Journal of Livestock Science and Technologies, **2018, 6 (1): 57-64** ***http://lst.uk.ac.ir***

DOI: 10.22103/jlst.2017.10987.1212

Evaluation of camel milk as an extender for short-term storage of ram semen at 15°C

A. Benmoula[1,2], A. Badi[1], A. Hilali[2], K. El Khalil[1], L. Allai[2], M. El Fadili[3] and B. El Amiri[1*]

[1]INRA–Centre Régional de la Recherche Agronomique de Settat, BP589, Settat, Morocco.

[2]Laboratoire d'agroalimentaire et santé, Faculté des Sciences et Techniques, Université Hassan1, BP 577, 26000 Settat, Morocco.

[3]INRA–Division Scientifique, Département des Productions Animales, BP 415 RP, Avenue Hassan II, Rabat, Maroc.

* Corresponding author, E-mail address: bouchraelamiri@hotmail.com

Abstract The purpose of the present study was to assess the effect of modified camel milk (MCAM) and skim cow milk (SCOM) on semen quality of INRA180 and Boujaâd ram breeds, stored at 15°C for up to 24 hours. Semen samples were collected from eleven mature rams (6 from INRA180 and 5 from Boujaâd rams) and extended in SCOM *vs* MCAM, to reach a final concentration of 0.8×10^9 spermatozoa/mL, and stored at 15°C for 24 hours. The quality assessment was performed at different storage periods (0, 2, 8 and 24 hours). The results showed that the stored sperm quality varied significantly from breed to breed and sperm response to milk extenders differed between ram breeds. The MCAM showed a clear beneficial effect on stored sperm quality, compared to SCOM especially for motility, lipid peroxidation, and membrane integrity. Additional studies are needed to evaluate the pregnancy rate after insemination with stored semen.

Keywords: camel milk, ram semen, ram breed, chilled semen

Received: 11 Nov. 2017, *accepted:* 02 Dec. 2017, *published online:* 23 Apr. 2018

Introduction

The dromedary camel is being used more and more in the intensified dairy system (Faye et al., 2014). Currently, camel milk products could not merely enrich the human being diet in arid and semi–arid areas, but also provide an important source of income for the nomadic herders (Faye et al., 2014). It has been reported that camel milk had been used for sperm liquid storage and artificial insemination in 1322 (Bowen, 1969). Seleem et al. (2009) recorded that in the rabbit, camel milk extender revealed results significantly better than saline solution or skim buffalo milk extenders for liquid storage and artificial insemination. Furthermore, El-Badry et al. (2007), suggested that buffalo chilled semen extended in camel milk had higher viability and fertilizing capacity than that offered in buffalo milk. The aim of this research was to determine if camel milk could be used as an extender instead of cow milk, for sperm preservation of other animal species such as sheep.

In all likelihood, when Colas et al. (1968) developed and optimized for the first time a skim cow milk based extender, his aim was to make it available over the world. Thus, using cow milk based extender for semen conservation at 15 °C remained a simple and economical procedure, with encouraging results in artificial insemination (Maxwell and Salamon, 1993), due to its high protein content (Khaskheli et al., 2005). On the other hand, camel milk can be stored for longer durations compared with the cow milk (Sboui et al., 2010). Camel milk has several particularities in physical and chemical composition, which possibly make it as a good alternative to skim cow milk for sperm cell conservation (Seleem et al., 2009). It contains higher levels of vitamin C (Al Haj and Al Kanhal, 2010), total cholesterol (Gorban and Izzeldin, 1999) and proteins (Khaskheli et al., 2005) compared to the cow milk. Therefore, the present work compared the effects of modified camel milk and skim cow milk as semen extenders in two Moroccan ram breeds when stored at 15°C for 2, 8 and 24 h.

Material and methods

Semen collection

Eleven rams (6 INRA180 and 5 Boujaâd rams) aged 2.5 to 3 years, were used for semen collection in 18 weeks during the breeding season. The rams were maintained at the National Institute of Agricultural Research Center

of Settat; Morocco. Ejaculates were collected from each ram once a week using an artificial vagina (40–42 °C). Immediately after collection, the ejaculates were placed in a water–bath at 37 °C. Semen volume, sperm concentration and subjective individual (400 x light microscopy) and mass motility (100 x light microscopy) were measured. Sperm concentration was assessed with a spectrophotometer previously calibrated using a hemocytometer. Samples with a concentration $>2.10^9$ spermatozoa/mL; mass motility >3+, and individual motility >70% were processed in this study. All acceptable ejaculates from each ram breed were pooled and extended to a final sperm concentration of 0.8×10^9 spermatozoa/mL in the extenders.

Extender preparation

The extenders were prepared just before semen collection. The cow milk based extender (SCOM, control extender) was prepared from skim milk powder (11 % w/v) and distilled water, heated to 95 °C for 10 min (Colas et al., 1968) and supplemented with penicillin (50 µg/mL). The modified camel milk (MCAM) extender was prepared using commercial pasteurized whole milk, heated to 95 °C for 10 min and centrifuged at 3000 *g* for 20 min at 4 °C. Then, the fat layer that tended to disrupt microscopic evaluation, was removed and 50 µg/mL penicillin added.

