3, plasma membrane integrity was significantly higher in TEY control group compared to that in TEY-1 group at the 0 hours. No differences were observed for membrane integrity in tris soybean lecithin groups (P>0.05). The plasma membrane integrity (Table 4) at 24 and 48 hours in TEY control group was lower than those in TSL control group, and TEY-0.25 group was lower than TSL-0.25 group. At the 48 hours, in TEY-0.50 group was lower than that in TSL-0.50 group (P<0.05).

Table 1. Effect of royal jelly supplementation on sperm motility of chilled buck spermatozoa during liquid storage periods (Mean±SE)

Groups		0 hours	12 hours	24 hours	36 hours	48 hours	60 hours	72 hours
TEY	Control	75.63±1.75^{a}	65.00±2.11^{a}	21.25±10.47^{b}	4.38±3.20^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	1% RJ	60.00±4.53^{b}	61.88±3.40^{a}	54.25±5.38^{a}	41.25±9.99^{a}	32.50±10.86^{a}	28.75±9.53^{a}	18.75±7.24^{a}
	0.75% RJ	71.25±2.80^{a}	68.13±3.26^{a}	62.63±5.73^{a}	32.50±10.69^{a}	17.50±11.46^{ab}	15.00±9.87^{ab}	10.00±7.56^{ab}
	0.50% RJ	73.13±2.10^{a}	66.38±3.23^{a}	52.50±8.81^{a}	27.50±10.65^{ab}	7.50±6.81^{b}	6.25±6.25^{b}	6.25±6.25^{ab}
	0.25% RJ	73.13±1.88^{a}	61.75±3.97^{a}	11.25±9.29^{b}	5.00±5.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	P	0.003	0.572	0.000	0.011	0.024	0.023	0.106
TSL	Control	69.38±2.40^{a}	64.38±2.74^{ab}	60.63±3.20^{a}	46.88±7.00^{a}	38.13±8.13^{a}	34.38±8.10^{a}	13.75±7.30^{a}
	1% RJ	63.13±4.11^{a}	56.25±3.87^{b}	50.00±2.83^{b}	48.13±3.53^{a}	45.63±3.33^{a}	35.00±5.00^{a}	19.38±6.51^{a}
	0.75% RJ	66.88±2.10^{a}	65.63±1.99^{a}	62.50±2.50^{a}	55.00±5.35^{a}	50.00±4.53^{a}	36.25±6.46^{a}	16.25±8.22^{a}
	0.50% RJ	69.38±1.75^{a}	63.75±2.45^{ab}	61.88±2.30^{a}	56.25±6.25^{a}	51.25±5.15^{a}	43.13±7.61^{a}	29.25±7.38^{a}
	0.25% RJ	70.00±1.64^{a}	65.63±2.90^{a}	62.50± 2.50^{a}	55.00±2.83^{a}	41.25±6.66^{a}	31.88±8.55^{a}	15.63±8.68^{a}
	P	0.313	0.135	0.009	0.596	0.458	0.850	0.633

a-b: Different letters within the same column show significant differences (P<0.05).

Table 2. Comparison of motility values in between groups (Mean±SE)

