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Seminal plasma Alters surface Glycoprofile of dromedary camel cryopreserved epididymal spermatozoa

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ABSTRACT

The high viscosity of Camelidae semen continues to present a major impediment for its application in assisted reproduction technology. The exposure of epididymal spermatozoa (ES) to seminal plasma (SP) may provide an approach to enhance the development of assisted reproductive techniques in these important domestic species. Since the sperm glycocalyx plays a key role in reproduction we aimed to evaluate whether SP exposure modifies the surface glycosylation patterns of cryopreserved dromedary ES. Epididymal sperm was collected through retrograde flushing of the cauda epididymidis that were obtained from orchidectomized mature dromedary bulls. The collected samples were then cryopreserved after dilution with a tris citrate clarified egg yolk extender, with and without the supplementation of 15% SP. Post-thaw carbohydrate surface profiles of both control and SP-treated spermatozoa were analyzed using 15 fluorescent lectins. Morpho-functional properties were also investigated via computer assisted sperm analysis. Lectin-binding analysis of the glycocalyx in control sperm revealed the presence of (1) N-glycans terminating with lactosamine (Con A, PHA-L, and RCA_{120}), in both acrosomal and tail regions. Whilst (2) α2,3-/α2,6-linked sialic acids (MALII, SNA), and O-linked glycans terminating with a single N-acetylgalactosamine residue (Tn antigen) (HPA, SBA) along with galactoseβ1,3N-acetylgalactosamine (T antigen) (PNA) were observed in the acrosomal cap. The expression of both N-acetylglucosamine (sWGA and GSA II) and terminalαgalactose (GSA I−B_4) residues was also noted in the acrosomal cap region of control sperm. Compared with controls, SP treated samples displayed: 1) the appearance of bisected di-triantennary complex-type N-glycans (PHA-E), terminating with lactosamine, as well as an increase of O-glycans terminating with Tn and T antigens in both the acrosomal and tail regions; 2) an increase in glycans containing α2,6-linked sialic acid, N-acetylglucosamine, and αgalactose in the tail region. The cytoplasmic droplets of both control and seminal plasma-treated sperm bound Con A, PHA-E, PHA-L, RCA_{120}, HPA, PNA, sWGA, GSA I−B_4, and GSA II. These results indicate that SP treatment affects the glycan composition of the dromedary camel ES glycocalyx. More comprehensive studies are required in order to evaluate the fertilization capacity of SP-treated ES in order to facilitate its application in dromedary camel assisted reproduction technology.

1. Introduction

The most noteable physical characteristic of the camelid ejaculate is its high viscosity [1]. This feature is considered to be the primary barrier for its effective application in assisted reproduction technologies (ARTs), as this quality complicates the processing of ejaculates and prevents adequate homogenisation of ejaculates with extenders. Despite considerable efforts in recent years to

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develop effective protocols for the adequate liquefaction of camelid semen, results have been largely inconsistent in this area [2,3].

Epididymal spermatozoa (ES) have been used in the assisted reproduction of humans [4] and of several domestic animal species [5–10]. In Camels, ES may offer an effective alternative to overcome the issues related to viscosity and could be of use in artificial insemination trials in this species [11,12].

It has been demonstrated that the exposure of ES to seminal plasma (SP) has positive effects on ES survival and cervical transit [10,13–15], oviduct epithelial cell binding [16], and fertilization capacity [10].

More recently, it has been reported that SP treatment can affect both the plasma membrane proteome [17] and the glycocalyx carbohydrate profile of ES [18,19], with the latter highlighting an important role for SP in sperm physiology and function. The sperm glycocalyx plays a key role in reproduction via involvement in sperm survival, transport in the oviduct, capacitation, sperm-egg interactions, the acrosome reaction, zona binding and fertilization, as well as having an immunoprotective function within the female reproductive tract [18,20,21]. Interestingly, SP components added to the glycocalyx maintain sperm in a non-capacitated, acrosome-intact state [18]. The presence of glycodelin S (GdS) (a male-specific glycodelin isoform that is highly enriched in SP) on the surface of ejaculated sperm is required to inhibit capacitation prior to its arrival at the site of fertilisation [22]. In camelids, SL15, a sugar-binding glycoprotein implicated in sperm reservoir formation, has been found on the surface of llama ejaculated sperm but not on the surface of those of epididymal origin [23].

Recently, we demonstrated that the supplementation of SP produces beneficial effects on post-thaw motility and the kinematic parameters of dromedary camel ES [24]. The aim of the current study was to examine the effect of SP on the composition of the glycocalyx of cryopreserved camel ES. Given that no studies are available on the glycocalyx composition of camel ES, the results of this study may provide useful information about the reproductive biology of the dromedary camel, which could give insights in the further improvement of ARTs in this species.