Semen processing

Ejaculate collected from each ram was divided into several aliquots, diluted (v/v) in SCOM and MCAM extenders, and placed in a water bath at 37 °C. After measuring the sperm concentration, the semen was diluted to a final concentration of 0.8×10^9 spermatozoa/mL. For each breed, equal volumes of diluted semen were pooled within the extender. The aliquots from each extender were taken to assess the initial sperm quality (0 h). Thereafter the samples were divided into three equal parts in closed Eppendorf tubes maintained at 15 °C for further analysis after storage at 2, 8, and 24 h.

Semen evaluation

1. Motility

A computer–assisted sperm motility analyzer (CASA; ISAS, version 1.0.17, Proiser, Valencia, Spain) was used to evaluate sperm total motility (MT %) and progressive motility (PM %) as described by Yániz et al. (2008). For each sample, the semen was diluted in phosphate-buffered saline supplemented with bovine serum albumin (1 mg/mL) to achieve 20×10^6 sperm/mL.

1. Viability

Sperm viability was assessed by using eosin–nigrosin staining. The stain was prepared by dissolving eosin–Y (1.67 g), nigrosin (10 g) and sodium citrate (2.9 g) in 100 mL distilled water. For each smear, 2.5 µL of diluted semen with 2.5 µL of the stain were mixed on a warm slide, and then smeared with another slide (Kulaksız et al., 2010). The viability was assessed by counting at least 200 cells (400 ×, light microscopy). Spermatozoa showing partial or complete purple stain were considered non–viable and only spermatozoa showing strict exclusion of the stain were considered to be alive.

2. Abnormalities

The percentage of abnormal cells was assessed using a Diff–Quik staining. All Diff–Quik solutions were obtained from Automatic Diagnostic Systems S.L., Barcelona (Spain). Briefly, a smear of 3 µL of diluted semen was prepared on a slide and air–dried for 4 min. Then, the slide was dipped for 1min in Diff–Quik fixative solution (0.002 g/L of Fast Green in methanol) prior to staining with Diff–Quik solution 1 (1.22 g/L of Eosin Y in phosphate buffer at pH 6.6 and 0.1 % (w/v) sodium azide as preservative) for 50 s and with Diff–Quik solution 2 (1.1 g/L of tiazine day in phosphate buffer at 6.6) for 50 s. At each step, the excess stain was dried by placing the slide vertically on absorbent paper. Sperm abnormality was assessed by counting at least 200 cells (400 ×, light microscopy).

3. Plasma membrane integrity

Sperm plasma membrane integrity was determined using the hypo-osmotic swelling test (HOST) in a hypo–osmotic solution (100 mOsM) containing 9 g fructose and 4.9 g sodium citrate in one liter distilled water (Revell, 2003). A portion of diluted semen (50 µL) was mixed with 500 µL HOS solution and incubated for 60 min at 37 °C. After incubation, 300 µL of the mixture was spread with a cover slip on a warm slide. The The percentage of spermatozoa with curled/swollen tail (Akhter et al., 2008) were counted among 200 sperm using a phase contrast microscope (400×, UB203 microscope).

4. Spontaneous lipid peroxidation

Lipid peroxidation (LPO) was determined in diluted sperm by measuring the amount of thiobarbituric acid reactive species (TBARS) formed, according to a modified procedure described by Maia (2006). One mL of the TBA reagent (trichloracetic acid 15 %, w/v, hydro-

chloric acid 0.25 N, thiobarbituric acid 0.375 % w/v in distilled water) and 1 % (v/v) of BHT (Butylatedhydroxytoluene) solution at 50 mM were added to the 250 µL samples diluted with (Tris–hydroxymethyl–aminomethane 1.8184 g, monohydrate citric acid 0.9901 g, double distilled water up to 50 mL, pH 7.4) freshly prepared to obtain a volume of 500 µL containing 10^8 spermatozoa/mL. The mixture was heated at 100°C for 15 min and then allowed to cool on ice. The sample was centrifuged at 1000 × g for 10 min. The supernatant was collected and TBARS were quantified with a spectrophotometer at 532 nm against a blank prepared under similar conditions. The amount of TBARS was calculated using a molar extinction coefficient of $1.56\times10^{-5}M^{-1}M^{-1}$ cm^{-1} for thiobarbituric acid and expressed in nmol TBARS/10^8 spermatozoa.

Statistical analysis

Statistical analyses were performed using JMP SAS 11.0.0 (SAS Institute Inc., Cory, NC, USA) program. The data of extended semen quality parameters were analyzed by a factorial design ANOVA. Two statistical models were performed in this study are; 1) The statistical model included the fixed effect of ram breed (Boujaâd *vs* INRA180) and storage periods (0, 8 and 24 h), and 2) the fixed effect of the base extender (SCOM *vs* MCAM) and storage periods (0, 8 and 24h) for each ram breed.

