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THE PRESENCE OF AQUAPORIN 9 IN THE VAS DEFERENS AND PROSTATE GLAND OF CAMELS (*Camelus dromedarius*) DURING AND AFTER RUTTING SEASON

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ABSTRACT

Aquaporins (AQPs) are small intrinsic membrane proteins found in many cell types of the male genital system that are involved in fluid transport. These proteins are required to provide the optimal luminal environment for sperm production, maturation, preservation and immigration. Aquaporin 9 (AQP9) is expressed in the vas deferens and prostate gland, among other parts of the male reproductive system in mammals and it permits water to pass through the epithelium rapidly. The current study employed immunohistochemistry to elucidate the expression of AQP9 in the vas deferens (initial, middle and ampullary parts) and prostate gland (corpus and disseminated parts) in dromedary camels over the year's rutting and non-rutting seasons. The outcomes showed that the lining epithelium and luminal spermatozoon of the vas deferens expressed AQP9 protein moderately to weak in the beginning and middle of the rutting season. This expression peaked at the end of the season and continued through the first period of the non-rutting season. The distribution showed erratic patterns in the middle months and ended with a mild reaction to APQ9 antibodies in September. In the prostate gland, AQP9 protein fluctuated relatively little over the year. In conclusion, AQP9-mediated transmembrane water and neutral solute transport is a vital physiological pathway for sperm immigration in the dromedary camel's vas deferens. Also, a low protein expression level in the prostate gland can mean that the cells there are normal.

Key words: Aquaporin 9, distribution, Dromedary camel, prostate gland, vas deferens

Once sperm exit the seminiferous tubules, they travel through the excurrent ducts (efferent ducts, epididymis and vas deferens), where the luminal fluid composition changes gradually and lumen concentration rises noticeably (Jones and Murdoch, 1996; Mahmud *et al,* 2015). The epithelium of the prostate gland produces a large amount of the prostatic fluid. Sperm receives nourishment and protection from this fluid, which supports their motility, viability and an inherent determinant of male fertility (Verze *et al,* 2016). Comparably, the shape of the accessory sex glands with other factors like sexual behaviour, desire and environmental variables was connected with the seasonal variations in camel semen quality (Al-Bulushi *et al,* 2019).

The aquaporins (AQPs) family of vital transmembrane proteins controls cell movement and proliferation, the balance of water in the body, the production of exocrine fluid, the entry of nutrients and other valuable molecules into cells and the elimination of metabolic waste products (Shivaraj *et al,* 2017; Meli *et al,* 2018; Azad *et al,* 2021; Ribeiro *et al,* 2021). Several AQPs in the male reproductive system may be necessary for regular reproduction. They may be biomarkers for sperm freezability and fertility (Yeste *et al,* 2017; Calamita *et al,* 2001; Althnaian, 2023; Elseory, 2024).

AQP9 is a prominent apical route of transmembrane fluxes of water and other solutes called aquaglyceroporins channel expressed in the vas deferens, efferent ducts and epididymis, among other parts of the male reproductive system (Tsukaguchi *et al,* 1998; Matsuzaki *et al,* 2002; Domeniconi *et al,* 2007).

Numerous investigations conducted over the last ten years have documented the expression of AQP9y in the male genital system of many species, mainly in the testis and epididymis (Schimming

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et al, 2015; Schimming *et al,* 2021; Mohamed *et al,* 2022; Martinez-Madrid *et al,* 2023; Oberska *et al,* 2024). However, the location of this protein in the vas deferens and male accessory glands has not been well studied (Pastor-Soler *et al,* 2001; Domeniconi *et al,* 2007; Jian-bo *et al,* 2008).

There is a paucity of information on AQP9's expression in the male reproductive system of camels. Our aim is to precisely locate AQP9 in various regions of the vas deferens and prostate gland of dromedary camels during the rutting season and non-rutting seasons using immunohistochemistry (IHC).

Materials and Methods

Sample collection

King Faisal University's ethical committee accepted the stringent animal protocol that was followed for every step involving animal samples. Samples were taken every two months for a year from eighteen mature, healthy local bread dromedary camels (aged 4≥ years old or older) at the Al Omran slaughterhouse in Al-Ahsa, Saudi Arabia. Tissue samples were obtained from the initial, body and ampulla of the vas deferens and the prostate gland (corpus and disseminated parts). For the IHC process, samples were stored in 10% buffered formalin.

