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LOCALISATION OF AQUAPORIN1 IN THE VAS DEFERENS AND PROSTATE GLAND OF THE DROMEDARY CAMEL (*Camelus dromedarius*) DURING RUTTING AND NON-RUTTING SEASON

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ABSTRACT

A membrane protein channel, Aquaporin 1 (AQP1), enables the fast water flow through the epithelium. Using the immunohistochemistry technique, the current work elucidated the presence of AQP1 in the vas deferens and prostate gland of dromedary camels in rutting and non-rutting seasons throughout the year. The immunohistochemistry of AQP1 revealed a strong immunoreactive in the epithelial cells of the initial vas deferens at the beginning of the rutting season (October and November). During December and January, this expression became moderate, and by its end (February and March), it seemed pale. The middle vas deferens epithelium and luminal sperms displayed high levels of AQP1 protein in the first and second third of the season. There has been little immunoreaction to the protein in recent months. At the start of the rutting season, the ampullary vas deferens moderately reacted to AQP1 antibodies; however, from December through March, this response decreased to a weak state.

In the non-rutting season, the middle part of the vas deferens exhibited a significant immunoreaction to AQP1. The initial vas deferens showed a strong immunoreaction in April and May before decreasing to appear mildly for the remainder of the season. AQP1 showed a weak expression in the ampulla at this time. AQP1 was not clearly expressed by the prostate gland over the year. In conclusion, it is possible that AQP1 has a role in spermatozoa migration via the male genitalia of camels and may even facilitate the flow of water, which is necessary for sperm motility.

Key words: Aquaporin 1, camel, immunohistochemistry, prostate gland, vas deferens

Aquaporins (AQPs) are membrane proteins that assist in moving water and other tiny solutes across biological membranes. They are a family of tiny, hydrophobic integral membrane channel proteins that speed up the passive transport of water. Mammals have been found to have 13 isoforms of AQPs (AQP0-AQP12), to date (Carrageta *et al,* 2020). Different AQPs have been found in several tissues of mammals including the male genital system (Stevens *et al,* 2000). In 1992, AQP1 was discovered in human erythrocytes (Preston *et al,* 1992). AQP1 was found to be expressed in the organs of the male reproductive tract of many mammals including the dromedary camel, human, horse, buffalo-bull, dog, cat, and fruit-eating bat (Ito *et al,* 2008; Lu *et al,* 2008; Domeniconi *et al,* 2008; Skowronski *et al,* 2009; Yeung *et al,* 2010; Oliveira *et al,* 2013; Klein *et al,* 2013; Arrighi and Aralla, 2014; Arrigh *et al,* 2016; An and Wang, 2016; Althnaian, 2023), where, water absorption and sperm concentration control in the male genital organs are major AQP1 functions (Brown *et al,* 1993; Nicotina *et al,* 2004; Nicotina *et al,* 2005; Lu *et al,* 2008; Arrighi *et al,* 2010b). According to the authors' knowledge, there is previous study about the expression of AQP1 in the dromedary's testis and epididymis. Thus, using the immunohistochemical technique, the current investigation was carried out to find AQP1 in the vas deferens and prostate gland of the dromedary camel throughout rutting and non-rutting seasons all year long to complete the view of this protein in the male genital system.

Materials and Methods

The samples protocol was accepted by the ethics committee of King Faisal University (Ref. No. KFU-REC-2023-MAY-ETHICS887). Samples were obtained from 36 healthy adult local breed dromedary camels (age 4-10 years) from local