Hours	Control		1% RJ		0.75% RJ		0.50% RJ		0.25% RJ	
	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL
0	75.63±1.75	69.38±2.40	60.00±4.53	63.13±4.11	71.25±2.80	66.88±2.10	73.13±2.10	69.38±1.75	73.13±1.88	70.00±1.64
P value	0.054		0.618		0.231		0.192		0.230	
12	65.00±2.11	64.38±2.74	61.88±3.40	56.25±3.87	68.13±326	65.63±1.99	66.38±3.23	63.75±2.45	61.75±3.97	65.63±2.90
P value	0.859		0.293		0.524		0.528		0.444	
24	21.25±10.47	60.63±3.20	54.25±5.38	50.00±2.83	62.63±5.73	62.50±2.50	52.50±8.81	61.88±2.30	11.25±9.29	62.50±2.50
P value	0.003		0.496		0.984		0.321		0.000	
36	4.38±3.20	46.88±7.00	41.25±9.99	48.13±3.53	32.50±10.69	55.00±5.35	27.50±10.65	56.25±6.25	5.00±5.00	55.00±2.83
P value	0.000		0.527		0.081		0.035		0.000	
48	0.00±0.00	38.13±8.13	32.50±10.86	45.63±3.33	17.50±11.46	50.00±4.53	7.50±6.81	51.25±5.15	0.00±0.00	41.25±6.66
P value	0.000		0.267		0.019		0.000		0.000	
60	0.00±0.00	34.38±8.10	28.25±9.53	35.00±5.00	15.00±9.87	36.25±6.46	6.25±6.25	43.13±7.61	0.00±0.00	31.88±8.55
P value	0.001		0.571		0.093		0.002		0.002	
72	0.00±0.00	13.75±7.30	18.75±7.24	19.38±6.51	10.00±7.56	16.25±8.22	6.25±6.25	29.25±7.38	0.00±0.00	15.63±8.68
P value	0.081		0.950		0.585		0.032		0.094	

Table 3. Effect of royal jelly supplementation on HOS test values of chilled buck spermatozoa during liquid storage periods (Mean±SE)

Groups		0 hours	24 hours	48 hours	72 hours
TEY	Control	74.38±1.65^{a}	20.88±10.05^{ab}	0.00±0.00^{b}	0.00±0.00^{b}
	%1 RJ	61.38±3.63^{b}	44.75±8.90^{a}	24.00±10.42^{a}	17.50±7.06^{a}
	%0.75 RJ	71.00±2.41^{a}	45.25±11.63^{a}	10.75±7.85^{ab}	6.38±6.38^{ab}
	%0.50 RJ	72.50±2.14^{a}	42.88±10.41^{ab}	7.50±5.22^{ab}	5.75±5.75^{ab}
	%0.25 RJ	70.25±2.08^{a}	12.13±9.79^{b}	0.38±0.38^{b}	0.00±0.00^{b}
	P	0.008	0.075	0.066	0.106
TSL	Control	67.00±3.17^{a}	58.13±3.19^{a}	28.50±8.60^{a}	14.75±6.89^{a}
	%1 RJ	62.50±3.32^{a}	48.38±3.59^{b}	38.13±6.26^{a}	16.63±5.41^{a}
	%0.75 RJ	67.38±2.55^{a}	60.00±3.70^{a}	33.75±8.82^{a}	16.13±7.49^{a}
	%0.50 RJ	67.00±2.79^{a}	59.38±2.68^{a}	34.75±8.91^{a}	20.00±7.51^{a}
	%0.25 RJ	66.38±2.60^{a}	59.88±3.59^{a}	26.38±8.13^{a}	16.50±8.30^{a}
	P	0.748	0.093	0.849	0.990

a-b: Different letters within the same column show significant differences (P<0.05).

Table 4. Comparison of HOS test values in between groups (Mean±SE)

Hours	Control		1% RJ		0.75% RJ		0.50% RJ		0.25% RJ	
	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL
0	74.38±1.65	67.00±3.17	61.38±3.63	62.50±3.32	71.00±2.41	67.38±2.55	72.50±2.14	67.00±2.79	70.25±2.08	66.38±2.60
P value	0.058		0.822		0.319		0.140		0.264	
24	20.88±10.05	58.13±3.19	44.75±8.90	48.38±3.59	45.25±11.63	60.00±3.70	42.88±10.41	59.38±2.68	12.13±9.79	59.88±3.59
P value	0.003		0.711		0.247		0.147		0.000	
48	0.00±0.00	28.50±8.60	24.00±10.42	38.13±6.26	10.75±7.85	33.75±8.82	7.50±5.22	34.75±8.91	0.38±0.38	26.38±8.13
P value	0.005		0.265		0.072		0.019		0.007	
72	0.00±0.00	14.75±6.89	17.50±7.06	16.63±5.41	6.38±6.38	16.13±7.49	5.75±5.75	20.00±7.51	0.00±0.00	16.50±8.30
P value	0.050		0.923		0.338		0.154		0.067	

