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Liquid storage of dromedary camel semen in different extenders

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ABSTRACT

This study was conducted to assess the effects of commercial extenders and storage temperature on dromedary camel sperm quality during liquid preservation. In Experiment 1, ejaculates (n = five males; replicated seven times) were split and diluted with synthetic (OPTIXcell, EquiPlus, INRA96, Bioxcell or AndroMed; Experiment 1a) or egg-yolk based (Biladyl, Green buffer or Triladyl; Experiment 1b) extenders and stored for 48 h at 4 °C. In Experiment 2, split ejaculates (n = five males; replicated six times) were used to directly compare Green buffer, OPTIXcell and Triladyl extenders over 48 h of storage at 4 °C. Ejaculates collected in Experiment 3 (n = five males; replicated five times) were diluted with Green buffer or Triladyl before chilled storage for 48 h at 4 or 15 °C. Sperm kinematics, viability and acrosome integrity were assessed during liquid storage. In Experiment 1a, there was the greatest total sperm motility (TM) in the OPTIXcell group following 24 and 48 h of storage, while in Experiment 1b, there was the greatest TM after 48 h of storage with Triladyl and Green buffer. In Experiment 2, there were greater TM and viable acrosome intact spermatozoa in the Triladyl and Green buffer than with OPTIXcell group. In Experiment 3, there was a greater TM in the Triladyl than Green buffer group at 24 and 48 h of storage regardless of storage temperature (which had no effect on sperm quality). In conclusion, camel sperm have greater viability when preserved in liquid form for 48 h following dilution with Triladyl and storage at either 4 or 15 °C.

1. Introduction

Artificial insemination (AI) is primarily used in domestic animals to rapidly disseminate superior male genetics. In dromedary camels, AI is not well developed and has only been used successfully on a small number of animals (Skidmore and Billah, 2006). This is largely due to the inability to consistently store and retain sperm viability of camel semen beyond 24 h (reviewed by Skidmore et al., 2018), which prevents convenient use in females distant from the site of semen collection or that are not in a suitable reproductive state when freshly collected semen is available.

Egg yolk-based extenders have been used successfully for liquid storage of semen in rams (Kasimanickam et al., 2011; Quan et al., 2016), stallions (Brogan et al., 2015) and buffalo bulls (Akhter et al., 2011). The low-density lipids (LDL) in egg yolk provide protection to spermatozoa during liquid storage or cryopreservation (Quinn et al., 1980; Graham and Foote, 1987; Bergeron and Manjunath, 2006). The chemically defined, soya-lecithin containing Bioxcell extender in buffalo (Akhter et al., 2011), caseinates containing EquiPlus and INRA96 extenders used for stallion semen storage (LeFrapper et al., 2010) and liposome containing

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OPTIXcell extender (Ansari et al., 2016) for protection of spermatozoa during liquid storage or cryopreservation.

The marked damaging effects of cryopreservation on camel sperm function (Crichton et al., 2016) make chilled, liquid preservation the most realistic current option for semen storage. This is a viable commercial option considering the main market for camel semen in the Gulf States of Middle East, which are all accessible well within a 48 h transit period.

Numerous extenders have been tested in attempts to preserve camel spermatozoa during liquid storage with varying results. In previous studies, there has been evaluation of the efficiency of egg yolk extenders containing Tris (Deen et al., 2004; Niasari-Naslaji et al., 2006; Wani et al., 2008; Panahi et al., 2017), citrate (Wani et al., 2008), sucrose (Wani et al., 2008), lactose (Hassan and Saeed, 1995), Green buffer (Morton et al., 2010, 2013), a milk-based Tris extender (Panahi et al., 2017), and other synthetic extenders such as Biociphos (Deen et al., 2004) and INRA96 (Morton et al., 2010, 2013) to store semen at 4 °C. Few of these extenders, however, had the capacity to maintain sperm motility ≥50% following 24 h of chilled storage (Tris based egg yolk extender, Niasari-Naslaji et al., 2006; Green buffer and INRA96, Morton et al., 2013). Beyond extender choice, storage temperature has also been investigated in an effort to improve sperm quality but no precise temperature has been identified as superior (Wani et al., 2008).

From an artificial breeding and AI perspective, it is imperative to identify an extender which has the capacity to maintain sperm motility for more than 24 h. This would facilitate semen transport during storage around the Gulf States to different camel studs. In many of the studies in which there has been investigation of extenders for liquid sperm preservation in this species, there has been a focus on traditional methods of sperm analysis, such as subjective motility assessment. The application of functional objective tests such as dynamic assessment of sperm motility and other kinematic patterns using advanced computer assisted sperm analysis (CASA) and assessment of membrane viability and acrosome integrity using fluorescent microscopy (reviewed by Rodríguez-Martínez, 1998) can result in a more detailed assessment of sperm quality.

While it is acknowledged that field studies investigating pregnancy rates following AI are integral to properly assess the effect of extenders on fertility, these studies are costly, labor intensive and time consuming. Before such studies can be performed, *in vitro* studies incorporating the previously described objective functional sperm assessments must be conducted to evaluate the effect of different extenders on the semen quality of chilled camel spermatozoa. This study, therefore, was designed to systematically evaluate a large number of extenders for the long-term (48 h) liquid storage of dromedary camel semen as well as the most desirable temperature for storage. It was hypothesized that egg-yolk based extenders would be superior to synthetic extenders and lesser storage temperatures would result in improved quality after long periods of chilled storage.

2. Materials and methods

2.1. Semen extenders

Eight commercial extenders were used in this study. All ingredient descriptions are based on supplier-provided information (Table 1). The preparation of each extender was conducted according to the instructions recommended by the manufacturer. Extenders were prepared fresh on the morning of each semen collection and total volumes of extenders were prepared based on the anticipated volume of semen to be processed. For egg yolk-based extenders (Green buffer, Triladyl, and Biladyl), 20% egg yolk was mixed at room temperature on the day of semen collection. After adding the egg yolk, the diluent was centrifuged at 10,000 × *g* for 20 min to remove the egg yolk particles and the supernatant was filtered through 0.45 µm filter.

2.2. Experimental design

The effect of commercial extenders on sperm motion kinematics during liquid storage was evaluated in two phases in Experiment 1. In Experiment 1a, semen was collected from five dromedary male camels. Each ejaculate was divided and diluted in five chemically defined (synthetic) extenders (OPTIXcell, EquiPlus, INRA96, Bioxcell or AndroMed). After complete liquefaction and adjusting the concentration of spermatozoa to 100×10^6 spermatozoa/mL with matching extenders, equal volumes of diluted semen from each male camel were pooled to make a single sample per extender treatment. This experiment was replicated seven times.

Table 1
Characteristics of commercial extenders evaluated in the study.

