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EPIDIDYMAL BASED CHANGES IN SPERMATOZOA OF DROMEDARY CAMELS

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ABSTRACT

This study was aimed to declare the role of epididymal passage on semen features, DNA integrity and ultra-structures changes in dromedary camel. The testes with attached epididymis of mature dromedary camels (n=50) were collected during the rutting season from December to April. The harvested spermatozoa from the three-main anatomical epididymal segments (Head, body and tail) were assessed for semen characters, DNA integrity as well as, ultra-structural changes. A significant difference in semen features (individual motility, sperm count, membrane and acrosome intactness, viability, normality, maturity, abnormalities and acrosome length and perimeter) among different epididymal segments. Spermatozoa from epididymal tail showed a significantly ($p<0.05$) lower DNA fragmentation than those collected from head segment while agarose gel electrophoresis revealed non-significant difference in DNA intensity between spermatozoa from the three epididymal regions. Ultra-structures of the epididymal spermatozoa showed changes in acrosome shape, sub-acrosomal space, chromatin condensation and protoplasmic droplet during epididymal passage. The protoplasmic droplets varied in size, density and position with the epididymal segments. In conclusion, epididymal passage (from the head to the tail) is an essential pre-request for dromedary camels' spermatozoa to attain high fertilising capacity through its great influence on spermatozoa characteristics and the fine cytological structures.

Key words: DNA integrity, dromedary camel, epididymis, spermatozoa, ultra-structures

Collection of epididymal spermatozoa received an increasing interest by many researchers to be adopted for several assisted reproductive techniques (El-Badry *et al*, 2015; Scholkamy *et al*, 2016). Sperm cells obtained from any epididymal segment have been assessed (Tajik *et al*, 2008). Epididymal spermatozoa recovery from slaughtered/dead animals, cryopreservation and subsequent IVF helps to preserve the genetic material either from highly productive animals and/or from endangered species (Martins *et al*, 2007a). Researchers studied acceptable motility and viability of spermatozoa recovered from the epididymis which have been maintained at room temperature or 5°C in camel (Waheed *et al*, 2011; Shahin *et al*, 2021), bull (Martins *et al*, 2009) and stallion (Muradás *et al*, 2006). However, researchers found that quality of epididymal spermatozoa varied according to breeding season (Abd and Ibrahim, 2014), temperature (Lone *et al*, 2011) and epididymal segment (Waheed *et al*, 2011; Rashad *et al*, 2018).

The current study was aimed to record the changes in semen features, DNA integrity and ultra-structures in relation to epididymal segment in mature dromedary camels during rutting season.

Materials and Methods

Epididymal semen collection and evaluation

Testes and epididymis (n=50) were collected from apparently healthy slaughtered camels aged 5 to 10 years during the rutting season (December – April). The epididymis was dissected, rinsed with 0.9% saline (Yu and Leibo, 2002) and epididymal anatomical segments (head, body and tail) were distinguished (Zayed *et al*, 2012). Each epididymal parts was incised and the spermatozoa were collected separately.

Semen evaluation

Sperm motility

The sperm individual progressive motility was determined by light microscope (×40) on a warm stage at 35°C (Melo *et al*, 2005).

Sperm count

Sperm count was determined with an improved Neubauer haemocytometer chamber after dilution with coloured hypertonic saline solution according to Atiq *et al* (2011).

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Sperm viability and abnormality

The sperm viability and morphology were evaluated in Eosin (5%) and Nigrosin (10%) stained film sand examined under oil immersion lens (×100) according to Skidmore *et al* (2013).

Plasma membrane integrity

Plasma membrane integrity was assessed by hypo-osmotic swelling test (HOS test) as described by Jeyendran *et al* (1984) with some modifications (Zubair *et al*, 2013). Briefly, 10 µl of each semen sample was mixed with 90 µl of a pre-warmed hypo-osmotic solution (0.735 g of sodium citrate dihydrate and 1.351 g of fructose in 100 ml of de-ionised water) and incubated at 37°C for one hour. The positive HOS cells indicated by swelling and coiling of the sperm tail (Lodhi *et al*, 2008).

