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Original article

EFFECTS OF HONEY ADDITION TO TRIS MEDIA ON OSMOLALITY, PH VALUES AND QUALITY PARAMETERS OF FRESH AND CHILLED BUCK SPERMATOZOA

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Summary

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The effects of honey addition to Tris media on osmolality, pH values and on the quality characteristics of fresh and chilled goat spermatozoa were studied. Semen samples were collected from five Shami bucks and incubated at 20 °C for the fresh samples and at 5 °C for the chilled ones in Tris-Based Medium (TBM) and Tris-Egg Yolk (TEY) medium without (controls) and with honey at ratios of 1%, 2% and 4% (v/v) respectively. Osmolality and pH levels were determined for all used media. Spermatozoa motility was analysed using the Computer Assisted Sperm Analyzer (CASA) system and the integrity of sperm plasma membrane was tested by the Hypo-Osmotic Swelling Test (HOST). Osmolality values were significantly increased ($P<0.05$) by honey addition reaching 558 mOsm/kg in TBM and 675 mOsm/kg in TEY with 4% of honey compared with 307 mOsm/kg and 312 mOsm/kg for the controls respectively. In contrast, pH values decreased ($P<0.05$) from 6.93 in TBM control medium to 6.4 in TBM with 4% of honey and from 6.83 in TEY control medium to 6.1 in TEY with 4% of honey. CASA motility parameters including the percent motility (MOT%) and the percent of sperm showing progressive motility (PMOT%) increased after adding 1% and 2% of honey while the addition of 4% led to a clear decrease ($P<0.05$) for the fresh and chilled samples. The percentages of spermatozoa subpopulations including the rapid % and the static % categories for fresh and chilled samples were significantly decreased and increased respectively after adding 4% honey. Moreover, for all media and for all incubation temperatures, the HOST percentage decreased significantly ($P<0.05$) by adding 4% honey. It was concluded that honey addition to Tris media influenced the osmolality and pH values, which in turn affected the quality characteristics of buck spermatozoa based on honey concentration regardless of the incubation temperature or the used media.

Key words: buck, honey, motility, osmolality, pH, spermatozoa

INTRODUCTION

Sperm cells are totally dependent on their storage media especially during *in vitro* incubation. That is why semen preservation media have been designed on an empirical basis to maintain spermatozoa during the storage process. Moreover, semen

media serve in providing energy substrates, conserve spermatozoa motility, control bacterial contamination and also in preventing the harmful effects of osmolarity and pH changes (Bustani & Baiee, 2021). It must be stressed out that these important properties enable the use of spermatozoa in different assisted reproductive technologies including artificial insemination (AI) and *in vitro* fertilisation (IVF). Generally, any semen media must maintain the pH at 6.8–7.2 (Liu *et al.,* 2016) and the osmolality values at 200–400 mOsm/kg (Alomar et *al.,* 2018). Several preservation media were used to extend both human and animal semen samples (Bustani & Baiee, 2021). In this regared, Tris-buffered media present one of the most used group of media to preserve the motility of animal spermatozoa at high extension rates for different species inculding rams (Alomar *et al.,* 2018) and bucks (Alomar, 2021). Beyond the advantages of being cost effective and easily available commercially, Tris media and especially the based ones, contain no insoluble particles or debris. Furthermore, the Tris-Egg Yolk (TEY) extender is commonly used for chilled, frozen semen, or both (Sun *et al.,* 2019).

