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IMMUNOHISTOCHEMICAL LOCALISATION OF MUCIN 1 IN MALE REPRODUCTIVE ORGANS OF DROMEDARY CAMELS DURING RUTTING AND NON-RUTTING SEASONS

Abdulhakeem Eljarah[1,2,*], Abdulrahman Alhaider[1], Marzook Al-Eknah[1] and Sabry M. El-Bahr[3,4]

[1]Department of Clinical Sciences, [3]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Ahasa 31982, Saudi Arabia

[2]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, 22110, Jordan

[4]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

ABSTRACT

This study was aimed to investigate the immune reactivity levels of anti-MUC1 (Mucin 1) antibody in male camel accessory sex glands (prostate and bulbourethral) in addition to the testes, epididymis and ductus deferens. This is potentially useful for an insight on the role of mucous secretion during rutting and non-rutting seasons in male camel. Therefore, it is important to check the immune reactivity levels of anti-MUC1 antibody as a marker for mucous secretion activity. To achieve that, testes (proximal part, distal part and rete testis), epididymis (head, body and tail), ductus deferens (initial, middle and ampullary part, prostate (compact and disseminated part) and bulbourethral gland were collected from 12 male camel during rutting and non-rutting seasons and were subjected to immunohistochemistry. Results showed that the higher immune reactivity levels of anti-MUC1 antibody during rutting season was in rete testis, proximal and distal part of the testes, head of epididymis, ampullary part of ductus deferens, prostate (compact and disseminated part) and bulbourethral gland. Whereas, the higher immune reactivity levels of anti-MUC1 antibody during non-rutting season was in rete testis, proximal and distal part of the testes, head of epididymis, ampullary part of ductus deferens and compact part of the prostate. Thus, the distribution of mucous secretion varied between rutting and non-rutting seasons and even among the reproductive organs itself. However, highest secretory activity was found during the rutting season in the ampulla of ductus deferens, disseminated part of the prostate and bulbourethral gland.

Key words: Accessory sex glands, *Camelus dromedarius*, epididymis, MUC1, testes

The viscosity of dromedary camels' semen complicates semen assessment when trying to separate spermatozoa from seminal plasma using routine methods for spermatozoal counting (Merkt *et al*, 1990; Marai *et al*, 2009; El-Kon *et al*, 2011), as well as motility. The viscosity causes oscillatory movement of the spermatozoa (Tingari *et al*, 1984; Elwishy, 1988; Merkt *et al*, 1990; Musa *et al*, 1990; El-Kon *et al*, 2011) rather than progressive motility like other domestic animals. The existence of mucopolysaccharides in the ejaculate, according to (Mann, 1964), may explain the high viscosity of camel semen. Mucins are glycoproteins with a high molecular weight that can be found on the apical surface of glandular epithelia including the gastrointestinal, respiratory, and reproductive tracts (Lichtenwalner *et al*, 1996). Mucin 1 (MUC1) is a Type I membrane glycoprotein that is expressed on the apical cell surface of many secretory epithelial cells. Its functions include preventing adhesion, lubricating and hydrating the epithelium, and protecting it from microbial attack (Walter Bravo *et al*, 1997; Zeidan *et al*, 2001). The high viscosity of male camel spermatozoa in the female reproductive tract, on the other hand, is important for their viability (Sumar and Garica, 1986). We hypothesised that the secretory activity and amount of mucous formed by the reproductive organs of the dromedary male camel have a distinct seasonal profile. As a result, the current research investigated the localisation of MUC1 in dromedary camels' testes, epididymis, ductus deferens, prostate, and bulbourethral glands. This could help with reproductive management by

SEND REPRINT REQUEST TO ABDULHAKEEM ELJARAH, SABRY M. EL-BAHR email: selbahar@kfu.edu.sa; aeljarah@kfu.edu.sa; sabryelbahr@hotmail.com

reducing and/or avoiding mucous secretory activity during times of the year when semen can be assessed and processed efficiently for artificial insemination in this species.