Acrosome integrity evaluation

The rate of spermatozoa acrosome integrity was determined using Giemsa stain (×100) as described by Chowdhury *et al* (2014).

Evaluation of DNA fragmentation

Acridine orange technique

Acridine orange stain was used to assess DNA integrity of the harvested epididymal spermatozoa as described by Martins *et al* (2007b). Damaged DNA gave red or orange fluorescence and normal double-strand DNA structure gave green fluorescence (Andrazek *et al*, 2014).

Gel electrophoresis

DNA was extracted from spermatozoa collected from the three regions of epididymis according to Trommelen *et al* (1993) with some modifications (Weyrich, 2012).The concentration and purity of extracted genomic DNA were determined spectrophotometrically (BIO RAD, USA).

Transmission electron microscopy (TEM)

Small blocks for spermatozoa from each epididymal segment were initially fixed for 2-3 h with 2% glutaraldehyde in PBS, washed three times with PBS (pH 7.4) for 5 min at 4°C and re-fixed in 1% osmium tetroxide for 1-2 h at 4°C (Boonkusol *et al*, 2010). All samples were dehydrated in ascending grade of ethanol (50, 70, 90 and 100%) and propylene oxide for 1 h and embedded in epoxy resin. Ultrathin sections were cut using the Leica EM UC6 ultramicrotome and stained with uranyl acetate and lead citrate.

Statistical analysis

Data (expressed as mean ± SE) were statistically analysed with one-way ANOVA followed by Tukey (HSD) test using IBM-SPSS for Windows (Ver. 21, 2017). The statistical significance was noted ($P<0.05$).

Results

Epididymal semen features

The epididymal semen characteristics in dromedary camels reorded in present study are presented in table 1.

Epididymal spermatozoa motility ($P< 0.01$), count ($P< 0.001$), membrane integrity ($P< 0.0001$), acrosome intactness ($P< 0.005$), viability ($P< 0.05$), maturity ($P< 0.0001$), normality ($P< 0.0001$) varied markedly between its compartments. There was a tremendous improvement in all previous mentioned parameters in epididymal tail compared with head and body parts. Moreover, the rate of sperm head ($P< 0.005$) and tail ($P< 0.001$) abnormalities greatly reduced in body and tail of epididymis in comparison with head segment. Acrosomal length and perimeter were highly significant ($P<0.001$) between epididymal segments.

Epididymal sperm DNA fragmentation

DNA fragmentation rate as examined by acridine orange showed a tendency ($P=0.099$) of variation between epididymal segment (Fig 1). Epididymal spermatozoa from head region possessed higher ($P<0.05$) fragmented DNA than those from the tail region.

The intensity of extracted sperm DNA did not show any variation between epididymal segments by gel electrophoresis (Fig 2).

Epididymal sperm ultra-structure

Alternations in spermatozoa ultra-structural during epididymal transit were mainly noticed in the acrosome shape, sub-acrosomal space, nuclear chromatin condensation and protoplasmic droplets (Figs 3-4). The acrosome appeared projected anteriorly at head segment, but the acrosome projection was absent at the tail segment. The sub-acrosomal space decreased progressively in spermatozoa from head to tail of epididymis (Fig 3 e, f). Plasma membrane of most spermatozoa was noticed somewhat elevated at epididymal tail region. Spermatozoa with a homogeneously densely packed nucleus were predominant in the tail of the epididymis than upper segments (Fig 4a-c). The protoplasmic droplets were numerous and dense in appearance in the head

Table 1. Epididymal spermatozoa features in dromedary camels.