IHC method

Tissue samples were fixed in formalin, dried in graded ethanol, washed in xylene and embedded in paraffin wax. Sections of 5 mμ were cut using a rotary microtome and put on Superfrost slides. Slides were dewaxed and rehydrated before being stained using the avidin-biotin-peroxidase complex technique (Adeghate *et al,* 2001). Using 0.01M PBS (pH 7.4), antigen retrieval was done in a microwave oven for fifteen minutes. The pieces were washed with PBS and chilled to 25°C. Endogenous peroxidase was inhibited for half an hour with 3% hydrogen peroxide. Goat serum (10%) was utilised for 20 minutes following three rounds of washing in PBS to prevent non-specific reactions. The material was incubated in a wet chamber for the whole night after applying the primary antibody, polyclonal rabbit anti-AQP9 (Abcam, dilution 1:200, Cambridge, Cambridgeshire, UK). Biotin-labeled secondary antibodies and avidin-horseradish peroxidase (HRP) were applied to the sections. Dibutyl phthalate polystyrene xylene (DAB) was used to determine the positive staining. Haematoxylin stain was used for section counter-staining. Except for omitting the primary antibody, the negative control sections adhere to the same methodology. Immunohistological examinations were performed and slides were photographed using light microscopy.

Results

Table 1 and Fig 1 display the strength of AQP9 in the different parts of the vas deferens and prostate gland during the rutting and non-rutting seasons.

Table 1. Showing the immunoreactive distribution of AQP9 in dromedary camels' vas deferens and prostate gland regions during the rutting and non-rutting seasons.

Part / Month	October	December	February	April	June	August
DI	++	+	+++	+++	+	++
DM	+	+	+++	++	+++	+
DA	++	+	+++	+++	++	++
PC	+	+	+	+	+	+
PD	++	+	++	+	++	++

DI, initial ductus deferens; DM, middle ductus deferens; DA, ampullary ductus deferens; PC, corpus prostate; PD, disseminated prostate; +, weak reaction; ++, moderate reaction; +++, strong reaction.

The vas deferens

Throughout the year, the different parts of the camel's vas deferens expressed AQP9 in diverse ways (Fig 1A).

Early in the rutting season (October), there was a range of moderate to mild reactivity to AQP9 antibodies. The lining epithelium of the initial and ampullary parts of the vas deferens was shown to be moderately immunoreactive. Meanwhile, the epithelial cells and luminal spermatozoon in the middle part showed weak expression (Fig 2).

AQPP9 immunolocalisation in the epithelial cells along the vas deferens and the luminal sperm demonstrated a mild reactivity in December, the middle of this season (Fig 2). In contrast, the reaction was strong in February, near the end of the meeting period (Fig 2).

During the non-rutting season, which spanned from April to September, AQP9 was expressed in all parts of the vas deferens, with its reactivity showing significant variations. In April, the lining epithelium cells in the initial and ampullary parts exhibited strong immunoreactivity to AQP9 antibodies. The reaction in the epithelial cells of the middle part was moderate at that time (Fig 3). The reaction fluctuated in June, the midpoint of this season, with a faint reaction in the initial part, a strong reaction in the