To promote the quality of semen media, several studies noted the use of materials and compounds such as milk (Amin *et al.,* 2018), fish oil (Khoshvaght *et al.,* 2010), plant origins (Gunawan *et al.,* 2020) and honey (Malik, 2019). Honey is a natural product which was used as supplement with different properties like energy source, non-permeant cryoprotectant and synergistic antioxidant for the improvement of semen quality in humans (Tartibian & Maleki, 2012; Fakhrildin *et al.,* 2014), bulls (El- Sheshtawy *et al.,* 2014; Yimer *et al.,* 2015), rams (Jerez-Ebenesperger *et al.,* 2015), buffalo bulls (El-Nattat *et al.,* 2016) and also bucks (Olayemi *et al.,* 2011). On average, honey is composed of 17.1% water, 82.4% carbohydrates (approximately 38.5% fructose, 31% glucose and 12.9 % other sugars) and 0.5% proteins, amino acids, vitamins, phenolic compounds, organic acids and multiple minerals (Kahn *et al.,* 2007; Estevinho *et al.* 2008). Furthermore, honey was reported to have an inhibitory effect on around 60 species of bacteria, some species of fungi and viruses (Aggad & Guemour, 2014). It must be however noted that the chemical composition of honey is very variable and depends on regional, climatic conditions and on the type of flowers visited by the bees. On the other hand, one of the very important points about honey is that this product is highly concentrated which provides a potential hyperosmotic extracellular environment around the spermatozoa enhancing the efflux of intracellular fluid, thereby minimising the formation of ice crystals inside the cytoplasm linked to sperm damage during cryopreservation (Fakhrildin & Alsaadi, 2014). This characteristic may be responsible in increasing the level of osmolality in any solution that contains honey, which in turn could cause an important effect on the qualitative properties of fresh and chilled spermatozoa incubated in such medium. Moreover, honey may change the pH degree of semen media with high probability of effecting sperm motility. For that, motility patterns of spermatozoa using computer aided sperm analyses (CASA) are very important to determine the effects of media on the motility of spermatozoa (Anand *et al.,* 2016). Moreover, the integrity of sperm membrane may be evaluated by HOST test and this assessment of sperm membrane could be directly associated

with motility results (Bustani & Baiee, 2021).

The aim of the present study was to determine the extention effects of adding different honey concentrations on the osmolality and pH values of Tris media and the effects of honey addition on the quality characteristics of fresh and chilled goat spermatozoa incubated in Tris media.

MATERIALS AND METHODS

Chemicals and media preparation

All chemicals were purchased from Roth (Carl Roth Gmbh-Karlsruhe-Germany). Two types of Tris media was employed in the present study – Tris-Based Medium (TBM) for the fresh semen samples and Tris-Egg Yolk (TEY) medium for the chilled ones. TBM was prepared as a 300 mOsm/Kg solution containing 2.44 g Tris (hydroxymethyl) aminomethane, 1.36 g citric acid monohydrate and 1 g glucose in 100 mL of distilled water. The TEY medium was prepared from 80 mL of the TBM and 20 mL of egg yolk was added to complete the final volume of this medium.

Site description, animals, semen collection and ethical approval

This study was carried out at Der-Al-Hajar Animal Production Research Station, 33 km south-east of Damascus in March and April. Semen was obtained from five sexually-experienced Shami bucks, aged between 3 and 4 years and average body weight 67.3±3.5 kg. Semen samples were collected with the aid of an electro-ejaculator (Minitube - Electro Ejaculator, Tiefenbach, Germany). Upon collection, the semen was evaluated for its general appearance and volume. Sperm concentrations were calculated using a haemocytometer (cell counting chamber; Neubauer Improved Marienfeld, Germany). An initial analysis of sperm motility was performed using CASA system (Hamilton Thorne Biosciences, Version 12 CEROS, Beverly, USA). Sperm samples with a motility score ≥75 % of motile sperm and a concentration of $\geq 1\times10^9$ spermatozoa/mL were employed. The average value for the volume of the collected ejaculates was 2.1±0.53 mL, while the average of sperm concentration in the ejaculate was $(1.7\pm0.2)\times10^9$ spermatozoa/mL. The present study was approved by the Local Scientific and Ethical Committee of the Atomic Energy Commission of Syria (AECS), Damascus, Syria (permit number 36/ZM1 - 2020).