	Contents	Manufacturer	PH
Chemically defined extenders			
OPTIXcell	Liposomes, carbohydrate, mineral salts, buffer, antioxidants, glycerol, tylosin, gentamicin, spectinomycin and lincomycin	IMV Technologies, France	6.8
EquiPlus	Caseinates derived from various fractions of milk casein, lincomycin and spectinomycin.	Minitub, Tiefenbach, Germany	6.9
INRA96	Purified fractions of the milk micellar protein, penicillin, gentamicin, and amphotericin B.	IMV Technologies, France	7.0
Bioxcell	Soybean derived components, tylosin, gentamicin, spectinomycin and lincomycin.	IMV Technologies, France	6.9
AndroMed	Soy-lecithin, phospholipids, tris, citric acid, antioxidants, glycerol, tylosin, gentamicin, spectinomycin and lincomycin.	Minitub, Tiefenbach, Germany	6.7
Egg yolk-based extenders			
Triladyl	Tris, citric acid, fructose, glycerol, tylosin, gentamicin, spectinomycin and lincomycin	Minitub, Tiefenbach, Germany	6.7
Biladyl (fraction A)	Tris, citric acid, fructose, tylosin, gentamicin, spectinomycin and lincomycin.	Minitub, Tiefenbach, Germany	6.7
Green buffer	Composition is undisclosed.	IMV Technologies, France.	6.9

Experiment 1b was conducted as described above, but compared three egg yolk-based extenders (Green buffer, Biladyl and Triladyl). In both Experiment 1a and 1b samples, sperm motion variables were immediately assessed at 37 °C, before storage at 4 °C for 48 h. Sperm motion assessments (CASA) were then repeated at 24 and 48 h of the storage period.

In Experiment 2, the most effective extenders for sperm processing from Experiment 1a and 1b were compared. Semen was collected from five dromedary male camels and each ejaculate was divided and diluted with OPTIXcell, Green buffer or Triladyl. After complete liquefaction and adjusting the concentration of sperm to 100×10^6 spermatozoa/mL, equal volumes of diluted semen from each male camel was pooled to make a single sample per extender treatment. Pooled semen samples were stored at 4 °C for 48 h. Sperm motion variables (CASA), viability and acrosome integrity (fluorescent microscopy) were assessed after 24 and 48 h of liquid storage. Experiment 2 was replicated six times.

In Experiment 3, the most effective extenders for semen processing in Experiment 2 were selected and effects on sperm function at 4 and 15 °C were compared. Semen was collected from five dromedary male camels and each ejaculate was divided and diluted with either Green buffer or Triladyl. After complete liquefaction and adjusting the concentration of spermatozoa to 100×10^6 spermatozoa/mL, equal volumes of diluted semen from each male camel was pooled to make a single sample per extender treatment. Samples were immediately evaluated for sperm motion variables, viability and acrosome integrity at 37 °C prior to being split and stored at 4 and 15 °C. Sperm motion variables, viability and acrosome integrity of each treatment (two extenders x two storage temperatures) was then evaluated after 24 and 48 h of liquid storage. This experiment was replicated five times.

2.3. Animals

This study was conducted at the Animal Research Centre, Royal Camel Corps, Muscat, Sultanate of Oman, during the peak breeding season of December to March (2015–2016 and 2016–2017). Five dromedary male camels aged between 10–14 years were selected for this study. Male camels were housed in pens and fed fresh green grass/dry fodder, as well as commercial concentrate pellets supplemented with minerals. All animal use and procedures were approved by the Animal Welfare Committee at the Animal Health and Research Centre of the Ministry of Agriculture and Fisheries of the Sultanate of Oman.

2.4. Semen collection

Semen was collected at weekly intervals as described previously (Al-Bulushi et al., 2018) using a bovine artificial vagina (AV: 30 cm length and 5 cm internal diameter). A smooth latex liner was mounted and fixed at both ends of the AV and a transparent graduated glass water-jacketed semen collection vessel was attached to the apex of the internal latex liner. The inner chamber of the AV was filled with water (45–48 °C) to maintain an internal AV temperature of 41–42 °C during semen collection. The water-jacketed semen collection vessel was then filled with warm water (37 °C) and the inner surface of the AV was lubricated. Prior to semen collection, male camels were exposed to a sexually receptive female for a period of 10–15 min, after which the male camel was allowed to approach and mount a female camel restrained in sternal recumbency. The technician positioned on the left side of the female then grasped the male's prepuce, cleaned the preputial orifice and directed the erect penis into the AV for copulation. During copulation, the pressure of the cervix was mimicked manually by holding the latex liner between the AV and semen collection vessel. The semen collection process was considered successful if the camel was sexually excited and copulated into the AV for at least 2 min with ejaculation occurring. If the first collection attempt was unsuccessful then a second attempt at collection occurred 30 min later.

2.5. Semen evaluation

2.5.1. Dilution and preparation of samples

Following collection, the samples were transferred immediately to a 35 °C water bath, diluted 1:1 with pre-warmed extender and incubated in a 35 °C water bath for liquefaction. Samples were pipetted at 5 min intervals to assess liquefaction (Al-Bulushi et al., 2018). After complete liquefaction, sperm concentration was determined using a Makler Counting Chamber (Sefi-Medical Instruments, Haifa, Israel) (Evans and Maxwell, 1987). Semen samples were further diluted to 100×10^6 spermatozoa/mL with the same extender. Samples were taken at 0 h for evaluation and then stored in a water jacket filled with 200 mL of 35 °C water and cooled over 2 h, in a cold cabinet (IMV Technologies) to 4 °C (except Experiment 3, where an additional aliquot was cooled over 1 h to 15 °C, Morton et al., 2009). Samples were then stored for 48 h. Aliquots of each sample were taken at 24 and 48 h of storage after gentle mixing of the sample and further diluted as subsequently described in this manuscript.

2.5.2. Assessment of sperm motion variables

Motion variables of spermatozoa were evaluated using computer assisted sperm assessment (CASA; CEROS, Version12, Hamilton Thorne Biosciences, USA) as described previously (Al-Bulushi et al., 2018). Settings of CASA system used in the study are presented in Table 2.

At each time point, samples were further diluted with matching extender at the same preservation temperature to 50×10^6 spermatozoa/mL and equilibrated in a 35 °C water bath for 5 min. Three microliters of semen was placed on a pre-warmed 20 µm standard count analysis chamber (Leja, Nieuw-Vennep, The Netherlands). Five randomly selected microscopic fields were scanned five times each and approximately 500 spermatozoa counted. The sperm total motility (TM), progressive motility (PM), average path velocity (VAP), progressive velocity (VSL), curvilinear velocity (VCL), lateral head amplitude (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) of spermatozoa were analysed (Al-Bulushi et al., 2018).

Table 2
Settings of the Hamilton Thorne CEROS animal software (Version 12) used to assess camel sperm kinematics.

