Iranian Journal of Veterinary Science and Technology

Received: 2023- May-22
Accepted after revision: 2023-Jul-30
Published online: 2023-Sep-17

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2023.82141.1247

Influences of Monosaccharides and Disaccharides Supplementations in Tris Media on the Motility Patterns of Fresh and Chilled Small Ruminant Spermatozoa

Mazen Alomar

Division of Animal Production, Department of Agriculture, Atomic Energy Commission, Damascus, Syria.

ABSTRACT

In this study, the effects of monosaccharides, including glucose and fructose, and disaccharides, namely sucrose and trehalose, in eight Tris media on the motility patterns of small ruminants spermatozoa were investigated. Fresh and chilled semen samples from five Awassi rams and five Shami bucks were diluted in TBM and TEY containing 50 mM of the four different sugar types. The characteristics of spermatozoa motility were analyzed using a computer-assisted sperm analyzer (CASA). Fresh ram spermatozoa incubated in a TBM-fructose medium had the highest CASA values with no differences between the motility values generated from the fructose- and glucose-supplemented media. Trehalose reduced the values of velocity parameters, including VAP, VCL, and VSL for fresh ram sperm. Sucrose was the most influential sugar in raising the values of motility parameters MOT%, PMOT%, VAP, VCL, and VSL for fresh bucks spermatozoa, while trehalose generally had an important positive effect on chilled buck sperms. No significant differences ($p > 0.05$) were recorded for sperm trajectory parameters where the values of STR% and LIN% for the two ruminant species and the two spermatozoa types did not significantly differ between the eight media. It was concluded that during the first hours of in vitro incubation and based on the incubation temperature, the velocity parameters of small ruminant spermatozoa were the most affected CASA characteristics by monosaccharides and disaccharides supplementations in Tris semen media.

Keywords

Spermatozoa, Motility, CASA, Glucose, Fructose, Sucrose, Trehalose

Number of Figures: 0
Number of Tables: 4
Number of References:: 37
Number of Pages: 9

Abbreviations

AECS :Atomic Energy Commission of Syria
CASA: computer-assisted sperm analyzer
GLM: general linear model procedure
IVF: in vitro fertilization
LIN%: linearity percent
MDA: malondialdehyde
MOT% :motility percent
PMOT%: percent of sperm showing progressive motility
STR%: straightness percent
TBM: Tris-based media

Corresponding author: *Mazen Alomar*
Email: ascientific15@aec.org.sy
Tel: +96(31) 1213-2580

Introduction

It is well known that semen preservation media are needed to maintain spermatozoa viability and motility of the different animal species [1]. However, to sustain the motility status, spermatozoa require proper nutritional agents in the media. In this regard, sugar is one of the main constituents in semen media which can be easily metabolized into energy [2, 3]. Motility has been considered one of the most important indicators of sugar utilization by sperm because sugars provide the external energy source necessary for maintaining the motility status [2, 4]. It must be noted that when the environment does not provide any external energy source for the semen, the spermatozoa could use sugars in two ways: 1) obtaining energy by the Krebs cycle [5] and 2) storing sugars in the form of glycogen which presents a middle- to long-term energy reserve that could maintain motility [4].

Generally, sugars are divided into three major groups: monosaccharide, disaccharide, and trisaccharide. Monosaccharides are the main energy source for mammalian sperm [3], and fructose and glucose are the most important members of this sugar group. Fructose is an essential source of spermatozoa energy as it is metabolized and converted to pyruvate and lactate to support both sperm motility and viability [6]. In certain conditions, fructose acts as an extracellular cryoprotectant agent to protect the sperm membrane from toxicity during storage [7]. Glucose is also essential for energy utilization by spermatozoa [8]. In humans, glucose is required to sustain an optimal ATP concentration and to support optimum sperm motility [9]. Moreover, according to previous studies, glucose can support significant levels of hyperactivated motility and at the same time can be substituted by fructose.

Sucrose and trehalose are two disaccharides with the same molecular formula ($C_{12}H_{22}O_{11}$) but with different geometrical structures. Sucrose is composed of two monosaccharides, including glucose and fructose, while trehalose consists of two molecules of glucose. Sucrose is produced naturally in plants, from which table sugar is refined, while some bacteria, fungi, plants, and invertebrate animals synthesize trehalose as a source of energy. Sperm quality parameters of chilled and cryopreserved semen were shown to improve by using trehalose. The protective effects of this sugar significantly enhanced the freezability of buck and ram spermatozoa [10, 11, 12]. However, when trehalose was added in high concentration in a culture medium, sperm movement was hampered [13].

