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

A. Benmoula¹², A. Badi¹, A. Hilali², K. El Khalil¹, L. Allai², M. El Fadili³ and B. El Amiri¹*

¹INRA–Centre Régional de la Recherche Agronomique de Settat, BP589, Settat, Morocco.
²Laboratoire d’agroalimentaire et santé, Faculté des Sciences et Techniques, Université Hassan1, BP 577, 26000 Settat, Morocco.
³INRA–Division Scientifique, Département des Productions Animales, BP 415 RP, Avenue Hassan II, Rabat, Morocco.

* 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 0.8×10⁹ 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 economic 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 >2.10⁹ 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 0.8×10⁹ 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 0.8×10⁹ 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 20×10⁹ sperm/mL.

2. **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 x, 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.

3. **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 x, light microscopy).

4. **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 (400x, UB203 microscope).

5. **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-
Evaluation of camel milk as an extender for ram semen

choloric acid 0.25 N, thiobarbituric acid 0.375 % w/v in distilled water) and 1 % (v/v) of BHT (Butylated-hydroxytoluene) solution at 50 mM were added to the 250 μL samples diluted with (Tris–hydroxymethyl–ammonomethane 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 10⁸ 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 × 10⁻⁵ M⁻¹ cm⁻¹ for thiobarbituric acid and expressed in nmol TBARS/10⁸ spermatozoa.

**Statistical analysis**

Statistical analyses were performed using JMP SAS 11.0.0 (SAS Institute Inc., Cary, 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 24 h) 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 breed or base extender at each storage duration point (P<0.05). However, sperm abnormality and LPO were not affected by the ram breed.

**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)

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Breed</th>
<th>TM (%)</th>
<th>PM (%)</th>
<th>Viability (%)</th>
<th>Abnormality (%)</th>
<th>Membrane integrity (%)</th>
<th>LPO (nmol TBARS/10⁸ sperm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>Boujaâd</td>
<td>91.27±0.41Ax</td>
<td>70.27±0.55Ax</td>
<td>93.83±0.57Ax</td>
<td>6.22±0.36Ax</td>
<td>87.05±0.32Ax</td>
<td>0.56±0.01Bx</td>
</tr>
<tr>
<td></td>
<td>INRA180</td>
<td>92.66±0.32Ax</td>
<td>69.44±1.09Ax</td>
<td>92.72±0.51Ax</td>
<td>6±0.37Ax</td>
<td>87.05±0.32Ax</td>
<td>0.52±0.03Bx</td>
</tr>
<tr>
<td>2 h</td>
<td>Boujaâd</td>
<td>87.5±0.89Bx</td>
<td>67.27±1.26AB</td>
<td>90.94±0.50Bx</td>
<td>10.55±0.38Cx</td>
<td>78.77±1.82Bx</td>
<td>1.12±0.05Cx</td>
</tr>
<tr>
<td></td>
<td>INRA180</td>
<td>91.83±0.38Ax</td>
<td>68.72±0.44Ax</td>
<td>92.38±0.54Ax</td>
<td>6.66±0.39y</td>
<td>86.11±0.34Ax</td>
<td>1±0.09Cx</td>
</tr>
<tr>
<td>8 h</td>
<td>Boujaâd</td>
<td>85.11±0.75Bx</td>
<td>64.5±1.15Bx</td>
<td>84.11±0.48y</td>
<td>22.55±0.61Bx</td>
<td>75.27±2.07Bc</td>
<td>1.76±0.08Bx</td>
</tr>
<tr>
<td></td>
<td>INRA180</td>
<td>85.61±0.53Bx</td>
<td>60.44±1.34Bx</td>
<td>88.77±0.45Bx</td>
<td>28.88±0.49Bx</td>
<td>81.5±0.58Bx</td>
<td>1.53±0.03B</td>
</tr>
<tr>
<td>24 h</td>
<td>Boujaâd</td>
<td>84.94±0.59Bx</td>
<td>58.05±0.53C</td>
<td>82.16±0.42Bx</td>
<td>25.88±0.81Bx</td>
<td>71.5±1.68C</td>
<td>2.38±0.08CA</td>
</tr>
<tr>
<td></td>
<td>INRA180</td>
<td>83.66±0.67C</td>
<td>55.38±1.14y</td>
<td>83.61±0.40C</td>
<td>29.88±0.94C</td>
<td>75.33±0.93C</td>
<td>2.2±0.16C</td>
</tr>
</tbody>
</table>

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)
a, 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.)