Materials and Methods

Pre-experimental ethics and experimental Sampling:

All sampling and experimental management were approved by King Faisal University Animal Care and Use Committee (KFU-ACUC). Samples collected during rutting and non-rutting season from adult dromedary camel bulls (*Camelus dromedarius*) form a local slaughterhouse at Al-Ahsa- Saudi Arabia (25° 21′ 52.45″ N and 49° 33′ 55.15″ E) with an age ranges from 4-16 years. The reproductive tissues samples were obtained from testis (proximal and distal parts and rete testis), epididymis (head, body, and tail), ductus deferens (initial, middle and ampullary parts), prostate gland (compact and disseminated parts) and randomly from the bulbourethral gland.

Immunohistochemistry paraffin protocol (IHC-P) for MUC1 detection

Approximately one centimetre of testes (proximal part, distal part and rete testis), epididymis (head, body and tail), ductus deferens (initial, middle and ampullary part, prostate (compact and disseminated part) and bulbourethral gland tissue was taken from each tissue and then fixed overnight in 4% paraformaldehyde. All the fixed samples were processed for immunohistochemical examination by dehydration, clearing, infiltration, and embedding. Tissue samples were sectioned at 5 µm using a microtome (Leica, Germany) and were deparaffinised by passing the Thermo Scientific TM Super Frosted TM Plus charged slides through xylene. Two changes were made for 5 min each followed by the hydration of the sections by dipping them for 30 s in degraded alcohol (100%, 100%, 95%, 80%, 70%). The slides were then washed for 2 x 5 min in tris-buffered saline (1X TBS) plus 0.025% triton X100 with gentle agitation. The slides were then blocked in 10% normal serum with 1% bovine serum albumin (BSA) in 1X TBS for 2 h at room temperature. The slides were left to drain for a few seconds and the sections were then wiped with tissue paper. Rabbit polyclonal Anti-MUC1 antibody (Abcam, ab15481) and a mouse- and rabbit-specific HRP/DAB (ABC) detection IHC kit (Abcam, ab64264) were used to detect MUC1. Slides were counterstained by immersing the tissue sections in Mayer's hematoxylin for 5 min and washing them under tap water for 10 min. Then sections were dehydrated by dipping them in graded alcohol (70%, 95%, 100%, 100%), just for seconds, and clearing them by using 2 changes of xylene for 5 min each. Finally, the tissue sections were mounted by using DPX with cover slides (22 x 40 mm) and the staining was observed by Leica ICC50 W light microscopy under 10x and 40x magnification powers. Wi-Fi-capable digital camera detector and Leica Air Lab App software were used. Reaction reactivity was taken using image processing and analysed using an imageJ 1.52a analyzer (Wayne Rasband, National Institute of Health, USA, http://imagej.nih.gov/ij).

Results

Testes

In rutting season, the immune reactivity levels of anti-MUC1 antibody (Table 1) was intense in the proximal part (Fig 1), rete testis (Fig 2) and distal part (Fig 3). The immunohistochemical detection of MUC1 localised in seminiferous tubules with normal spermatogenesis in the proximal part (Fig 1) and distal part (Fig 3) of testes. While in non-rutting male camel testes, the immune reactivity of anti-MUC1 antibody was intense in rete testis and moderate in both proximal and distal parts of the testes as shown in Table 1. The immunohistochemical detection of MUC1 localised in the interstitial tissue of testes surrounding seminiferous tubules in the proximal part (Fig 1) and distal part (Fig 3) of testes. Whereas in rete testis, the immunohistochemical detection of MUC1 localised in interconnecting tubules in both rutting and non-rutting season as shown in Fig 2.

Epididymis

In rutting season, the immune reactivity levels of anti-MUC1 antibody was intense in the head and weak in both body and tail. The signal was strong in the basal cell (stem cell) layer and sterocelia of the columnar cell of the head of the epididymis (Fig 4) and weak positivity of the columnar cell in both body (Fig 5) and tail (Fig 6). While in non-rutting male camel epididymis, the immune reactivity of anti-MUC1 antibody was moderate in the head and very weak in both body and tail as shown in Table 1. The signal shows a moderate positivity in both basal cell and columnar cell (Fig 4) and very weak signal in columnar cell in both body (Fig 5) and tail (Fig 6).