Item	Head	Body	Tail	P value
Spermatozoa motility (%)	13.33±2.25^{c}	39.17±1.68^{b}	58.13±2.82^{a}	0.01
Sperm cell count (×10^{6}/ml)	58.75±10.87^{b}	96.63±16.64^{b}	224.70±24.86^{a}	0.001
Membrane integrity (%)	70.58±2.06^{b}	83.71±0.99^{a}	87.28±0.87^{a}	0.0001
Acrosome integrity (%)	90.61±0.45^{b}	91.53±0.58^{b}	95.68±0.99^{a}	0.005
Livability (%)	49.95±1.35^{c}	73.65±1.58^{b}	82.84±2.97^{a}	0.05
Immaturity (%)	19.08±1.06^{a}	11.60±0.89^{b}	8.00±0.78^{c}	0.0001
Sperm normality (%)	47.07±2.31^{c}	55.73±1.78^{b}	66.67±2.47^{a}	0.0001
Head abnormality (%)	10.21±2.50^{a}	4.87±0.68^{b}	2.94±0.46^{b}	0.005
Tail abnormality (%)	41.91±1.82^{a}	39.02±1.75^{a}	30.38±2.24^{b}	0.001
Acrosome length (µm)	4.76±0.13^{a}	4.27±0.06^{b}	4.00±0.07^{c}	0.001
Acrosome perimeter (µm)	19.02±0.28^{a}	17.70±0.14^{b}	17.00±0.18^{c}	0.001
DNA fragmentation %	1.62±0.14^{a}	1.42±0.21^{ab}	1.09±0.36^{b}	0.005

Data was presented as mean ± SE (n=50). Values with different superscript letters within the same row were significantly different.

region, few and dark in the body region and few and light in the tail region. Protoplasmic droplets were situated at a higher position of sperm tail and centered around axoneme at head and body segments. Yet, few spermatozoa showed eccentric position around axoneme. Protoplasmic droplets at tail segment mostly situated at distal position and often at the mid-way of sperm tail and eccentrically placed around the axoneme (Fig 4d-e).

Discussion

The current investigation proved that progressive sperm motility radically increased from the epididymal head to tail and this came in accordance with former studies in camel (Waheed *et al*, 2011; El-Badry *et al*, 2015), bull and ram (Amann, 1987), horse (Johnson *et al*, 1980) and donkey (Contri *et al*, 2012). These findings slightly matched with that mentioned by El-Badry *et al* (2015) for the spermatozoa from the epididymal tail and body and slightly lower for spermatozoa from epididymal head. The motility percentage of spermatozoa from tail value was also close to those reported by Turri *et al* (2013).

The present study showed that epididymal sperm count was higher in tail segment than in body and head segments. These values matched with those of Ibrahim *et al* (2012), who claimed that the epididymal tail acts as sperm depot in dromedary camel. This finding was in a strong agreement with Bitto and Okpale (2006), Ahemen and Bitto (2007), Ugwu (2009) and Ibrahim *et al* (2012), but not in agreement with finding of Osman and El-Azab (1974), who indicated that the camel epididymal body incubated more spermatozoa than head and tail. The variations were perhaps due to different harvesting method where dissection was associated with high sperm count.

In our study, there was significant difference in the sperm livability percentage among the three epididymal segments. This finding was not in agreement with Tajik *et al* (2008) and El-Badry *et al* (2015) in dromedary camel. Moreover, in the present study, live sperm percentage from epididymal tail was slightly higher than that recorded by Ziapour *et al* (2014) and El-Badry *et al* (2015). Nevertheless, the mean sperm livability rate herein was slightly lower in harvested spermatozoa from epididymal head and body than that recorded by El-Badry *et al* (2015).