2. Materials and methods

2.1. Sampling

All testicles used in these experiments resulted from routine orchidectomies carried out on healthy mature dromedary bulls (n=5) within the breeding season (April 2017) at the Oasispark Fuerteventura, Spain. Animals underwent castration to prevent rutting, aggressive and restless behavior during touristic activities (Caravan tours) and all animals displayed normal sexual behavior prior to castration (Poll gland secretion, tail flapping, dulaa extrusion). Bulls ranged in age from 6 to 10 years, and were fed with a mix of alfa-alfa and hay, supplemented with concentrates, vitamins and minerals. Surgical procedures were performed in conformity with European Union Directive 2010/63/EU and all applicable international, national, and/or institutional guidelines for the care and use of animals were strictly followed.

Seminal plasma was collected from two adult males, of proven fertility, operating as stud bulls in a camel farm located in Gran Canaria (Spain) with the use of an artificial vagina. Assessment of ejaculates showed no contamination, volume ≥4 mL, sperm concentration >100 × 10^6 spermatozoa (spz)/mL and total motility >80%. The collected ejaculates were allowed to spontaneously liquefy at 4 °C, pipetted to reduce residual viscosit, and then centrifuged twice at 1000 xg for 30 min. Processed SP samples from each bull were checked for the absence of spermatozoa, then mixed with each other in equal volumes and stored in aliquots at −80 °C until use, in concordance with procedures described by Monaco et al. [24].

2.2. Collection, evaluation, freezing and thawing of epididymal spermatozoa

Testes, including the epididymides and vas deferens, were collected during surgeries and stored at 4 °C for 24 h until isolation and flushing of the epididymides and deferens ducts.

Epididymal spermatozoa were collected through retrograde flushing of the cauda epididymis. Briefly, after cannulation of the deferens ducts with a blunt needle, flushing was conducted (direction from deferens to epididymal tail) with a Tris-citrate buffer (tris 247 mM; citric acid 87 mM; cefalexin 0.1%); obtained samples were assessed for volume, sperm viability, concentration, and morphology [24,25].

After evaluation, samples were split into two aliquots and were processed in an extender of the following composition: Tris 268.28 mM/L, citric acid 79.7 mM/L, lactose 152.64 mM/L, glucose 27.75 mM/L, 20% clarified egg yolk, glycerol 4% (modified from El-Bahrawy [26]), and the final concentration of 50 × 10^6 spz/mL. One aliquot was left as a control (C) whereas the other one was supplemented with 15% SP.

Freezing was performed, after a 180-min equilibration period, according to the procedure described by Monaco et al. [24], whereas thawing was performed at 46 °C for 20 s [27].

The post-thaw sperm evaluation included sperm viability (eosin/nigrosin staining) as well as motility and kinematic parameters assessed through computer-assisted sperm analysis (CASA) [24]. The setting of the CASA motility parameters was performed according to Malo et al. [28].

2.3. Statistical analysis

Post-thaw semen parameters of control and SP-treated samples were analyzed using the non-parametric Wilcoxon test (P-value 0.05). All statistical analyses were performed using SPSS® statistical software (ver. 20) and results were expressed as the mean ± with standard deviation (SD).

2.4. Lectin binding

After thawing, spermatozoa of control and SP-treated samples were prepared as previously described [29]. Spermatozoa were fixed in 4% (v/v) buffered paraformaldehyde, pH 7.4, for 45 min at room temperature (RT) and then pelleted using centrifugation at 800 x g for 5 min. After supernatant removal, the sperm pellets were washed twice with 0.01 M phosphate-buffered saline (PBS, pH 7.4), then smeared on poly-L-lysine coated glass slides and fixed by air-drying. Dried slides were subsequently rinsed in 0.05 M Tris–HCl-buffered saline (TBS) (pH 7.4) and incubated at RT for 1 h in the dark with appropriate dilutions of 15 fluorescent lectins (Table 1) diluted in TBS. All lectins were obtained from Vector Laboratories (Burlingame, CA, USA) except for MAL II which was obtained from Glycomatrix (Dublin, Ohio, USA). After incubation, the slides were rinsed three times in the same buffer and mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, USA).