Osmolarity and pH analysis of Tris media

The osmolarity of the different Tris media was determined using an osmometer (Osmomat 030, Gonotec, Germany). The instrument was calibrated with 100, 300, and 1000 mOsm/kg standards before use and each solution was assayed in duplicate or triplicate. The pH of each Tris media was determined using a pH meter (Bibby Scientific, Model 3505, United Kingdom), each solution was assayed in duplicate or triplicate.

Experimental design

Four experiments were conducted in the present work using 30 ejaculates. To diminish the effect of interindividual variation, a mixture of semen from five animals was used in each assay. The semen dilution rate was 1:10 (semen sample:medium) where the sperm concentration was 25×10^6/mL. In the first and second experiments, motility status was assessed by CASA technology for fresh spermatozoa incubated at 20 °C for 60 minutes in TBM without honey (controls) and with honey ratios of 1 %, 2% and 4% (v/v) and for the

chilled samples incubated in TEY media with the same honey concentrations at 5 °C for 180 minutes. These two experiments were replicated three times.

In the other two experiments, HOST tests for the fresh spermatozoa incubated in TBM without honey (controls) and with honey ratios of 1%, 2 % and 4% (v/v) and for the chilled samples incubated in TEY media with the same honey concentrations at 5 °C for 180 minutes were conducted. These two experiments were replicated three times.

Motility analyses

The motility characteristics of the spermatozoa were assessed by computer aided sperm analyzer (CASA), using the Hamilton-Thorne motility analyzer (HTM; v.12, USA). Five microliter aliquots of diluted spermatozoa 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: percent motility (MOT, %), average path velocity (VAP, μm/s), percent linearity (LIN, %), percent straightness (STR, %), and the percent of sperm showing progressive motility (PMOT, %; VAP ≥75 μm/s and straightness STR ≥80 %).

The HTM system settings of ram spermatozoa are presented in Table 1. Spermatozoa subpopulations were defined in four categories by the CASA system: rapid (4): fraction of all cells moving with VAP > path velocity (VAP=25 μm/s); medium (3): fraction of all cells moving with VAP cutoff (5 μm/s) < VAP < path velocity (VAP=25 μm/s); slow (2): fraction of all cells moving with VAP < VAP cutoff (5 μm/s) or VSL < VSL cutoff (11 μm/s); and static (0-1) fraction of all cells that is not moving at all.

The HTM settings used for goat spermatozoa were negative phase contrast optics at a recording rate of 60 frame/s, temperature of analysis 37 ºC, light adjustment 90–110, minimum cell size 5 pixels, non motile head size 5 pixels, non motile head intensity 55, low VAP cut off 21.9 μm/s, low VSL cut off 6 μm/s, static size limit 0.60/8 (min/max), static intensity limit 0.25/1.50 (min/max), static elongation 0/95 (min/max).

Hypoosmotic swelling test

After conducting the incubation process in TBM and TEY media, the fresh and chilled goat spermatozoa samples were subjected to hypoosmotic swelling test (HOST %). For each sample, 0.1 mL of liquefied semen was added to 1 mL of warmed 100 mOsm hypoosmotic swelling solution containing sodium citrate (25 mmol/L) and fructose (75 mmol/L) and incubated for 30 min at 37 °C in a water bath. Sample aliquots were placed on a clean glass slide with a cover slip within 5 min and observed by phase-contrast microscopy. Sperm swelling was observed according to changes in the shape of the tail. The response to hypoosmotic stress resulted in spermatozoa with either no tail swelling or spermatozoa that responded with various degrees of swelling from the distal end of the flagellum (Stranger *et al.,* 2010). For each sample, a total of 200 spermatozoa were examined and the final HOST score was calculated. HOST scores were expressed as a percentage of total swollen spermatozoa (HOST, %).

Statistical analysis

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 (ANOVA, general linear model procedure, GLM) followed by multiple pairwise comparisons using a post-hoc (Tukey test). The threshold of significance was set at $P<0.05$.