When statistically significant differences were detected, the Tukey's post hoc test was applied to compare the means at $P < 0.05$ for the effect of storage du ration, and Student's t test was applied to compare the means at $P<0.05$ for the effect of breed or base extender at each storage duration point. Data are expressed as mean ± SE.

Results

The effect of ram breed and storage duration on semen quality are presented in Table 1. Table 2 shows the effect of semen extender and storage time on semen quality in INRA180 rams. In Table 3, the effects of storage time and extender on semen quality in Boujaâd rams are shown.

Effect of storage duration on ram semen quality parameters

There was a significant effect of storage time and breed on semen parameters (Table 1), although semen quality did not differ between breeds at 0 h ($P>0.05$).

At 2 h of storage, the sperm from Boujaâd rams showed a significant decrease in terms of TM, PM, viability, and HOST, and a significant increase in percent sperm abnormality and LPO ($P<0.05$). In INRA180 rams, only the LPO increased ($P< 0.05$). Sperm TM and HOST were higher in INRA180 compared to Boujaâd rams ($P<0.05$). However, sperm abnormality was significantly higher in Boujaâd rams. Progressive motility, viability and LPO were not affected by the ram breed.

At 8 h of storage, the difference in sperm quality between the two breeds continued to appear. For Boujaâd ram semen a significant ($P<0.05$) decrease in viability and a significant increase in LPO and abnormalities were recorded. However, for INRA180 rams sperm a significant decline in TM, PM, viability, and HOST and a significant increase in abnormality and LPO were recorded. Sperm viability and membrane integrity were significantly higher in INRA180 compared to Boujaâd ram. However, progressive motility and LPO were higher in Boujaâd rams compared to INRA180 rams ($P<0.05$).

Table 1. Effect of storage duration (0, 2, 8 and 24 h) at 15°C on total motility (TM), progressive motility (PM), viability, abnormality, membrane integrity and lipid peroxidation (LPO) of Boujaâd and INRA180 ram semen (Mean±S.E.M)

Storage duration	Breed	TM (%)	PM (%)	Viability (%)	Abnormality (%)	Membrane integrity (%)	LPO (nmol TBARS/10^8 sperm)
0 h	Boujaâd	91.27 ± 0.41^{Ax}	70.27 ± 0.55^{Ax}	93.83 ± 0.57^{Ax}	6.22 ± 0.36^{Dx}	87.05 ± 0.32^{Ax}	0.56 ± 0.01^{Dx}
	INRA180	92.66 ± 0.32^{ax}	69.44 ± 1.09^{ax}	92.72 ± 0.51^{ax}	6 ± 0.37^{cx}	87.05 ± 0.32^{ax}	0.52 ± 0.03^{dx}
2 h	Boujaâd	87.5 ± 0.89^{By}	$67.27\pm1.26A^{ABx}$	90.94 ± 0.50^{Bx}	10.55 ± 0.38^{Cx}	78.77 ± 1.82^{By}	1.12 ± 0.05^{Cx}
	INRA180	91.83 ± 0.38^{ax}	68.72 ± 0.44^{ax}	92.38 ± 0.54^{ax}	6.66 ± 0.39^{cy}	86.11 ± 0.34^{ax}	1 ± 0.09^{cx}
8 h	Boujaâd	85.11 ± 0.75^{Bx}	64.5 ± 1.15^{Bx}	84.11 ± 0.48^{Cy}	22.55 ± 0.61^{Bx}	75.27 ± 2.07^{BCy}	1.76 ± 0.08^{Bx}
	INRA180	85.61 ± 0.53^{bx}	60.44 ± 1.34^{by}	88.77 ± 0.45^{bx}	22.88 ± 0.49^{bx}	81.5 ± 0.58^{bx}	1.53 ± 0.03^{by}
24 h	Boujaâd	84.94 ± 0.59^{Bx}	58.05 ± 0.53^{Cx}	82.16 ± 0.42^{Dy}	25.88 ± 0.81^{Ay}	71.55 ± 1.68^{Cx}	2.38 ± 0.08^{Ax}
	INRA180	83.66 ± 0.67^{cx}	55.38 ± 1.14^{cy}	83.61 ± 0.40^{cx}	29.88 ± 0.94^{ax}	75.33 ± 0.93^{cx}	2.2 ± 0.16^{ax}

$^{a, b, c}$ Different superscripts within column indicate an effect of storage duration on INRA180 ram semen ($P<0.05$, Tukey's post hoc test)
$^{A, B, C}$ Different superscripts within column indicate an effect of storage duration on Boujaâd ram semen ($P<0.05$, Tukey's post hoc test)
$^{x, y}$ Different superscripts within column indicate an effect of breed for each storage duration point ($P<0.05$, Student's t test).