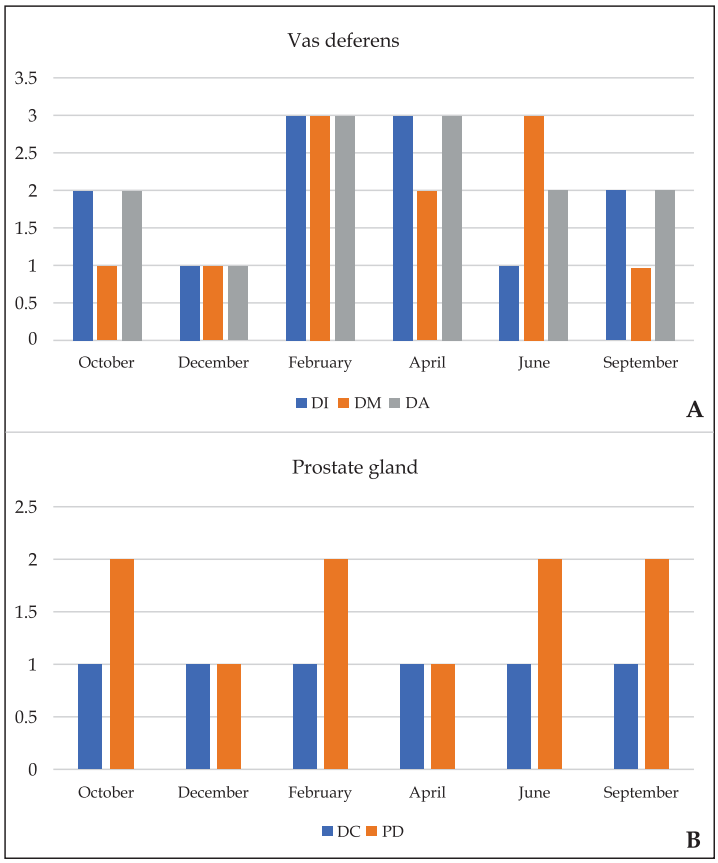


Fig 1. Showing the distribution of AQP9 in the different parts of the dromedary camel's vas deferens (A) and prostate gland (B) throughout the rutting and non-rutting seasons. VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; PC, corpus prostate; PD, disseminated prostate; 1, weak reaction; 2, moderate reaction; 3, strong reaction.

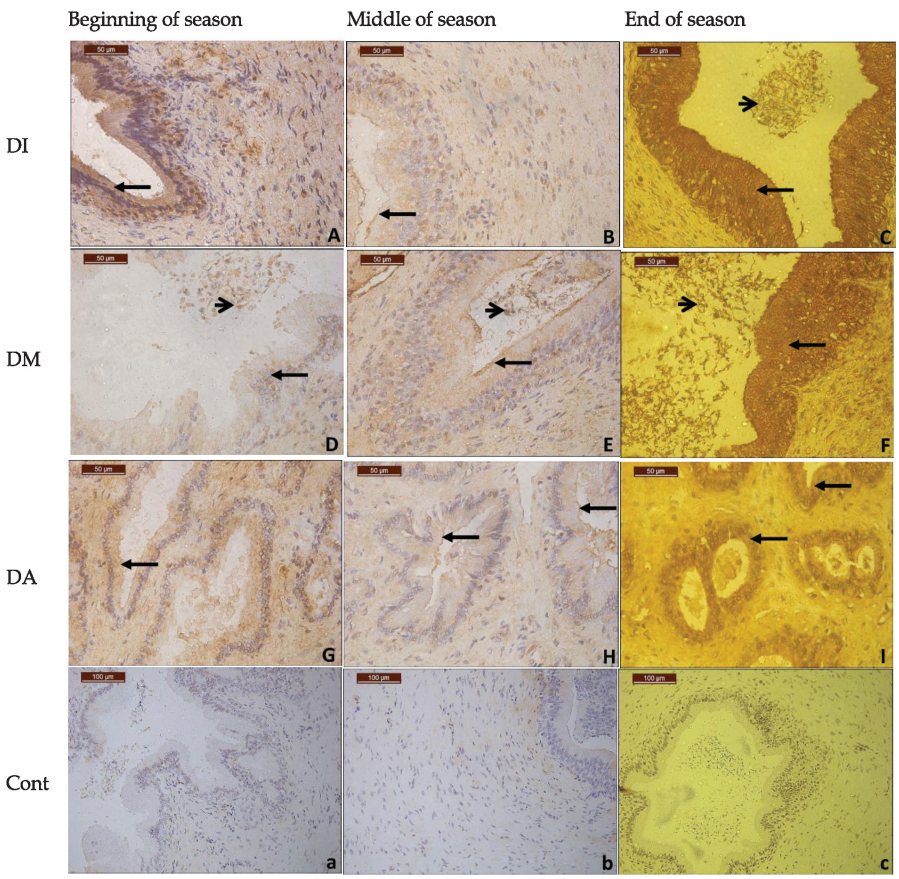


Fig 2. AQP9 immunolocalisation in the different parts of the camel's vas deferens during the rutting season. The reaction was observed variously in the epithelial cells (arrow). The response to AQP9 antibodies at the beginning of this season was between moderate and weak reactions (A, D, G). It reacted moderately in the middle of this period (B, E, H). Meanwhile, AQP9 immunoreaction at the end of the season clarified an extreme reaction (C, F, I). The luminal sperm shows a similar reaction (arrowhead). X40. (a), (b) and (c) negative control 20X.VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; Cont, negative control.

