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Original article

INFLUENCES OF BOVINE SERUM ALBUMIN ON FRESH AND CHILLED RAM SPERM MOTILITY ASSESSED BY COMPUTER AIDED SPERM ANALYSER

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Summary

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The present work was designed to study the effect of bovine serum albumin (BSA) on the motility characteristics of fresh ram spermatozoa collected at different periods of the year and on their motility status under stress conditions. Moreover, the ability of BSA to replace egg yolk in semen medium was assessed using chilled spermatozoa. Fresh Awassi ram semen samples were collected in April and in June and incubated with two BSA levels (5 mg/mL and 10 mg/mL). Motility parameters of fresh spermatozoa samples treated or not with hydrogen peroxide (H_2O_2), with 5 mg/mL or 10 mg/mLof BSA were compared. The effects of partial and total replacement of egg yolk by 5 mg/mL of BSA on motility characteristics of chilled spermatozoa were assessed by computer-aided sperm analyser (CASA). The addition of BSA significantly increased ($P<0.05$) the values of CASA parameters in April, while the same values did not significantly changed during June. BSA improved the motility parameters ($P<0.05$) in the samples treated with H_2O_2. Replacing a part of egg yolk by BSA enhanced the values of velocity parameters, while the total substitution resulted in a significant decrease ($P<0.05$) in all CASA motility parameters. It was concluded that BSA had the ability to improve the motility of fresh spermatozoa at certain periods of the year and the motility of spermatozoa under stress conditions. BSA was capable to replace an important part of egg yolk in semen preservation media for the chilled ram spermatozoa.

Key words: albumin, hydrogen peroxide, ram, sperm

INTRODUCTION

Serum albumin is the most abundant protein in circulatory system and is also present in the genital tract. Moreover, this protein plays a key role in the transport of a large number of metabolites, fatty acids and hormones to the appropriate cellular targets. On the other hand, albumin was effective in maintaining sperm motility in different preservation media and protected spermatozoa from detrimental effect of dilution (Nair *et al.,* 2006). According to Kreider *et al.* (1985), when albumin was added to either seminal plasma or diluents, the maintenance of motile equine

spermatozoa was enhanced compared to the same diluents without albumin. In bovine, it has been demonstrated that sperm penetration of cumulus free oocytes in protein free media was significantly lower compared with other media supplemented with bovine serum albumin (Tajik *et al.,* 2003).

It is well known that sperm plasma membrane is rich in polyunsaturated fatty acids and is therefore susceptible to peroxidative damage with consequent loss of decreased sperm motility resulting from reactive oxygen species (ROS) generation (Nair *et al.,* 2006). Antioxidants play an important role in scavenging ROS agents which could largely protect sperm plasma membranes (Baumber *et al.,* 2005). It was shown that in an aerobic *in vitro* system spermatozoa underwent spontaneous lipid peroxidation. However, when BSA was added to the incubation medium, lipid peroxidation of the liquid stored rabbit semen was inhibited (Sarıözkan *et al.,* 2013). Nevertheless, the effects and the role of BSA as antioxidant are still controversial and need more profound research especially on small ruminant's spermatozoa.

On the other hand and based upon the experimental media, fresh and chilled spermatozoa were able to fertilise oocyte after several hours to several days of storage. In this respect, cervical artificial insemination in sheep with fresh semen yields a much higher pregnancy rate than when frozen-thawed semen is used, while frozen semen had only acceptable results when laparoscopic insemination was applied (Gourley & Riese, 1990). For that, improvement in the field of freezing-free semen preservation including both fresh and chilled ones was highly needed. This can be done by supplementation of compounds such as BSA that can maintain and improve the quality of stored spermatozoa (Osman *et al.,* 2012). Moreover, Matsuoka *et al.* (2006) reported that BSA can substitute egg-yolk in the ram semen diluents, and may improve the motility and viability of ram spermatozoa following the freezing-thawing process of frozen samples. Anyhow, more research is clearly needed to confirm any positive BSA effects on the quality of ram spermatozoa especially the motility status. In this respect, computer-aided sperm analysis (CASA) technology facilitated the analysing of motility in larger population of spermatozoa (Amann & Waberski, 2014). The use of CASA for sperm analyses in human, rodents and other animal species including sheep has been described (Cancel *et al.,* 2000; Verstegen *et al.,* 2002; Alomar *et al.,* 2018b). Thus, application of CASA technology for sperm analysis may be very beneficial in the evaluation of BSA effects on sperm motility status. The present study was designed to examine the effect of bovine serum albumin (BSA) addition on the motility status of fresh and chilled ram spermatozoa assessed by CASA.