Table 2. Effect of storage duration (0, 2, 8 and 24 h) and milk-based extenders (SCOM and MCAM) at 15°C on total motility (TM), progressive motility (PM), viability, abnormality, membrane integrity and lipid peroxidation (LPO) of INRA180 ram semen (Mean±S.E.M)

Storage periods	Extender	TM (%)	PM (%)	Viability (%)	Abnormality (%)	Membrane integrity (%)	LPO (nmol TBARS/10^8 sperm)
0 h	MCAM	92.55 ± 0.47^{Ax}	70.88 ± 0.93^{Ax}	93.33 ± 0.79^{Ax}	5.66 ± 0.66^{Cx}	87 ± 0.5^{Ax}	0.49 ± 0.04^{Bx}
	SCOM	92.77 ± 0.46^{ax}	68 ± 1.91^{ax}	92.11 ± 0.63^{ax}	6.33 ± 0.33^{cx}	87.11 ± 0.45^{ax}	0.55 ± 0.03^{dx}
2 h	MCAM	91.88 ± 0.65^{Ax}	69.88 ± 0.53^{Ax}	93.22 ± 0.75^{Ax}	6.66 ± 0.66^{Cx}	86.66 ± 0.54^{Ax}	0.63 ± 0.05^{By}
	SCOM	91.77 ± 0.46^{ax}	67.55 ± 0.47^{ay}	91.55 ± 0.72^{ax}	6.66 ± 0.47^{cx}	86 ± 0.54^{ax}	1.37 ± 0.05^{cx}
8 h	MCAM	85.11 ± 0.63^{Bx}	63.22 ± 2.36^{Bx}	89.44 ± 0.64^{Bx}	23.11 ± 0.75^{Bx}	83.22 ± 0.46^{Bx}	1.41 ± 0.02^{Ay}
	SCOM	86.11 ± 0.87^{bx}	57.66 ± 0.44^{by}	88.11 ± 0.58^{bx}	22.66 ± 0.66^{bx}	79.77 ± 0.70^{by}	1.66 ± 0.02^{bx}
24 h	MCAM	84 ± 1.09^{Bx}	55.11 ± 2.75^{Cx}	84.55 ± 0.44^{Cx}	29.33 ± 1.29^{Ax}	78.77 ± 0.64^{Cx}	1.68 ± 0.18^{Ay}
	SCOM	83.33 ± 0.84^{cx}	55.66 ± 0.57^{bx}	82.66 ± 0.52^{cy}	30.44 ± 1.44^{ax}	71.88 ± 0.58^{cy}	2.73 ± 0.07^{ax}

MCAM: Modified camel milk; SCOM: Skim cow milk

[a, b, c] Different superscripts within column indicate an effect of storage duration on INRA180 ram semen extended with SCOM (P<0.05, Tukey's post hoc test).

[A, B, C] Different superscripts within column indicate an effect of storage duration on INRA180 ram semen extended with MCAM (P<0.05, Tukey's post hoc test).

[x, y] Different superscripts within column indicate an effect of milk extender for each storage duration point (P<0.05, Student's t test).

Table3. Effect of storage duration (0, 2, 8 and 24 h) and milk-based extenders (SCOM and MCAM) at 15°C on total motility (TM), progressive motility (PM), viability, abnormality, membrane integrity and lipid peroxidation (LPO) of Boujaâd ram semen (Mean±S.E.M)

Storage duration	Extender	TM (%)	PM (%)	Viability (%)	Abnormality (%)	Membrane integrity (%)	LPO (nmol TBARS/10^8 sperm)
0h	MCAM	91.22 ± 0.64^{Ax}	71.88 ± 1.08^{Ax}	94.33 ± 0.86^{Ax}	5.88 ± 0.58^{Dx}	87.44 ± 0.47^{Ax}	0.55 ± 0.01^{Dx}
	SCOM	91.33 ± 0.55^{ax}	70.66 ± 0.6^{ax}	93.33 ± 0.76^{ax}	6.55 ± 0.44^{cx}	86.66 ± 0.44^{ax}	0.57 ± 0.02^{dx}
2h	MCAM	90.66 ± 0.64^{Ax}	69.88 ± 0.96^{Ax}	91.11 ± 0.75^{Bx}	11.11 ± 0.58^{Cx}	85.66 ± 0.54^{Bx}	0.98 ± 0.06^{Cy}
	SCOM	84.33 ± 0.70^{by}	62.66 ± 0.57^{by}	90.77 ± 0.74^{ax}	10 ± 0.47^{bx}	71.44 ± 0.70^{by}	1.25 ± 0.04^{cx}
8h	MCAM	87.66 ± 0.60^{Bx}	68.88 ± 0.48^{Ax}	84.77 ± 0.72^{Cx}	21.11 ± 0.75^{By}	83.66 ± 0.37^{Cx}	1.43 ± 0.05^{By}
	SCOM	83.11 ± 0.48^{by}	60.11 ± 0.80^{cy}	83.44 ± 0.60^{bx}	24 ± 0.72^{ax}	66.88 ± 0.77^{cy}	2.1 ± 0.03^{bx}
24h	MCAM	86.77 ± 0.66^{Bx}	59.22 ± 0.68^{Bx}	82.77 ± 0.6^{Cx}	25.77 ± 1.43^{Ax}	78.33 ± 0.52^{Dx}	2.04 ± 0.03^{Ay}
	SCOM	82.55 ± 0.66^{by}	56.88 ± 0.63^{dy}	81.55 ± 0.55^{bx}	26 ± 0.86^{ax}	64.77 ± 0.49^{cy}	2.72 ± 0.04^{ax}