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slaughterhouse in Al-Ahsa, Kingdoms of Saudi Arabia, on a regular monthly basis over the course of a year. These samples were taken from three parts of the vas deferens (initial, middle and ampulla) and prostate gland (corpus and disseminate parts) for immunohistochemically (IHC) analysis. Tissue samples were fixed in 10% buffered formalin for 36 hours, then were thoroughly washed in phosphate-buffered saline (PBS), embedded in paraffin wax after being dehydrated in graded ethanol. A rotary microtome was used to cut 5 micrometre-thick slices from each tissue. After that, tissue sections were deparaffinised by xylene, washed in ethanol alcohol and rehydrated in PBS. For fifteen minutes, antigen retrieval was carried out in a microwave oven using 0.01M PBS (pH 7.4). Thereafter, the sections were cooled at 25°C and washed again in PBS. 3% hydrogen peroxide was used for 30 minutes to block endogenous peroxidase. To prevent any non-specific reactions, the goat serum (10%) was utilised for 20 minutes after three rounds of washing in PBS. Then, the primary antibody, polyclonal rabbit anti-AQP1 was applied (Abcam, dilution 1:200) and incubated overnight in a wet chamber. Sections were incubated with biotin-labelled secondary antibodies and avidin-HRP third antibodies, DAB was used to detect the positive staining. Hematoxylin stain was used for section counter-staining. Negative control sections have the same procedure except for skipping the primary antibody. Slides were examined under light microscopy for histological studies, and photo-micrographs were taken.

Results

Expression of AQP1 immunoreactive protein was carried out using immunohistochemistry in the vas deferens and prostate gland of the dromedary camel during rutting and non-rutting season over a period of 12 months. Localisation and intensity of AQP1 in these organs were recognised and recorded in Tables 1 and 2, for the rutting and non-rutting seasons, respectively. Both seasons were clarified in Fig 1.

Table 1. Displaying AQP1 localisation throughout the **rutting season** in the different parts of the vas deferens and prostate gland of the dromedary camel.

Month Part	October- November	December- January	February- March
VI	+++	++	+
VM	+++	+++	+
VA	++	+	+
PD	+	+	+
PC	+	+	+

VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; PD, disseminated prostate; PC, corpus prostate; -, negative reaction; +, weak reaction; ++, moderate reaction; +++, strong reaction.

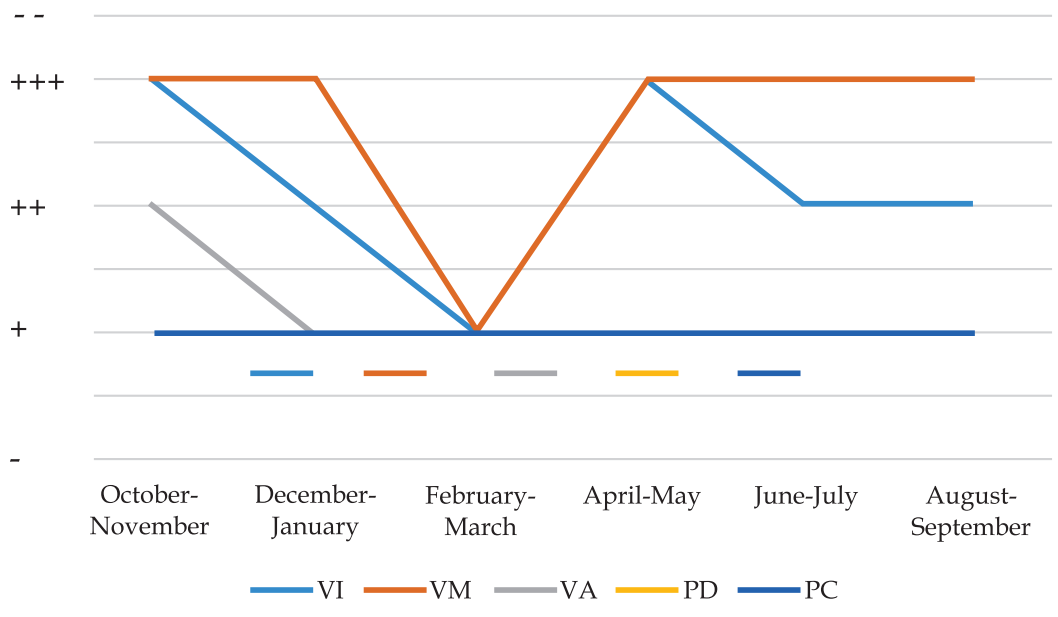

Fig 1. localisation of AQP1 in the vas deferens and prostate gland of the dromedary camel through the rutting and non-rutting seasons.

VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; PD, disseminated prostate; PC, corpus prostate

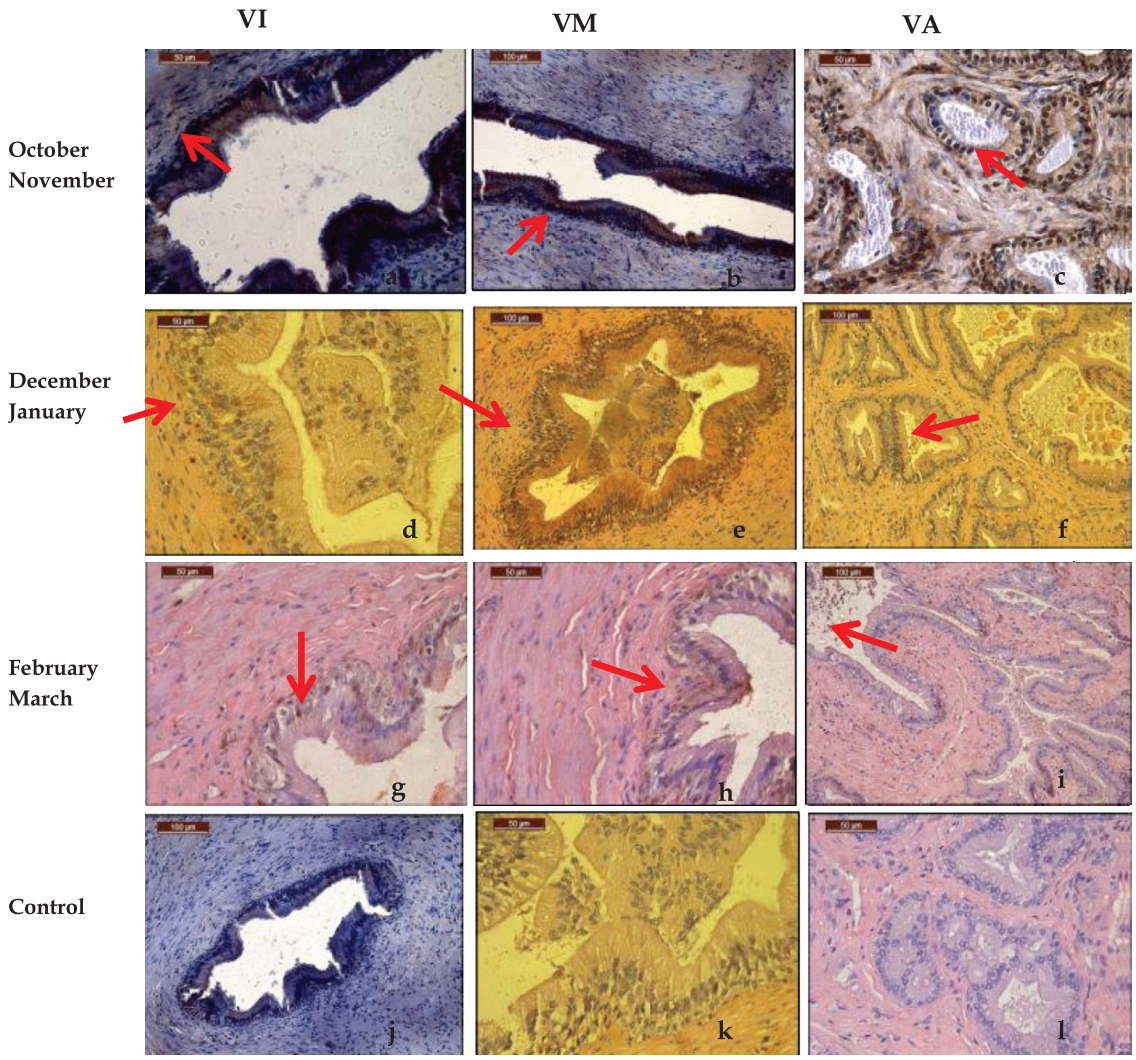

Fig 2. Micrograph of the dromedary camel's vas deferens at the beginning of the rutting season (October) showing a strong immunoreactive of AQP1 in the lining epithelium (arrow) of the initial (a) and middle (b) parts of the vas deferens, while, the ampullary vas deferens (c) showed a moderate reaction to the AQP1 antibodies. In December and January, the VI (d) lining epithelium (arrow) showed a moderate immunoreactive, which increased in the epithelium of VM (e) (arrow), while in VA (f), the epithelial cells had a weak immunoreaction to the AQP1 antibodies. At the end of the rutting season (February and March), a weak immunoreaction of AQP1 was recognized in the lining epithelium of all parts of the vas deferens VI (g), VM (h) and VA (i). (j, k, l) Negative control for initial, middle and ampullary parts of the vas deferens, respectively.

Table 2. Displaying AQP1 localisation throughout the **non-rutting season** in the different parts of the vas deferens and prostate gland of the dromedary camel.

Month Part	April- May	June- July	August-September
VI	+++	++	++
VM	+++	+++	+++
VA	+	+	+
PD	+	+	+
PC	+	+	+

VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; PD, disseminated prostate; PC, corpus prostate; -, negative reaction; +, weak reaction; ++, moderate reaction; +++, strong reaction.

Rutting season

The different parts of the vas deferens of the dromedary camel at the beginning of the rutting season (October and November) showed variety in reaction to AQP1 antibodies (Fig 2). A strong immunoreactive was recognised in the lining epithelium of the initial and middle parts of the vas deferens (Figs 2a, b), while, the ampullary vas deferens had a moderate reaction to the AQP1 antibodies (Fig 2c). In the second two months of the season, December and January, the lining epithelium of the initial part revealed a moderate immunoreactive (Fig 2d), which increased in the middle part of the organ (Fig 2e). While, the epithelial