RESULTS

Compared to control media, significant differences were found for the osmolality levels between TBM and TEY media supplemented with honey (1 and 2 and 4 %) at 20 ^{o}C and 5 ^{o}C respectively (Fig. 1 and 2). Osmolality values were significantly increased ($P<0.05$) by honey addition reaching 558 mOsm/kg in TBM with 4% of honey and 675 mOsm/kg in TEY compared to 307 mOsm/kg and 312 mOsm/kg for the controls. Also, the pH values decreased from 6.93 in TBM control medium to 6.4 in TBM with 4% of honey and from 6.83 in TEY control medium to 6.1 in TEY with 4% of honey, with significant differences between all media supplemented with honey.

The effects of honey supplementations on CASA motion characteristics of fresh and chilled buck spermatozoa are depicted in Tables 1 and 2. TBM and TEY media supplemented with 1% and 2% of honey demonstrated higher percentages of motility (MOT) and progressive motility (PMOT) in comparisons to control ($P<0.01$). No differences were noted for VAP parameter for the sperm samples incubated in these two media. In contrast,

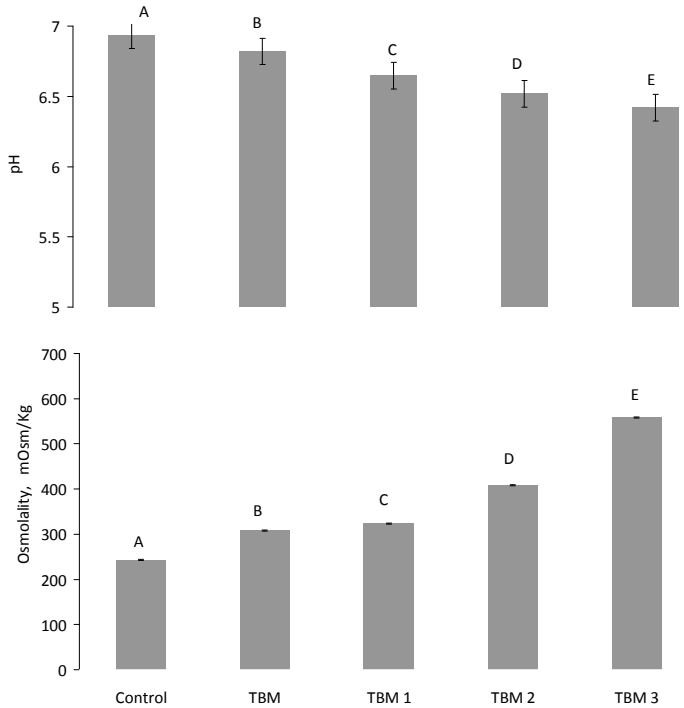

Fig. **1.** pH and osmolality values (mean±SD, n=15) of different TBM media including Tris (control), Tris glucose-based medium (TBM) and Tris-based medium with 1% of honey (TBM 1), 2% of honey (TBM 2) and 4 % of honey (TBM 3). Values with different superscript letters differ significantly at $P<0.05$.