MATERIALS AND METHODS

Site description, animals and semen processing

This study was carried out at Der-Al-Hajar Animal Production Research Station, 33 km south-east of Damascus. The study was approved by the Local Scientific and Ethical Committee of the Atomic Energy Commission of Syria (AECS), Damascus, Syria (permit number 36 Z/M 2-2018).

Semen was obtained from five sexually-experienced local Syrian Awassi rams, aged between 3 and 4 years. Semen samples were collected with the aid of an

electro-ejaculator (Minitube - Electro Ejaculator, Germany) administrating a series of 20 cycles pulses of short electrical stimuli with each cycle (two seconds impulse, then two seconds interval) delivering a slightly higher intensity (from 0 to 20 V maximum) until semen production. Upon collection, the semen was immediately evaluated for its general appearance (including the milky colour and absence of any urinary contamination) and volume. Sperm concentration was estimated using a haematocytometer. An initial analysis of sperm motility was performed using CASA system (Hamilton Thorne Biosciences, USA). Sperm samples with a motility score ≥ 70 % of motile sperm and a concentration of ≥ 1×10^9 spermatozoa/mL were used. Three experiments were conducted using a total of 45 ejaculates. To diminish the effect of individual variation between the rams, a mixture of semen from five animals was used in each assay.

Chemicals, media preparation and experimental design

All the chemicals in this study were purchased from Roth (Carl Roth Gmbh-Karlsruhe-Germany). Bovine serum albumin fraction (V) was provided from PSPARK (Scientific Limited Northampton, UK).

Two media were used, the first was Tyrode's albumin-lactate-pyruvate (TALP) and the second was TRIS-egg-yolk (TEY). The TALP solution was prepared as a 300 mOsm/kg solution containing (in g/L) 6.6 g NaCl, 0.24 g KCl, 0.04 g NaH_2PO_4, 1.92 g $NaHCO_3$, 0.1 g $MgCl_2.6H_2O$, 0.3 g $CaCl_2.2H_2O$, 0.11 g $C_3H_3O_3Na$, 0.5 g $C_3H_5NaO_3$. The TEY medium also prepared as a 300 mOsm/kg solution containing 2.44 g of TRIS (hydroxymethyl) aminomethane, 1.36 g citric acid monohydrate and 1 g of glucose in 80 mL of distilled water, plus 20 mL of egg yolk, bringing the total volume to 100 mL. The two media components were held at constant pH – 7.0.

Three experiments were conducted. In the first experiment, sperm motility of fresh collected semen samples of Awassi males was analysed during two periods: the first was in April and the second in June, the month when testosterone reaches its highest peak in Awassi rams in Syria (Alomar *et al.,* 2016b). In this experiment, fresh sperm samples at 50×10^6 sperm/mL were incubated in TALP medium at 37 °C for 60 minutes with 0 (control), 5 or 10 mg/mL of BSA. This experiment was replicated three times in three different weeks for each period of semen collection.

In the second experiment, the sperm motility was analysed in TALP medium by incubating fresh sperm samples of 50×10^6 sperm/mL with and without 1 mM of hydrogen peroxide (H_2O_2). In this experiment samples were incubated in four groups: 1) without H_2O_2 and without BSA (control), 2) with 1 mM H_2O_2 without BSA, 3) with 1 mM H_2O_2 + 5 mg/mL of BSA and 4) with 1 mM H_2O_2 + 10 mg/mL BSA. Sperm motility was analysed after 60 minutes of incubation at 37 °C. This experiment was repeated three times in three different weeks.

In the third experiment, the effects of partial or total replacement of egg yolk by BSA were studied. Sperm motility was analysed in TEY medium at 5 °C. Sperm samples at 50×10^6 sperm/mL were incubated in this experiment in four groups: 1) with TRIS-based solution containing 20 % of egg yolk (control), 2) TRIS-based solution containing 10% of egg yolk + 5 mg/mL of BSA, 3) TRIS-based solution containing 5% egg yolk + 5 mg/mL of BSA and 4) TRIS-based solution without

egg yolk + 5 mg of BSA. Sperm motility status was analysed after sperm samples incubation in each medium during 6, 24, 48 and 72 hours of incubation at 5 °C. This experiment was replicated 3 times over a period of three different weeks.