Variable	Setting
Frame rate	60 Hz
Number of frames acquired	45
Minimum contrast	55
Minimum cell size (pixels)	6
Progressive path velocity cutoff	30 μm/s
Progressive straightness	60%
Slow average path velocity cutoff	10 μm/s
Slow straight line velocity cutoff	5 μm/s
Static average path velocity cutoff	4 μm/s
Static straight line velocity cutoff	1 μm/s
Non-motile head size (pixels)	0.5 to 4.8
Non-motile head intensity	0.25 to 1.8
Magnification	1.87x
Video frequency	60
Illumination intensity	2300
Temperature	37 °C

2.5.3. Assessment of sperm viability and acrosome integrity

Sperm viability and acrosome integrity was evaluated as described previously (Kershaw-Young et al., 2013). Briefly, 90 μL of semen (50×10^6 spermatozoa/mL) was mixed with 10 μL of 1% neutral buffered formalin at room temp to fix and immobilize spermatozoa. Fixed spermatozoa (100 μL) were stained with 6 μL of fluorescent isothiocyanate-conjugated lectin from Arachis hypogaea (working concentration 40 μg/mL; FITC-PNA; Sigma) and incubated at 37 °C for 10 min in a darkened area. Propidium iodide (0.5 μL; working concentration 0.6 mM; PI, Sigma) was then added and the sample incubated for another 5 min. Stained sperm (20 μL) was then placed onto a glass slide and covered with a 22 × 40 mm coverslip. At least 200 spermatozoa per sample were examined with an Olympus BX51 epifluorescence microscope. Spermatozoa stained with both PI and FITC-PNA were classified as non-viable with damaged membranes and a non-intact acrosome. Spermatozoa stained with only PI were considered as non-viable with intact acrosomes. Unstained spermatozoa were classified as viable with intact membranes and acrosomes. Spermatozoa stained with FITC-PNA alone were classified as viable but with a non-intact acrosome.

2.6. Statistical analysis

All statistical analyses were performed in GENSTAT (Version 17, VSN International, Hemel Hempstead, UK). Data recorded on the motion variables and membrane acrosome status were analyzed using repeated measures linear mixed model, utilizing arc-sin transformations to attain normality where necessary. Time (0, 24 and 48 h), extender (OPTIXcell, EquiPlus, INRA96, Bioxcell, AndroMed, Green buffer, Biladyl and Triladyl) and incubating temperature (4 and 15 °C) were specified as fixed effects. Replicate was specified as a random effect. For all models, $P < 0.05$ was considered statistically significant and if there were no significant interactions between fixed factors, the interaction was dropped from the model.

3. Results

3.1. Experiment 1a. Effect of chemically defined extenders on sperm motion variables

The average values for sperm motion variables of spermatozoa following 0, 24 and 48 h of liquid storage at 4 °C are presented in Table 3. There was an interaction of extender and time on the TM, PM, VAP, VSL, VCL, BCF, ALH and STR of spermatozoa ($P < 0.001$). There was an effect of time only on LIN ($P < 0.001$).

The TM of spermatozoa did not differ among extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in OPTIXcell had the greatest TM, followed by AndroMed, EquiPlus and INRA96 while TM was the least when sperm were extended in Bioxcell ($P < 0.05$). At 48 h, TM of spermatozoa diluted in OPTIXcell was greater in comparison to the other extenders ($P < 0.05$), while there was no difference among AndroMed, EquiPlus, INRA96 and Bioxcell ($P > 0.05$; Table 3).

The percentage of spermatozoa having PM at 0 h was greater when diluted in INRA96 and EquiPlus compared to spermatozoa diluted in OPTIXcell, AndroMed and Bioxcell ($P < 0.05$). At 24 h, there was a greater PM of spermatozoa when diluted in OPTIXcell compared to AndroMed and Bioxcell ($P < 0.05$), while there was no difference among OPTIXcell, EquiPlus and INRA96 ($P > 0.05$). At 48 h, the PM of spermatozoa was greatest in OPTIXcell compared to all other extenders ($P < 0.05$; Table 3).

At 0 h, the VAP and VCL of spermatozoa diluted in AndroMed was greater than with OPTIXcell ($P < 0.05$) and there was no difference among other extenders ($P > 0.05$). At 24 h, the VAP and VCL of spermatozoa extended in Bioxcell was less compared to all other extenders ($P < 0.05$). By 48 h, spermatozoa diluted in OPTIXcell had the greatest sustained VAP and VCL ($P < 0.05$), however, there was a decrease for EquiPlus, AndroMed and INRA96 which were similar to that with Bioxcell at this time point ($P > 0.05$; Table 3).

Table 3
Average values for sperm motion variables when diluted in commercial extenders, following liquid storage at 4 °C (Experiment 1a).