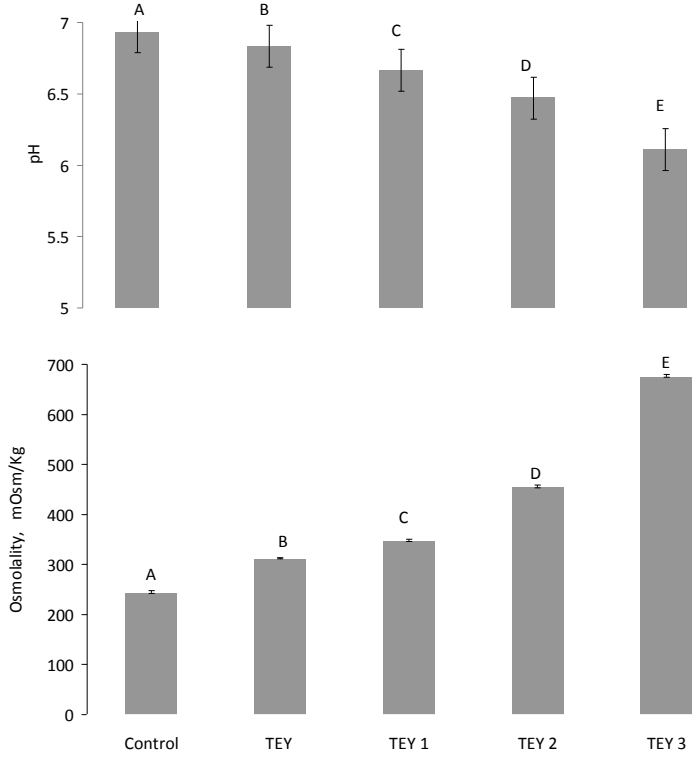

Fig. **2.** pH and osmolality values (mean±SD, n=15) of different TEY media including Tris (control), Tris Egg-Yolk (TEY) medium and Tris Egg-Yolk with 1% of honey (TEY 1), with 2% of honey (TEY 2) and 4% of honey (TEY 3). Values with different superscript letters differ significantly at $P<0.05$.

when honey concentration was increased to 4%, the three previous sperm motion characteristics decreased significantly ($P<0.01$). However, for both STR and LIN, no differences were noted in between the different media containing honey and the controls.

A positive effect was evident by increasing the percentage of rapid spermatozoa after adding 1% of honey in TBM medium, while the percentages of static, slow and medium categories did not differ between control and the media containing 1% and 2 % of honey. When both TBM and TEY media were supplemented with 4% of honey, the percentage of rapid sperm subpopulation category decreased ($P<0.05$) and the percentage of static sperm category increased significantly ($P<0.05$) compared to control and to the media containing 1% and 2 % of honey.

Fig. 3 shows the HOST values of fresh bucks spermatozoa incubated in TBM medium (control) and in TBM media supplemented with 1%, 2% and 4% honey at 20 °C for 60 minutes and chilled bucks spermatozoa incubated in TEY (control) and in TEY media supplemented with 1%, 2% and 4% honey at 5 °C for 180 minutes. No differences were noted for the spermatozoa incubated in TBM and TEY control media and those incubated in the same media containing 1% and 2% of honey. In contrast, when both media were

Table 1. Effects of honey addition on CASA sperm motion characteristics (mean±SD, n=15) of fresh bucks spermatozoa incubated in Tris-Based Medium (Control), Tris-Based Medium with 1% of honey (TBM 1), 2% of honey (TBM 2) and 4% of honey (TBM 3) at 20 °C for 60 minutes.

CASA parameters	Control	TBM 1	TBM 2	TBM 3
MOT (%)	77.4 ± 2.9 [a]	83.3 ±5.9 [b]	82.11 ± 4.4 [b]	68.6 ± 6.1 [c]
PMOT (%)	15.1 ± 2.1 [a]	17.9 ±1.8 [b]	17.7± 2.5 [b]	12 ± 4.5 [c]
VAP (µm/s)	83.9 ± 6.1 [a]	90.9 ± 11.9 [a]	89.2 ± 9.6 [a]	77.3 ± 10.5 [c]
STR (%)	56.4 ± 2.5 [a]	56.8 ± 3 [a]	57.8 ± 2.4 [a]	56.23 ± 9 [a]
LIN (%)	29.1 ± 1.9 [a]	31.6 ± 3.9 [a]	31.3 ± 1.6 [a]	31.1 ± 3.1 [a]
Motility of subpopulations				
Static %	21.33± 2.90 [a]	18.7 ± 2.2[a]	22.78 ± 1.94 [a]	30.78 ± 3.11 [b]
Slow %	7.11 ± 2.04 [a]	5 ± 2.11 [a]	5.44 ± 1.6 [a]	7.33 ± 3.08 [a]
Medium %	16.11± 3.40 [a]	14.22 ± 1.92 [a]	15.44± 3.53 [a]	18.9 ± 3.21 [b]
Rapid %	55.44 ± 5.02 [a]	62.11 ± 3.03 [b]	56.33 ± 4.8 [ab]	43 ± 5.7 [c]