Ductus deferens

In rutting and non-rutting season, the intense immune reactivity of anti-MUC1 antibody was only

Table 1. The semi-quantitatively immunohistochemical reactivity to Anti-MUC1 antibody in testis, epididymis, ductus deferens, prostate and bulbourethral gland.

Immunoreactivity of Rabbit Polyclonal Anti-MUC1 Antibody												
Tissue sample	Testis			Epididymis			Ductus Deferens			Prostate gland		Bulbourethral gland
	TP	TR	TD	EH	EB	ET	DI	DM	DA	PC	PD	Random part
Rutting season	+ + + +	+ + + +	+ + +	+ + + +	+ +	+ +	+	+	+ + + +	+ + +	+ + + +	+ + + +
Non-Rutting season	+ + +	+ + + +	+ + +	+ + +	+	+	+	+	+ + +	+ +	+	+

(TP): Testis Proximal Part, (TR): Rete testis, (TD): Testis Distal Part, (EH): Epididymis Head, (EB): Epididymis Body, (ET): Epididymis Tail, (DI): Ductus Deferens initial part, (DM): Ductus Deferens Middle part, (DA): Ductus Deferens Ampullary part, (PC): Prostate Compact Part and (PD): Prostate Disseminated Part. (+) = very weak reactivity. (+ +) = weak reactivity, (+ + +) = moderate reactivity and (+ + + +) = intense reactivity.

in ampullary part of ductus deferens when compared to initial and middle parts, in which they had a very weak reactivity to anti-MUC1 antibody as shown in Table 1. The signal showed a very weak positivity in epithelium and basal cell of the initial (Fig 7) and middle parts (Fig 8). The signal showed strong positivity in epithelial columnar and basal cell in the ampullary part (Fig 9).

Prostate and bulbourethral glands

In rutting season, the highest immune reactivity of anti-MUC1 antibody was in the disseminated part of prostate and bulbourethral gland compared to moderate reaction in the compact part of prostate gland as shown in Table 1. The signal showed a moderate positivity in the acinar cells of the compact part (Fig 10) and intense signal in the acinar cells and secretory alveoli of the disseminated part of prostate (Fig 11) and bulbourethral gland (Fig 12). On the other hand, the immune reactivity of anti-MUC1 antibody in non-rutting season was very weak in disseminated part of prostate and bulbourethral gland compared to moderate reaction in the compact part of prostate gland as shown in Table 1. The signal showed a moderate positivity in the acinar cells of the compact part (Fig 10) and very weak in the acinar cell and secretory alveoli of disseminated part of prostate (Fig 11) and basal cell of the bulbourethral gland (Fig 12).

Discussion

Camelid ejaculate is highly viscous, grey to milky white in colour, with low volume and concentration of spermatozoa (Marai *et al*, 2009). Compared to stallion ejaculate; the gel fraction cannot be separated from the sperm-rich fraction making sperm cells motility assessment very difficult and highly variable (Skidmore, 2005). Moreover, the seminal plasma is considered the best media for the liveability of sperms, so great variation in seminal plasma constituents such as mucous concentration may play a major role in spermatozoal motility and liveability (Kershaw-Young and Maxwell, 2012). Mucins are categorised into membrane associated and secreted mucins and contain 17 genes: MUC 1-4, 5AC, 5B, 6-13, 15-17, 19 and 20. The large gel-forming mucins and small soluble mucins are two types of secreted mucins (Russo *et al*, 2006). Secretory mucins are released into the environment, where they can form extremely large and viscous gels, which are then cleared by net fluid flow through the lumina of various mucosa (Forstner, 1995; Hattrup and Gendler, 2008). MUC1 is a large transmembrane mucin glycoprotein that is expressed on the apical surface of a variety of reproductive tract epithelia and acts as a lubricant, hydrant of cell surfaces, and antimicrobial and degradative enzyme protectant (Brayman *et al*, 2004). Furthermore, the cytoplasmic tail of MUC1 has been found to be associated with β-catenin (Yamamoto *et al*, 1997) and other signaling molecules, such as Grb2/Sos (Pandey *et al*, 1995), implying that MUC1 can play a role in cell signaling (Gendler, 2001). We found that during rutting season, immune reactivity levels of anti-MUC1 antibody in male camel testes, epididymis, ductus deferens, and accessory sex glands were significantly higher than during non-rutting season, which was consistent with suggestion of Gendler (2001). Moreover, during rutting season, anti-MUC1 antibody immune reactivity was intense in the seminiferous tubules of the proximal and distal parts. Anti-MUC1 antibody immune reactivity