Variable	Time (h)	OPTIXcell	EquiPlus	INRA96	Bioxcell	AndroMed
Total motility (TM, %)	0	86.6 ± 1.3	79.1 ± 2.3	77.1 ± 1.5	82.7 ± 1.6	81.0 ± 2.2
	24	71.0 ± 2.6 [a]	41.7 ± 6.0 [b]	42.1 ± 9.4 [b]	12.6 ± 8.6 [c]	41.4 ± 9.9 [b]
	48	61.1 ± 3.0 [a]	8.6 ± 5.0 [b]	13.1 ± 7.7 [b]	2.7 ± 2.7 [b]	5.4 ± 3.5 [b]
Progressive Motility (PM, %)	0	23.0 ± 2.3[a]	29.4 ± 2.6[b]	29.0 ± 2.9[b]	23.7 ± 1.2[a]	22.0 ± 1.4[a]
	24	14.0 ± 1.1 [a]	11.7 ± 1.4 [ab]	10.1 ± 2.2 [ab]	2.6 ± 1.6 [c]	7.4 ± 2.1 [bc]
	48	12.1 ± 1.5 [a]	3.1 ± 1.9 [b]	2.0 ± 1.4 [b]	0.3 ± 0.3 [b]	0.7 ± 0.5 [b]
Path velocity (VAP, μm/s)	0	92.2 ± 7.1 [a]	100.9 ± 6.6 [ab]	95.3 ± 8.9 [ab]	107.3 ± 6.7 [ab]	117.7 ± 5.5 [b]
	24	79.6 ± 4.7 [a]	65.0 ± 7.5 [a]	70.4 ± 14.0 [a]	25.6 ± 13.3 [b]	55.0 ± 11.8 [a]
	48	70.4 ± 6.6 [a]	25.5 ± 13.0 [b]	23.3 ± 12.3 [b]	8.6 ± 8.6 [b]	11.6 ± 7.5 [b]
Progressive velocity (VSL, μm/s)	0	46.2 ± 3.8 [a]	60.7 ± 3.3 [b]	55.1 ± 5.3 [ab]	56.8 ± 3.9 [ab]	59.7 ± 3.6 [b]
	24	36.0 ± 2.3 [a]	35.4 ± 1.8 [a]	37.1 ± 6.8 [a]	13.3 ± 6.5 [b]	24.8 ± 5.1 [b]
	48	34.4 ± 3.1 [a]	12.4 ± 6.2 [b]	13.1 ± 7.4 [b]	3.4 ± 3.4 [b]	5.0 ± 3.2 [b]
Curvilinear velocity (VCL, μm/s)	0	182.0 ± 14.0 [a]	219.4 ± 12.3 [ab]	195.6 ± 18.8 [ab]	224.9 ± 14.7 [ab]	235.3 ± 13.5 [b]
	24	164.9 ± 9.6 [a]	143.8 ± 11.5 [a]	147.9 ± 28.8 [a]	50.9 ± 27.2 [b]	101.8 ± 21.5 [a]
	48	145.8 ± 7.3 [a]	58.0 ± 29.0 [b]	52.5 ± 27.7 [b]	20.2 ± 20.2 [b]	19.7 ± 12.8 [b]
Lateral head amplitude (ALH, μm)	0	9.9 ± 0.6 [a]	12.9 ± 0.7 [b]	11.5 ± 0.6 [ab]	12.3 ± 0.9 [ab]	11.7 ± 0.8 [ab]
	24	9.1 ± 0.6 [a]	10.6 ± 0.6 [a]	9.1 ± 1.6 [a]	2.5 ± 1.3 [b]	5.9 ± 1.2 [c]
	48	9.4 ± 0.4 [a]	3.9 ± 1.9 [b]	3.4 ± 1.7 [b]	1.0 ± 1.0 [b]	1.3 ± 0.8 [b]
Beat cross frequency (BCF, Hz)	0	29.1 ± 0.8	28.4 ± 0.9	27.0 ± 0.9	27.9 ± 1.0	26.5 ± 0.5
	24	30.8 ± 0.8 [a]	28.3 ± 1.5 [a]	18.1 ± 4.1 [b]	8.2 ± 5.7 [c]	15.5 ± 3.5 [bc]
	48	30.5 ± 1.3 [a]	13.8 ± 5.8 [b]	13.9 ± 6.6 [b]	4.9 ± 4.9 [b]	7.1 ± 4.6 [b]
Straightness (STR, %)	0	48.7 ± 1.0	56.4 ± 1.9	55.7 ± 1.7	51.9 ± 0.8	47.9 ± 1.5
	24	46.8 ± 1.0 [a]	52.9 ± 1.7 [a]	46.3 ± 8.2 [a]	21.9 ± 10.4 [b]	41.7 ± 7.1 [a]
	48	46.7 ± 1.0 [a]	24.7 ± 11.7 [b]	22.6 ± 11.1 [b]	5.6 ± 5.6 [c]	12.7 ± 8.2 [bc]
Linearity (LIN, %)	0	26.1 ± 0.4	26.3 ± 0.4	28.0 ± 0.7	26.4 ± 0.5	25.7 ± 0.5
	24	23.9 ± 0.5	24.1 ± 1.0	23.6 ± 4.4	12.7 ± 6.1	24.1 ± 4.2
	48	23.4 ± 0.6 [a]	11.3 ± 5.4 [ab]	11.0 ± 5.4 [ab]	2.66 ± 2.6 [b]	8.0 ± 5.2 [ab]

Values within a row with different superscripts differ ($P < 0.05$).

At 0 h, the VSL of spermatozoa diluted in AndroMed and EquiPlus was greater than OPTIXcell ($P < 0.05$), but there was no difference among other extenders ($P > 0.05$). At 24 h, spermatozoa diluted in OPTIXcell, INRA96 and EquiPlus had the greatest VSL, compared to both Bioxcell and AndroMed ($P < 0.05$). At 48 h, spermatozoa diluted in OPTIXcell had a greater sustained VSL compared to that with all other extenders ($P < 0.05$; Table 3).

The BCF of spermatozoa did not differ among extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in OPTIXcell and EquiPlus had the greatest BCF compared to AndroMed and Bioxcell and INRA96 ($P < 0.05$). At the same period of time, spermatozoa diluted in INRA96 had a greater BCF compared to Bioxcell ($P < 0.05$). At 48 h, the BCF of spermatozoa diluted in OPTIXcell was greater than spermatozoa diluted with any other extender ($P < 0.05$; Table 3).

The ALH of spermatozoa diluted in EquiPlus was greater at 0 h compared to OPTIXcell ($P < 0.05$). By 24 h, spermatozoa diluted in AndroMed had a greater ALH than Bioxcell, but was less compared to EquiPlus, INRA96, OPTIXcell ($P < 0.05$). At 48 h, spermatozoa diluted in OPTIXcell had a greater ALH than with the other extenders ($P < 0.05$; Table 3).

The STR of spermatozoa did not differ among the extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in AndroMed, INRA96, OPTIXcell and EquiPlus had a greater STR than Bioxcell ($P < 0.05$). At 48 h, spermatozoa diluted in OPTIXcell had a greater STR compared to all other extenders ($P < 0.05$; Table 3).

The LIN of spermatozoa did not differ among the extenders at 0 and 24 h ($P > 0.05$). At 48 h, spermatozoa diluted in OPTIXcell had a greater LIN than with Bioxcell ($P < 0.05$), but there was no difference when compared to other extenders ($P > 0.05$; Table 3).

3.2. Experiment 1b. Effect of egg yolk-based extenders on the sperm motion variables

The average values for motion variables of spermatozoa following 0, 24 and 48 h of liquid storage at 4 °C are presented in Table 4. There was an interaction of extender and time on sperm TM ($P < 0.001$). There was an effect of extender only on PM, VAP, VSL, VCL ($P < 0.001$). There was an effect of time only on PM, VAP, VSL, VCL, ALH, LIN and STR ($P < 0.001$). There was no effect of extender or time on the BCF of spermatozoa ($P > 0.05$).

The TM, PM and VAP of spermatozoa did not differ among extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in Green buffer and Triladyl had a greater TM and PM compared to Biladyl ($P < 0.05$). The VAP of spermatozoa diluted in Triladyl was greater compared to Biladyl at 24 h ($P < 0.05$). At 48 h, spermatozoa diluted in Triladyl had the greatest TM, PM and VAP followed by Green buffer and then Biladyl ($P < 0.05$; Table 4).

The VSL of spermatozoa did not differ among extenders at 0 and 48 h ($P > 0.05$), but spermatozoa diluted in Triladyl had a greater VSL compared to Biladyl ($P < 0.05$). The VCL of spermatozoa did not differ among extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in Triladyl had a greater VCL compared to Biladyl and at 48 h spermatozoa diluted in Triladyl had a greater VCL compared to Biladyl and Green buffer ($P < 0.05$). The ALH of spermatozoa did not differ among extenders at 0 and 24 h ($P > 0.05$). At 48 h, spermatozoa diluted in Triladyl had a greater ALH compared to Biladyl ($P < 0.05$). The BCF, STR and LIN of spermatozoa,

Table 4
Average values for motion variables of spermatozoa diluted in egg-yolk based commercial extenders, following liquid storage at 4 °C (Experiment 1b).