Controls included (1) a substitution of the substrate medium with a buffer lacking lectins; and (2) incubation with each lectin in the presence of its hapten sugar (0.2 mM Tris buffer) (Table 1). Both control experiments produced negative staining results. Slides were observed and photographed at 100x magnification under a light photomicroscope (Eclipse Ni–U, Nikon, Japan) equipped with a digital camera (DS-U3, Nikon, Japan) with a 1 s exposure time.

Table 1
Lectin used, their sugar specificities and the inhibitory sugars used in control experiments.

Lectin abbreviation	Source of lectin	μg/ml	Sugar specificity	Inhibitory sugar
MAL II	*Maackia amurensis*	25	NeuNAcα2-3Galβ1-3GalNAc	NeuNAc Lac
SNA	*Sambucus nigra*	25	Neu5Acα2,6Gal/GalNAc	NeuNAc Lac
RCA_{120}	*Ricinus communis*	25	Terminal Galβl,4GlcNAc	Galactose
Con A	*Canavalia ensiformis*	25	Terminal/internal αMan>αGlc	Mannose
PHA-E	*Phaseolus vulgaris E*	30	bisected complex GlcNAcβ1,2Man	Mannose
PHA-L	*Phaseolus vulgaris* L	30	GlcNAc βl,2Man	Mannose
sWGA*	*Triticum vulgaris*	25	Terminal/internal βGlcNAc	GlcNAc
GSA I–B_4	*Griffonia simplicifolia*	25	Terminal αGal	Galactose
HPA	*Helix pomatia*	25	GalNAcαl,3GalNAc	GalNAc
DBA	*Dolichos biflorus*	30	Terminal GalNAcαl,3(LFucαl,2)Galβl,3/4GlcNAcβl	GalNAc
SBA*	*Glycine max*	25	Terminal α/βGalNAc	GalNAc
PNA*	*Arachis hypogaea*	25	Terminal Galβl,3GalNAc	Galactose
GSA II	*Griffonia simplicifolia*	25	Terminal D-GlcNAc	GlcNAc
LTA	*Lotus tetragonolobus*	30	Terminal *a* L-Fuc	Fucose
UEA I	*Ulex europaeus*	30	Terminal L-Fucαl,2Galβl,4GlcNAcβ	Fucose

Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuNAc, N-acetyl neuraminic (sialic) acid; s, succinylated. *, TRITC (rhodamine)-labeled lectins. Non-marked lectins were FITC (fluorescein isothiocyanate)-labeled lectins.

Exposition conditions were maintained for all images. The following fluorescence filter sets were used: 1) FITC fluorescence filter: 465–495 nm excitation filter (Ex); 505 nm dichroic mirror (Dm); 515–555 nm emission filter (Em); 2) TRITC fluorescence filter: 540/25 nm Ex; 565 nm Dm; 605/55 nm Em. The resulting images were analyzed using the NIS Elements BR software (Ver. 4.20; Nikon, Japan).

3. Results

3.1. Semen evaluation

The parameters of the collected ES are reported in Table 2. Morphological evaluation revealed that 91% of all sperm showed normal morphology, 1% of spermatozoa displayed head abnormalities, 3% of spermatozoa were with abnormal midpieces, and 5% of sperm cells were found to display coiled tails. Moreover, 48% of the spermatozoa displayed the presence of cytoplasmic droplets (CDs).

Post-thaw kinematic parameters of both the control and SP-treated samples are reported in Fig. 1. For the majority of sperm parameters under consideration, which included viable sperm (Control 66 ± 7.21 *vs* SP 67.6 ± 6.9), amplitude of lateral head displacement (ALH) (Control 4.1 ± 0.9 vs SP 5 ± 0.9), beat cross frequency (BCF) (Control 19.1 ± 3.8 *vs* SP 16.92 ± 6.6), straightness (STR) (Control 47 ± 2.4 *vs* SP 51.4 ± 3.6), and linearity (LIN) (Control 28 ± 2.9 *vs* SP 30.4 ± 6), a non-significant increase in SP-treated sperms relative to the control was observed.

3.2. Sperm surface glycopattern

As summarized in Table 3, the SP supplementation induced important changes in the lectin binding pattern of sperm surfaces.

Control spermatozoa displayed four different lectin-binding patterns: 1) Con A bound to the entire surface of spermatozoa, showing more intense labelling of the head than the tail (Fig. 2a); 2) MAL II labeled only to the sperm head (Fig. 2b); 3) PHA-L (Fig. 2c) and RCA_{120} (Fig. 3a) reacted with the acrosomal cap and the tail; with the acrosomal cap displaying much more intense labelling with RCA_{120} than with PHA-L, whereas the tail exhibited a faintly visible staining with both lectins; 4) GSA II (Fig. 2d), HPA, PNA (Fig. 3b), PHA-E (Fig. 3c), succinyl (s) WGA (Fig. 4a), GSA I–B_4 (Fig. 4b), SBA and SNA (Fig. 4c) displayed affinity restricted to the acrosomal cap; with the latter two lectins showing fewer binding sites than other lectins.