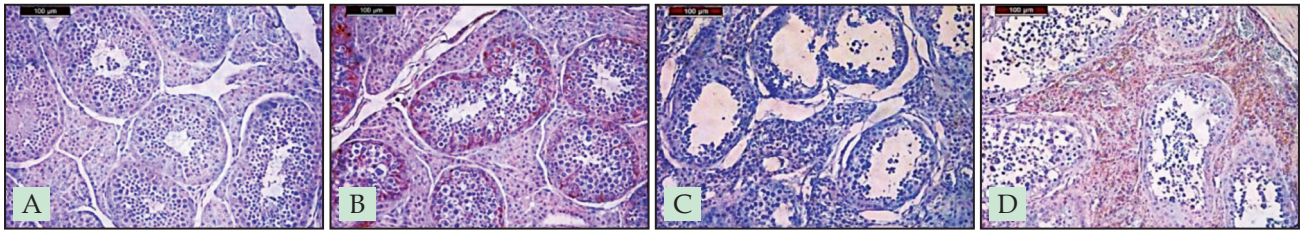

Fig 1. Chromogenic immunohistochemistry of anti-MUC1 antibody in the proximal part (TP) of the testes. (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season-mucin localised in seminiferous tubules with normal spermatogenesis. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows mucin localised in interstitial tissue of the testes. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

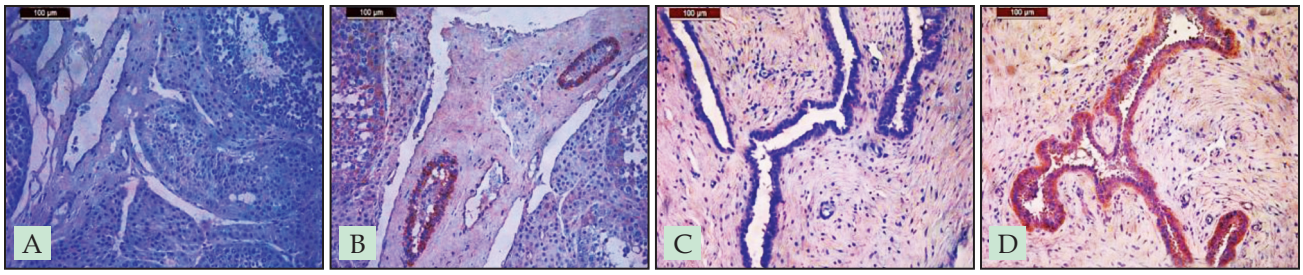

Fig 2. Chromogenic immunohistochemistry of anti-MUC1 antibody in the rete testis (TR). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong interconnecting tubules positivity. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows strong cytoplasmic positivity in myocytes in both muscle fibre and its nucleus interconnecting tubules. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

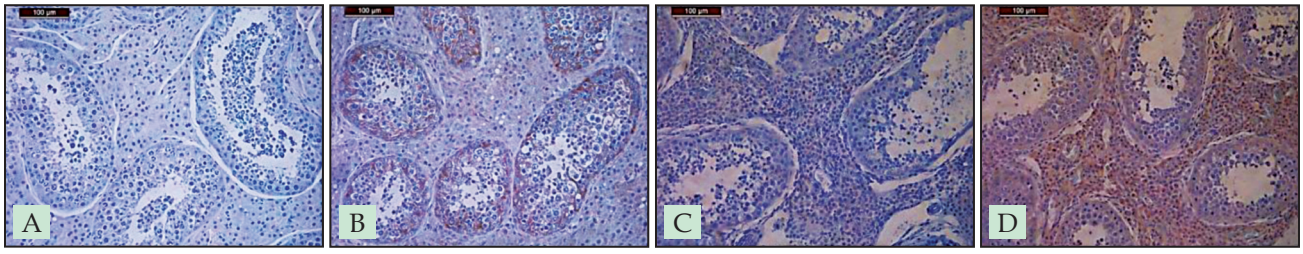

Fig 3. Chromogenic immunohistochemistry of anti-MUC1 antibody in the distal part (TD) of the testes. (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows mucin localised in seminiferous tubules with normal spermatogenesis. (**C**) Negative control of MUC1 during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows mucin localised in interstitial tissue of the testes. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