As represented in Table 5, TEY-1 group revealed a higher percentage of abnormal spermatozoa compared to TEY-0.50 and TEY-0.25 groups in tris egg yolk groups at the 0 hours. At the 24 hours, the percentage of abnormal spermatozoa was the highest in TEY-0.25 group, while higher abnormality was detected in the highest RJ supplemented group (1%) in tris soybean lecithin extenders. TEY-0,25 group was significantly higher than TSL-0.25 group at the 24 hours (P <0.05). The percentage of dead spermatozoon in TEY control and TEY-0.25 groups were higher than those in TEY-1, TEY-0,75, and TEY-0.25 groups at the 24 hours. In TSL control group, the dead spermatozoon rate was significantly lower than that in TSL-1 group at the 24 hours (Table 6). As represented in Table 7, the 24-hour TEY control group was higher than TSL control group. At the 48 hours, TEY-0.50 group was higher than TSL-0.50 group. At the 24 and 48 hours, TEY-0.25 groups were higher than TSL-0.25 groups.

Table 5. Effect of royal jelly supplementation on abnormal spermatozoa percentage of chilled buck spermatozoa during liquid storage periods

Groups		0 hours	24 hours	48 hours	72 hours
TEY	Control	14.00±1.19^{ab}	13.85±1.10^{b}	13.50±1.50	-
	1% RJ	18.62±3.13^{a}	17.28±1.40^{ab}	16.33±1.72	20.80±4.44
	0.75% RJ	14.25±1.23^{ab}	17.33±1.76^{ab}	20.40±3.74	13.50±5.50
	0.50% RJ	11.62±0.99^{b}	15.33±0.61^{b}	21.25±4.76	19.00±-
	0.25% RJ	11.62±1.19^{b}	21.57±2.36^{a}	16.00±-	-
	P	0.047	0.021	0.631	0.679
TSL	Control	15.25±2.44^{a}	18.50±2.20^{ab}	17.57±1.78^{a}	21.14±2.89^{a}
	%1 RJ	14.62±2.05^{a}	19.75±1.38^{a}	15.71±1.56^{a}	24.00±3.97^{a}
	%0.75 RJ	13.62±1.36^{a}	17.00±1.83^{ab}	12.83±2.27^{a}	22.16±2.89^{a}
	%0.50 RJ	13.50±1.55^{a}	14.00±1.14^{b}	15.33±2.38^{a}	20.00±2.86^{a}
	%0.25 RJ	13.50±2.04^{a}	13.75±0.92^{b}	14.66±1.02^{a}	22.00±3.50^{a}
	P	0.952	0.036	0.497	0.937

a-b: Different letters within the same column show significant differences (P<0.05).

In terms of osmolarity (Figure 1), the TEY control group was higher than all groups with royal jelly supplementation at the 0 hours. It was seen that when the amount of royal jelly increased, osmolarity values significantly decreased. At the 24 hours, the TEY control and TEY-0.25 groups were significantly higher than the other royal jelly groups (P <0.05). The TEY control group at the 48 hours was significantly higher than TEY-1 and TEY-0.75 groups. The TSL control group was significantly higher than trial groups at the 0 and 24 hours, and the TSL control group was significantly higher than the TSL-1, TSL-0.75, and TSL-0.50 groups between 48 and 72 hours. In the intergroup evaluation, there were no significant differences (P >0.05). In the pH evaluation, pH values were measured between 6.35 and 6.56. There was no statistical difference between the control and experimental groups in both diluent groups and among the extenders groups (P>0.05).