Seminal plasma supplementation did not affect Con A, MAL II, PHA-L, and GSA II affinity (Fig. 2a–d), whereas it caused: 1) an increase of RCA_{120} binding sites in both acrosomal and tail regions (Fig. 3d); 2) an increase of HPA, PNA, and PHA-E binding sites on the acrosomal cap as well as the appearance of these binding sites on the tail (Fig. 3e and f); 3) the appearance of sWGA, GSA I–B_4, SBA, and SNA binding sites were detected only on the tail (Fig. 4d,e,f).

Cytoplasmic droplets of both C and SP-treated sperm bound Con A, PHA-L, GSA II (Fig. 2 a,c,d), RCA_{120}, HPA, PNA, PHA-E (Fig. 3a,b,c), sWGA, and GSA I–B_4 (Fig. 4a and b). Cytoplasmic droplets of SP-supplemented spermatozoa showed an increase in the RCA_{120}, PNA, PHA-E (Fig. 3d,e,f), and sWGA (Fig. 4d) reactivity.

Table 2
Parameters of dromedary camel epididymal spermatozoa samples (n = 5)after collection through retrograde flushing.

Parameter	Unit	Mean	St. Dev.	Median	Q1	Q3
Volume	mL	1.66	0.31	1.80	1.35	1.90
Sperm concentration	$x10^6$spz/mL	485.60	279.12	384.00	323.50	698.50
Total Recovered Sperms	$x10^6$spz	743.30	243.44	637.20	607.25	932.40
Normal sperm cells[a]	%	91.00	5.05	92.00	87.00	94.50
Abnormal Heads	%	1.00	0.71	1.00	0.50	1.50
Abnormal Midpieces	%	3.00	2.12	3.00	1.00	5.00
Abnormal Tails	%	5.00	3.54	4.00	2.50	8.00
Proximal cytopl drop	%	37.40	5.98	38.00	32.00	42.50
Distal cytopl drop	%	11.00	5.00	11.00	6.00	16.00

[a] proximal and distal cytoplasmic droplets included.

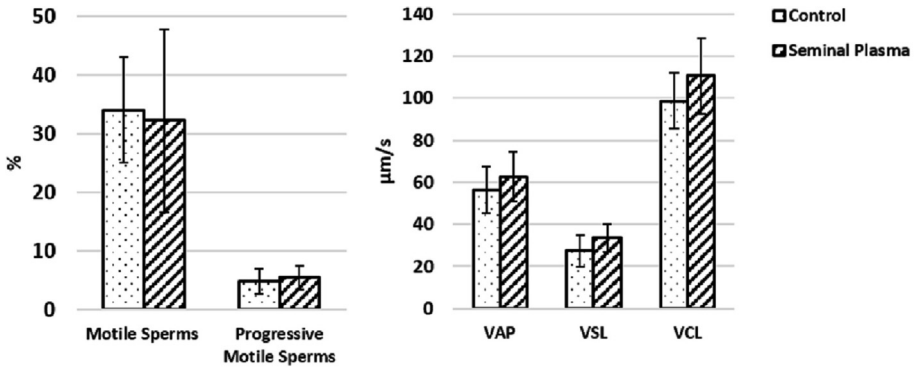

Fig. 1. Post-thaw motility and kinematic parameters (mean ± SD) of dromedary camel epididymal spermatozoa frozen with and without 15% seminal plasma. VAP: average path velocity; VSL straight line velocity; VCL: curvilinear velocity.

DBA, LTA, and UEA I produced negative reactions with both C and SP-treated spermatozoa.

4. Discussion

The ejaculates of camelids are most notably characterized by a highly viscous consistency. This feature limits an operator's capacity to process the ejaculate (pipetting, slide preparation, evaluation of concentration and motility of spermatozoa) as well as disrupting the blending of semen with extenders (see Ref. [3] for references). Consequently, semen viscosity continues to present a major impediment for its application in ARTs in Camelidae species. Despite continuous efforts and the use of multiple techniques, this constraint has yet to be overcome. The use of epididymal spermatozoa has recently been proposed as a possible tool to understand the effect of SP on sperm physiology and investigated for its possible use in sperm cryopreservation protocols [24].