Sugar consumption by spermatozoa depends on both sugar type and sugar concentration. Matos-Brito et al. [14] showed that when extenders were used with appropriate concentrations of carbohydrates, goat sperm remained viable regardless of the initial concentration of fructose in goat seminal plasma. Moreover, Salamon and Ritar [15] noted that glucose and fructose addition to Tris buffer extenders resulted in higher post-thaw motility. Despite the importance of all the previously mentioned studies, the direct actions of sugars on spermatozoa are still little understood. In addition, to achieve the most efficacious use of fresh and chilled small ruminant semen, it is important to study the influence of diverse sugars on the motility status of these two spermatozoa types preserved in different semen media. Thus, the main objective of the present study was to assess the effects of using monosaccharides and disaccharides in Tris semen preservation media on the motility patterns of small ruminants spermatozoa, including fresh and chilled rams and bucks spermatozoa, during the first hours of *in vitro* incubation.

Result

Table 1 shows CASA motility values of fresh spermatozoa from rams incubated in TBM media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 37°C for 60 min. The highest values of MOT% and PMOT%, as well as the velocity parameters VAP, VSL, and VCL were recorded when spermatozoa were incubated in the TBM solution containing fructose without recording any significant differences for MOT% and PMOT% between the four media. The addition of trehalose to the TBM solution led to a significant ($p < 0.05$) decrease in the values of VAP, VSL, and VCL in comparison with the spermatozoa incubated in TBM-sucrose, TBM-glucose, and TBM-fructose media. No significant differences ($p > 0.05$) were recorded between the four media containing monosaccharides and disaccharides for the trajectory parameters STR% and LIN%.

Table 2 shows CASA motility values of fresh spermatozoa from bucks incubated in TBM media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 37°C for 60 min. The effect of sucrose was evident by increasing the values of MOT%, PMOT%, VAP, VSL, and VCL parameters in comparison with the other three sugar types. The clearest difference in motility values was between the medium containing sucrose and the base solution containing trehalose

Abbreviations-Cont'd

TEY: Tris-egg yolk
VAP: average path velocity VCL:curvilinear velocity
VSL: straight line velocity.

Table 1.
CASA sperm motion characteristics of fresh spermatozoa from rams incubated in Tris based medium (TBM) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 37°C for 60 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	89.44 ± 2.92^{a}	93.5 ± 1.88^{a}	91.89 ± 2.26^{a}	89.55 ± 0.73^{a}
Progressive motility %	20.44 ± 1.33^{a}	22.56 ± 2.88^{a}	19.44 ± 1.42^{a}	18.66 ± 7.3^{a}
VAP (µm/s)	115.11 ± 4.01^{ab}	120.6 ± 11.9 ^{a}	114.89 ± 5.98^{ab}	109.33 ± 8.26^{b}
VSL (µm/s)	74.89 ± 4.04^{ab}	78.44 ± 9.25^{a}	69.67 ± 3.73^{ab}	67.89 ± 4.26^{b}
VCL (µm/s)	226.44 ± 7.32^{ab}	234.6 ± 11.4 ^{a}	221.44 ± 7.52^{ab}	210 ± 5.35^{b}
STR %	61.33 ± 2.65^{a}	59.88 ± 3.01^{a}	58.22 ± 1.79^{a}	60.44 ± 1.51^{a}
LIN %	33.22 ± 2.04^{a}	32.56 ± 2.3^{a}	30.33 ± 1.80^{a}	33 ± 1^{a}
Motility subpopulations				
Static %	11.22 ± 2.89^{a}	7.89 ± 3.05^{a}	8.89 ± 1.85^{a}	11.31 ± 1.73^{a}
Slow %	5.44 ± 2.89^{a}	4.55 ± 1.51^{a}	5.33 ± 1.69^{a}	10.48 ± 3.28^{b}
Medium %	23.77 ± 3.53^{a}	22.44 ± 2.52^{a}	26.22 ± 2.92^{a}	24.64 ± 1.92^{a}
Rapid %	59.55 ± 1.36^{ab}	65.11 ± 3.05^{a}	59.56 ± 1.37^{ab}	53.55 ± 1.37^{b}

