Influence of Honey Addition As a Natural Energy Source On Cooled Camel Spermatozoa

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ABSTRACT:

Camel is an important source of food as well as of transport for large communities in sub-Saharan Africa, the Middle East, and the Indian subcontinent. The present study aimed to evaluate the effect of different concentrations of honey (0.0, 1.0, 2.5, or 5.0%) addition to Tris-extender on epididymal camel semen quality during storage at 5º C for up to 48 hrs. Eleven healthy dromedary camels (*Camelus dromedarius*), aged between 8 to 15 years, were used in the present study during the rutting season. Percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, acrosome damage, and membrane integrity were determined. The obtained results showed that the extended semen supplemented with honey at levels of 1%, and 2.5% significantly (P<0.01) improved percentages of sperm motility, intact acrosome, and membrane integrity, while decreased the percentages of dead and abnormal camel spermatozoa during storage at 5ºC for up to 48 hrs. Moreover, the addition of 5.0% honey to the extender significantly (P<0.01) tended to be a deleterious effect on camel semen quality. In conclusion, Tris-extender added with 2.5% honey improved camel semen quality during storage at 5º C for up to 48 hrs.

Keywords: Camel; Chilled Semen; Honey; Spermatozoa; Tris extender.

INTRODUCTION

Camel is an important source of food as well as of transport for large communities in sub-Saharan Africa, the Middle East, and the Indian subcontinent (Wilson, 1984). In general, artificial insemination (AI) is an important technique in all domestic species to ensure rapid genetic progress. The use of AI has been reported in camels although insemination trials are rare. The epididymal camel spermatozoa after slaughtered or recently died animals will increase the opportunities to create semen and to establish their use for artificial insemination (AI), *in vitro* fertilization (IVF), or intracytoplasmic insemination (Turri *et al.,* 2013).

Good extender must contain energy source, phospholipids for cold shock protection, buffering system, and antibiotics (Jerez-Ebensperger *et al.,* 2015). On the other hand, about 95% of the honey is composed of carbohydrates, mainly of fructose and glucose. 5-10 % of the total carbohydrates are oligosaccharides in total about of 25 different di- and tri-saccharides. Honey, in addition, contains a variety of substances including amino acids, organic acids, proteins, lipids, enzymes, trace of pollen, vitamins, minerals, polyphenols, and aroma compounds that can act as a source of energy to support spermatozoa survival and motility during cryopreservation. Moreover, honey contains also tiny amounts of several compounds thought to function as antioxidants, including glucose oxidase, catalase, flavonoids, ascorbic acid, phenolic acids, carotenoid derivatives etc., Honey has been shown to possess antiviral, antimicrobial, antiparasitic, antitumor and anti-inflammatory effects (Bogdanov *et al.,* 2008). Honey flavonoids possess free radicals scavenging activity, thereby inhibiting free radicals induced DNA damage (Chen *et al.,* 2004).

Many researchers have reported that the benefits of honey as a supplementation in the cryopreservation media in different species like rabbit (El-Sherbiny, 2013), cattle (El-Sheshtawy *et al*., 2014; Chung *et al*., 2019), sheep (Jerez-Ebensperger *et al.,* 2015), buffaloes (El-Nattat *et al*., 2016), and stallion (El-Sheshtawy *et al*., 2016). However, studies on the effect of honey addition to the extender on camel spermatozoa during storage at 5º C are not available.

Therefore, the present study aimed to define the effect of honey addition of concentrations at 0.0, 1.0, 2.5, and 5.0 ml/100 ml to Tris-yolk extender on camel semen quality during storage at 5º C for up to 48 hours.

MATERIALS AND METHODS

Eleven pairs of testes were collected from apparently healthy slaughtered camels aged 8 to 15 years, from Qalyub abattoir, Qalyubia

Governorate during the breeding season. The processing of the samples was carried out directly at arrival to the laboratory as soon as possible. The samples were transported to the laboratory on an icebox according to El-Harairy *et al*. (2016) and then the epididymis and vas deferens were dissected and separated from the testis. The epididymis was sectioned into three parts (caput, corpus, and cauda) in sterile Petri dishes using a sterile scalpel and forceps according to Zeidan *et al*. (2001) in the dromedary camels. Semen was collected from the cauda region using the retrograde flushing method with Tris-extender as the method described by Turri *et al*. (2013). The content of the epididymis was emptied into a clean test tube.