Motility assay

The motility characteristics of the ram sperm were assessed by CASA system, using the Hamilton-Thorne motility analyzer (HTM version 12.3, USA). Aliquots of diluted semen (5 microliters) were placed in the system slide and loaded into the analyser. At least three fields were counted for each sample. The motility characteristics included in the analysis were: the percent motility (MOT%), the percent of sperm showing progressive motility (VAP ≥75 µm/s and STR ≥80 %), average path velocity (VAP, µm/s), curvilinear velocity (VCL, µm/s) and straight line velocity (VSL, µm/s). The HTM system settings of ovine spermatozoa are presented in Table 1.

Statistical analyses

Statistical analysis was conducted with the Minitab program (Minitab Coventry, United Kingdom). The normality of values distribution was first tested with the Shapiro-Wilk test. Data were subjected to a factorial analysis of variance general linear model procedure, (GLM) followed by multiple pairwise comparisons using a post-hoc (Tukey) test. The threshold of signification was set at $P<0.05$.

RESULTS

The bovine serum albumin effects on CASA motility parameters of fresh ram spermatozoa collected during April and June are presented in Table 2. In April and compared to control, the values of CASA parameters increased significantly ($P<0.05$) when ram sperm samples were treated with 5 mg/mL of BSA, while only VAP, VCL and VSL values increased ($P<0.05$) when 10 mg/mL BSA was added. In June, no significant differences ($P>0.05$) were noted between the values of CASA parameters of semen treated with either 5 or 10 mg/mL of BSA compared to control values.

The effects of hydrogen peroxide (H_2O_2) and BSA on CASA motion characteristics of fresh sperm samples assessed in TALP medium at 37 °C are illustrated

Table 1. The settings for the Hamilton Thorne Biosciences system version 12.3 used to evaluate ram semen

Parameters	Settings
Frame rate (Hz)	60
Frames acquired (no)	30
Minimum contrast	60
Minimum cell size (pixels)	5
Low VAP cut off	21.9
Low VSL cut off	6
Non-motile head size (pixels)	5
Non-motile head intensity	55
Static size limit (min/max)	0.60/8
Static intensity limit (min/max)	0.25/1.50

Table 2. Effect of BSA on CASA sperm motion characteristics for fresh ram semen collected at two different time periods. Values are presented as mean±SD (n=15)

Treatment/ CASA prameter	MOT (%)	PMOT (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)
			Fresh semen collected in April		
Control	77.67 ±5.61 [a]	13.89 ±3.65 [a]	72.22 ±6.22 [a]	57.44 ±6.50 [a]	133.78 ±2.82 [a]
+ 5 mg/mL BSA	86.33±5.43 [b]	18.88 ±2.03[b]	83.22 ±14.5 [b]	63.78 ±8.91 [b]	150.78 ±11.44[b]
+ 10 mg/mL BSA	79.89±6.17[ab]	15.11 ±1.83 [a]	77.01 ±8.20 [c]	61.11±7.84 [b]	143.56 ±11.21[ab]
			Fresh semen collected in June		
Control	91.67±2.18 [a]	22.22±4.06 [a]	99.63 ±14.6 [a]	67.66±10.24[a]	202.22 ±17.39 [a]
+ 5 mg/mL BSA	90.11±5.28 [a]	22.77±2.16 [a]	106.22±8.87[a]	74.11±8.13 [a]	200±13.69 [a]
+ 10 mg/mL BSA	91.78±2.49 [a]	20.33±3.67 [a]	100.11±12.9[a]	68.56±5.85 [a]	199±18.12 [a]

MOT % = percent motility; PMOT % = percent of sperm showing progressive motility; VAP = average path velocity ; VSL = straight line velocity ; VCL = curvilinear velocity . Values for each semen collection period with different letters within columns differ significantly ($P<0.05$).