MCAM: Modified camel milk; SCOM: Skim cow milk

[a, b, c] Different superscripts within column indicate an effect of storage duration on Boujaâd ram semen extended with SCOM (P<0.05, Tukey's post hoc test).

[A, B, C] Different superscripts within column indicate an effect of storage duration on Boujaâd ram semen extended with MCAM (P<0.05, Tukey's post hoc test).

[x, y] Different superscripts within column indicate an effect of milk extender for each storage duration point (P<0.05, Student's t test).

At 24 h of storage, TM showed a significant decrease in INRA180 ram sperm only, while sperm PM, viability, and HOST were decreased in both ram breeds. Sperm abnormality and LPO increased in both ram breeds (P<0.05). Sperm PM was significantly higher in Boujaâd compared to INRA180 rams, but sperm viability and abnormality were higher in INRA180 rams (Table 1).

Effect of storage duration and extender type on INRA180 ram sperm quality

The combination of storage duration and extenders for INRA180 ram breed (Table 2) showed that; sperm TM, PM, viability, abnormality and membrane integrity were unchanged from 0 h to 2 h of storage regardless of the extender type used. The LPO was not affected by 2 h of storage in MCAM, while it was significantly increased in SCOM from 0 h to 2 h of liquid storage.

At 8 h of storage, a significant decrease in sperm TM, PM, viability and HOST and a significant increase in sperm abnormality and LPO were recorded in both extenders.

At 24 h, sperm TM and LPO were unchanged in MCAM while a decrease in sperm PM, viability, HOST and an increase in sperm abnormality were recorded. The sperm stored in SCOM exhibited a significant decrease in TM, PM, viability and HOST, and a significant increase in abnormality and LPO.

Sperm TM and abnormality in INRA180 rams were

not affected by the extender type at different times of storage. Sperm PM was higher in MCAM than in SCOM from 2 h to 8 h of liquid storage. Sperm viability was higher in MCAM at 24 h of storage compared to SCOM. However, the HOST was intact in MCAM from 8 h of storage onwards compared to SCOM. Lipid peroxidation decreased also by MCAM from 2 h of storage onwards, compared to SCOM (Table 2).

Effect of storage duration and extender type on sperm quality in Boujaâd rams

In Boujaâd rams, sperm TM and PM were not affected by storage duration from 0 h to 2 h in MCAM (Table 3). However, a significant decrease in sperm viability and HOST, and a significant increase in abnormality and LPO were recorded after 2 h of storage in MCAM.

Sperm that were stored in SCOM showed lower TM, PM and HOST, and higher abnormality and LPO. Sperm viability was not affected up to 2 h of storage. At 8 h of storage, the sperm extended in MCAM recorded a decrease in TM, and HOST and an increase in abnormality and LPO, while no significant change was recorded in viability. Sperm extended in SCOM showed a decrease in PM, viability and HOST, and an increase in abnormality and LPO, however, sperm viability was not affected ($P>0.05$).

At 24 h of storage in MCAM, sperm TM and viability were unchanged, while a decrease in PM and HOST and an increase in abnormality and LPO were recorded. The samples extended in SCOM showed a decrease in PM, and a significant increase in LPO, whereas TM, viability, abnormality and HOST were not affected ($P>0.05$).

The extender MCAM recorded higher TM, PM and membrane integrity and lower LPO level compared to SCOM from 2 h of storage onwards ($P<0.05$) but sperm viability and abnormality were not affected by the type of extender whatever the time of storage was (Table 3).