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

Table 2.
CASA sperm motion characteristics of fresh spermatozoa from bucks incubated in Tris based medium (TBM) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 37 °C for 60 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	87.67 ± 4.24^{ab}	89.6 ± 2.1^{ab}	92.77 ± 2.58 ^{a}	85.11± 3.98^{b}
Progressive motility %	20.77 ± 1.86^{b}	25 ± 1.73^{ab}	29.11 ± 1.61^{a}	21.11 ± 2.89^{b}
VAP (µm/s)	102.33 ± 5.34 ^{b}	114.4 ± 4.21^{a}	130.11± 8.79 ^{c}	107.66 ± 7.26^{b}
VSL (µm/s)	65.89 ± 2.26 ^{b}	75 ± 4.39^{a}	92.33 ± 7.78 ^{c}	71.33 ± 6.32 ^{a}
VCL (µm/s)	209 ± 5.12 ^{b}	215.6 ± 8.59^{a}	228.11± 12.25^{a}	211.22 ± 8.3^{ab}
STR %	58.66 ± 2.45^{a}	60.00 ±1.53^{a}	63.33 ± 1.23^{a}	60.77 ± 2. 6 ^{a}
LIN %	31.66 ± 1^{a}	34.11± 0.93 ^{a}	36.55 ± 2.06 ^{a}	34.77 ± 1.92 ^{a}
Motility subpopulations				
Static %	12.01 ± 3.46^{a}	11.66 ± 3.84 ^{a}	7.44 ± 2.13 ^{b}	14.77 ±3.11 ^{a}
Slow %	5.66 ± 1.72^{a}	4.33 ± 0.77 ^{a}	3.88 ± 0.38^{a}	3.11 ± 0.86 ^{a}
Medium %	14.01 ± 2.11^{a}	11.11 ± 0.96 ^{a}	9 ± 1.61^{b}	12.88 ± 3.57^{a}
Rapid %	68.33 ± 2.31^{a}	73.11 ± 3.07 ^{a}	79.77 ± 2.94^{b}	69.33 ± 4.35^{a}

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

(29.11 vs. 21.11 for PMOT%, and 130.11 vs. 107.66 for VAP, respectively), while no significant differences were recorded between the four TBM media for STR% and LIN%. Moreover, sucrose was also able to significantly raise ($p < 0.05$) the percentage of rapid sperm subpopulation compared to the other three media.

Table 3 shows CASA motility values of chilled spermatozoa from rams incubated in TEY media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 5°C for 180 min. No differences were noted for none of the CASA motility characteristics between the chilled ram spermatozoa samples treated with different sugar types. Table 4 shows CASA motility values of chilled spermatozoa from bucks incubated in TEY media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 5°C for 180 min. No significant differences ($p > 0.05$) were recorded for sperm incubated within TEY media containing the four types of supplemented sugars for both MOT% and PMOT% parameters. An increase in VAP, VCL, and VSL values was observed for the spermatozoa incubated in TEY medium containing trehalose, while there were no significant differences ($p > 0.05$) in the values of STR% and LIN % between the four different TEY media. Moreover, compared to the incubated spermatozoa in the TEY-fructose medium, a rise in the percentage of the rapid subpopulation category was caused by trehalose supplementation, and a decrease in the percentage of static sperm was recorded in this medium.

Discussion

Our present study is the first investigation that simultaneously shows the direct effects of monosaccharides and disaccharides on the spermatozoa motility pattern of two small ruminant species, including sheep and goat, and two spermatozoa types, including fresh and chilled samples. It must be stressed that motility is the main function of sperm cells and the main aim of energy obtainment. In this respect, sugar can be easily changed into energy, and the use of sugars, such as fructose, glucose, sucrose, and trehalose in semen media could increase sperm motility. It is well known that spermatozoa is a strict, glycolytic cell. Furthermore, the predominant metabolic pathways through which spermatozoa produce ATP, necessary for sperm motility, are mitochondrial oxidative phosphorylation and glycolysis [9]. In sperm cells, sugar, and especially glucose, is the main substrate for glycolysis, where it is metabolized to pyruvate and/or lactate to obtain cellular energy in the form of ATP. Generally, the effects of sugars may largely vary between species due to the differences in the chemical and physical composition of the sperm [16, 8]. Moreover, the differences in spermatozoa motility status may be due to several factors, including individual

Table 3.
CASA sperm motion characteristics of chilled spermatozoa from rams incubated in Tris egg-yolk medium (TEY) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 5 °C for 180 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	91.55 ± 2.3[a]	91.11 ± 1.96[a]	92.67 ± 2.5[a]	91.56 ± 1.94[a]
Progressive motility %	16.55 ± 2.45[a]	16.44 ± 2.24[a]	16.11 ± 3.01[a]	15.11 ± 2.52 [a]
VAP (µm/s)	105.55 ± 7.23[a]	107.3 ± 6.42[a]	100.77 ± 4.52 [a]	99.11± 3.88 [a]
VSL (µm/s)	67 ± 5.12[a]	67.33 ± 4.58[a]	65.89 ± 4.25[a]	64.89 ± 4.98[a]
VCL (µm/s)	205.44 ± 8.35[a]	207.11 ± 6[a]	203.55 ± 4.36 [a]	199 ± 8.1 [a]
STR %	58.88 ± 1.76 [a]	59.33 ± 1.73[a]	58.77 ± 1.72[a]	60 ± 2.4 [a]
LIN %	31.33 ± 1[a]	31.78 ± 1.30[a]	31.55 ± 1.9[a]	32 ± 2.18 [a]
		Motility subpopulations		
Static %	9.77 ± 1.71[a]	9.11 ± 1.39[a]	8 ± 1.89[a]	9.78 ± 2.58[a]
Slow %	6.22 ± 1.84[a]	7.55 ± 2.04[a]	6.88 ± 1.66[a]	8.22 ± 2.71[a]
Medium %	29.44 ± 2.93[a]	27.77 ± 2.15[a]	29.88 ± 1.40[a]	29.66 ± 1.24 [a]
Rapid %	54.55 ± 4.72[a]	55.55 ± 2.77[a]	55 ± 1.59[a]	52.11 ± 2.57[a]