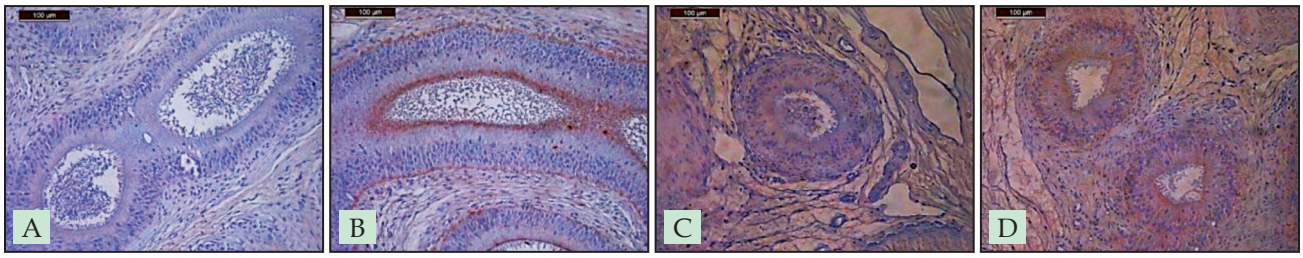

Fig 4. Chromogenic immunohistochemistry of anti-MUC1 antibody in the head of epididymis (EH). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong positivity in basal cell (stem cell) layer and sterocelia of the columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate positivity in both basal cell and columnar cell. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

was moderate in the interstitial tissue surrounding seminiferous tubules in both the proximal and distal parts during the non-rutting season. The differences in MUC1 localisation in testes during rutting and non-rutting season could be linked to the physiological state of the male in addition to steroidal hormone

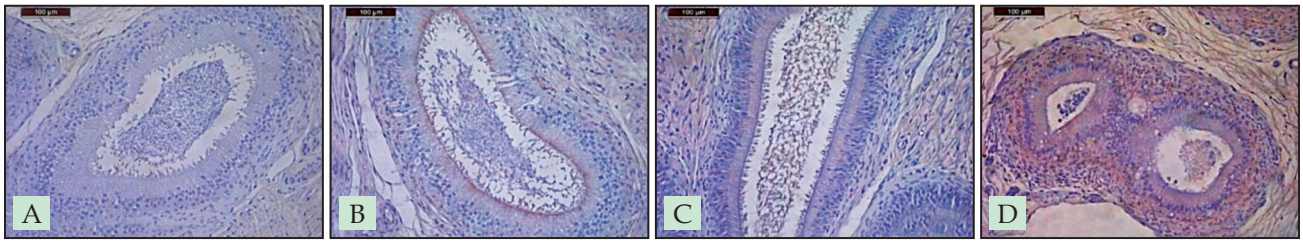

Fig 5. Chromogenic immunohistochemistry of anti-MUC1 antibody in the body of epididymis (EB). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows weak positivity sterocelia of the columnar cell. (**C**) Negative control of MUC1 in the body of epididymis during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows very weak positivity of MUC1 in the body of epididymis. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

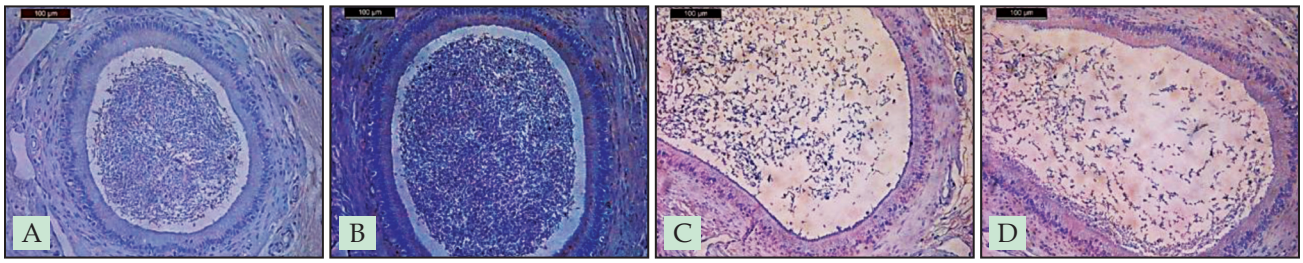

Fig 6. Chromogenic immunohistochemistry of anti-MUC1 antibody in the tail of epididymis (ET). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows weak positivity of columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows very weak positivity of MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