Table 6. Effect of RJ supplementation on dead spermatozoa percentage of chilled buck spermatozoa during liquid storage periods (Mean±SE)

Groups		0 hours	24 hours	48 hours	72 hours
TEY	Control	17.50±2.41^{b}	71.57±13.71^{a}	98.50±1.50^{a}	-
	%1 RJ	28.87±7.01^{a}	36.42±6.89^{b}	52.85±12.87^{a}	88.50±1.50^{a}
	%0.75 RJ	20.00±1.95^{ab}	31.33±7.82^{b}	72.50±17.39^{a}	86.00±5.00^{a}
	%0.50 RJ	17.00±1.91^{b}	27.83±5.93^{b}	75.25±17.32^{a}	85.00±5.00^{a}
	%0.25 RJ	16.25±1.85^{b}	84.57±11.71^{a}	95.00±5.00^{a}	-
	P	0.109	0.001	0.424	0.889
TSL	Control	22.00±3.44^{ab}	27.62±3.32^{b}	48.50±10.68^{a}	75.42±11.20^{a}
	%1 RJ	29.37±4.18^{a}	38.50±3.07^{a}	45.62±4.26^{a}	68.57±7.65^{a}
	%0.75 RJ	24.50±2.37^{ab}	27.12±1.15^{b}	39.14±6.66^{a}	66.83±10.08^{a}
	%0.50 RJ	19.25±2.63^{b}	26.75±2.86^{b}	37.42±7.14^{a}	56.20±10.15^{a}
	%0.25 RJ	19.75±2.80^{ab}	28.75±2.61^{b}	48.57±9.37^{a}	67.20±13.46^{a}
	P	0.168	0.021	0.792	0.797

a-b: Different letters within the same column show significant differences (P<0.05).

Table 7. Comparison of percentage of dead spermatozoa in between groups (Mean±SE)

Hours	Control		1% RJ		0.75% RJ		0.50% RJ		0.25% RJ	
	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL	TEY	TSL
0	17.50±2.41	22.00±3.44	28.87±7.01	29.37±4.18	20.00±1.95	24.50±2.37	17.00±1.91	19.25±2.63	16.25±1.85	19.75±2.80
P value	0.303		0.952		0.166		0.501		0.316	
24	71.57±13.71	27.62±3.32	36.42±6.89	38.50±3.07	31.33±7.82	27.12±1.15	27.83±5.93	26.75±2.86	84.57±11.71	28.75±2.61
P value	0.006		0.779		0.549		0.861		0.000	
48	98.50±1.50	48.50±10.68	52.85±12.87	45.62±4.26	72.50±17.39	39.14±6.66	75.25±17.32	37.42±7.14	95.00±5.00	48.57 ±9.37
P value	0.056		0.582		0.084		0.041		0.041	
72	-	75.42±11.20	88.50±1.50	68.57±7.65	86.00±5.00	66.83±10.08	85.00±5.00	56.20±10.15	-	67.20±13.46
P value	-		0.416		0.418		0.399		-	

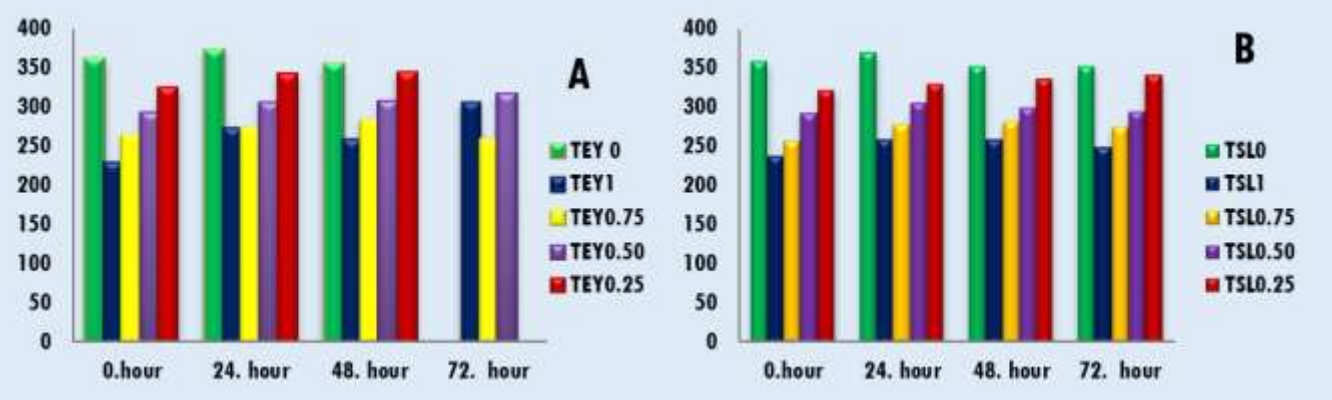

Figure 1: Effect of different concentrations of royal jelly on osmolarity at different incubation periods in TEY (A) and TSL (B) extenders.