Semen was collected, pooled, motility was evaluated, and divided into four equal aliquots and then extended with tris extender according to Zeidan *et al*. (2014) with different honey concentrations (Table 1). Only samples with at least 70% progressively motile spermatozoa extended with the different media to provide final concentration of (40 $\times 10^6$ spermatozoa /ml).

Chilling of semen at 5° C

The test tubes containing extended semen were placed in a 500 ml beaker containing water at 37° C with a thermometer to facilitate periodic checking of the temperature during the cooling period. The beaker was placed in a refrigerator and gradually cooled till its temperature reached 5º C within 2 hours as the method described by Salisbury *et al*. (1978). The cooled spermatozoa were kept at 5º C for up to 48 hours. After each storage time (0, 6,12, 24, and 48 hours), percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, acrosome damage, and intact membrane of spermatozoa were recorded.

Semen quality assays

Percentage of sperm motility was estimated by adding one droplet of the diluted semen on a dry, clean, and pre-warmed (37°C) glass slide and covered with a warmed coverslip and immediately examined using high power magnification (X400). Sperm motility was estimated by observing the approximate percentage of spermatozoa moving advanced motion across the field of vision with a normal vigorous swimming motion according to El-Nady. (2017).

The percentage of dead spermatozoa was stained with Eosin-Nigrosin and examined microscopically at X 1000 magnification according to Swanson and Bearden (1951).

Assessment of abnormal spermatozoa (%) was determined in the same smears prepared for live/dead spermatozoa ratio (Brito, 2007).

Acrosome damage of spermatozoa was determined using a Giemsa stain procedure as the method described by Zeidan *et al*. (2019).

The hypo-osmotic solution (150 mOsm/L) was prepared by dissolving 0.735g sodium citrate and 1.35g fructose in 100 ml of distilled water. The solution was stored at 4º C till it is used. A 1.0 ml of hypoosmotic solution was mixed with 0.1 ml of semen and incubated at 37ºC for 60 min. Spermatozoa with intact membranes are identified by changes in the shape of the cell, as indicated by coiling of the tail according to Zeidan *et al*., (2008).

Statistical analysis

Data were statistically analyzed as a split plot design using a mixed model (ANOVA). Data were statistically analyzed using of SPSS Statistical Software version 20. Significant differences between each to means were done according to Duncan's multiple range test (Duncan, 1955) at P< 0.05.

The statistical model for trial was as follows:

$$y_{ijkl} = \mu + \alpha_i + \eta_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{kij}$$

Where: y_{ijkl} observation

μ Overall mean

α_i Effect of the i^{th} concentration, a fixed effect, i = 1,2,3,4

η_i Whole-plot error assumes N (0, σ^2_η)

β_j Effect of the j^{th} time, a fixed effect, i = 1,2,3,4,5

$(\alpha\beta)_{ij}$ Fixed effect for interaction between concentration and incubation time

ε_{kij} Effect of random error assumes N (0, σ^2)

RESULTS

The effect of different levels of honey on the percentage of sperm motility is shown in table (2). The percentage of sperm motility significantly (P<0.01) increased with honey at 1.0 %, or 2.5 % as compared with control. However, 5.0 % of honey addition significantly (P<0.01) tended to deteriorate sperm motility.

The percentages of the dead, abnormal, and acrosome damage of camel spermatozoa is shown in tables (3- 5). The tris-extender

supplemented with 1.0 % and 2.5 % honey led to lower percentages of the dead, abnormal, and acrosome damage compared to control. However, when the amount of honey used at 5.0 % increased the percentages of dead, abnormal, and acrosome damaged significantly (P<0.001).

The percentage of membrane integrity of dromedary camel spermatozoa extended with Tris and honey is shown in table (6). Addition of honey at concentration of 1.0 or 2.5% showed significantly (P< 0.001) improved the percentage of intact sperm membrane than control, where the maximal values were recorded with the semen contained 2.5 %.

The percentages of camel spermatozoa motility and membrane integrity significantly (P<0.01) decreased, while the percentages of dead spermatozoa, abnormal spermatozoa, and acrosome damage increased significantly (P<0.01) as time of storage increased.

DISCUSSION

In the present study, we added honey to camel semen extender as an energy substrate, and protector against cold shock, besides its powerful antioxidant effect against spermatozoa damage during cooling. The results of the current study concluded that adding of honey to extender significantly (p<0.01) improved sperm motility, viability, intact membrane, and acrosome integrity of camel spermatozoa during storage at 5° C.