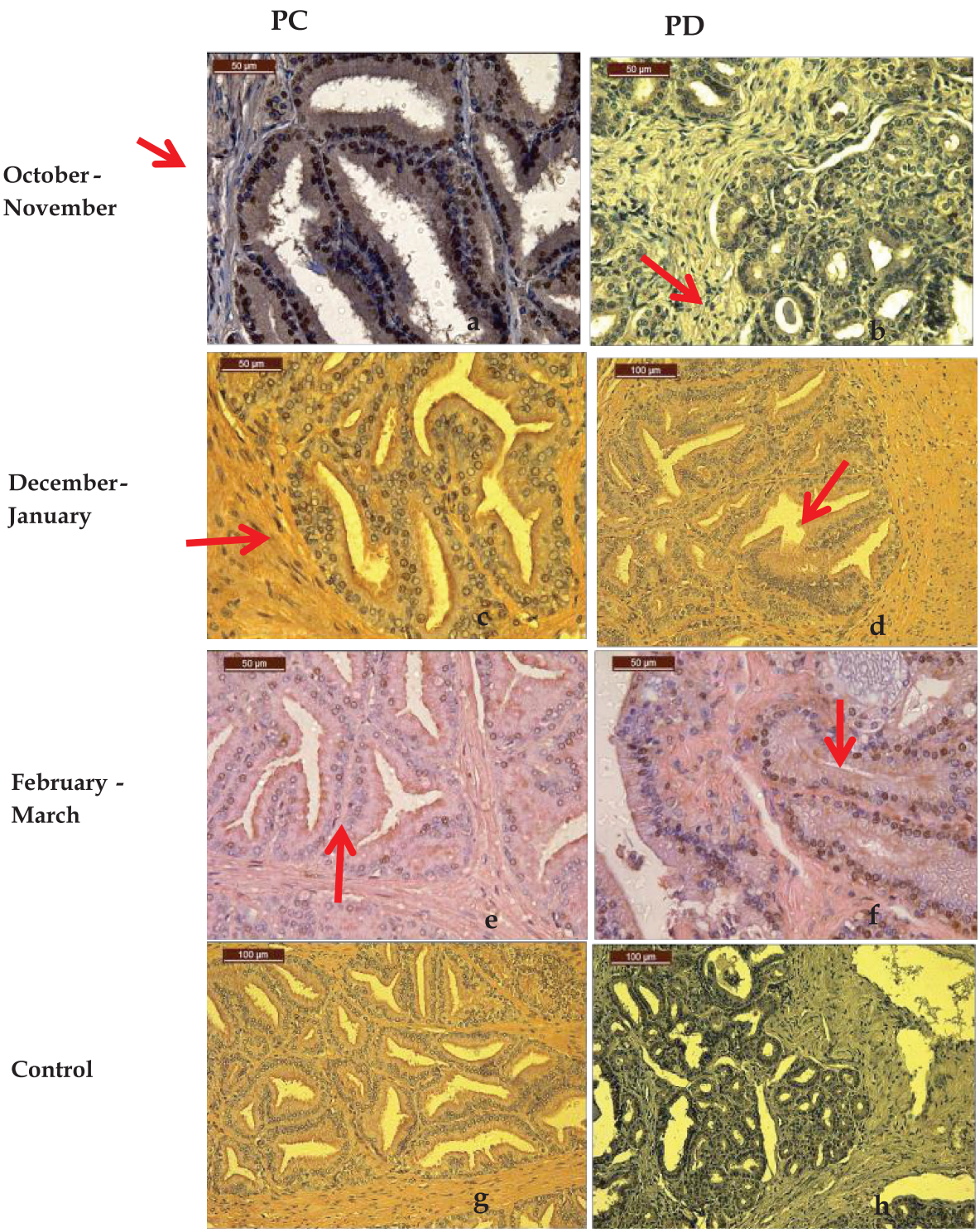

Fig 3. A micrograph showing a weak AQP1 immunoreactive in the lining epithelium (arrow) of the prostate gland in the dromedary camel during the rutting season. This immunoreactivity is recognized in both; the corpus prostate (PC) (a, c, e) and disseminated prostate (PD) (b, d, f) in October (a, b), December (c, d), and February (e, f), respectively. Negative control for the corpus prostate (g) and disseminated prostate (h).

cells of the ampullary vas deferens showed a weak immunoreactive to the protein (Fig 2f). At the last two months of the rutting season (February and March), the lining epithelium in all parts of the vas deferens demonstrated a weak immunoreaction to AQP1 antibodies (Fig 2g, h, i).

The prostate gland, both corpus and disseminate parts, in the dromedary camel during the