Discussion

The study showed that the storage duration and extender type affected the sperm quality. It was also found that the semen from the two breeds were not similarly affected as also previously reported by Kasimanickam et al. (2007). The recorded differences could not be attributed to factors such as high ambient temperature leading to heat stress, semen collection frequency, and age as the rams were treated in the same manner. Most studied parameters decreased from 8 h of storage. These findings are in agreement with López–Sáez et al., (2000), who reported that regardless of diluents, dilution rate, temperature, or storage conditions, the quality of spermatozoa decreased with the advancement of storage time. The main changes that occur during storage include a reduction in motility, membrane integrity and viability and an increase in lipid peroxidation, abnormalities and DNA fragmentation (Allai et al., 2017; 2016; 2015; Bucak and Tekin, 2007). The possible physiological reasons for this decline might be related to extracellular oxidative stress (Hong et al., 2010; Lamirande et al., 1997; Maxwell and Salamon, 1993; Vishwanath and Shannon, 2000). To solve such problems, camel milk was selected in this experiment for its physical–chemical particularities. It showed a clear beneficial effect compared to SCOM, especially on motility, lipid peroxidation, and membrane integrity. Previous studies showed that camel milk is rich in vitamin C, cholesterol, and proteins (Al haj and Al Kanhal, 2010, Gorban and Izzeldin, 1999; Khaskheli et al., 2005) known to induce good effects on maintaining sperm quality. For instance, vitamin C which is a major chain–breaking antioxidant neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination (Agarwal et al., 2004). Its level in camel milk is three times higher than in cow milk (Farah et al., 1992). Moreover, when ascorbic acid was added to the cow skim milk extender, it prevented membrane lipid peroxidation during storage and thus had protective effects on the sperm membrane integrity (Aurich et al., 1997). Like vitamin C, cholesterol has been found to be higher in the camel milk than in the cow milk (Gorban and Izzeldin, 1999). This compound is known to maintain the quality of spermatozoa, especially when cholesterol to phospholipids ratio is low and cells become more sensitive to low temperature damage than those with higher ratios (Watson, 1981; Hartwig et al., 2014). Furthermore, it was shown that extender supplemented with cholesterol protected the ovine sperm membrane structure during cooling (Mocé et al., 2010). Another compound of interest is lactoferrin which may be useful in the preservation of spermatozoa. Previous studies showed that lactoferrin is present higher concentration in the camel milk than in the bovine milk (El–Hatmi et al., 2006; Konuspayeva et al., 2007). It is considered as a source of free radical–scavenging peptide having a beneficial effect as an antioxidant in semen (Lien, 2003) with the possibility to improve the quality of preserved ram sperm (Ollero et al., 1998). Likewise, the most protective constituent of milk seems to be casein micelles (the major proteins of milk). In fact, it has been shown that these micelles isolated from milk can protect ram

sperm (Choong and Wales, 1962), by preventing the binding of the sperm to the major proteins of ram seminal plasma (Yue et al., 2009). Furthermore, it is reported that the molecular weights of caseins in camel milk are higher compared to that of cow milk (Salmen et al., 2012) which may impart protective characteristics of the former.

Lactose is considered to be a source of energy for spermatozoa (Salamon and Maxwell, 2000). However, lactose is more abundant in camel milk than cow's milk (Khaskheli et al., 2005). Paradoxically, in the present work, the motility of sperm stored in MCAM was higher compared to that in SCOM. This may be due to an increase in lactic acid accumulated by time caused by the sperm anaerobic metabolism leading to changes in both osmotic pressure and pH in the media exerting a deleterious effect on the sperm cells (Seleem et al., 2009); this may in turn cause a decrease in content of adenosine triphosphate resulting in inactivation of spermatozoa (Zeidan et al., 2002).

The level of magnesium is also higher in camel milk than in cow milk (Sawaya et al., 1984; Abu–Lehia, 1987). Moreover, a positive correlation between magnesium in seminal plasma and sperm motility in ram was observed (Abdel–Rahman et al., 2000). Elagamy (2000) underlined that camel milk contained high levels of antimicrobial agents such as lysozyme, lactoferrin, immunoglobulins, lacto–peroxidase/thiocyanate/hydrogen peroxide system (LSP) and free fatty acids. These may have beneficial effects on diluted semen quality (Sone et al., 1982).

The richness of camel milk in terms of antioxidative and antimicrobial compounds compared to cow milk (Kula, 2016) can explain the advantage of this milk in maintaining the quality (mainly HOST and LPO) of ram sperm during liquid storage, compared to the cow milk. Altogether, the above-mentioned substances make camel milk as an alternative to cow's milk for ram sperm storage at 15°C during 24 h. However, in this study, camel milk was processed to eliminate the lipid fraction, which hinders the microscopic evaluation; this may or not influence the ability of camel milk for sperm storage. For this reason, studies are needed to optimize its use of ram sperm liquid storage. Further studies should focus on heat treatment, antibiotic addition, centrifugation speed and storage temperature combined with field fertility evaluation.

Conclusions

Sperm quality parameters declined by increasing the storage time for both breeds and in the two milk extenders, but not with the same tendency. This could be due to differences in seminal plasma composition between the two ram breeds. The findings introduce new perspectives in ram semen storage as that seminal plasma has a critical role in semen storage. Modified camel milk can be used as a main extender instead of skim cow milk extender for ram semen liquid storage in geographical locations producing large amounts of camel milk. In addition, a full fertility trial will be necessary to confirm the beneficial effects of modified camel milk as an extender in ram semen liquid preservation.

Acknowledgments

This work was carried out at the Regional Center for Agricultural Research in SETTAT (INRA Morocco). The authors are grateful to ALF SAHL for proving a commercial food for animals. We thank the staff of this Center for their cooperation and CNRST of Morocco and the Ministry of high education in Morocco for supporting a part of this work (PPR15/47). Anass Benmoula has a CNRST (National Center for Scientific and Technical Research) Scholarship (003UHP2014).