The mammalian sperm glycocalyx plays a key role in sperm motility, maturation and fertilization (see Ref. [18] for review). Therefore, the evaluation of sperm surface glycans could facilitate a better understanding of sperm biology and the changes that occur under different physiological, pathological and extender-induced conditions. In this study we report for the first time the effect of SP supplementation on the surface glycopattern of cryopreserved dromedary ES.

Table 3
Lectin-binding pattern of control and seminal plasma (15% supplementation) dromedary camel epididymal frozen-thawed spermatozoa samples.

Lectin	Head		Tail	
	C	SP	C	SP
Con A	++	++	+	+
MAL II	+	+	−	−
PHA-L	±ac	±ac	±	±
RCA_{120}	++ac	+++ac	±	+
GSA II	+ac	+ ac	−	−
HPA	+ac	++ac	−	+
PNA	+ac	+++ac	−	+
sWGA	+ac	+ac	−	+
GSA I–B_4	+ac	+ac	−	±
SBA	+ac	+ac	−	+
SNA	±ac	±ac	−	±
PHA-E	±ac	++ac	−	+
DBA	−	−	−	−
LTA	−	−	−	−
UEA I	−	−	−	−

ac: acrosomal cap; C: control; s: succinylated; SP: seminal plasma. Results are expressed by a subjective scale: , negative reaction; ±, faintly visible reaction; +, ++, +++, weak, moderate, strong reactions.

Assessments of dromedary ES indicate that SP supplementation increases both viability and motility parameters, as recently demonstrated by Monaco et al. [24], though the present study did not observe a significant difference between control and SP-treated spermatozoa in this regard, possibly because of low sample numbers. Nonetheless, the current sperm glycopattern investigation remains valid, since the kinematic analysis in this study was performed merely for the purpose of evaluating the quality of the samples used. Previous studies on *Lama glama* have demonstrated that SP effects epididymal sperm motility [30,31] and the interactions of sperm with oviductal epithelial cells, thus participating in the formation of sperm reservoirs [32]. In addition, beneficial effects of SP on epididymal sperm motility, sperm penetration of cervical mucus and pregnancy rates have been demonstrated in the ram [10,33]. Seminal plasma also enhances the ability of human [13], macaque [34] and buffalo [14] spermatozoa to migrate through cervical mucus. This advantage in cervical transit could depend on the capacity of SP to limit non-opsonin-mediated binding which has inhibitory effects on complement-mediated phagocytosis [35]. Moreover, exposure to selected fractions of SP improves boar sperm cryo-survival more than exposure to SP obtained from whole ejaculates [36].

In our analysis, lectin-binding patterns indicated that a modification of epididymal sperm plasma membrane glycoproteins took place after exposure to SP. The acrosomal cap of control spermatozoa was found to be covered with a glycocalyx containing 1) high mannose and complex-type N-glycans (Con A and PHA-L affinity), terminating with lactosamine, α2,6-linked sialic acids (RCA_{120} and SNA binding, respectively), 2) O-linked glycans terminating with the Tn antigen (the simplest mucin O-glycan made by a single N-acetylgalactosamine linked to serine or threonine) (HPA and SBA affinity), a core 1 disaccharide, galactoseβ1,3N-acetylgalactosamine (named T antigen) (receptors for PNA), and disialyl T sequence sialic acid, α2,3galactoseβ1,3(±sialic acidα2,6)N-acetylgalactosamine (MAL II), 3) oligosaccharides containing N-acetylglucosamine (sWGA and GSA II affinity) and terminal αgalactose (GSA I-B_4 binding). The acrosomal regions of SP supplemented spermatozoa revealed the appearance of lactosamine terminating bisected di-triantennary complex-type N-glycans (RCA_{120}, PHA-E) and an increase of N-linked glycans and O-glycans terminating with N-acetylgalactosamine and galactoseβ1,3N-acetylgalactosamine respectively. Interestingly, the PNA staining pattern, used to assess acrosomal status, demonstrated that SP treatment does not appear to affect acrosomal integrity [37].