Table 4.
CASA sperm motion characteristics of chilled spermatozoa from bucks incubated in Tris egg-yolk medium (TEY) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 5 °C for 180 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	90.77 ± 3.15[a]	90.44 ± 2[a]	89.77 ± 1.56 [a]	93.22 ± 2.28[a]
Progressive motility %	20.11± 2.82[a]	20 ± 2.7 [a]	19.44 ±1.23 [a]	22.11 ± 2.57[a]
VAP (µm/s)	86.56 ±9.14[b]	87.8± 5.31[ab]	92.22 ± 4.9 [ab]	96.77 ± 5.26 [a]
VSL (µm/s)	60.22 ± 6.30[a]	60.44 ± 3.17[a]	62.44 ± 2.9 [ab]	65.89 ± 5.13 [b]
VCL (µm/s)	184.66 ±10.16[a]	186.4 ± 9.72[a]	189.22 ± 7.07[a]	197.22 ± 4.57 [b]
STR %	61.66 ± 1.5 [a]	61.11± 2.93[a]	60.44 ± 0.72[a]	61.78 ± 2.28[a]
LIN %	32.78 ± 2.39 [a]	32.44 ± 1. 9[a]	32.22 ±1.09[a]	32.77 ± 1.72[a]
		Motility subpopulations		
Static %	10.78 ± 2.38 [a]	10.33 ± 0.96 [a]	11.11 ± 1.36 [a]	8 ± 0.91[b]
Slow %	8.77 ± 1.94 [a]	8.44 ± 1.19 [a]	8.55 ± 1.59[a]	6.78 ±1.03[a]
Medium %	21.78 ± 1.93[a]	22.55 ± 1.72 [a]	21 ± 2.73[a]	20.88 ±3.29[a]
Rapid %	58.66 ± 2.80[ab]	58.22 ± 2.10 [a]	59.44 ± 2.66[ab]	64.33 ±3.70[b]

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

variation [17, 18], storage temperature [19], type of media [20], and also the differences in motility estimation methods (subjective or objective).

As sperm cells are dependent on their storage medium, different semen media, such as TBM and TEY, were developed to be used in assisted reproductive technologies. In this respect, sugar concentration is one of the most important points that should always be carefully considered during the preparation of any semen media, especially TBM and TEY. In the present study, a concentration of 50 mM for each sugar type was adopted in TBM and TEY media, as one of the concentrations usually used in Tris media [21]. Better maintenance of canine spermatozoa motility was noted when increased amounts of sugars were added to the semen extender. In this respect, the supplementation of TEY extender with 70 mM of sugars had notable beneficial effects on chilled canine spermatozoa compared to 10 mM, and this concentration resulted in significantly higher values for the percent motility and VAP parameter over the experiment period [22]. Moreover, compared to the freshly pooled canine semen, the mean values of VAP, VSL, and VCL increased significantly, suggesting that sugars activate sperm velocity [22]. However, in the present case, any higher concentrations over the 50 mM level of monosaccharides or disaccharides may cause osmolarity to become too high and in such a situation, substantial osmotic damage could be produced. It is well known that sperm cells are sensitive to osmotic stress [21, 23]. However, spermatozoa can tolerate a moderate range of osmolarities without a reduction in fertility. In this study, the osmolarity of the eight Tris media was within the range of physiological values (300-330 mOsm/Kg), which is the physiological osmolarity of most physiological fluids. Therefore, the osmolarity levels in our Tris media are not expected to have any significant effect on the motility status of rams and bucks spermatozoa.

The effects of monosaccharides on the metabolism of freshly ejaculated spermatozoa are very important for motility status. In dogs, sperm metabolize fructose and glucose in separate pathways resulting in separate systems of energy management indicated by different motility patterns and different roles in glycogen metabolism [2, 4]. The activating role of fructose and glucose on dog sperm was initiated by the intense and rapid increase in the tyrosine phosphorylation of some specific proteins [2]. Sperm functions could be modulated and modified immediately after ejaculation. However, further research in protein phosphorylation in the spermatozoa of small ruminant species treated by different sugar types must be conducted in the future.