Table 3. Effects of hydrogen peroxide (H_2O_2) and BSA on CASA sperm motion characteristics of fresh ram semen. Values are presented as mean±SD (n=15)

Treatment/ CASAparameter	MOT (%)	PMOT (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)
Control	91.66±2.18[a]	22.22±4.06 [a]	99.33±14.06 [a]	67.66±10.24 [a]	200.22±17.39 [a]
+ 1 mM H_2O_2	50.66±6.44 [b]	9.00±2.29 [b]	66.01±9.72 [b]	45.11±6.35 [b]	148.22±15.55 [b]
+ 1 mM H_2O_2+5 mg/mL BSA	80.01±7.33 [c]	17.78±3.90 [a]	91.55±11.85[ac]	64.11±10.95 [a]	183.56±14.05 [c]
+ 1 mM H_2O_2 +10 mg/mL BSA	81.33±5.29 [c]	15.33±4.53[a]	88.06±12.90 [c]	59.00±11.69 [a]	181.55±12.73 [c]

MOT % = percent motility; PMOT % = percent of sperm showing progressive motility; VAP = average path velocity ; VSL = straight line velocity ; VCL = curvilinear velocity . Values for each semen collection period with different letters within columns differ significantly ($P<0.05$).

in Table 3. One mM of hydrogen peroxide decreased significantly ($P<0.05$) the values of CASA parameters compared to control. When either 5 and 10 mg/mL of BSA were added to the H_2O_2-treated samples, the values of CASA parameters increased significantly ($P<0.05$) compared to those treated with hydrogen peroxide without BSA, while the control samples had always the highest values for all CASA parameters.

The comparison between the motility values of chilled ram sperm incubated in TRIS-based solution supplemented with different percentages of egg yolk and 5 mg/mL BSA is presented in Table 4. With the exception of VCL, no differences between CASA values were observed after 6 hours of incubation at 5 ºC between TRIS-based medium supplemented with 20% egg yolk and the TRIS media supplemented with either 10% and 5% of egg yolk + 5 mg/mL BSA. At the same end time point and when TRIS based solution was supplemented with 5 mg/mL BSA alone, all CASA values significantly decreased ($P<0.05$).

After 24 and 48 hours of sperm incubation at 5 ºC, the values of velocity parameters VAP, VSL and VCL were significantly higher in the egg yolk media supplemented with 5 mg/mL BSA compared to 20% egg yolk medium.

After 72 hours of incubation, all CASA parameters were significantly higher in the egg yolk media supplemented with 5 mg/mL BSA compared to the TRIS + 20% of egg yolk. However, in all the end time points, the supplementation of TRIS-based solution with BSA without egg yolk significantly decreased the values of all motility parameters compared to the other TRIS based media supplemented with the different egg yolk concentrations.

DISCUSSION

The primary focus of the present investigation was to explore the extent to which bovine serum albumin involves motility changes in both fresh and chilled ram spermatozoa assessed by CASA technology. The presence of BSA in cryopreservation extenders augmented motility values of turkey, bull and ram spermatozoa (Matsuoka *et al.,* 2006; Ashrafi *et al.,* 2013). Unlike this study, the previous investigations were conducted using frozen spermatozoa. Unfrozen ram sperm (fresh and chilled) are important spermatozoa types for the artificial insemination (AI) technique. However, improving the motility of both frozen and unfrozen ram sperm by different additives such as BSA could be very useful for sheep semen preservation and for the AI process.

In this study, two concentrations of BSA (5 mg/mL and 10 mg/mL) were used, but relatively better results were obtained from 5 mg/mL level. In agreement with our results, Gokce *et al.* (2017) noted that 5 mg/mL of BSA supplemented to lecithin based medium preserved chilled ram sperm motility, plasma membrane functional integrity and acrosome integrity, and the effect of this concentration was better than both BSA-free or higher dose BSA-supplemented media. The data of Osman *et al.* (2012) on bull spermatozoa indicated that BSA, given at high concentration, seemed to have a harmful effect on spermatozoa viability and DNA integrity. Moreover, and according to the previous authors, supplementation of BSA at low concentration was not able to provide enough energy supply and antioxidant protection to the stored sperm. However, for any sperm type, choosing the effective concentration of BSA is considered to be one of the most important factors to obtain the ap-

Table 4. Effect of partial and complete egg yolk (EY) substitution by BSA on CASA sperm motion parameters during 6, 24, 48 and 72 hours of incubation at 5 ºC in TRIS media. Values are presented as mean±SD (n=15)