Variable	Time (h)	Biladyl	Green buffer	Triladyl
Total motility (TM, %)	0	85.9 ± 1.0	87.4 ± 0.9	88.6 ± 1.0
	24	72.4 ± 1.8 [a]	78.9 ± 1.7 [b]	83.0 ± 1.2 [b]
	48	54.4 ± 2.0 [a]	64.4 ± 2.7 [b]	74.1 ± 1.6 [c]
Progressive Motility (PM, %)	0	24 ± 1.3	27.0 ± 1.5	29.1 ± 2.4
	24	14.4 ± 0.7 [a]	18.4 ± 0.8 [b]	21.1 ± 1.1 [b]
	48	8.9 ± 0.8 [a]	12.7 ± 0.7 [b]	16.4 ± 0.9 [c]
Path velocity (VAP, μm/s)	0	94.8 ± 8.0	102 ± 3.8	104.2 ± 3.9
	24	88.5 ± 4.5 [a]	94.7 ± 3.2 [ab]	103.8 ± 2.0 [b]
	48	57.6 ± 3.5 [a]	69.9 ± 3.9 [b]	83.3 ± 2.9 [c]
Progressive velocity (VSL, μm/s)	0	50.5 ± 3.7	56.4 ± 3.3	56.0 ± 1.8
	24	43.5 ± 1.5 [a]	47.1 ± 1.4 [ab]	50.8 ± 1.8 [b]
	48	29.3 ± 1.2	33.3 ± 1.7	41.0 ± 1.3
Curvilinear velocity (VCL, μm/s)	0	187.8 ± 18.3	197.3 ± 7.8	211.9 ± 5.4
	24	175.9 ± 7.6 [a]	190.6 ± 6.2 [ab]	200.3 ± 5.6 [b]
	48	118.1 ± 5.2 [a]	152.8 ± 3.7 [a]	171.9 ± 5.7 [b]
Lateral head amplitude (ALH, μm)	0	11.1 ± 0.9	11.5 ± 0.8	11.7 ± 0.3
	24	10.2 ± 0.3	10.9 ± 0.4	10.7 ± 0.4
	48	8.9 ± 0.3 [a]	9.5 ± 0.2 [ab]	10.5 ± 0.4 [b]
Beat cross frequency (BCF, Hz)	0	30.3 ± 0.7	28.3 ± 1.2	29.1 ± 0.6
	24	28.0 ± 0.5	28.1 ± 0.5	27.3 ± 0.6
	48	29.5 ± 1.0	29.3 ± 1.2	27.8 ± 0.8
Straightness (STR, %)	0	50.6 ± 0.9	51.9 ± 1.3	51.1 ± 1.5
	24	46.4 ± 0.6	47.0 ± 0.3	47.3 ± 0.6
	48	47.6 ± 1.2	46.6 ± 0.8	47.3 ± 0.8
Linearity (LIN, %)	0	26.4 ± 0.7	27.7 ± 1.4	26.0 ± 0.5
	24	25.3 ± 0.4	25.0 ± 0.4	25.6 ± 0.4
	48	24.1 ± 0.5 [a]	23.8 ± 0.6 [b]	24.1 ± 0.5 [a]

Values within a row with different superscripts differ ($P < 0.05$).

however, did not differ among extenders at 0, 24 and 48 h ($P > 0.05$; Table 4)

When pooled over time, the PM, VAP, VSL and VCL of spermatozoa diluted in Triladyl was greater than that of Green buffer, which was greater than Biladyl ($P < 0.05$; Table S1). When pooled over extender, the PM, VSL and LIN of spermatozoa was greater at 0 h compared to 24 h ($P < 0.05$; Table S2). Similarly, kinematics recorded at 24 h were greater than those recorded at 48 h ($P < 0.05$; Table S2). The VAP and VCL of spermatozoa at 0 and 24 h were greater than 48 h ($P < 0.05$), but there was no difference between 0 and 24 h ($P > 0.05$; Table S2). The ALH of spermatozoa at 0 h was greater than at 48 h ($P < 0.05$), but there was no difference between 0 and 24 h ($P > 0.05$; Table S2). The STR of spermatozoa at 0 h was greater than at 24 and 48 h ($P < 0.05$), but there was no difference between 24 and 48 h ($P > 0.05$; Table S2).

3.3. Experiment 2: Effect of OPTIXcell, Triladyl and Green buffer on the sperm motion variables and acrosome integrity

The average values for the variables of sperm motion, viability and acrosome integrity following 0, 24 and 48 h of liquid storage at 4 °C are presented in Table 5. There was an interaction of extender and time on the TM, PM, LIN and percentage of viable, acrosome intact sperm ($P < 0.001$). There was an effect of extender only on VAP, VSL, VCL and BCF ($P < 0.05$). There was an effect of time only on VAP, VSL and VCL ($P < 0.05$). There was no effect of extender or time on ALH or STR ($P > 0.05$).

The TM and PM did not differ among extenders at 0 h ($P > 0.05$). At 24 and 48 h, there were a greater TM and PM of spermatozoa diluted in Triladyl in comparison to spermatozoa diluted with OPTIXcell or Green buffer ($P < 0.05$; Table 5). The VAP of spermatozoa diluted in Triladyl was greater than in Green buffer ($P < 0.05$), but there was no difference among extenders at 48 h ($P > 0.05$; Table 5). The VSL and VCL of spermatozoa did not differ among extenders at 0 and 48 h ($P > 0.05$). At 24 h, spermatozoa diluted in Triladyl had a greater VSL and VCL compared to OPTIXcell and Green buffer ($P < 0.05$; Table 5). The LIN of spermatozoa did not differ among extenders at 0 h ($P > 0.05$). At 24 h, spermatozoa diluted in Triladyl had a greater LIN compared to other extenders ($P < 0.05$). At 48 h, spermatozoa diluted in Triladyl had a greater LIN compared to Green buffer ($P < 0.05$; Table 5). The ALH, BCF and STR of spermatozoa, however, did not differ among extenders at 0, 24 and 48 h ($P > 0.05$; Table 5). The percentage of viable, acrosome intact sperm did not differ among extenders at 0 h ($P > 0.05$). At 24 and 48 h, spermatozoa diluted in OPTIXcell had a lesser percentage of viable, acrosome intact sperm compared to other extenders ($P < 0.05$; Table 5).

When pooled over time, spermatozoa diluted in Triladyl had a greater VAP, VSL, VCL and BCF compared to Green buffer and OPTIXcell ($P < 0.05$; Table S3). The ALH and STR of spermatozoa did not differ among extenders ($P > 0.05$; Table S3).

When pooled over extender, spermatozoa had a greater VAP, VSL and VCL at 0 h than 24 h ($P < 0.05$; Table S4). Similarly, kinematics recorded at 24 h were greater than those recorded at 48 h ($P < 0.05$; Table S4). The STR of spermatozoa at 0 h was greater than 24 and 48 h ($P < 0.05$), but there was no difference between 24 and 48 h ($P > 0.05$; Table S4). The ALH and BCF did not differ between 0, 24 and 48 h ($P > 0.05$; Table S4).