On the other hand, is it possible to make a preference for a specific type of monosaccharides to be used in semen solutions? In general, the sperm of many mammals use glucose in preference to fructose when both substrates are available [24, 25]. Rogers and Perreault [26] observed that glucose supported better progressive human sperm motility than fructose, while Williams and Ford [9] found both sugars to be equally effective. In contrast, fructose has been found beneficial for frozen-thawed sperm in bovine [27] and ram [28]. It must be noted that in goat seminal plasma, fructose was the primary substrate for glycolysis [29] and the end product of the glycolysis pathway produces more ATP, necessary to support sperm motility. However, the combination of glucose and fructose as a supplement in the TALP medium improved the progressive motility of boar sperms [30]. In our study and during the first hours of in vitro incubation, we did not notice any clear differences between glucose and fructose, especially for the fresh and chilled ram spermatozoa. In agreement with these results, the survival of unfrozen ram sperms did not improve when ten different sugars were added [20]. Moreover, no effect was noted for sugar type (i.e., glucose, fructose, sucrose, lactose, and trehalose) on the motility of the post-thaw ram spermatozoa [31]. It must be pointed out that the monosaccharide metabolization pathways in mammalian spermatozoa depend on several factors, such as the medium composition, energy consumption rhythm, and most importantly the studied species where the sperm come from [3].

Diverse sperm types, semen media, and sugar groups used for supplementation could result in different motility patterns. In this study and unlike the motility status in the case of fresh spermatozoa types, trehalose had positive effects on CASA velocity parameters and the rapid subpopulation category of chilled goat spermatozoa. In contrast to our findings concerning chilled ram spermatozoa, the results of Zhao et al. [12] indicated that the addition of trehalose to Tris diluent improved the quality of long-preserved ram semen under low-temperature conditions. According to the previous authors, trehalose addition to Tris-fructose egg yolk medium at a concentration of less than 20 mM did not directly increase the progressive motility of sperm but significantly raised the integrity of the preserved sperm acrosome. In humans, superior post-thaw sperm parameters were observed by using 50 mM trehalose over sucrose and other trehalose concentrations [32]. It must be noted that trehalose exerts an indirect antioxidant effect by augmenting the level of glutathione and reducing lipid peroxide [33]. Chhillar et al. [34] documented that trehalose decreased H_2O_2 and MDA in frozen-thawed bull semen to the levels of fresh semen, while Badr et al. [35] reported similar results in buffalo. However, the antioxidant potential of trehalose on the spermatozoa of small ruminants and its relation to sperm motility pattern needs further research.

In the present study, we focused on fresh and chilled spermatozoa types. It must be pointed out that these two types could be used in different assisted reproductive technologies and they present a very important option for using cryopreserved ones. As the cryoprotective effects of monosaccharides and disaccharides on spermatozoa were well documented in the literature [36, 37], our present study was more focused on the motility aspect. However, the main result demonstrated that regardless of temperature, both monosaccharides and disaccharides could support the motility of bucks and rams spermatozoa during the first three hours of in vitro incubation in the Tris media.

Finally, two major limitations could be noted for the current study. The first is that sperm CASA motility parameters may not accurately predict the in vivo fertility results or IVF outcome. Therefore, other assessments of semen quality, including sperm viability, membrane, and acrosome integrity are needed. The second limitation is that motility status in this study was assessed during the first hours of in vitro incubation. In fact, we were interested in these particular incubation time points for both fresh and chilled spermatozoa samples because they represent the time window by which spermatozoa of such types could be directly used in different assisted reproduction technologies, especially AI. However, during the first three hours of in vitro incubation, our initial data clearly showed that the four sugars only affected the velocity, and not the spermatozoa trajectory of the small ruminants.

Conclusion

Taken together and by using CASA technology, this research showed the direct effects of monosaccharides and disaccharides supplementations in different Tris media on the spermatozoa motility patterns of small ruminants. It was clear that the sperm motility parameters of both rams and bucks were affected by monosaccharides and disaccharides based on the incubation temperature. Moreover, CASA velocity parameters were the most affected motility characteristics by sugars, while no differences were recorded for the sperm trajectory parameters. Such data could assist in the selection of the most appropriate sugar compatible with the temperature of keeping small ruminant spermatozoa within the semen media to achieve the best motility status. However, further studies are needed to test the in vivo and in vitro fertilization of small ruminants' spermatozoa incubated in different

media supplemented with different sugars.

Materials and Methods

Animals and ethical approval

This study was carried out at Der-Al-Hajar Animal Production Research Station, 33 km southeast of Damascus. Semen was obtained from five adult Awassi rams and five adult Shami bucks (two local Syrian small ruminant species). The animals aged 3-4 years, and were fed a diet based on concentrate, wheat straw, and barley, with water available ad libilum. It must be noted that the experiments for this study were approved by the Local Scientific and Ethical Committee of the AECS, Damascus, Syria (permit number 36-Z/M4 - 2019).