References

Abdel-Rahman, H.A., El-Belely, M.S., Al-Qarawi, A.A., El-Mougy, S.A., 2000. The relationship between semen quality and mineral composition of semen in various ram breeds. *Small Ruminant Research* 38, 45-49.

Abu–Lehia, I.H., 1987. Composition of camel milk. *Milchwissenschaft - Milk Science International* 42, 368-371.

Agarwal, A., Said, T.M., 2004. Carnitines and male infertility. *Reproductive BioMedicine Online* 8, 376-384.

Akhter, S., Ansari, M.S., Andrabi, S.M.H., Ullah, N., Qayyum, M., 2008. Effect of antibiotics in extender on bacterial and spermatozoal quality of cooled buffalo (Bubalus bubalis) bull semen. *Reproduction in Domestic Animals* 43, 272-278.

Al haj, O.A., Al Kanhal, H.A., 2010. Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal* 20, 811-821.

Allai, L., Druart, X., Contell, J., Louanjli, N., Benmoula, A, Badi, A., Essamadi, A., Nasser, B., El Amiri, B., 2015. Effect of argan oil on liquid storage of ram semen in Tris or skim milk based extenders. *Animal Reproduction Science* 160, 57-67.

Allai, L., Druart, X., Louanjli, N., Contell, J., Nasser, B., El Amiri, B., 2017. Improvements of ram semen quality using cactus seed oil during liquid preservation in Tris egg yolk and skim milk based extenders. *Small Ruminant Research* 151, 16-21.

Allai, L., Druart, X., Öztürk, M., Benmoula, A., Nasser, B., El Amiri, B., 2016. Protective effects of Opuntia ficus–indica extract on ram sperm quality, lipid peroxidation and DNA fragmentation during liquid storage. *Animal Reproduction Science* 175, 1-9.

Aurich, J.E., Schonherrt, U., Hoppe, H., Aurich C, 1997. Effect of antioxidants on motility and membrane integrity of child– stored stallion semen. *Theriogenology* 48, 185-192.

Bowen, J.M., 1969. Artificial insemination in the horse. *Equine Veterinary Journal* 1, 98-110.

Bucak, M.N., Tekin, N., 2007. Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. *Small Ruminant Research* 73, 103-108.

Choong, C.H., Wales, R.G., 1962. The effect of cold shock on spermatozoa. *Australian Journal of Biological Science* 15,543-551.

Colas, G., Dauzier, L., Courot, M., Ortavant, R., Signoret, J.P., 1968. Résultats obtenus au cours de l'étude de quelques facteurs importants de l'insémination artificielle ovine. *Annales de zootechnie* 17, 47-57.

Elagamy, E.I., 2000. Effect of heat treatment on camel milk proteins with respect to antimicrobial factors: a comparison with cows' and buffalo milk proteins. *Food Chemistry* 68, 227–232.

El–Badry, D.A., Gomaa, A.M., Shaker, M.H., 2007. The use of camel's milk as extender for buffalo semen. Proceedings of the 19th Congress on Animal Reproduction and Fertility, Hurghada, Egypt.

El–Hatmi, H., Levieux, A., Levieux, D., 2006. Camel (*Camelus dromedarius*) immunoglobulin G, alpha–lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early post-partum period. *Journal of Dairy Research* 73, 288-293.

Farah, Z., Rettenmaier, R., Atkins, D., 1992. Vitamin content of camel milk. *International Journal for Vitamin and Nutrition Research* 62, 30-33.

Faye, B., Jaouad, M., Bhrawi, K., Senoussi, A., Bengoumi, M., 2014. Elevage camelin en Afrique du Nord : état des lieux et perspectives. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* 67, 213-221.

Gorban, A.M.S., Izzeldin, O.M., 1999. Study on cholesteryl ester fatty acids in camel and cow milk lipid. *International Journal of Food Science & Technology* 34, 229-234.

Hartwig, F.P., Lisboa, F.P., Hartwig, F.P., Monteiro, G.A., Maziero, R.R.D., Freitas–Dell'Aqua, C.P., Alvarenga, M.A., Papa, F.O., Dell'Aqua, J.A., 2014. Use of cholesterol–loaded cyclodextrin: an alternative for bad cooler stallions. *Theriogenology* 81, 340–346.

Hong, Z., Hailing, L., Hui, M., Guijie, Z., Leyan, Y., Dubing, Y., 2010. Effect of vitamin E supplement in diet on antioxidant ability of testis in Boer goat. *Animal Reproduction Science* 117, 90-94.

Kasimanickam, R., Kasimanickam, V., Pelzer, K.D., Dascanio, J.J., 2007. Effect of breed and sperm concentration on the changes in structural, functional and motility parameters of ram–lamb spermatozoa during storage at 4 °C. *Animal Reproduction Science* 101, 60-73.