The tails of control frozen-thawed ES displayed N-glycans terminating with lactosamine. This glycan pattern was enriched with

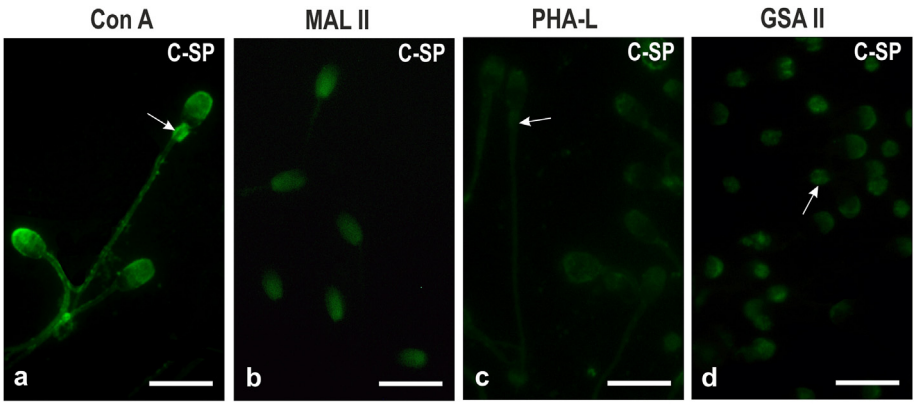

Fig. 2. Con A (a), MAL II (b), PHA-L (c), and GSA II (d) binding pattern of post-thaw dromedary camel epididymal spermatozoa. The sperm affinity of these lectins was not affected by seminal plasma treatment. C, control; SP, seminal plasma. Arrows, cytoplasmic droplets. Bar: 10 μm.

glycans containing T and Tn antigen, as well as lactosamine, N-acetylglucosamine, αgalactose, and α2,6-linkedsialic acid residues after exposure to SP.

Changes in lectin binding patterns have been reported on ram epididymal sperm surfaces after SP treatment [19]. In the ram study, which did not report the regional distribution of lectin-binding sites, it was demonstrated that SP exposure increases WGA binding sites whilst decreasing LPA binding sites on sperm membranes. It is well known that SP components are also secreted by the seminal vesicles, prostate, as well as bulbourethral and

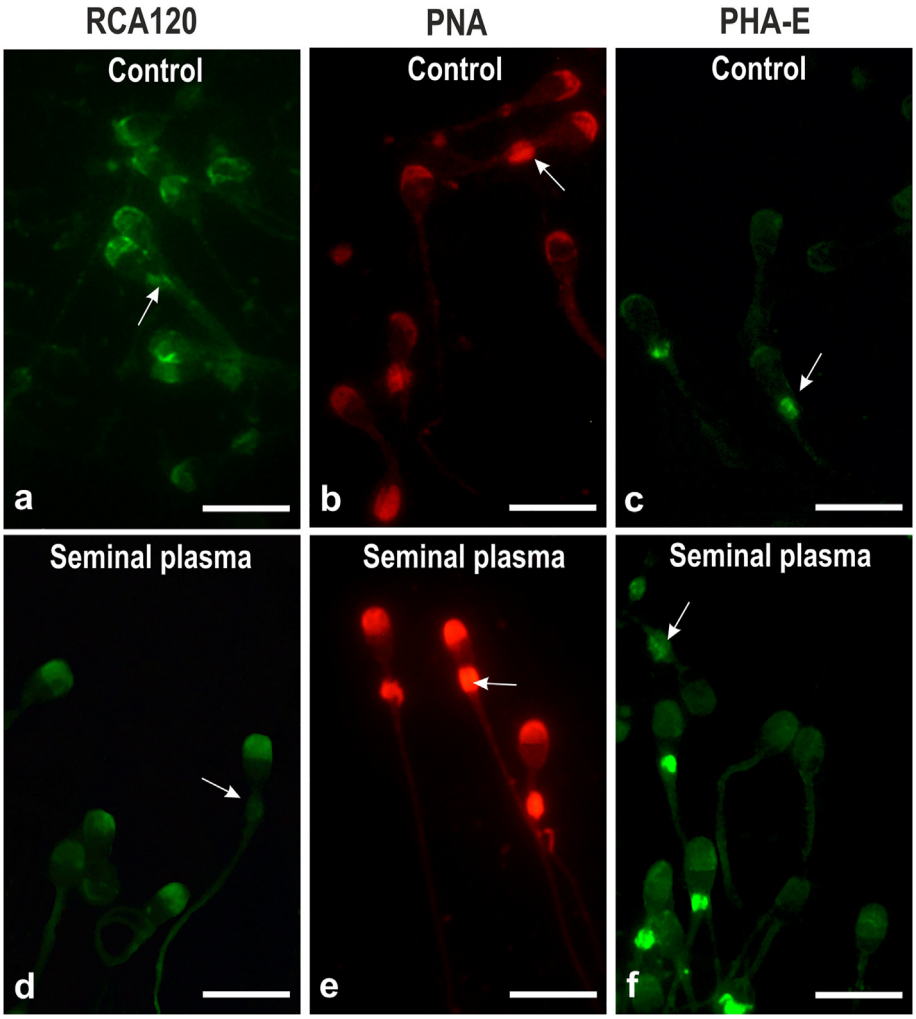

Fig. 3. RCA_{120}, PNA, and PHA-E binding pattern of post-thaw control (a,b,c) and seminal plasma-treated (d,e,f) dromedary camel epididymal spermatozoa. Seminal plasma-treated spermatozoa show an increase of RCA_{120}, PNA and PHA-E binding sites on the acrosomal region as well as the increase of RCA_{120} staining and appearance of PNA and PHA-E affinity on the tail region. Arrows, cytoplasmic droplets. Bar: 10 μm.