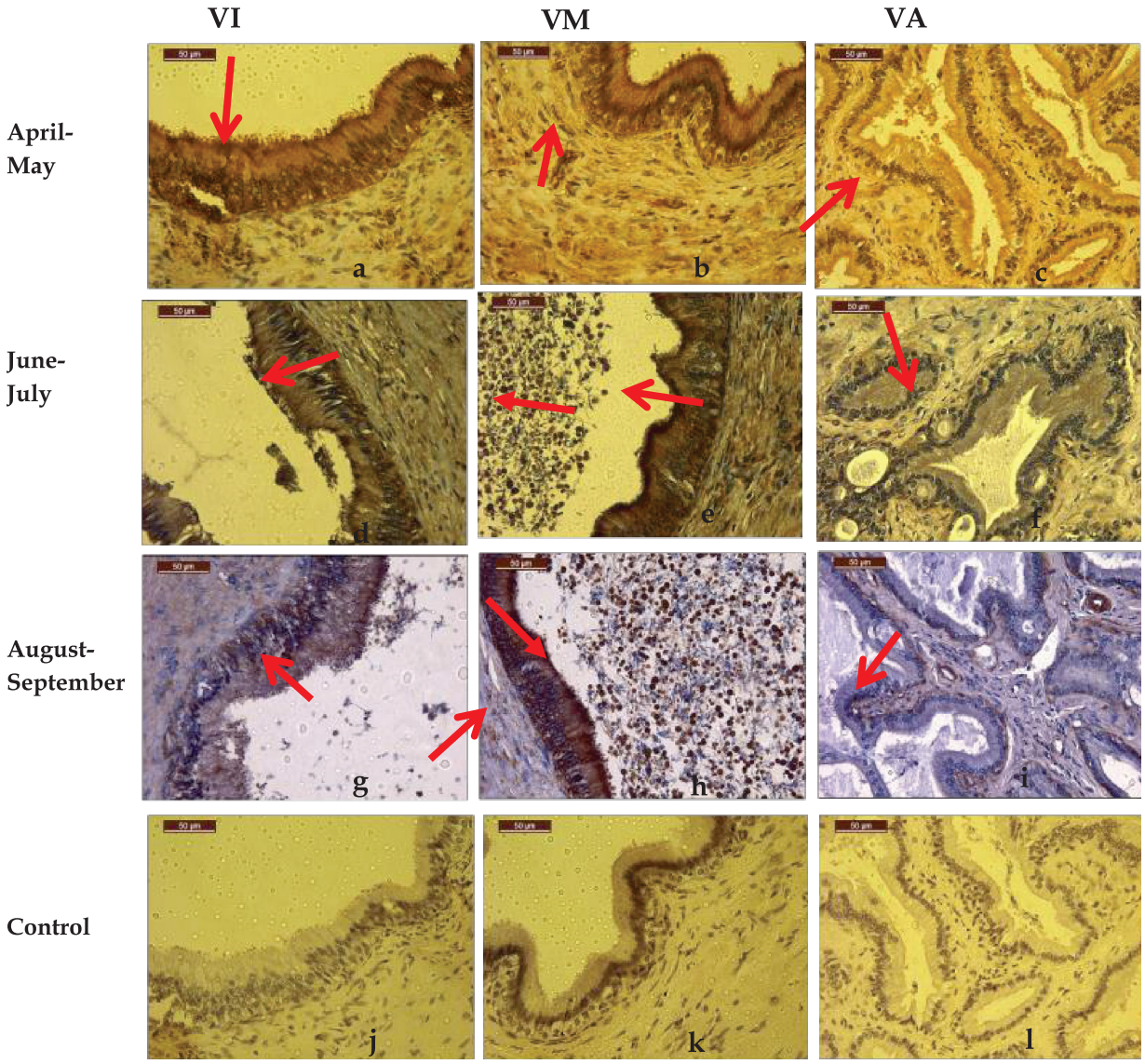

Fig 4. Micrograph of the dromedary camel's vas deferens at the beginning of the non-rutting season (April) showing a strong immunoreactive of AQP1 in the lining epithelium (arrow) of the initial (VI)(1) and middle (VM) (2) parts of the vas deferens, while, the ampullary vas deferens (VA) (3) showed a moderate reaction to the AQP1 antibodies. In the rest of the season, from June to September, the VI (4, 7) lining epithelium (arrow) showed moderate immunoreactive, whereas the epithelial cells of VM (5, 8) (arrow) verified a strong immunoreactive to AQP1 antibodies. The ampullary part (VA) (3, 6, 9) of the vas deferens showed weak immunostaining for AQP1 antibodies during the whole season. Negative control for initial (j), middle (k) and ampullary (l) vas deferens.

rutting season showed a weak AQP1 immunoreactive in their lining epithelium throughout the period (Fig 3a- f).