Table 5
Average values for motion variables, viability and acrosome integrity of spermatozoa diluted in commercial extenders, following liquid storage at 4 °C (Experiment 2).

Variable	Time (h)	OPTIXcell	Triladyl	Green buffer
Total motility (TM, %)	0	84.7 ± 1.5	84.8 ± 0.9	82.3 ± 1.1
	24	73.3 ± 2.3 [a]	81.7 ± 0.9 [b]	70.7 ± 2.5 [a]
	48	58.7 ± 1.1 [a]	70.5 ± 2.4 [b]	61.3 ± 1.7 [a]
Progressive Motility (PM, %)	0	23.8 ± 1.6	22.8 ± 0.9	22.7 ± 1.3
	24	14.8 ± 0.9 [a]	18.3 ± 0.9 [b]	16.0 ± 1.0 [a]
	48	10.7 ± 0.6 [a]	15.5 ± 1.1 [b]	12.0 ± 0.9 [a]
Path velocity (VAP, μm/s)	0	98.8 ± 5.1 [ab]	105.6 ± 1.8 [a]	89.7 ± 4.2 [b]
	24	88.2 ± 6.2 [ab]	102.3 ± 2.0 [a]	82.9 ± 5.7 [b]
	48	69.5 ± 7.4	83.0 ± 4.7	69.3 ± 2.8
Progressive velocity (VSL, μm/s)	0	49.8 ± 3.8	54.8 ± 2.1	48.3 ± 1.7
	24	38.8 ± 2.8 [a]	48.1 ± 1.7 [b]	39.7 ± 3.0 [a]
	48	34.9 ± 2.9	41.3 ± 2.5	33.2 ± 1.7
Curvilinear velocity (VCL, μm/s)	0	195.5 ± 14.7	208.2 ± 4.6	182.2 ± 6.9
	24	178.6 ± 9.8 [a]	198.8 ± 5.4 [b]	174.6 ± 8.0 [a]
	48	145.3 ± 8.0	166.6 ± 6.5	149.9 ± 7.3
Lateral head amplitude (ALH, μm)	0	10.6 ± 0.7	11.5 ± 0.4	11.3 ± 0.6
	24	10.1 ± 0.3	10.7 ± 0.3	13.1 ± 2.9
	48	9.6 ± 0.4	10.1 ± 0.5	9.3 ± 0.4
Beat cross frequency (BCF, Hz)	0	29.1 ± 0.5	29.8 ± 0.5	29.9 ± 0.4
	24	30.4 ± 1.0	27.1 ± 0.6	29.2 ± 1.0
	48	30.9 ± 1.2	28.7 ± 0.8	28.7 ± 1.5
Straightness (STR, %)	0	49.8 ± 1.0	49.5 ± 1.5	51.7 ± 1.2
	24	46.1 ± 0.5	46.7 ± 0.4	46.4 ± 0.7
	48	46.2 ± 0.4	48.0 ± 0.6	45.0 ± 1.9
Linearity (LIN, %)	0	26.3 ± 0.5	26.2 ± 0.5	26.3 ± 0.3
	24	23.8 ± 0.3 [a]	25.5 ± 0.2 [b]	24.0 ± 0.4 [a]
	48	23.5 ± 0.4 [ab]	24.4 ± 0.3 [a]	21.8 ± 0.9 [b]
Viable-intact acrosome (VIA, %)	0	62.7 ± 1.4	67.0 ± 2.1	63.0 ± 1.7
	24	49.2 ± 1.5 [a]	59.5 ± 1.7 [b]	57.2 ± 1.7 [b]
	48	38.5 ± 1.2 [a]	52.8 ± 0.9 [b]	48.5 ± 0.8 [b]

Values within a row with different superscripts differ ($P < 0.05$).

3.4. Experiment 3: Effect of storage temperature (4 °C compared with 15 °C) on the sperm motion variables and acrosome integrity

The average values for sperm motion variables, viability and acrosome integrity of spermatozoa following 0, 24 and 48 h of liquid storage at 4 and 15 °C are presented in Table 6. There was no interaction between sperm storage temperature and duration on semen storage in Triladyl or Green buffer extenders ($P > 0.05$) The sperm motion variables, viability and acrosome integrity of spermatozoa diluted in Triladyl or Green buffer did not differ between storage temperatures at 24 and 48 h ($P > 0.05$; Table 6). There was also no interaction between storage temperature and extenders ($P > 0.05$).

When data were pooled over storage temperature, there was an interaction of extender and time only on the TM, PM, VCL and ALH ($P < 0.05$; Fig. 1). There was an effect of extender only on percentage of viable-acrosome intact cells ($P < 0.05$). There was an effect of time only on VAP, VSL, BCF, LIN and percentage of viable-acrosome intact cells ($P < 0.05$).

The TM and PM of spermatozoa did not differ among extenders at 0 h ($P > 0.05$; Fig. 1a-b). After 24 and 48 h of storage, spermatozoa diluted in Triladyl, however, had a greater TM than those stored in Green buffer ($P < 0.05$; Fig. 1a). The VCL and ALH did not differ among extenders at 0 h and 24 h ($P > 0.05$). After 48 h of storage, spermatozoa diluted in Triladyl had a greater VCL and ALH than those stored in Green buffer ($P < 0.05$; Fig. 1c-d).

4. Discussion

In the present study, there was evaluation of the effect of different extenders and storage temperatures on the semen quality of dromedary camels following liquid preservation. Overall, results indicate that Triladyl is a superior extender for maintaining sperm viability, with semen diluted in this extender having the greatest percentages of sperm motility and spermatozoa with viable and intact acrosomes compared to the other extenders at 24 and 48 h of storage. Furthermore, the results of this study indicate the temperature at which camel semen is liquid stored in Triladyl does not alter its quality when there is a 48 h storage period. These results supported the initial hypothesis of the superiority of egg-yolk based extenders for liquid preservation of camel semen, but disproved the hypothesis that lesser temperatures of chilled storage would provide additional benefits for sperm viability. To our knowledge, the results of this study are the first where there is reported camel sperm quality (as assessed by motility and viability/acrosome integrity) following 48 h of liquid storage.

This is first study in which there has been systematic evaluation of the suitability of a wide range of commercial extenders for the liquid storage of dromedary camel semen. In Experiment 1a, there was the greatest sperm quality at 24 and 48 h of storage with use of

Table 6
Average (± SEM) values for motion variables, viability and acrosome integrity of spermatozoa diluted in commercial extenders, following liquid storage at 4 and 15 °C (Experiment 3).