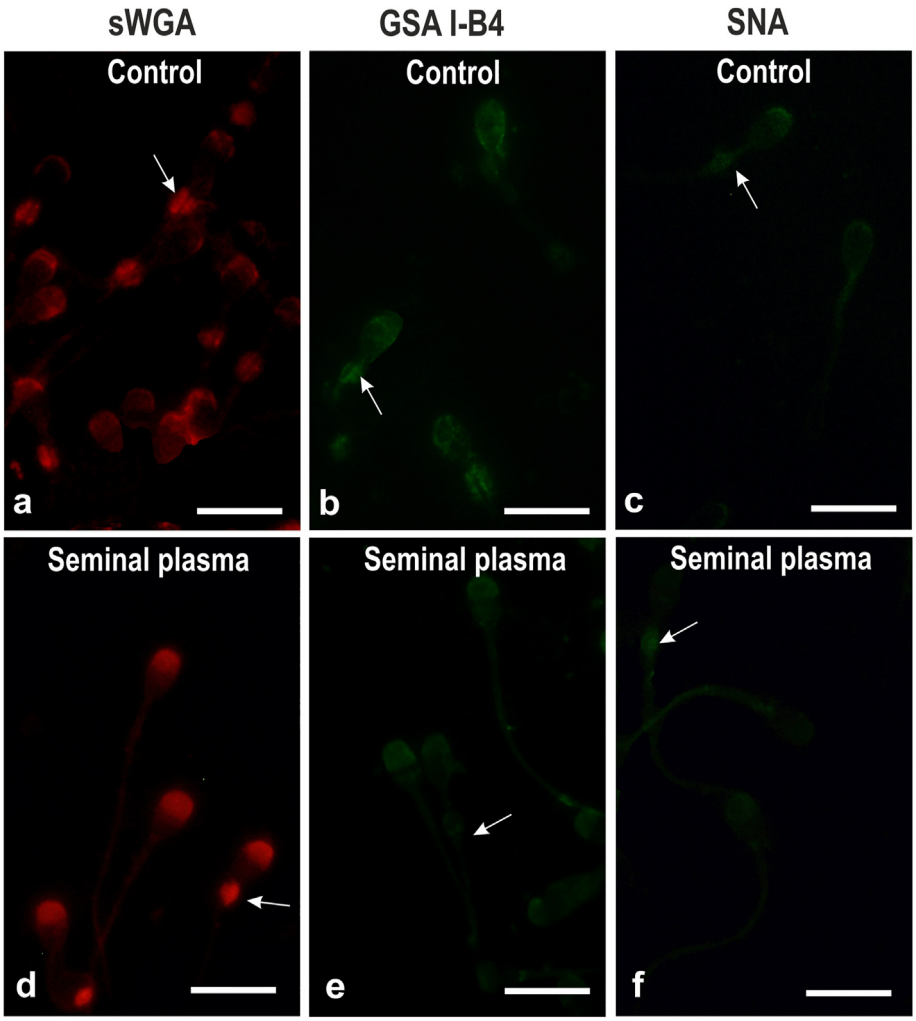

Fig. 4. Succinyl (s) WGA, GSA I–B_4, and SNA binding pattern of post-thaw control (a,b,c) and seminal plasma-treated (d,e,f) dromedary camel epididymal spermatozoa. Seminal plasma treatment induced the appearance of binding sites only on the tail region. Arrows, cytoplasmic droplets. Bar: 10 μm.