Non-rutting season

In the first third of this season, April and May, the initial and middle parts of the vas deferens of the dromedary camel showed a strong immunoreactive of AQP1 in their lining epithelium (Fig 4a, b). Whereas, the ampullary vas deferens revealed a moderate reaction to this protein (Fig 4c). In the rest of the season (from June until September), a moderate reaction was recognised in the lining epithelium of the first part of the vas deferens (Fig 4d, g), while, the middle part of this organ showed a strong immunoreaction to AQP1 antibodies (Fig 4e, h). The reaction appeared faint in the ampulla of the vas deferens during the whole season (Fig 4c, f, i).

Throughout the whole months of the non-rutting season, the two parts of the prostate gland, corpus prostate (Figs 5a, c, e) and diffused prostate (Fig 5b, d, f) of the dromedary camel demonstrated limited immunoreactivity for AQP1 antibodies.

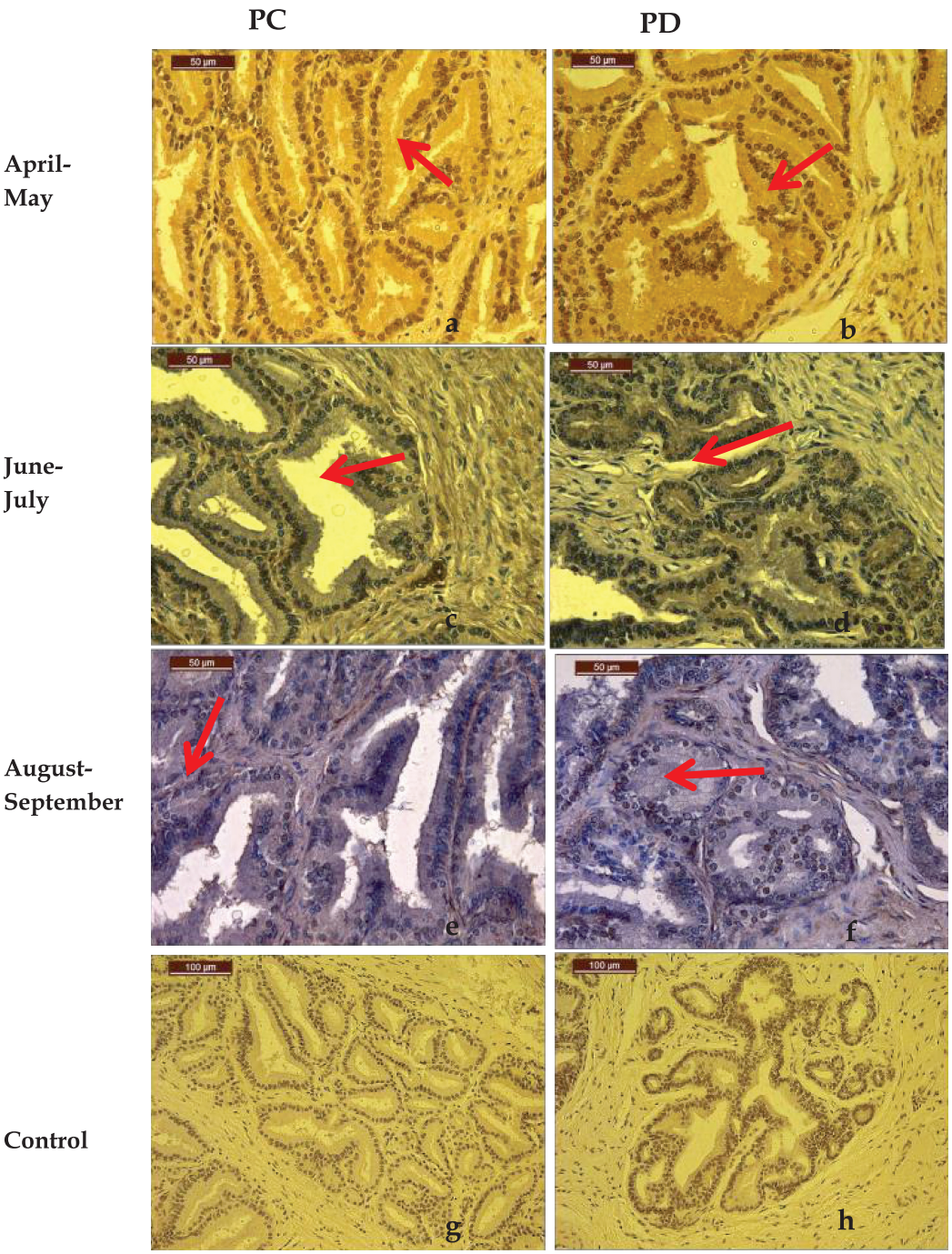

Fig 5. A micrograph showing a weak AQP1 immunoreactive in the lining epithelium (arrow) of the prostate gland in the dromedary camel during the non-rutting season. The immunoreactivity stain is seen in both; the corpus prostate (PC) (1, 3, 5) and disseminated prostate (PD) (2, 4, 6) throughout the whole season. (g, h) Negative control for the corpus and disseminated prostate, respectively.

Discussion

In the current study, the different parts of the dromedary camel's vas deferens exhibited a range of reactions to AQP1 antibodies throughout the year. In the months that started the rutting season (October and November), The lining epithelial cells of the initial vas deferens showed a strong immunoreactive stain to AQP1. This expression became moderate in the middle months of the season and at the end of this period appeared weak. In the same sequence of months, AQP1 protein expressed strongly in the epithelium and luminal sperms of the middle vas deferens in the first and second third of the season. While the last months showed a weak

immunoreaction to the protein. The ampullary vas deferens responded moderately to AQP1 antibodies at the beginning of the rutting season which reduced to become weak from December until March.