Time (h)	Extender	Temperature (°C)	TM (%)	PM (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	VIA (%)
0	Triladyl	37	89.6 ± 0.7	26.2 ± 1.5	105.8 ± 4.0	53.9 ± 1.2	207.5 ± 4.6	10.3 ± 0.4	27.4 ± 0.6	47.8 ± 0.5	25.0 ± 0.5	66.6 ± 1.2
	Green buffer	37	88 ± 0.8	26.4 ± 1.2	109.9 ± 5.3	58.4 ± 5.6	223.3 ± 17.2	11.6 ± 1.1	28.0 ± 0.9	49.6 ± 1.5	25.4 ± 0.5	64.0 ± 1.6
24	Triladyl	4	83.4 ± 0.9	21.2 ± 1.1	105.0 ± 2.7	52.9 ± 2.8	207.8 ± 5.0	11.4 ± 0.2	27.8 ± 0.6	47.4 ± 0.9	24.8 ± 0.4	60.4 ± 1.3
		15	81.0 ± 1.3	19.8 ± 0.7	95.7 ± 3.7	47.7 ± 0.8	187.7 ± 11.3	11.5 ± 0.4	28.7 ± 0.6	47.8 ± 1.4	24.6 ± 0.7	56.6 ± 2.0
	Green buffer	4	80.4 ± 0.7	18.8 ± 0.8	94.0 ± 6.0	48.2 ± 3.6	192.0 ± 15.1	10.9 ± 0.9	28.9 ± 0.8	48.0 ± 1.8	25.0 ± 0.3	58.2 ± 1.8
		15	78.4 ± 0.7	18.2 ± 1.3	87.2 ± 8.2	43.9 ± 5.2	185.9 ± 17.9	10.7 ± 1.0	29.9 ± 0.9	48.6 ± 1.8	25.0 ± 0.6	55.8 ± 1.5
48	Triladyl	4	75.6 ± 1.3	16.8 ± 0.4	90.1 ± 3.2	45.0 ± 0.7	183.6 ± 6.6	10.7 ± 0.5	25.3 ± 0.6	48.2 ± 0.9	25.0 ± 0.5	54.6 ± 1.1
		15	72.6 ± 1.3	15.2 ± 0.4	84.9 ± 7.2	42.4 ± 2.6	179.3 ± 8.1	10.9 ± 0.5	26.9 ± 1.1	47.8 ± 1.5	24.6 ± 0.7	52.4 ± 0.9
	Green buffer	4	64.2 ± 2.2	13.2 ± 0.6	76.3 ± 7.5	35.9 ± 2.4	145.0 ± 14.2	9.1 ± 0.7	25.0 ± 1.6	48.6 ± 1.2	26.6 ± 1.1	50.4 ± 2.6
		15	63.2 ± 2.6	12.8 ± 0.7	75.3 ± 2.0	40.2 ± 2.5	158.9 ± 11.0	10.0 ± 0.7	27.5 ± 1.5	48.6 ± 1.4	26.0 ± 1.0	50.2 ± 1.0

TM, total motility; PM, progressive motility; VAP, average path velocity; VSL, progressive velocity; VCL, curvilinear velocity; ALH, lateral head amplitude; BCF, beat cross frequency; STR, straightness; LIN, linearity and VIA, viable-intact acrosome.
Values within a column at each time point with different superscripts differ ($P < 0.05$).

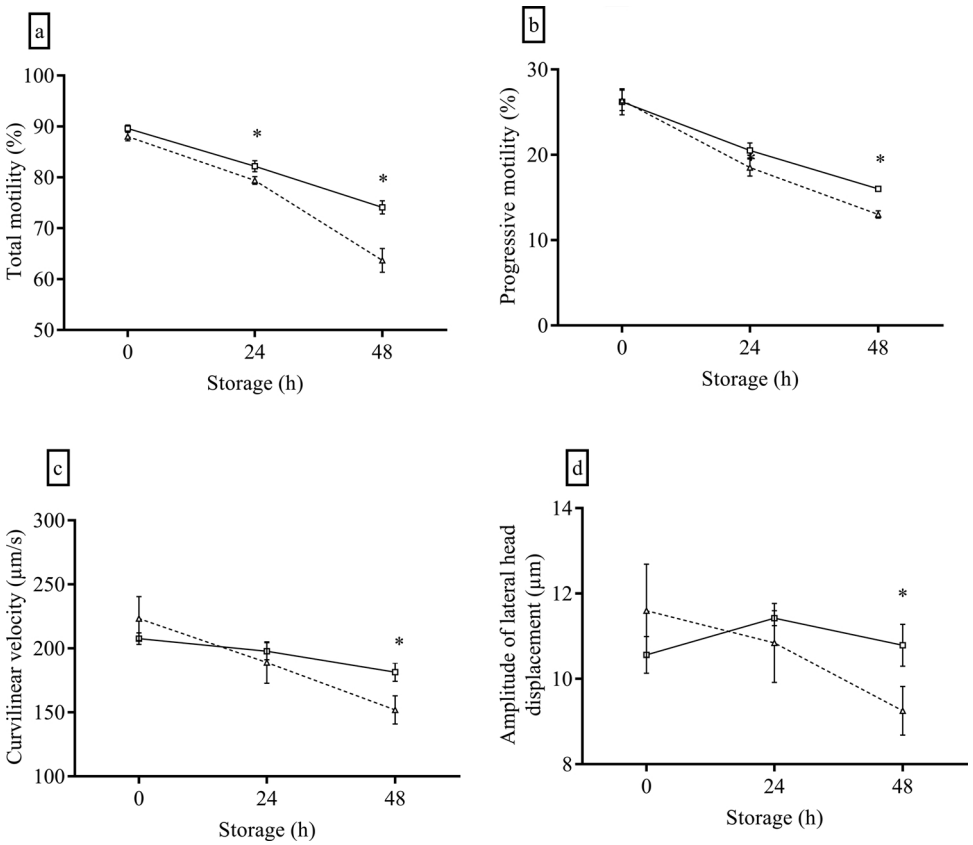

Fig. 1. Percentage of total motility (a), progressive motility (b), curvilinear velocity (c) and amplitude of lateral head displacement (d), of camel spermatozoa diluted in two different extenders (Green buffer; Δ and Triladyl; □) (Experiment 3; data were pooled for all storage temperatures); Values are averaged for five replicates, ± SEM; *Indicates differences ($P < 0.05$) between the means (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

OPTIXcell compared to other extenders. To our knowledge this is first study where the use of OPTIXcell for liquid storage of camel semen was reported. This diluent contains liposomes which have been reported to be beneficial for semen cryopreservation in other species (Wilhelm et al., 1996; He et al., 2001; Zeron et al., 2002; Kumar et al., 2015; Ansari et al., 2016). Although there is lack of information regarding the effect of OPTIXcell's liposomes in the success of chilled storage of sperm from any species, there is a decreased the sensitivity of sperm to chilling during this part of the cryopreservation process (Zeron et al., 2002) and this could be due to the incorporation of liposomes into and stabilization of the sperm membrane (Ansari et al., 2016). Studies into the mechanism of activity of liposomes in camel spermatozoa and the extent to which liposomes alter the cholesterol/phospholipid ratio in this species have not yet occurred.