The present research showed that sperm normality rate was higher in epididymal cauda than corpus and caput while the sperm deformities were higher in epididymal head and body than the tail. Tingari *et al* (1986) found that the percentage of spermatozoa with protoplasmic droplet was higher in epididymal head than body and tail. This finding was in a strong agreement with McKinnon *et al* (1994). In contrary, El-Badry *et al* (2015) recorded that the morphologically abnormal sperm percentage did not vary between the three epididymal segments and the proportion of spermatozoa with protoplasmic droplets was higher in the cauda than in the corpus or caput epididymis. Nevertheless, Tajik *et al* (2008) found no significant difference in the percentage of spermatozoa with protoplasmic droplets between three regions of the epididymis. Some authors reported that cytoplasmic droplets in sperm cells might be considered as an abnormality (Bravo *et al*, 1997; Flores *et al*, 2002), while others didn't agree with them (Tingari *et al*, 1986).

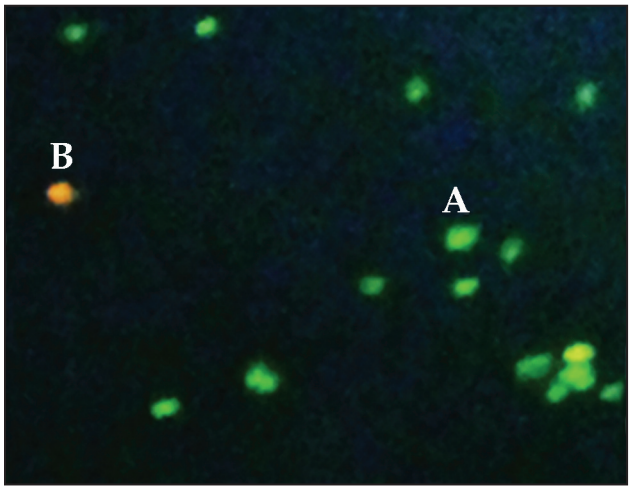

Fig 1. Representative photomicrograph for sperm DNA fragmentation stained with acridine orange stain and examined by florescent microscope. (A) refers to sperm with non-fragmented (emitted green fluorescence) DNA while (B) refers to sperm with fragmented DNA (emitted variable shades of fluorescence from yellow-green to red).

Former works considered the assessment of sperm plasma membrane integrity by the HOS test an indication of male fertility (Revell and Mrode, 1994; Perez-Llano *et al*, 2001). The present study showed that the proportion of sperm cells with an intact plasma membrane was higher for sperms from epididymal tail than head and body. The recorded values here were higher than that reported by El-Badry *et al* (2015) for camel epididymal sperm cells and Ziapour *et al* (2014) for camel ejaculatory sperms. This difference may be due to the method of evaluation.

The current study showed that percentage of spermatozoa with intact acrosome was higher in epididymal tail than those from body and head. These results were marginally higher than that recorded by El-Badry *et al* (2015), who noticed that there were no significant differences among spermatozoa with an intact acrosome between epididymal parts. Also, Morton *et al* (2010) recorded that large proportion of alpaca epididymal semen had an intact acrosome. Our study revealed that there was a very highly significant difference between the acrosome lengths of spermatozoa from different epididymal regions. Similar values were recorded by Osman and Plöen (1986), although these values were lower than that reported by Abdel-Raouf and El-Naggar (1965).

In this study, the epididymal sperm DNA didn't show differences in DNA intensity between epididymal compartments though nuclear