These results are in agreement with those of Olayemi *et al*. (2011), Jerez *et al*. (2013), El-Sheshtawy *et al.* (2014), and El-Sheshtawy *et al*. (2016), who found that the presence of honey in cooling and freezing extenders increased sperm motility and improved sperm quality in goat, ram, cattle bull, and Arabian stallion, respectively. Moreover, Banday *et al.* (2017) concluded that honey can be used as an energy source in semen extenders of ram at 2.5% but high dose of honey (5 %, and 7 %) caused deleterious effect of semen. Malik (2018) reported also that honey addition with different semen extenders improved sperm motility in both chilled and frozen bull semen.

The improvement in sperm motility gained with the addition of 2.5 % honey might be attributed to the wide variety of properties that honey possessed. The main source of energy in the semen extender is sugar. It is known that honey mainly contains sugars such as oligosaccharides, disaccharides, and polysaccharides that can act as a source of energy to aid sperm survival and motility during cryopreservation (Yimer *et al.,* 2015). As a result of the strong antioxidant capability, sufficient nutrients available in the honey could boost the metabolic activity of spermatozoa, resulting in fewer dead spermatozoa (Chung *et al.,* 2019).

Improvement of sperm motility is an important feature associated with the fertilizing capacity of spermatozoa (Verstegen *et al.,* 2002; Cassani *et al.,* 2005). Sugars have a lot of functions in cryopreservation in the first place being the major energy source, as they improve the viability of spermatozoa, integrity of acrosomes, and maintaining osmotic balance (Jerez-Ebensperger *et al.,* 2015). On the other hand, the decrease in sperm motility and semen quality with an increase in the honey supplement of more than 2.5% might be associated with the excess hyperosmotic around spermatozoa created due to the high level of honey that can lead to decrease in cell volume due to transport of water outside the cell like the effect of a high concentration of non-penetrating cryoprotectants (Lemma, 2011).

High concentration of honey may cause a hyperosmotic extracellular environment in spermatozoa which increase intracellular dehydration. Osmotic stress that occurs will change the organization membrane and permeability which compromised the sperm function (Watson, 2000). As a result, too high concentrations of honey at a level of 5.0% with tris extender were not satisfactory and causes poor epididymal camel semen quality.

On the other hand, a decrease in the quality of camel spermatozoa as time storage increases may be due to the accumulation of lactic acid and reactive oxygen species which exerts a toxic effect on spermatozoa. Shannon and Curson (1972) found that the percentage of dead spermatozoa was a source of acid oxidase which causes the production of H_2O_2, consequently, increased the percentage of dead spermatozoa as the time of storage increases. A similar trend was reported by (Zeidan, 2008; Zeidan *et al.*, 2014) in the dromedary camels.

El-Sheshtawy *et al*. (2014) found that the addition of 10.0% honey solution to cattle bull semen extender improved sperm motility (%) in chilled and frozen semen and improved the conception rate. In addition, Olayemi *et al.* (2011) concluded that, the addition of honey at 5.0% to egg yolk extender improved motility and live dead ratio and viability of liquid storage goat semen.

Improve semen quality with honey as extender supplementation might be due to the powerful antioxidant capacity of honey as it contains a mixture of antioxidants, carbohydrates, enzymes, amino acids, and vitamins (El-Nattat *et al.,* 2016).

El-Sherbiny (2013) found that addition of honey at level of 1.0% to 5.0% to replaced part of egg yolk to rabbit semen extender improved the percentage of progressive motility, viability, and normal spermatozoa pre- or post-thawing.

Addition of honey as a supplementation in skimed milk extender improved the chilled and frozen semen quality of Egyptian buffaloes (Kandiel, and Elkhawagah, 2017). Moreover, addition of 10.0% natural honey to cryopreservation solution improved post-thawing motility of infertile human spermatozoa (Fakhrildin and Alsaadi, 2014). The same later authors reported also that the presence of monosaccharides such as fructose and glucose and disaccharides as sucrose and other sugars in honey may protect sperm during cryopreservation and enhanced sperm parameters.