Other chemically defined soya-lecithin based AndroMed and Bioxcell extenders as well as the caseinate-based EquiPlus and INRA96 extenders failed to preserve sperm quality longer than 24 h of storage in the present study. The INRA96 and EquiPlus used for stallion semen storage (LeFrapper et al., 2010) and Bioxcell in buffalo (Akhter et al., 2011) have been used successfully for liquid storage of semen. These results differ from those reported by Morton et al. (2013), where there was a motility-preserving effect of the caseinate based extender, INRA96 in camels. The positive effect observed by Morton et al. (2013), however, was only recorded after 24 h, while in the current study, sperm motility was assessed after 48 h of storage. Other reasons for this difference could be due to variance in experimental conditions, the quality of the initial semen collected and the methods of semen assessment used. In Experiment 1b, spermatozoa diluted in Triladyl had a greater TM at 48 h of storage than Green buffer and Biladyl. The greater sperm-preserving capacity of Triladyl as compared with other egg-yolk based extenders (Green buffer and Biladyl) in the present study could be due to the presence of glycerol. Similar to findings in the present study, results of a study in rams with the addition of glycerol indicated there was a decreased damage to spermatozoa after 120 h of refrigeration in tris-based egg yolk media, thus preserving total motility and plasma and acrosomal integrity (Crespilho et al., 2008). In bulls, fertility rates were improved after insemination with samples which were cooled with glycerol to 5 °C (Papa et al., 2015).

In Experiment 2, spermatozoa diluted in Triladyl had greater values for motion variables (TM, PM and LIN) and percent viable intact acrosome spermatozoa at 24 and 48 h of storage when compared to storage with OPTIXcell, and also indicated there were greater values for motion variables (TM, PM, VCL and ALH) at 48 h of storage when compared to use of Green buffer in Experiment 3. The more desirable sperm-preserving capacity of Triladyl as compared with other egg-yolk based extenders (Green buffer and

Biladyl) and OPTIXcell (chemically defined) in the present study could be due to the combined presence of both glycerol and egg yolk. Egg yolk is also thought to have a synergistic effect with other penetrating cryoprotectants like glycerol (Pace and Graham, 1974). The low-density lipids (LDL) of egg yolk could interact with lipid-binding proteins of seminal plasma (Vishwanath et al., 1992) that could induce cholesterol and phospholipid removal from the sperm membrane, resulting in enhancement of sperm viability during storage in liquid or frozen states (Bergeron and Manjunath, 2006). While the benefits of glycerol and egg yolk during cryopreservation are well known, caution is warranted in relation to the concentration of these components in extenders. Both glycerol and egg yolk at relatively greater concentrations can be deleterious to spermatozoa, especially during fresh incubation or liquid storage (Shannon, 1972; Lopez Saez et al., 2000; Gil et al., 2003). Glycerol concentrations of greater than 6% can be toxic to sperm cells, while greater concentrations of egg yolk can sequest seminal plasma proteins, preventing sperm from benefiting from these compounds during cryopreservation (Bergeron and Manjunath, 2006; Bergeron et al., 2007). In the current study, Triladyl was used at a concentration of 20% egg yolk and 6% glycerol. At these concentrations, egg yolk and glycerol provided a protection to camel spermatozoa during cooling and preserved sperm quality to the 48 h time point post collection. Even though the results from the present study are encouraging, it should be considered that the use of egg yolk in extenders is limited due to an increased risk of microbiological contamination, export quarantine restrictions, disease transmission, a known wide variability in the composition and the capacity to increase viscosity of samples due to the presence of particulate debris (Vishwanath and Shannon, 2000). As such, for the last two decades, continuous efforts have been made to develop chemically defined or synthetic extenders, like OPTIXcell for use in sperm preservation protocols, which contain liposomes and are free from proteins of animal origin.

In the present study, Green buffer and OPTIXcell extenders maintained sperm motility are rates of 58.7% and 61.3%, respectively, following 48 h storage (Table 5). While Triladyl was not superior to these diluents for maintaining sperm viability, the biosecurity benefits of OPTIXcell, an egg yolk free extender, cannot be overlooked nor should the relatively greater sperm quality values with use of Green buffer be ignored. Suitability of these two extenders for liquid preservation should, therefore, only be absolutely excluded following *in vivo* artificial insemination trials.

In the present study, the motion variables and percentage of viable acrosome-intact spermatozoa were not affected by storage temperature (4 °C compared with 15 °C) when extended in either Triladyl or Green buffer. Similar results have been reported in alpacas (Morton et al., 2009), where sperm motility and acrosome integrity were not affected by storage temperature (4 °C compared with 15 °C) for as long as 48 h of storage. Furthermore, Wani et al. (2008) reported a similar result in camels when comparing storage temperatures of 4 and 23 °C. In this study, there was no effect of storage temperature on acrosomal integrity (Wani et al., 2008). Additionally, it is worth noting that the glycerol in Triladyl did not have any detrimental effects on semen variables evaluated during liquid storage of semen at 15 °C. While a promising result for future *in vivo* use, fertility trials of spermatozoa stored in these conditions with Triladyl or Green buffer and studies on effect storage temperature (4 and 15 °C) on semen quality during liquid storage in other extenders (OPTIXcell, AndroMed, EquiPlus, Bioxcell and Biladyl) are required. Nonetheless, in the present study, it was determined that dromedary camel semen can be stored in liquid form for up to 48 h *in vitro*, and this may facilitate semen transport domestically and internationally for artificial insemination.

The relationship between *in vitro* semen variables assessed using CASA and *in vivo* fertility has been established in a variety of species (Farrell et al., 1998; Silva et al., 2006; Gillan et al., 2008; Samardžija et al., 2008; Akhter et al., 2010; Bezerra et al., 2012; Dorado et al., 2013; Nagy et al., 2015). In dromedary camels, CASA has been used to record sperm kinematics variables (Al-Qarawi et al., 2002; Rateb, 2016; Malo et al., 2019) and FITC-PNA stain has been used to assess acrosome status (Morton et al., 2011, 2013). Unfortunately, at present the relationship between CASA variables or other *in vitro* variables and fertility in camels is unknown. As the success of artificial insemination in camels improves and associations between *in vitro* sperm variables (like CASA) and fertility can be made, the results of the present study will further increase in the relevance of findings in such studies and for commercial applications.

In conclusion, the results of this study indicate Triladyl is the most suitable extender for liquid storage of dromedary camel semen following 48 h of collection. Semen quality over this time period was equally sustained when stored in Triladyl at a temperature of either 4 or 15 °C. While these results are encouraging for the camel industry, the *in vivo* fertility of liquid stored camel spermatozoa extended in Triladyl must be assessed before its use is recommended in commercial artificial insemination programs.

Declaration of competing interest

The authors do not have any conflict of interest potentially influencing the results of this study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2019.06.008.

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