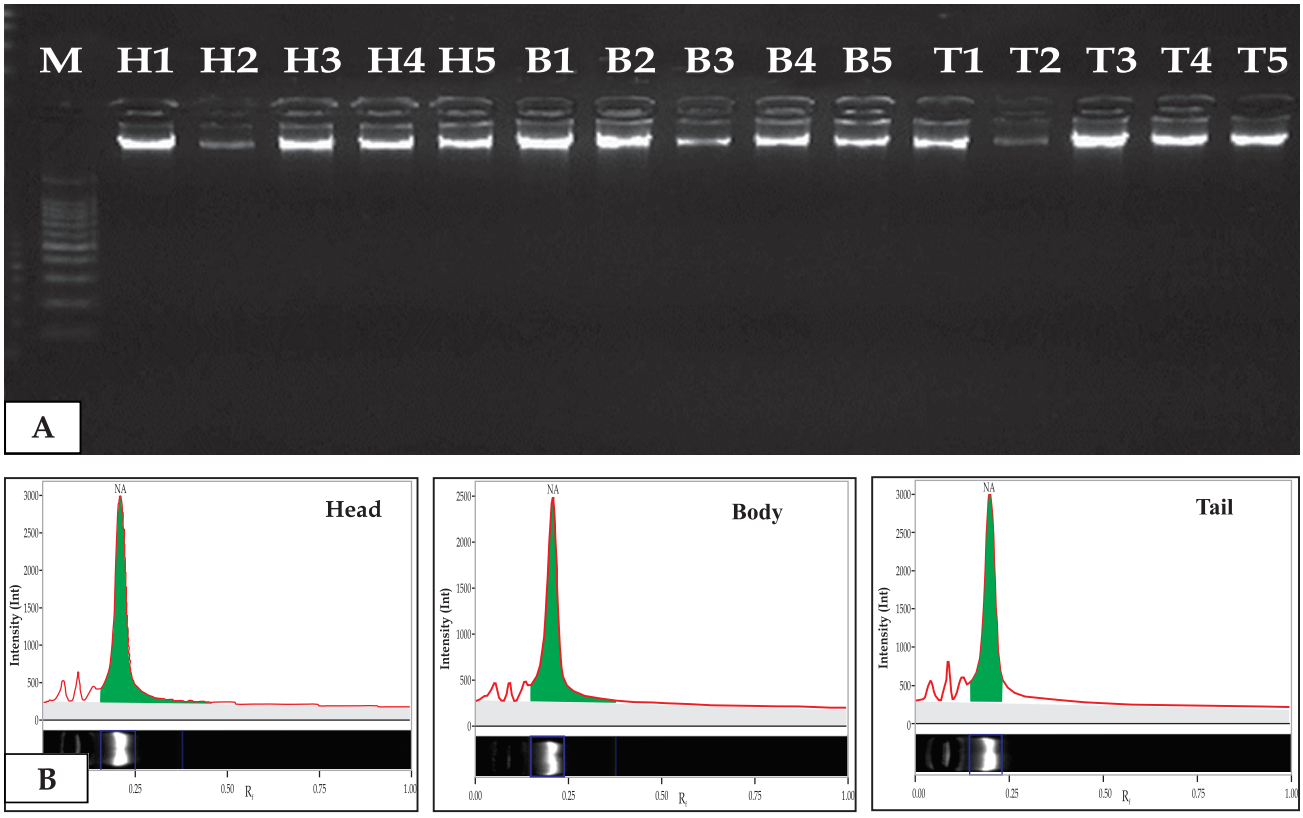

Fig 2. (A)Agarose gel stained with ethidium bromide showed sperm cell DNA extraction product. M: 100-bp ladder. Lanes: H (1-5), B (1-5) and T (1-5) represented DNA extracted from spermatozoa collected from head, body and tail of camel epididymis, respectively. Fig 2(B): Computer aided DNA band density assessment of sperm cell DNA extraction product.