Jerez-Ebensperger *et al.* (2015) concluded that fructose can be replaced by honey, that contains monosaccharides such as fructose and glucose leading improve ram semen quality after thawing. In buffalo, El-Nattat *et al.* (2016) found that supplementation of honey at level of 1% (in chilling for up to 7 days) and 2-5% (in freezing) caused a significantly ($p<0.05$) improvement of bull semen quality (percentage of motility, viability, abnormalities, and intact membrane) compared to free- honey semen (control). Moreover, honey has also been praised as a potent noble antioxidant in protecting cells of the various organs in the body from damage due to oxidative stress reactive oxygen species (Erejuwa *et al.,* 2012).

Yimer *et al.* (2015) revealed that when honey was added to Tris-extender at a level of 2.5% improved bull semen quality after cryopreservation comparable to Bioxcell® extender, which was superior to all other extenders. On the other hand, when honey was added at 5 and 10%, it was not acceptable because it resulted in much worse quality sperm post-cryopreservation.

In the Arab stallion, El-Sheshtawy *et al*., (2016) found that the addition of honey at levels of 2 %, 3 %, and 4 % in INRA-82 extenders significantly ($p < 0.01$) improved semen quality (sperm motility, viability index, membrane integrity and intact acrosome during storage at 0, 1, 2 and 3-hrs post-thawing. While the use of low (1 %) or high (5 %) concentration of honey showed insignificant ($P< 0.05$) differences. The previous studies are in agreement with our results which revealed the increase of sperm quality added with honey.

CONCLUSION

In conclusion, addition of honey at level of 2.5 % significantly enhanced sperm motility, viability, intact membrane, and acrosome integrity of dromedary camel epididymal spermatozoa during storage at 5 °C.

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Table 1: Compositions of the buffered tris extender

Components	Grams /100 ml of distilled water			
	Control	1.0 % honey	2.5 % honey	5.0 % honey
*Tris	3.028	3.028	3.028	3.028
Sodium citrate dihydrate	2.9	2.9	2.9	2.9
Citric acid	0.04	0.04	0.04	0.04
Fructose	1.25	-	-	-
Egg yolk (ml)	10	10	10	10
Gentamicin (μ/ml)	400	400	400	400
Honey (ml)	0.0	1.0	2.5	5.0

*Tris(hydroxymethyl) aminomethane, Aldrich Chemical Co. Ltd., Gillingham, Dorest England.

Table 2: Mean percentage of motile dromedary camel spermatozoa with different honey concentrations, during storage at 5 °C for up to 48 hours

Storage times (hours)	Honey level (%)				Mean
	0.0	1.0	2.5	5.0	
0	65.00	69.42	70.42	64.71	67.39^{a}
6	62.28	64.71	69.14	60.28	64.10^{b}
12	56.71	63.57	64.42	53.42	59.53^{c}
24	50.00	54.28	59.28	45.14	52.17^{d}
48	40.71	48.28	53.85	36.00	44.71^{e}
SE±	1.58	1.58	1.58	1.58	0.79
Mean	54.94^{B} ±0.70	60.05^{A} ±0.70	63.42^{A} ±0.70	51.91^{B} ±0.70	57.58
F value			1.867		
Sig			***		

Values with different superscripts (A-B) within a row, and (a-e) within a column were significantly (P<0.01).

Table 3: Mean percentage of the dead dromedary camel spermatozoa with different honey concentrations, during storage at 5 °C for up to 48 hours

Storage times (hours)	Honey level (%)				Mean
	0.0	1.0	2.5	5.0	
0	13.00	11.28	10.85	13.85	12.25^{d}
6	15.00	13.28	12.42	17.00	14.42^{d}
12	18.71	16.42	15.28	21.14	17.89^{c}
24	22.42	19.71	18.14	25.28	21.39^{b}
48	26.42	23.71	21.28	30.00	25.35^{a}
SE±	0.45	0.45	0.45	0.45	0.22
Mean	19.11^{B} ±0.20	16.88^{C} ±0.20	15.59^{D} ±0.20	21.45^{A} ±0.20	18.26
F value			4.210		
Sig			***		

Values with different superscripts (A-D) within a row, and (a-d) within a column were significantly (P<0.01).