periurethral glands [38] and that SP also contains species-specific glycoproteins which bind to spermatozoal surfaces during transit through the male tract [18]. This may explain the increased binding sites for HPA, PNA and PHA-E found in the acrosomal region and for increased sites for RCA_{120}, HPA, PNA, sWGA, GSA I–B_4, SBA, and SNA that was observed within the tail regions of SP-treated spermatozoa. These observed regional changes may depend on the presence, absence, or abundance of region-specific glycan-modifying enzymes. Glycosyl transferase and glycosidase have both been detected in mammalian seminal fluid [18]. Whilst the roles of sperm surface glycans have not been completely understood, it is known that O-linked (mucin-type) glycans are important in sperm-zona binding during the process of fertilization. Moreover, the contribution of mucin-type glycans to male fertility has been reported in impaired spermatogenesis correlated to the absence of a functional MUC1 gene [39]. Interestingly, the present study demonstrated an increase of O-linked glycans (HPA and PNA affinity) on the acrosomal and tail regions of SP treated epididymal spermatozoa. N-glycans contribute greatly to the physicochemical properties of the glycocalyx due to their extraordinary flexibility (high degree of potential rotational movement around each glycosidic bond) and micro-heterogeneity (variation in chain length, branching, monosaccharide composition and terminal sialylation) [40]. It has been suggested that oligomannosidic chains may provide a recognition signal for the selective elimination of incompetent sperm during their transit through the female reproductive tract [41], and may also participate in the binding of sperm to the zona pellucida in humans [42]. The importance of N-glycans synthesis in sperm has been demonstrated in both mice and humans [43]. Male Basigin knockout mice, characterized by a significant reduction of N-acetylglucosamine terminated N-glycans, are sterile due to the absence of mature sperm in the testis and epididymis [44]. Moreover, spermadhesins, a family of SP N-glycosylated proteins, have been found to be associated with sperm surfaces in the boar, the stallion, the bull (see Ref. [45] and cited references), and in the ram [46]. Spermadhesins have been implicated in the binding of sperm to the zona pellucida in the sow [45] and may prevent premature acrosome reactions by forming a protective coat around the acrosomal regions of sperm [47]. Furthermore, GdS, another SP N-glycosylated protein, inhibits both innate and adaptive immune responses [18] as well as inhibiting premature sperm capacitation during the passage from the cervical mucus and the uterus prior to entering the oviduct in humans [22]. Lastly, SP N-glycosylated SL15 is probably involved in the oviductal sperm reservoir of South American camelids via sperm binding to GalNAc of the surface of oviductal epithelium [23]. Therefore, the increase of PHA-E and RCA_{120} binding sites on SP-exposed

spermatozoa observed in the present study may be ascribed to the presence of SL15 in camelid SP.

As for sialic acids, they contribute to the net negative charge of the sperm surface [48]. The presence of sialoglycans on the plasma membrane is indicative of uncapacitated sperm in boars, bulls [49,50], mice [51] and humans [51–53]. Moreover, sialic acids inhibit the autoagglutination of spermatozoa in goats [54], are implicated in sexual selection by female immunity against paternal antigens [55], and are immunoprotective for sperm in the female reproductive tract [15]. Furthermore, sperm transit through cervical mucus is known to require the presence of sialylated glycoproteins in primates [18,34].

Cytoplasmic droplets are a common feature in mammalian epididymal spermatozoa [56] including dromedary camels [57]. The present study detected the presence of N- and O-linked glycans (Con A, RCA_{120}, PHA-L, PHA-E, sWGA, HPA, PNA, GSA II, SNA and GSA I–B_4 reactivity) in the cytoplasmic droplets of dromedary epididymal spermatozoa. The presence of a glycosilated protein, specifically TEX101 (a GPI-anchored protein) has been found in the plasma membrane of mouse CD, although its role is not known [58]. The effect of CDs on sperm quality is heavily species dependent. Mouse cauda epididymal sperm containing CDs are more motile than those without [59], whereas CDs' retention has been associated with reduced fertility in the boar [60,61] and bull [62,63], whilst cytoplasmic droplets are not considered detrimental to the vitality or motility of human ejaculated sperm [64]. Although the functional role of the cytoplasmic droplets in mature sperm is not currently understood [65], it has been demonstrated that CDs represents an energy source during epididymal sperm maturation in mice [59,66] and play a role in regulating ion homeostasis and the volume of spermatozoa during the epididymal transit in several mammals [65].

5. Conclusions

The present study investigated, for the first time, the glycosylation patterns of the glycocalyx of cryopreserved dromedary epididymal spermatozoa and demonstrated that seminal plasma incubation modifies the plasma membrane glycan pattern. These results provide useful information about the reproductive biology of this species and may facilitate further progress towards the application of ARTs in Camelidae. Due to the key role of the sperm glycocalyx in reproduction, further functional studies are needed to evaluate the fertilization capacity of SP-treated epididymal spermatozoa and to illucidate further application of this knowledge in dromedary camel ART.

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