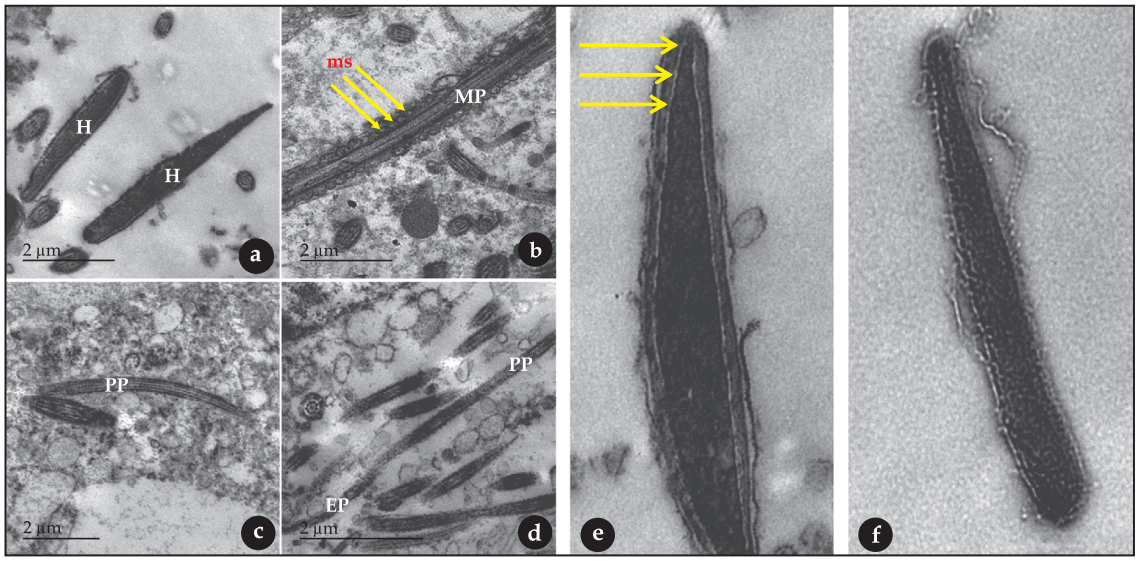

Fig 3. Representative transmission electron micrographs showed sagittal sections at camel epididymal spermatozoa parts. (H) sperm head, (mp) mid piece, (ms) mitochondrial sheath, (pp) principle piece and (EP) end piece. Notice the wide sub-acrosomal space (Fig 3e) in camel epididymal spermatozoa (arrows) that became ultimately disappeared at final epididymal transit (Fig f).