Khaskheli, M., Arain, M.A., Chaudhry, S., Soomro, A.H., Qureshi, T.A., 2005. Physico-chemical quality of camel milk. *Journal of Agriculture and Social Sciences* 1, 2.

Konuspayeva, G., Faye, B., Loiseau, G., Levieux, D., 2007. Lactoferrin and immunoglobulin contents in camel's milk (Camelus bactrianus, Camelus dromedarius, and Hybrids) from Kazakhstan. *Journal of Dairy Science* 90, 38-46.

Kula, J., 2016. Medicinal values of camel milk. *International Journal of Veterinary Science and Research* 2, 018–025.

Kulaksız, R., Çebi, Ç., Akçay, E., Daşkın, A., 2010. The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. *Small Ruminant Research* 88, 12-15.

de Lamirande, E., Leclerc, P., Gagnon, C., 1997. Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Molecular Human Reproduction* 3, 175-194.

Lien, E.L., 2003. Infant formulas with increased concentrations of alpha–lactalbumin. *The American Journal of Clinical Nutrition* 77, 1555-1558.

López–Sáez, A., Ortiz, N., Gallego, L., Garde, J.J., 2000. Liquids storage (5°C) of ram semen in different diluents. *Archives of Andrology* 44, 155-164.

Maia, M.S, 2006. Sperm Viability and Reactive Oxygen Species (ROS) Generation in ram semen cryopreserved in extenders supplemented with sodium lauryl sulfate (OEP), Trolox–C and catalase. Ph.D. Thesis, Faculty of Veterinary Medicine and Animal Science, São Paulo State University.

Maxwell, W.M., Salamon, S., 1993. Liquid storage of ram semen: a review. *Reproduction Fertility Development* 5, 613-638.

Mocé, E., Purdy, P.H., Graham, J.K., 2010. Treating ram sperm with cholesterol–loaded cyclodextrins improves cryosurvival. *Animal Reproduction Science* 118, 236-247.

Ollero, M., Perez–Pe, R., Muiño–Blanco, T., Cebrian–Perez, J.A., 1998. Improvement of ram sperm cryopreservation protocols assessed by sperm quality parameters and heterogeneity analysis. *Cryobiology* 37, 1-12.

Revell, S., 2003. Assessment of the morphology of frozen–thawed bull sperm in relation to its cryopreservation for artificial insemination. *Veterinary Record* 152, 765-767.

Salamon, S., Maxwell, W.M.C., 2000. Storage of ram's semen. *Animal Reproduction Science* 62, 77-111.

Salmen, S.H., Abu–Tarboush, H.M., Al–Saleh, A.A., Metwalli, A.A., 2012. Amino acids content and electrophoretic profile of camel milk casein from different camel breeds in Saudi Arabia. *Saudi Journal of Biological Science* 19, 177-183.

Sawaya, W.N., Khalil, J.K., Al–Shalhat, A., Al–Mohammad, H., 1984. Chemical composition and nutritional quality of camel milk. *Journal of Food Science* 49, 744-747.

Sboui, A., Khorchani, T., Djegham, M., Belhadj, O., 2009. Comparaison de la composition physicochimique du lait camelin et bovin du Sud tunisien ; variation du pH et de l'acidité à différentes températures. *Afrique science : revue internationale des sciences et technologie* 5.

Seleem, T.S.T., Rowida., Riad, M., El–Badry, D.A., Gomaa, A.M., 2009. Effect of camel milk diluent with or without antibiotics on fertilizing ability of rabbit spermatozoa. *Journal of the Egyptian Veterinary Medical Association* 69, 97-110.

Sone, M., Ohmura, K., Bamba, K., 1982. Effects of various antibiotics on the control of bacteria in boar semen. *Veterinary Record* 111, 11-14.

Vishwanath, R., Shannon, P., 2000. Storage of bovine semen in liquid and frozen state. *Animal Reproduction Science* 62, 23-53.

Watson, P.F., 1981. The effects of cold shock on sperm cell membranes. In: Morris, G.J., Clarke, A. (Eds), The Effects of Low Temperatures on Biological Membranes. Academic Press, London, pp. 189-218.

Yániz, J.L., Santolaria, P., Marco–Aguado, M.A., López–Gatius, F., 2008. Use of image analysis to assess the plasma membrane integrity of ram spermatozoa in different diluents. *Theriogenology* 70, 192-198.

Yue, W., Shi, L., Bai, Z., Ren, Y., Zhao, Y., 2009. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of ram seminal plasma proteins and their correlation with semen characteristics. *Animal Reproduction Science* 116, 386-391.

Zeidan, A.E.B., Daader, A.H., El-Darawany, A.A., Abd El-Salaam, A.M., 2002. Effects of different extenders and cryoprotective agents on the frozen rabbit semen quality. Proceedings of the 3rd Scientific Conference on Rabbit Production in Hot Climates, Hurghada, Egypt.

Communicating editor: *Mohammad Javad Zamiri*