DISCUSSION

Soybean extracts are used as a component of extenders, especially during long-term storage (Salamon and Maxwell, 2000). In recent decades, soybean lecithin was reported to have neither a cytotoxic effect nor a negative effect on sperm motility has been demonstrated to be safer than egg yolk, and it is used for sperm cryopreservation in different species (Hong et al., 1986; Fiume, 2001). The results of the present study indicated that Tris soybean lecithin-based extender was better than the tris egg yolk based extender in preserving Damascus goat semen. Fathi et al. (2019) revealed that Tris with 3% of soybean lecithin, showed higher post-thaw sperm quality in Damascus goats. This concentration of lecithin was higher than our soybean lecithin concentration. Chelucci et al. (2015) reported that the best concentration of soybean lecithin was 1%. Salmani et al. (2014) reported that optimal soybean lecithin concentration in tris extender was 1.5% for motility during cryopreservation of caprine semen. These concentrations are compatible with our study. Previous studies suggested that soybean lecithin extender provided better protection in goats (Jiménez-Rabadán et al., 2012; Vidal et al., 2013; Salmani et al., 2014; Chelucci et al., 2015; Lekshmi Bhai et al., 2015). Soybean lecithin contains antioxidant composites as glutathione, which helps to the reduction of lipid peroxidation and inhibition of MDA generation during semen processing (Salmani et al., 2013). The Tris egg yolk extender is more viscous than the Tris soybean extender. Because high viscosity could decrease the velocity and movement characteristics of spermatozoa (Aires et al., 2003). These findings are consistent with our results. Contrary to our findings, Yodmingwan et al. (2016) and Debbarma et al. (2019) reported higher sperm motility and viability in egg yolk based extender in goats. It may be the result of the difference in goat breeds, the protocol of semen dilution, the concentration of egg yolk, or the presence of seminal plasma (Ritar and Salamon, 1982; Yodmingwan et al., 2016).

Abd-Allah (2010) reported that a high concentration of RJ in Tris extender was able to maintain better motility of bovine spermatozoa. Inversely, the results of our experiment showed that motility values were not improved with RJ in TSL extender while being compatible with TEY extender, which had better motility in high and medium doses of RJ during storage. Alcay et al. (2019) reported that the lowest RJ supplementation in extenders yielded successful motility results during cryopreservation of honey bee drone spermatozoa. As proposed by Moradi et al. (2013), the RJ supplementation at lower concentrations improves the ram spermatozoa motility during the liquid storage at 4°C in TEY extender, and it is thought that the high concentration of exogenous antioxidants may disrupt redox balance. These results are opposite to our study. Alcay et al. (2017) reported that the addition of 0.50 and 0.75% RJ in soybean lecithin based extenders led to higher motility following the post-thaw process in goats. In our study, motility values of TSL0.50 and TSL0.75 groups were found to be similar to those of the control group, and the RJ additions did not improve this parameter, probably due to the different protocols for preparing extenders, storage temperatures, ingredients of royal jelly. Amini et al. (2019) reported that addition of medium concentration of RJ into Tris egg yolk extender was found to result in the best sperm quality after cooling in rams. These results are similar to our results in terms of it shows good results in average doses. Shahzad et al. (2016) reported that supplementation of lower levels of RJ (0.1 and 0.3%) in freezing extender could improve the motility of bull semen while the high concentration of RJ did not provide any effect on sperm quality. Atalla et al. (2019) reported that the sperm motility during post-thawing could be enhanced by adding a dose of 0.5% RJ in post-thawing media in contrast, in groups with the addition of 1% and 2% RJ were lower motility rates in rams. Higher concentrations of RJ may negatively alter the osmolarity, which decreases motility rates. When the RJ concentrations were compared among each other, increasing concentrations of RJ caused a gradual decrease in motility. Our study shows that the highest concentration of RJ resulted in reduced motility and survival of chilled sperm compared to medium and low concentrations of RJ in Tris soybean lecithin extender. While, motility was terminated earlier in the TEY control and the lowest RJ added groups.