Media preparation, semen collection, and experimental design

All chemicals were purchased from Roth (Carl Roth Gmbh-Karlsruhe-Germany). Eight Tris media were prepared in the present study, including four TBM and four TEY. TBM prepared as a 300 mOsmol/Kg solution contained 200 mM tris (hydroxymethyl) aminomethane, 64.7 mM citric acid monohydrate, and 50 mM of each sugar type (glucose, fructose, sucrose, and trehalose), which were separately added to each base solution. For the TEY media, 20% of egg yolk was added to the four tris-based solutions with conserving the same previous concentrations of tris (200 mM), citric acid (64.7 mM), and the four sugar types (50 mM for each) in each TEY medium. It must be noted that the eight media were always held constant at pH 7.

In this study, a total of 60 ejaculates were collected from ten experimental animals (30 ejaculates from rams and 30 from bucks). Semen samples were collected using an electro-ejaculator (Minitube-Electro Ejaculator, Tiefenbach, Germany). Upon collection, semen specimens were immediately evaluated for general appearance and volume. For each animal and after semen collection, spermatozoa concentration was estimated using a haematocytometer (Neubauer Improved Marienfeld, Germany). An initial analysis of sperm motility was performed using the CASA system (Hamilton Thorne Biosciences, Version 12 CEROS, Beverly, USA). Sperm samples with a motility score ≥ 75% of motile spermatozoa and a concentration of ≥ 1×10^9 spermatozoa/ml were utilized. All ejaculations with no or poor motility status were immediately excluded before conducting the analyses. The collected ejaculates from each species were mixed in each replicate to isolate the individual effect of males. The fresh spermatozoa samples (25×10^6/ml) of both bucks or rams were incubated in TBM containing the four sugar types at 37°C in a water bath for 60 min, while the chilled spermatozoa samples (25×10^6/ml) of bucks or rams were incubated in TEY media containing the four sugar types at 5°C in the refrigerator for 3 h. Each experiment for each animal species and each semen media was repeated three times.

Motility assessment

The motility characteristics of the spermatozoa were assessed by CASA, using the Hamilton-Thorne motility analyzer (Hamilton Thorne Biosciences, Version 12 CEROS, Beverly, USA). For each sperm sample, three fields were selected, counted randomly, and assessed to generate data from at least 200-250 sperm/samples. The CASA characteristics included in the analysis were the MOT%, VCL (µm/s), VAP (µm/s), VSL (µm/s), LIN%, STR%, and PMOT% (VAP ≥ 75 µm/s and STR ≥ 80 %; HTM-CEROS; installation getting started guide version 12 CEROS). Spermatozoa subpopulations were defined in four categories by CASA as Rapid (4): fraction of all cells moving with VAP = 25 µm/s; Medium (3): fraction of all cells moving with 5 µm/s < VAP ≤ 25 µm/s; Slow (2): fraction of all cells moving with VAP < (5 µm/s or VSL < 11 µm/s; and Static (0-1): fraction of all cells not moving at all.

The Hamilton-Thorne motility analyzer settings used for goat spermatozoa were negative phase contrast optics at a recording rate of 60 frame/s, temperature of analysis 37°C, light adjustment 90-110, minimum cell size 5 pixels, non-motile head size 10 pixels, non-motile head intensity 80, low VAP cut off 20 µm/s, low VSL cut off 5 µm/s, static size limit 0.60/4.32 (min/max), and static intensity limit 0.20/1.92 (min/max). While the Hamilton-Thorne motility analyzer settings used for sheep spermatozoa were negative phase contrast optics at a recording rate of 60 frame/s, temperature of analysis 37°C, light adjustment 90-110, minimum cell size 5 pixels, non-motile head size 10 pixels, non-motile head intensity 80, low VAP cut off 21.9 µm/s, low VSL cut off 6 µm/s, static size limit 0.60/8 (min/max), and static intensity limit 0.25/1.50 (min/max).

Statistical analysis

Statistical analysis was conducted using the Minitab program (Minitab Coventry, United Kingdom, Version 13.31, 2000). Motility data were subjected to a factorial analysis of variance (ANOVA) for the four sugar types by GLM, followed by multiple pairwise comparisons using the Tukey posthoc test. The threshold of signification was set at $p < 0.05$.

Acknowledgements

The author would like to thank the Atomic Energy Commission of Syria for their support while conducting the experiments.

Competing Interests

The author declare that there is no conflict of interest.