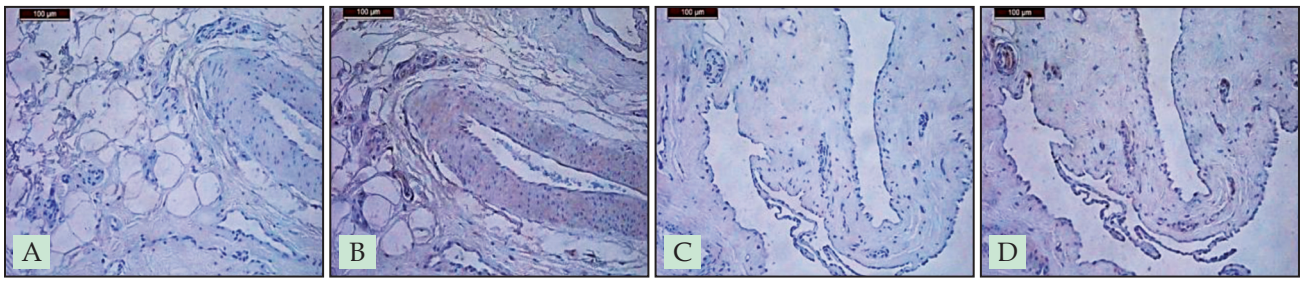

Fig 7. Chromogenic immunohistochemistry of anti-MUC1 antibody in the initial part of ductus deferens (DI). (**A**) Negative control of MUC1 during rutting season. (**B**) Very weak positive reaction of MUC1 during rutting season. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Very weak positive reaction of MUC1 during non-rutting season. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

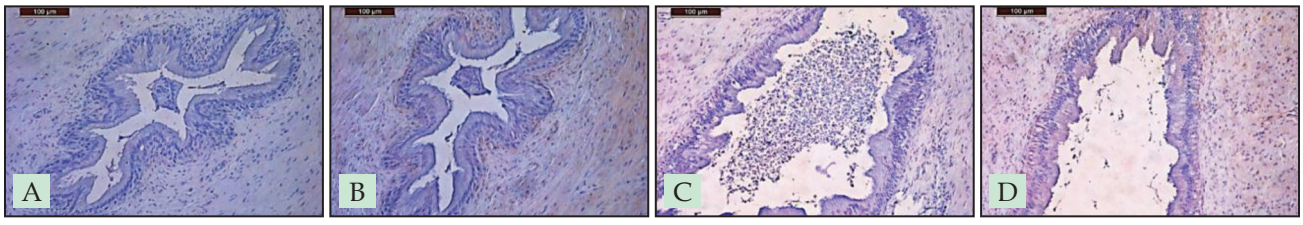

Fig 8. Chromogenic immunohistochemistry of anti-MUC1 antibody in the middle part of ductus deferens (DM). (**A**) Negative control of MUC1 during rutting season. (**B**) Very weak positive reaction of MUC1 during rutting season. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Very weak positive reaction of MUC1 during non-rutting season. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

level during rutting and non-rutting season. During rutting season, the intense immune reactivity levels of anti-MUC1 antibody within seminiferous tubules of the proximal and distal parts may be linked to increased spermatogenesis and sperm hydration. Under these conditions, the molecular mechanism of MUC1 regulation is unclear. Our findings suggest that testosterone hormone regulates MUC1 gene transcription in testicular epithelial cells during the rutting season. In this regard, further research