Treatment/ CASA prameter	MOT (%)	PMOT (%)	VAP (µm/s)	VSL (µm/s)	VCL (µm/s)
			Incubation for 6 hours		
TRIS + 20% EY	88.22±5.47 [a]	16.66±4.90 [a]	90.00±15.09 [a]	64.44±6.69 [a]	186.67±18.13 [a]
TRIS + 10% EY + 5 mg/mL BSA	91.11±3.72 [a]	18.55±3.53 [a]	102.33±12.6 [a]	69.33±6.12 [a]	206.66±17.47[b]
TRIS + 5% EY+ 5 mg/mL BSA	90.22±4.29 [a]	19.33±3.08 [a]	98.88±8.29 [a]	68.55±6.93 [a]	195.67±6.02 [a]
TRIS + 5 mg/mL BSA	53.55±5.34 [b]	6.22±0.83 [b]	68.33±6.85 [b]	43.44±4.58 [b]	138.00±11.28 [c]
			Incubation for 24 hours		
TRIS + 20% EY	88.77±3.34 [a]	15.33±3.38 [a]	90.22±5.28 [a]	58.22±7.82 [a]	184.67±9.93 [a]
TRIS + 10% EY + 5 mg/mL BSA	92.22±2.58 [a]	18.33±4.18 [a]	102.55±11.07[b]	71.22±9.73 [b]	198.33±16.46[b]
TRIS + 5% EY+ 5 mg/mL BSA	90.44±4.32 [a]	20.22±3.01 [a]	101.77±10.45[b]	70.11±9.93 [b]	200.22±13.29[b]
TRIS + 5 mg/mL BSA	24.77±6.35 [b]	1.23±2.55 [b]	62.33±2.78[c]	39.11±3.29 [c]	128.89±9.34 [c]
			Incubation for 48 hours		
TRIS + 20% EY	85.11±4.34 [a]	12.33±1.65 [a]	90.01±10.58 [a]	57.33±3.93 [a]	178.55±4.25 [a]
TRIS + 10% EY + 5 mg/mL BSA	90.55±2.65 [a]	19.22±0.97 [b]	94.33±2.95 [b]	69.55±6.48 [b]	192.44±8.21 [b]
TRIS + 5% EY+ 5 mg/mL BSA	89.84±3.46 [a]	18.44±2.74 [b]	96.44±4.21 [b]	69.33±5.22 [b]	196.55±5.85 [b]
TRIS + 5 mg/mL BSA	24.55±2.69 [b]	1.55±0.01 [c]	43.78±2.48 [c]	35.11 ±6.23 [c]	80.12±8.89 [c]
			Incubation for 72 hours		
TRIS + 20% EY	83.77±4.35 [a]	11.89±1.16 [a]	81±5.74 [a]	54.11±3.59 [a]	178.44±9.87 [a]
TRIS + 10% EY + 5 mg/mL BSA	90.11±1.90 [b]	15.77±1.98 [b]	97±5.33 [b]	64.67±3.81 [b]	197.00±4.44 [b]
TRIS + 5% EY+ 5 mg/mL BSA	89.77±3.83 [b]	17.00±1.22 [b]	99.33±3.27 [b]	62.00±3.31[b]	199.44±4.15 [b]
TRIS + 5 mg/mL BSA	8.88±4.62 [c]	0 [c]	41.23±3.42 [c]	23.55±2.18 [c]	71.11±3.33 [c]

MOT % = percent motility ; PMOT %= percent of sperm showing progressive motility; VAP= average path velocity; VSL = straight line velocity; VCL = curvilinear velocity. Values for each semen collection period with different letters within columns differ significantly (P<0.05).

propriate influences on motility characteristics.

Our results showed that the addition of BSA to fresh ram semen collected in April had a higher positive effect compared to those collected in June. The high rates of sperm motility in June which corresponded to the testosterone peak in the plasma of Awassi rams (Alomar *et al.,* 2016b) may mask any possible positive influences of BSA on sperm motility at this period of the year. It must be noted that, breeding season of Awassi sheep lasts from late June/early July through December, peaking between August and September. Moreover, Awassi rams had satisfactory sexual activity and performance during the spring and were capable of mating with fat-tailed ewes (Kridli *et al.,* 2007). Based on our finding, it may be useful to compare BSA effect on sperm motility during either breeding season or non-breeding season. BSA appears to have higher ability to improve sperm motility during non-breeding season. Thus, BSA addition to semen preservation media may be useful to improve the motility status of spermatozoa collected in such critical period of the year.