MOT: percent motility spermatozoa, PMOT: percent of sperm showing progressive motility, VAP: average path velocity, STR: percent straightness, LIN: percent linearity. Values with different super-script letters (a, c) within rows differ at P<0.05.

Table 2. Effects of honey addition on CASA sperm motion characteristics (mean±SD, n=15) of chilled bucks spermatozoa incubated in Tris Egg Yolk (Control), Tris Egg Yolk with 1% of honey (TEY 1), 2% of honey (TEY 2) and with 4% of honey (TEY 3) at 5 °C for 180 minutes.

CASA parameters	Control	TEY 1	TEY 2	TEY 3
MOT (%)	77.5 ± 4.7 [a]	83.1 ± 5.7 [b]	79.8 ± 6.1 [b]	59.4 ± 11.8 [c]
PMOT (%)	11.7 ± 2.1 [a]	15.8 ± 3 [b]	13.2 ± 1.8 [b]	6.4 ± 2.2 [c]
VAP (µm/s)	71.2 ±11.3 [a]	78.3 ± 9.6 [b]	76.4 ± 7.3 [b]	53.1 ± 6.1 [c]
STR (%)	56.3 ± 2.00 [a]	58.44 ± 5 [a]	55.5 ± 9.2 [a]	58.1 ± 3.5 [a]
LIN (%)	29 ± 2 [a]	31.7 ± 6 [a]	29.5 ± 4.4 [a]	30.7 ± 4.1 [a]
Motility of subpopulations				
Static %	20.80 ± 4.42 [a]	17.8 ±0 4.66 [a]	23.67 ± 4.59 [a]	38.22 ± 11.8 [b]
Slow %	11.33 ± 5.96 [a]	9.89 ± 3.86 [a]	11.67 ± 4.24 [a]	17.56 ± 3.45 [b]
Medium %	24.67 ± 3.07 [a]	23.89± 5.92 [a]	22.75 ± 3.43 [a]	24.56 ±4.51 [a]
Rapid %	43.22 ± 7.28 [a]	48.44± 5.57 [a]	41.89 ± 3.90 [a]	19.67 ± 2.24 [b]

MOT: percent motility spermatozoa, PMOT: percent of sperm showing progressive motility, VAP: average path velocity, STR: percent straightness, LIN: percent linearity. Values with different super-script letters (a, c) within rows differ at P<0.05.

supplemented with 4% of honey, HOST values decreased significantly (P<0.05).

DISCUSSION

In present study demonstrated that honey addition to different Tris media was able to modify significantly osmolality and pH values. Moreover, by using CASA technology and HOST test the changes in motility status and membrane integrity of fresh and chilled buck spermatozoa incubated in TBM and TEY media supplemented with different concentrations of