Alcay et al. (2019) and Moradi et al. (2013) reported that low concentrations of RJ in extenders improved plasma membrane integrity during cryopreservation of honey bee drone spermatozoa and improved the plasma membrane functionality of ram sperm during the liquid storage at 4°C, respectively. Alcay et al. (2017) reported that the addition of 0.50 and 0.75% RJ in soybean lecithin based extender led to higher HOS test value following post-thaw process in goats. In our study, no benefit was found in the trial groups in the TSL extender. Amini et al. (2019) reported that the chilled sperm membrane integrity was significantly higher in Tris egg yolk 3% RJ group in rams and addition of medium concentration of RJ into Tris egg yolk extender was found the best sperm quality after cooling. This study is not consistent with these results. Shahzad et al. (2016) reported that supplementation of lower levels of RJ (0.1 and 0.3%) in freezing extender enhanced plasma membrane integrity while the high concentration of RJ did not provide any effect on sperm quality. Atalla et al. (2019) reported that the plasma membrane integrity during post-thawing could be enhanced by adding a dose of 0.5% RJ in post-thawing media in contrast, 1 and 2% of RJ were lower membrane integrity rates in rams. This result is consistent with our study. Amini et al. (2019) reported that the chilled sperm abnormality was significantly lower the medium dose of RJ supplement in Tris egg yolk in rams while the high dose was the highest abnormality. These findings are in contradiction with our study. Abd-Allah (2010) reported that the viability was superior to the high concentration of RJ in tris extender during post-thaw incubation in bovine spermatozoa while Amini et al. (2019) and Moradi et al. (2013) reported that the chilled sperm viability was significantly higher than the medium dose of RJ supplement and was superior to the low dose of RJ in Tris egg yolk in rams, respectively. The variations could be due to the result of the cryopreservation procedure. Shahzad et al. (2016) reported that the sperm viability was significantly higher than the low dose of RJ supplement in Tris extender in bulls. The negative effect on cooled semen quality with a higher level of RJ in the current study in TSL extender is in agreement with this report, while the TEY extender control and TEY-0,25 groups were found to have a shorter life span. In terms of osmolarity, it decreases as the concentration of RJ increased. When osmotic pressure declined in the extender, it led to damage to sperm cells (Moussa et al., 2002). Increasing the concentration of RJ in extender may lead to changes in the biochemical pathways, imbalance in redox reactions following the adding of the extender with higher concentrations of RJ (Silici et al., 2009). This result is compatible with the TSL groups, which was high dose RJ associated with increased abnormal and dead spermatozoa rate while it is not compatible with the TEY groups, which was control and low concentration of RJ groups were revealed the lowest viability and motility. The variations could be due to the difference in the semen collection method, storage of semen, extender composition, breed of the animals used for the study (Hafez and Hafez, 2000).

CONCLUSIONS

Based on the results, it can be concluded that an extender containing soybean lecithin as the lipid/lipoprotein source can be used to prepare the Damascus goat semen for chilling and the RJ supplementation did not achieve any success during the liquid storage at 4°C. High dose of RJ caused an increase of percentages of abnormal and dead spermatozoa in TSL extender while in TEY extender, control and 0.25% royal

jelly groups had the lowest motility and viability values and the addition of high and medium concentration of RJ was found to be beneficial to sperm motility and plasma membrane integrity. Future studies are needed to focus on low doses of RJ supplement in TSL extender and to evaluate fertility capacity with artificial insemination.

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