Several studies showed that albumin protected the spermatozoa of bull and rabbit from free radicals (Ashrafi *et al.,* 2013; Sarıözkan *et al.,* 2013). Furthermore, the results of Ashrafi *et al.* (2013) indicated that antioxidant enzyme activities increased in bovine semen by the inclusion of BSA in the freezing medium. Our present work is the first one which shows the capacity of BSA in improving the motility of fresh ram spermatozoa after hydrogen peroxide (H_2O_2) incubation. It is worth noting that H_2O_2 is one of the most important members of reactive oxygen species (ROS) family, having a high oxidant potential and can freely cross cell membranes (Thannickal & Fanburg, 2000). We have previously documented the generation of H_2O_2 from live and dead spermatozoa of different species including bull, bucks and ram (Alomar & Donnay, 2006; Alomar *et al.,* 2016a; Alomar, 2019). We also showed the positive and negative effects of hydrogen peroxide on the motility of fresh Awassi ram sperm (Alomar *et al.,* 2018b). However, the exact mechanism by which BSA may increase sperm motility after H_2O_2 incubation is unknown. Clearly, further studies are required to obtain more concrete results on the determination of the antioxidant capacities of BSA on ram semen.

Egg yolk is a common lipid additive to different semen media and extenders. It has been previously evidenced that low-density lipoproteins (LDL) fraction off egg yolk is the main cryoprotective component in egg yolk (Moussa *et al.,* 2002). However, development of semen media without egg yolk is very important to avoid the disadvantages of this complex additive as well as the elimination of various pathogens (Muller-Schlosser *et al.,* 1995). Such development was previously carried out in many species using soya bean lecithin (Aires *et al.,* 2003) and also by adding BSA (Yamashiro *et al.,* 2006; Gokce *et al.,* 2017). Our data clearly showed the ability of the partial replacement of egg yolk by BSA in improving the motility of chilled ram spermatozoa after 72 hours of incubation, whilst the complete replacement was not useful in our present experimental condition. The stabilising phase of motility during the first 6 hours of incubation in TRIS-based supplemented with egg yolk-BSA compared to control, and then the improvement at 48 and 72 hours might occur because of the better adaptation of spermatozoa to cool environment in presence of BSA.

Sperm membrane cholesterol may be removed by BSA in the diluents resulting in increasing membrane fluidity and regulation the susceptibility of sperm to cold shock. Some studies provided direct evidence that BSA adheres rapidly to the sperm membrane, modifies lipid composition of the sperm and promotes plasma membrane protein hydrolysis, leading to calcium ions influx into the cytoplasm and reduction of the cholesterol and phospholipids ratio in the plasma membrane (Singleton & Killian, 1983). On the other hand, Tchacarof & Mollova (1980) suggested that a BSA-sperm surface interaction rendered ram sperm more resistant to deleterious effect of a high rate of semen dilution.

In contrast to our finding and for cooled Boer goat spermatozoa, Memon *et al.* (2013) reported that TRIS based extender with 18% egg yolk concentration was better for sperm motility than the extenders containing 12% and 6% of egg yolk. It must be noted that the previous study was based on decreasing egg yolk concentration in this medium without any accompanying additives as it was the case in our study. Anyhow, there is a need to find additional additives which may help BSA to complete his replacement role of egg yolks in semen solutions. The use of low-density lipoprotein (LDL) derived from egg yolk may be one of the possible alternatives. The synergistic effect of BSA and LDL on conducting a total replacement of egg yolk in different semen solutions may require further research.

CONCLUSION

Taken together, the data suggest that BSA has the ability to improve the motility parameters of fresh spermatozoa collected during non-breeding season and when the spermatozoa are under stress conditions. Moreover, the addition of 5 mg/mL BSA to TRIS-egg yolk media had a positive effect on the sperm cells kinematics during preservation at 5 °C and can replace an important part of egg yolk in semen extenders for the chilled ram spermatozoa. Anyhow, it was clear from the present results that this protein can largely participate in improving the motility characteristics of both fresh and chilled ram spermatozoa.

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