Reference

1. Bustani GS, Baiee FH. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. Vete World 2021; 14(5): 1220-1233. Doi: 10.14202/vetworld.2021.1220-1233

2. Rigau T, Rivera M, Palomo MJ, Fernandez-Novell, JM, Mogas T, Ballester J. Differential effects of glucose and fructose on hexose metabolism in dog spermatozoa. Reproduction 2002; 123: 579–91. Doi: 10.1530/rep.0.1230579

3. Rodríguez- Gil JE. Mammalian sperm energy resources management and survival during conservation in refrigeration. Reprod Domest Anim 2006; 41(2): 11-20. Doi:10.1111/j.1439-0531.2006.00765

4. Rigau T, Farre M, Ballester J, Mogas T, Peiia A, Rodriguez-Gil JE. Effects of glucose and fructose on motility paterns of dog spermatozoa from fresh ejaculates. Theriogenology 2001: 56: 601-615. Doi: 10.1016/s0093-691x(01)00609-4

5. Mann T. Biochemistry of semen. In: Greep RO, Astwood EB

(ed): Handbook of Physiology. Washington DC, American Physiology Society, Section, 1975; 67(5): 321- 347.

6. Nagai T, Yamaguchi K, Moriwaki C. Studies on the effects of sugars on washed human sperm motility. J Pharmacobiodyn, 1982; 5: 564–7. Doi: 10.1248/bpb1978.5.564.

7. Johnson LA, Welch GR, Rens W. The Beltsville sperm sexing technology: high-speed sperm sorting gives improved sperm output for in vitro fertilization and AI. J Anim Sci 1999; 2: 213-220. Doi: 10.2527/1999.77suppl_2213x

8. Naing SW, Wahid H, Mohd Azam K, Rosnina Y, Zuki AB, Kazhal S, et al. Effect of sugars on characteristics of Boer goat semen after cryopreservation. Anim Reprod Sci 2010; 122: 23–28. Doi: 10.1016/j.anireprosci.2010.06.006

9. Williams AC, Ford WC. The Role of Glucose in Supporting Motility and Capacitation in Human Spermatozoa. J Androl 2001; 22(4): 680-695. Doi:10.1002/j.1939-4640.2001.tb02229

10. Aboagla EME, Terada T. Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. Biol Reprod 2003; 69: 1245 – 1250. Doi:10.1095/biolreprod.103.017889

11. Aboagla EME, Terada T. Effects of the supplementation of trehalose extender containing egg yolk with sodium dodecyl sulphate on the freezability of goat spermatozoa. Theriogenology 2004; 62: 809 – 818. Doi:10.1016/j.theriogenology.2003.12.003

12. Zhao J, Xiao G, Zhu W, Fang D, Li N, Han C, et al. Trehalose addition to a Tris-fructose egg yolk extender on quality of ram sperm preserved at 0 ◦ C. Rev Bras Zootec 2020; 49: e20200061. Doi:10.37496/rbz4920200061.

13. Athurupana R, Takahashi D, Ioki S, Funahashi H. Trehalose in glycerol-free freezing extender enhances post-thaw survival of boar spermatozoa. J Reprod Dev 2015; 61:205-210. Doi:10.1262/ jrd.2014-152

14. Matos-Brito BG, Lima ICS, Pereira JF, Barboza FM, Linard MAB, Aguiar GV, et al. Effect of initial seminal plasma fructose concentration on goat semen storage at 5oC. Arch Zootec 2013; 62; Doi:10.4321/S0004-05922013000100015

15. Salamon S, Ritar AJ. Deep freezing of Angora goat semen: Effects of diluent composition and method and rate of dilution on survival of spermatozoa. Aust J Biol Sci 1982; 35: 295–303.

16. Purdy PH. A review on goat sperm cryopreservation. Small Rumin Res 2006; 63(3): 215–225. Doi:10.1016/j.smallrumres.2005.02.015

17. Aisen EG, Medina VH, Venturino A. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 2002; 57: 1088–1801. Doi: 10.1016/s0093-691x(02)00653-2

18. Garde JJ, del Olmo A, Soler AJ, Espeso G, Gomendio M, Roldan ERS. Effect of egg yolk, cryoprotectant, and various sugars on semen cryopreservation in endangered Cuvier's gazelle (Gazella cuvieri). Anim Reprod Sci 2008; 108(3–4): 384–401.Doi:10.1016/j.anireprosci.2007.09.010

19. Lapwood KR, Martin ICA. The use of monosaccharides, disaccharides and trisaccharides in synthetic diluents for the storage of ram spermatozoa at 37 ◦ C and 5 ◦ C. Aust J Biol Sci, 1966; 19: 655–671.

20. Abdelhakeam AA, Graham EF, Vazquez IA, Chaloner KM. Studies on the absence of glycerol in unfrozen and frozen ram semen:development of an extender for freezing: effects of osmotic pressure, egg yolk levels, type of sugars, and the method of dilution. Cryobiology 1991; 28(1): 43–49. Doi:10.1016/0011-2240(91)90006-A

21. Alomar M, Zarkawi M, Alzoabi M.A. Analysis of Awassi sperm motility in two media at different levels of temperature, pH and osmolality. Iran J Appl Anim Sci 2018; 8(3): 167-172.