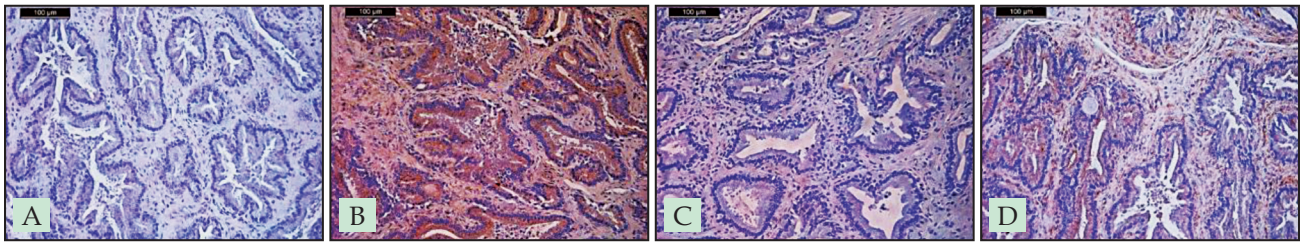

Fig 9. Chromogenic immunohistochemistry of anti-MUC1 antibody in the ampullary part of ductus deferens (DA). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong positivity of the columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate positivity of the columnar cell. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

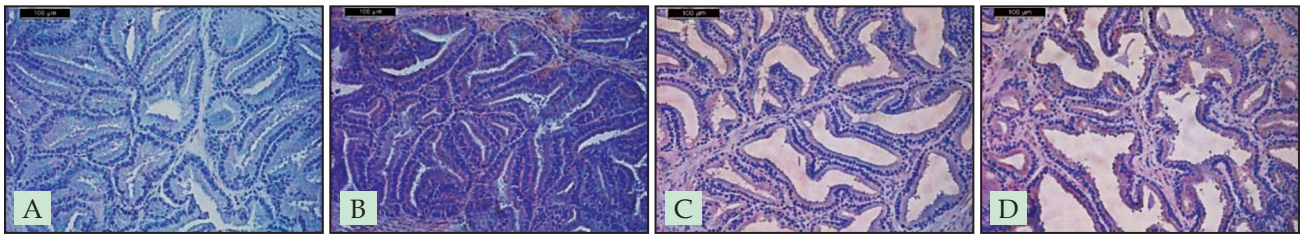

Fig 10. Chromogenic immunohistochemistry of anti-MUC1 antibody in the compact part of the prostate gland (PC). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows moderate acinar cell positivity of anti-MUC1. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate acinar cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

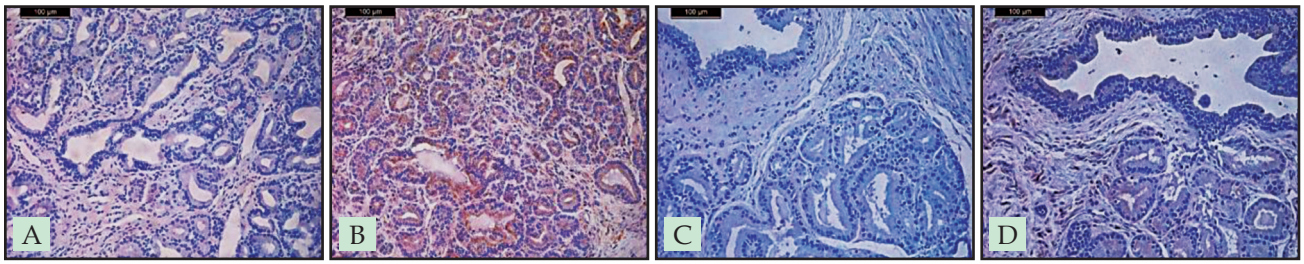

Fig 11. Chromogenic immunohistochemistry of anti-MUC1 antibody in the disseminated part of the prostate gland (PD). (**A**) Negative control of MUC1 during rutting season. (**B**) positive reaction of MUC1 during rutting season shows strong acinar cell and secretory alveoli positivity of anti-MUC1. (**C**) Negative control of MUC1 during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows very weak acinar cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