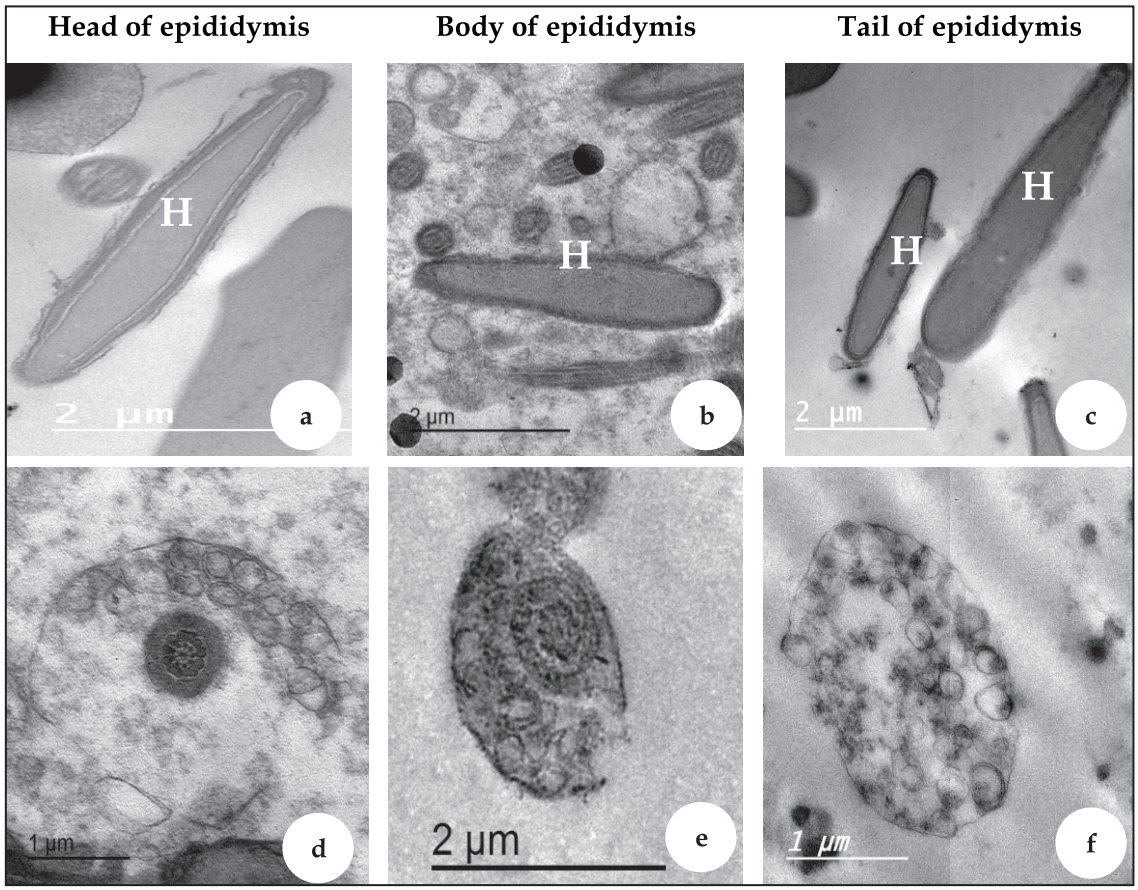

Fig 4. Representative transmission electron microscopic images of the changes in camel spermatozoa nuclear DNA decondensation (a-c) and protoplasmic droplets (d-f) during epididymal passage. There was little variation in nuclear decondensation in samples obtained from the head (a) and body (b) epididymal regions. Spermatozoa from tail of the epididymis showed a homogeneously dense nucleus (c). Protoplasmic droplet appeared proximal eccentric position (d), distal eccentric position (e) and shed-off (f) as spermatozoa passed from head to body and tail segments, respectively.

fragmentation was higher in head than tail of the epididymis. These findings indicated high stability of camel sperm DNA during epididymal passage. Aberrant chromatin packing during spermatogenesis results in sperm DNA fragmentation (Gorczyca *et al*,1993; Sailer *et al*, 1995). Accordingly, El-Badry *et al* (2015) reported that dromedary camel epididymal spermatozoa from cauda epididymis had higher DNA integrity in comparison to corpus and caput epididymal spermatozoa. Our findings agreed with Yanagimachi (1994), who stated that the mammalian spermatozoa nuclei were very stable and highly condensed with a unique DNA organisation (a six-fold more compact than somatic cells). This unique DNA packing was important to protect the sperm cell and minimise damages caused by exogenous agents before fertilisation.

Electron microscope application in reproductive research was helpful in recognising the monomorphic and polymorphic sperm defects beside understanding of spermatozoa physiology and pathology (Moretti *et al*, 2016). In the current study, camel epididymal spermatozoa showed ultra-structural variations between epididymal segments in association with the maturational changes and most of these modifications were confined to acrosome, plasma membrane and protoplasmic droplet. In epididymal head, acrosome projected anteriorly with wider sub-acrosomal space and the protoplasmic droplets were numerous, condensed and dark in appearance at a higher position of sperm tail. On the other hand, in epididymal tail, acrosome projection faded with a very narrow sub-acrosomal space, plasma membrane was somewhat elevated and the protoplasmic droplet were few and light in colour at the midway of sperm tail and eccentrically placed around the axoneme. Osman and Plöen (1986) reported that the cytoplasmic droplet was proximal immediately behind the neck region at the initial epididymal segment and centered around the axoneme then became more eccentric, migrated distally and was eventually shed as the spermatozoa move through the camel epididymis.

Conclusions

Semen characteristics as well as spermatozoa morphology were greatly modified during epididymal passage and this was an essential pre-request for successful fertilisation by the ejaculated semen. Epididymal tail spermatozoa showed an improved fertilising capacity (cytologically and molecularly) that ensure its suitability for assisted reproductive techniques e.g., IVF or ICSI in camels.

Conflict of interest

The Authors declare that there is no conflict of interest.

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