In the non-rutting season, generally, the middle part of the vas deferens showed strong immunoreaction to AQP1, while, the initial part revealed a strong reaction to it in April and May and decreased to appear moderately at the rest of the season. AQP1 expressed weakly in the ampulla during this period.

The presence of AQP1 in the vas deferens in this study is confirmed with the previous studies in the vas deferens of the Bactrian camel (An and Wang, 2016), mice (Zhou *et al,* 2001; Lu *et al,* 2008), cats (Arrighi *et al,* 2010b; Arrighi and Aralla, 2014) and buffalo (Arrighi *et al,* 2016). Furthermore, the location of AQP1 in the vas deferens is also widely agreed upon (Badran and Hermo, 2002; Da Silva *et al,* 2006).

On the other hand, the variety in the distribution of this protein in the parts of the vas deferens is in contrast with its function, where, the fluid reabsorption is regulated by steroids and may be facilitated by a variety of AQPs (Huang *et al,* 2006). Moreover, Da Silva *et al* (2006) and others reported that the vas deferens can modify its luminal environment other than simply transit spermatozoa.

The current investigation revealed that the dromedary camel's prostate gland expressed AQP1 faintly all year long. This expression resembles to the prostate gland of Bactrian camel and mice (Zhou *et al,* 2001; Lu *et al,* 2008; An and Wang, 2016). The localisation of AQP1 may play a critical role in water secretion into the seminal and prostatic fluid as well as the removal of water from the inter-tubular region. Since AQP1 is constitutively generated in epithelial cells and is less responsive to androgen or oestrogen regulation (Oliveira *et al,* 2005), the distribution of AQP1 in the prostate gland and ampulla of dromedary camels in this study remained constant in both seasons.

In conclusion, the current data support AQP1's primary function as a selective water channel, suggesting that AQP1 may be important for spermatozoa migration via the camel's male genital system and may even permit the outflow of water needed for sperm motility. As a result, AQP1 may someday function as a unique biomarker of male fertility and infertility.

Conflicts of Interest

The authors declare no conflict of interest.

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