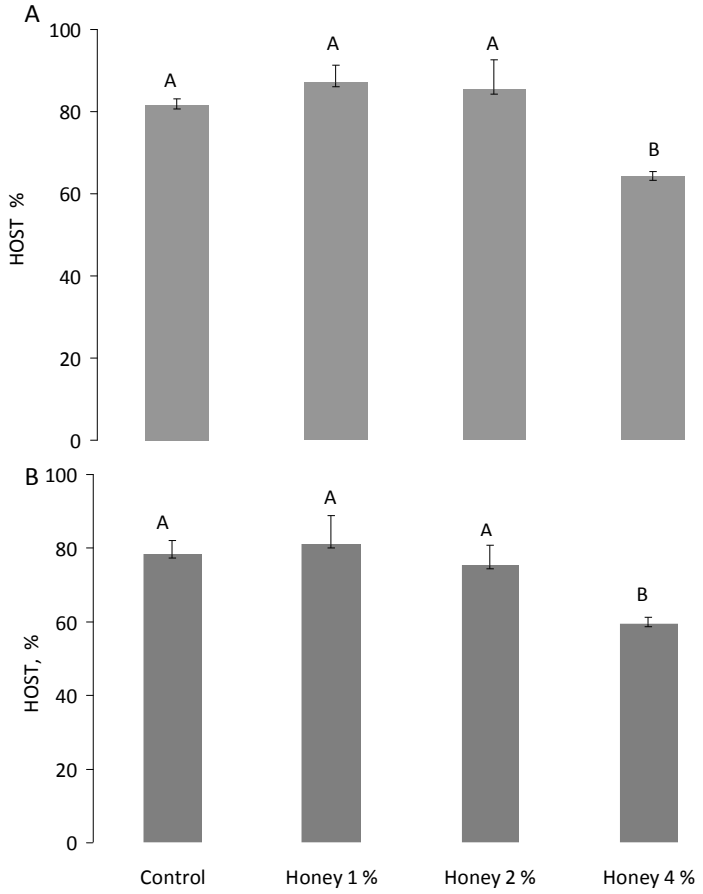

Fig. **3.** HOST % values (mean±SD, n=15) of fresh bucks spermatozoa incubated in TBM medium (control) and in TBM media supplemented with 1%, 2% and 4% honey at 20 °C for 60 minutes (A) and chilled bucks spermatozoa incubated in TEY (control) and in TEY media supplemented with 1%, 2% and 4% honey at 5 °C for 180 minutes (B). Values with different superscript letters differ significantly at P<0.05.

honey were clearly showed. It is well known that the effects of media and extenders on spermatozoa quality rely upon osmolality, pH and incubation temperature (Curry *et al.,* 1994; Varisli *et al.,* 2009; Contri *et al.,* 2013; Alomar *et al.,* 2018). Anyhow, the present research is the first which simultaneously showed the changes of osmolality and pH values of semen media supplemented with honey and the effects of honey addition on the quality parameters of fresh and chilled buck spermatozoa. Moreover, almost all documented literature reports had neglected the reference to fresh spermatozoa type incubated in media containing honey. However, the effect of honey additon on such important type of goat spermatozoa at 20 °C was clarified in the current work.

Generally, any Tris medium promotes sperm motility as it is particle-free and with low viscosity. Therefore, TBM basic

medium has several advantages for routine evaluation of fresh semen. On the other hand, if spermatozoa are to be cooled and stored at 5 °C, then protective substances as egg yolk are clearly required. In this respect, TEY medium was reported to maintain buck and ram sperm motility for a longer period of time following semen preservation (Paulenz *et al.,* 2005; Alomar *et al.*, 2018). These two Tris media have been in use on our laboratory for several years and were compared for their value in processing ram and buck for CASA analysis (Alomar, 2021; 2022). Moreover, in a previous report, it was noted that osmolality and pH of semen media affected significantly ram sperm motility patterns assessed by CASA and that this was partially related to the employed medium, but also to the incubation temperature (Alomar *et al.,* 2018).