22. Ponglowhapan S, Esseen-Gustavsson B, Forsberg C.L. Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. Theriogenology 2004; 62: 1498–1517.

23. Curry MR, Kleinhans FW, Watson PF. Measurement of the water permeability of the membranes of boar, ram, and rabbit spermatozoa using concentration-dependent self-quenching of an entrapped fluorophore. Cryobiology 2000; 41: 167–73. Doi:10.1006/cryo.2000.2277

24. Bedford JM, Hoskins, DD. The mammalian spermatozoon: morphology, biochemistry and physiology. In Marshall's Physiology of Reproduction 1990; 379–568 Ed. GE Lamming. Churchill Livingstone, Edinburgh.

25. Jones AR, Connor DE. Fructose metabolism by mature boar spermatozoa. Reprod Fertil Dev 2000; 12: 355–359. Doi:10.1071/RD00116

26. Rogers BJ, Perreault SD. Importance of glycolysable substrates for in vitro capacitation of human spermatozoa. Biol Reprod 1990; 43: 1064–1069. Doi:10.1095/biolreprod43.6.1064

27. Garcia MA, Graham EF. Development of a buffer system for dialysis of bovine spermatozoa before freezing. II. Effects of sugars and sugar alcohols on post thaw motility. Theriogenology 1989; 31: 1029–37. Doi:10.1016/0093-691X(89)90486

28. Molinia FC, Evans G, Maxwell WM. In vitro evaluation of zwitterion buffers in diluents for freezing ram spermatozoa. Reprod Nutr Dev 1994; 34: 491–500. Doi: 10.1051/rnd:19940510

29. Pellicer-Rubio MT, Magallon T, Combarnous Y. Deterioration of goat sperm viability in milk extenders is due to a bulbourethral 60-kilodalton glycoprotein with triglyceride lipase activity. Biol Reprod, 1997; 57(5): 1023-1031. Doi: 10.1095/biolreprod57.5.1023

30. Malo C, Gil L, Gonzalez N, Cano R, de Blas I, Espinosa, E.

Comparing sugar type supplementation for cryopreservation of boar semen in egg yolk based extender. Cryobiology 2010; 61(1): 17–21. Doi: 10.1016/j.cryobiol.2010.03.008.

31. Molinia FC, Evans G, Quintana Casares PI, Maxwell W.M.C. Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim Reprod Sci 1994; 36(1–2): 113–122. Doi:10.1016/0378-4320(94)90058-2

32. Suksai M, Dhanaworavibul K. Effects of Trehalose and Sucrose on Human Sperm Motility, Vitality and Morphology after Cryopreservation. J Health Sci Med Res, 2019; 37(2): 101-107. Doi:10.31584/jhsmr.201945

33. Aisen EG, Quintana M, Medina V, Morello H, Venturino A. Ultramicroscopic and biochemical changes in ram spermatozoa cryopreserved with trehalose based hypertonic extenders. Cryobiology 2005; 50(3): 239-249. Doi: 10.1016/j.cryobiol.2005.02.002

34. Chhillar S, Singh VK, Kumar R, Atreja SK. Effects of Taurine or Trehalose supplementation on functional competence of cryopreserved Karan Fries semen. Anim Reprod Sci, 2012; 135(1): 1-7. Doi: 10.1016/j.anireprosci.2012.08.029

35. Badr MR, Mary G, Hassan M.H. Effect of trehalose on cryopreservation, oxidative stress and DNA integrity of buffalo spermatozoa. J Reprod Infertil 2010; 1: 50-57.

36. Stanishevskaya O, Silyukova Y, Pleshanov N, Kurochkin A. Role of Mono- and Disaccharide Combination in Cryoprotective Medium for Rooster Semen to Ensure Cryoresistance of Spermatozoa. Molecules. 2021; 29;26(19): 5920. Doi: 10.3390/molecules26195920.

37. Malo C, Gil L, Gonzalez N, Cano R, de Blas I, Espinosa, E. Comparing sugar type supplementation for cryopreservation of boar semen in egg yolk based extender. Cryobiology 2010; 61(1): 17–21. Doi: 10.1016/j.cryobiol.2010.03.008.

How to cite this article
Alomar M. Influences of Monosaccharides and Disaccharides Supplementations in Tris Media on the Motility Patterns of Fresh and Chilled Small Ruminant Spermatozoa. Iran J Vet Sci Technol. 2023; 15(3): 7-15.
DOI: https://doi.org/ 10.22067/ijvst.2023.82141.1247
URL:https://ijvst.um.ac.ir/article_44302.html