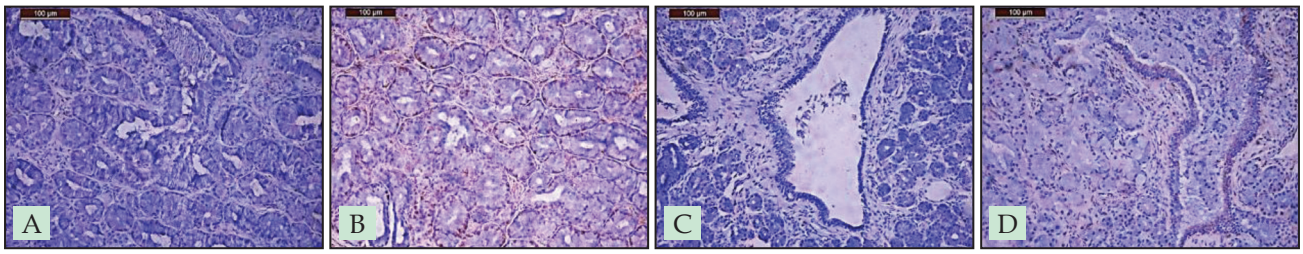

Fig 12. Chromogenic immunohistochemistry of anti-MUC1 antibody in random parts of the bulbourethral gland (BU). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong basal cell positivity of anti-MUC1 (brown colour). (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows very weak basal cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

is needed to demonstrate and understand the mechanisms underlying steroid hormone regulation and signaling during rutting season on MUC1 and other mucin genes transcriptional regulation.

By detecting immune reactivity levels of anti-MUC1 antibody, we attempted to identify the source of MUC1 secretion in male camel prostate and bulbourethral gland. The highest immune reactivity

of anti-MUC1 antibody was found in the ampullae of ductus deferens, acinar cells, and secretory alveoli of the disseminated portion of prostate, as well as the bulbourethral gland, during rutting season. In the non-rutting season, however, anti-MUC1 antibody immune reactivity was very weak in the initial and middle part of the ductus deferens, as well as the acinar cell and secretory alveoli of the disseminated part of the prostate and bulbourethral gland. In conclusion, the main source of MUC1 secretion among male camel reproductive and accessory sex glands comes from ampullae of ductus deferens, acinar cell and secretory alveoli of the disseminated portion of prostate and the basal cells of bulbourethral gland. However, the highest secretory activity occurs during the rutting season, which could be used to reduce the effect of the gel fraction in camel semen when collected outside of the rut season, though this needs to be investigated further.

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Camel-based baby formula to hit shelves in Dubai

A UAE-based company has unveiled what it calls the world's first camel-based baby formula, an instant powder mix aimed mainly at infants allergic to cow's milk. The Emirates Industry for Camel Milk & Products, or "Camelicious," introduced the product at Gulfood 2018, an international food and beverage trade event. The milk, which it says is suitable for children ages one to three, primarily targets infants allergic to cow's milk. The company calls its latest product the world's first instant baby milk processed from camel milk. This milk genuinely complements the UAE values and traditions that are handed down from one generation to another.

(Arab News, Saturday. May 01, 2021)

Camel milk consumption drops over MERS fears

There has been a 65 percent decline in demand for camel milk in the past year, forcing some stores to stop selling the product, while many camel owners have now put a halt to this once-thriving commercial activity. This coincides with increased efforts being made by the Ministry of Health to warn the public about the serious dangers of drinking camel milk without boiling it. Scientists have linked camels and camel milk to the deadly MERS coronavirus. In spite of the Health Ministry's warnings, some camel milk consumers remain adamant. The ministry has clearly said that many are unaware that camel herders or milk consumers themselves may be carrying the coronavirus without knowing it or experiencing any symptoms. The Health Ministry warned that such infected persons could transmit the virus to one of their family members who has a poor immune system without even knowing it, putting their whole family at risk.

(Arab News, Saturday. May 01, 2021)

Dubai cafe introduces camel products on ITS menu

A Dubai cafe, trying to give a modern twist to an old Bedouin tradition, has started putting camel products on its menu. Cafe2Go, launched in September last year by an Emirati entrepreneur as part of a scheme to revive Bedouin traditions, now features camel-lattes, camel-ccinos and camel-meat fajitas. He launched Camellos — a brand name for his products derived from the Spanish word for camel. Café owner wanted our younger generation to start drinking it again by mixing it with modern drinks.

(Arab News, Saturday. May 01, 2021)