A clear reduction of pH values was noted when honey was added to TBM and TEY media. The acidic pH of honey is normally 4.31–6.02 and its content of sugars may be responsible for the pH changes in these media. The present results made obvious that the observed decrease in motility status and membrane integrity at 4% of honey was in some way related to the clear reduction in pH of TBM and TEY media. Indeed, human sperms exhibited a greater reduction in motility in response to acidic than to alkaline conditions (Makler *et al.,* 1981). The relationship between intracellular pH and motility was previously studied, and the link between these parameters was suggested to be protein phosphorylation (Carr & Acott, 1989). However, the maintenance of a stable pH during liquid storage of goat semen improved dramatically sperm viability and fertilising potential (Liu *et al.,* 2016). Moreover, monitoring media pH at different times and modeling its variation according to nonlinear models was important to achieve optimisation of the protocol for liquid semen preservation (Liu *et al.,* 2016). It must be stressed out that the incubated spermatozoa may also play a role in changing pH of the media. When glucose was present in the extender, boar spermatozoa as well as the extender were acidified to pH 6.2 (Kamp *et al.,* 2003) from the initial pH 7.2 to 7.5 in freshly ejaculated semen (Johnson *et al.,* 2000). Spermatozoa can metabolise glucose through glycolysis for energy supply producing lactate. Lactate, and other permeant weak acids, have been shown to reduce the intracellular pH of bovine spermatozoa and many other types of cells (Acott & Carr, 1984). For that, one of the important point to be analysis in the future is the pH of the media with the incubated spermatozoa to determine the exact role of the spermatozoa in changing the pH of any given media supplemented with honey.

In the different Tris media assessed in this study, higher osmolality values were related to the increased concentration of added honey. Generally, the high sugar contents in honey may create a high osmotic pressure. In the present work, CASA motility parameters increased when 1% of honey was added and decreased with 4% honey addition to TBM and TEY media. In fact, spermatozoa motility was clearly related to their sensitivity to media osmolality (Alomar *et al.,* 2018). Moreover, after the exposure to solutions with 600 and 1200 mOsm, almost all rat spermatozoa lost motility and the motility did not improve after return to isosmotic conditions (Si *et al.,* 2006). Similar to the decrease of sperm motility, plasma membrane integrity of rat sperm declined rapidly at hyperosmotic conditions (Si *et al.,* 2006). These observations concerning

rats' spermatozoa are in agreements with our findings for goat spermatozoa incubated in Tris media supplemented with 4% of honey.

Motility is an important indicator of sugar utilisation by spermatozoa as the sugars provide the external energy source essential for maintaining motility. The energy required by the spermatozoa is provided by simple sugars (monosaccharides). Honey contains the monosaccharides needed by spermatozoa to keep alive. Furthermore, honey addition to egg yolk was found to improve spermatozoa motility and viability of chilled goat semen (Olayemi *et al.,* 2011). Our results clearly showed the positive effects of 1% of concentration of honey addition on fresh and chilled buck spermatozoa motility. In line with these results, the supplementation of honey to Tris-extenders was able to enhance sperm motility of chilled and frozen bull semen and to increase the conception rate (El-Sheshtawy *et al.,* 2014). Moreover, El-Nattat *et al.* (2016) obtained better post-thaw motility and viability of buffalo sperm when honey was added at 2% rate compared to 3%, 4% and 5%. Also El-Sheshtawy *et al.* (2014) reported better post-thaw sperm motility and live sperm count at 1% level for cryopreserved bull sperm and at 3% level for chilled bull semen. Addition of honey to egg yolk-based extender improved motility, viability and fertilizing ability of New Zealand white rabbit semen, and this effect was concentration dependent, whereas higher concentrations of honey had negatively affect sperm motility and viability (El-Sherbiny, 2013). It must be pointed out that all the previous reports did not report the osmolality and pH values of used media as was the case in the present work. These two important factors may be the most influential ones in both the motility and the plasma membrane integrity of buck spermatozoa, especially when the concentration of added honey exceeds 2%.

CONCLUSION

The results of the current study suggested that osmolality and pH values of Tris media were significantly affected by honey addition whereas the osmolality values increased parallelly with honey concetration while at the same time the pH values siginficantly decreased. Increasing the concentration of added honey to 4% had a clear negative effect on both sperm motility and the integrity of its plasma membrane whatever the temperature. Despite the interesting results presented here, the topic merits further research, particularly to understand the specific mechanisms of action through which natural honey could affect the quality of spermatozoa preserved in different semen media.

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