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

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).

xy Different superscripts within column indicate an effect of milk extender for each storage duration point (P<0.05, Student’s t test).

Table 3. 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 Boujaad ram semen (Mean±SEM)

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Extender</th>
<th>TM (%)</th>
<th>PM (%)</th>
<th>Viability (%)</th>
<th>Abnormality (%)</th>
<th>Membrane integrity (%)</th>
<th>LPO (nmol TBARS/10^8 sperm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>MCAM</td>
<td>91.22±0.64Ax</td>
<td>71.88±1.08Ax</td>
<td>94.33±0.86Ax</td>
<td>5.88±0.58Dx</td>
<td>87.44±0.47Ax</td>
<td>0.55±0.01Ax</td>
</tr>
<tr>
<td></td>
<td>SCOM</td>
<td>91.33±0.55Ax</td>
<td>70.66±0.6Ax</td>
<td>93.3±0.76Ax</td>
<td>6.55±0.44Ax</td>
<td>86.66±0.44Ax</td>
<td>0.57±0.02Ax</td>
</tr>
<tr>
<td>2h</td>
<td>MCAM</td>
<td>90.66±0.64Ax</td>
<td>69.88±0.76Ax</td>
<td>91.11±0.75Bx</td>
<td>11.11±0.58Cy</td>
<td>85.66±0.54Bx</td>
<td>0.98±0.06Cy</td>
</tr>
<tr>
<td></td>
<td>SCOM</td>
<td>84.33±0.70Bx</td>
<td>62.66±0.57Bx</td>
<td>90.77±0.74Ax</td>
<td>10±0.47Bx</td>
<td>71.44±0.70Bx</td>
<td>1.25±0.04Ax</td>
</tr>
<tr>
<td>8h</td>
<td>MCAM</td>
<td>87.66±0.60Bx</td>
<td>68.88±0.48Ax</td>
<td>84.77±0.72Cx</td>
<td>21.11±0.75By</td>
<td>83.66±0.37Cy</td>
<td>1.43±0.05By</td>
</tr>
<tr>
<td></td>
<td>SCOM</td>
<td>83.11±0.48Bx</td>
<td>60.11±0.80By</td>
<td>83.44±0.60Bx</td>
<td>24±0.72Bx</td>
<td>66.88±0.77By</td>
<td>2.1±0.03Bx</td>
</tr>
<tr>
<td>24h</td>
<td>MCAM</td>
<td>86.77±0.66Bx</td>
<td>59.22±0.68Bx</td>
<td>82.77±0.6Cx</td>
<td>25.77±1.43Ax</td>
<td>78.33±0.52Dx</td>
<td>2.04±0.03Cy</td>
</tr>
<tr>
<td></td>
<td>SCOM</td>
<td>82.55±0.66Bx</td>
<td>56.88±0.63Bx</td>
<td>81.55±0.55Bx</td>
<td>26±0.86Ax</td>
<td>64.77±0.49y</td>
<td>2.72±0.04Ax</td>
</tr>
</tbody>
</table>

MCAM: Modified camel milk; SCOM: Skim cow milk

a, b, c Different superscripts within column indicate an effect of storage duration on Boujaad 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 Boujaad ram semen extended with MCAM (P<0.05, Tukey’s post hoc test).

xy 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 Boujaad 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 TM, 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 Kasimianickam 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 spermatzoa 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 (Khashkeli 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
Evaluation of camel milk as an extender for ram semen


*Communicating editor: Mohammad Javad Zamiri*