Table 4: Mean percentage of abnormal camel spermatozoa with different honey concentrations, during storage at 5 °C for up to 48 hours

Storage times (hours)	Honey level (%)				Mean
	0.0	1.0	2.5	5.0	
0	10.28	8.57	7.57	12.28	9.67^{e}
6	12.71	10.71	9.85	15.14	12.10^{d}
12	16.00	13.85	12.71	18.57	15.28^{c}
24	20.85	17.85	15.85	23.71	19.57^{b}
48	25.71	22.42	19.71	29.28	24.28^{a}
SE±	0.76	0.76	0.76	0.76	0.38
Mean	17.11^{B} ±0.34	14.68^{C} ±0.34	13.14^{C} ±0.34	19.80^{A} ±0.34	16.18
F value			1.320		

Sig			***		

Values with different superscripts (A-C) within a row, and (a-e) within a column were significantly (P<0.01).

Table 5: The percentage of acrosome damage of dromedary camel spermatozoa with different honey concentrations, during storage at 5 °C for up to 48hours

Storage times (hours)	Honey level (%)				Mean
	0.0	1.0	2.5	5.0	
0	4.28	3.14	3.01	5.14	3.89^{d}
6	5.28	4.42	4.24	6.57	5.12^{c}
12	7.57	6.42	5.28	9.03	7.07^{bc}
24	10.31	8.57	7.16	11.85	9.47^{b}
48	13.14	11.14	9.85	15.72	12.46^{a}
SE±	0.20	0.20	0.20	0.20	0.10
Mean	8.11^{B}±0.09	6.74^{C}±0.09	5.91^{D}±0.09	9.66^{A}±0.09	7.60
F value			7.884		
Sig			***		

Values with different superscripts (A-D) within a row, and (a-d) within a column were significantly (P<0.01).

Table 6: The percentage of the intact membrane of the dromedary camel spermatozoa with the different honey concentrations, during storage at 5 °C for up to 48hours

Storage times (hours)	Honey level (%)				Mean
	0.0	1.0	2.5	5.0	
0	72.71	76.27	79.28	70.71	74.74^{a}
6	68.42	71.71	73.85	66.42	70.10^{b}
12	64.14	66.57	69.57	60.73	65.25^{c}
24	58.57	60.28	63.57	53.41	58.96^{d}
48	52.01	54.85	58.14	45.28	52.57^{e}
SE±	1.60	1.60	1.60	1.60	0.80
Mean	63.17^{C} ±0.71	65.93^{B} ±0.71	68.88^{A} ±0.71	59.31^{D} ±0.71	64.32
F value			0.408		
Sig			***		

Values with different superscripts (A-D) within a row, and (a-e) within a column were significantly (P<0.01).

تأثير إضافة العسل كمصدر طبيعي للطاقة على الحيوانات المنوية للإبل المحفوظة بالتبريد

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الملخص العربي:

تهدف هذه الدراسة الي معرفة تأثير إضافة العسل بنسب مختلفة (0.0؛ 1.0؛ 2.5؛ 5.0 %) الي مخفف التريس علي جودة الحيوانات المنوية البربخية للإبل وحيدة السنام عند حفظها عند درجة حرارة 5 درجة مئوية لمدة 48 ساعة. استخدم في هذه الدراسة أحد عشر ذكرا من الإبل وحيدة السنام تتراوح أعمارهم من 8- 15 سنة خلال موسم النشاط الجنسي. تم تقدير النسبة المئوية لكل من حركة الحيوانات المنوية؛ ونسبة الشواذ؛ والشذوذ الأكروسومي؛ وسلامة الغشاء البلازمي. أظهرت النتائج أن إضافة العسل الي مخفف التريس بنسبة 1.0% و2.5% أدي الي زيادة حركة الحيوانات المنوية بدرجة معنوية عند مستوي (P<0.01)؛ في حين انخفضت النسبة المئوية لكل من نسبة الشواذ؛ وتلف غشاء الأكروسوم؛ وتلف الغشاء البلازمي. سجلت أفضل النتائج مع نسبة إضافة 2.5% مقارنة بباقي المعاملات. أظهرت النتائج أيضا ان استخدام العسل بنسبة 5.0% كان له تأثير ضار على خصائص الحيوانات المنوية. من خلال النتائج المتحصل عليها فإننا نوصي باستخدام العسل مع مخفف التريس بنسبة 2.5% عند حفظ الحيوانات المنوية للإبل بالتبريد عند درجة حرارة 5 درجة مئوية.

الكلمات الاسترشادية: الجمال، السائل المنوي المبرد, عسل نحل, حيوانات منوية, مخفف التريس.