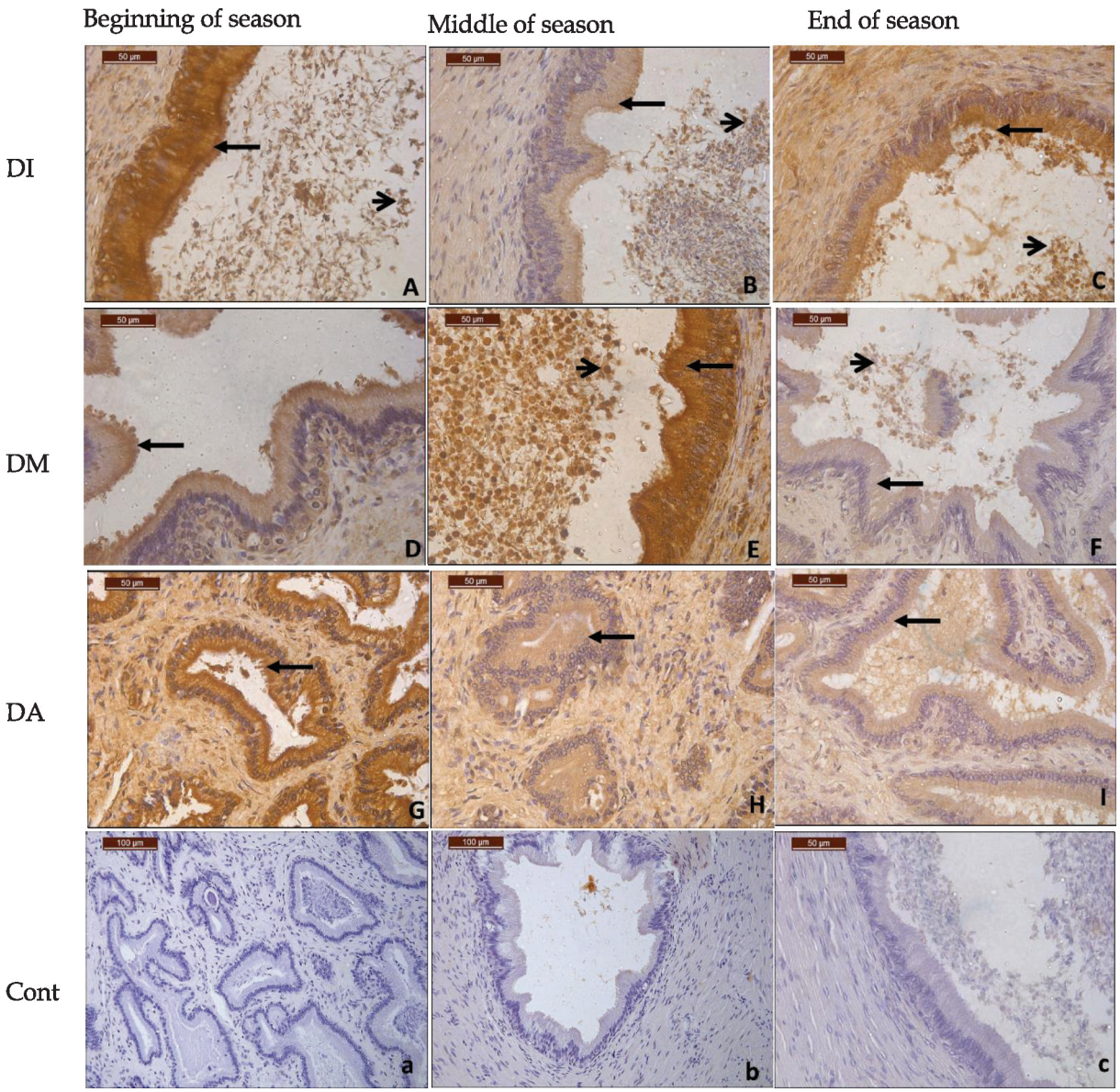


Fig 3. AQP9 immunolocalisation in the different parts of the vas deferens throughout the non-rutting season of the dromedary camel. Different reactions were seen in the epithelial cells (arrow). At the start of this season, the AQP9 immunoreactivity is generally strong (A, D, G). In the middle of this season, its reaction is inconsistent in the different parts (B, E, H). A moderate reaction is observed at the end of the season (C, F, I). A similar response is seen in the luminal sperm (arrowhead). X40. (a), (b) and (c) negative control 20X. VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; Cont, negative control.

second part and a moderate reaction at the ampulla (Fig 3). In August, when the season was almost over, the epithelium of the organ revealed a mild reactivity (Fig 3).

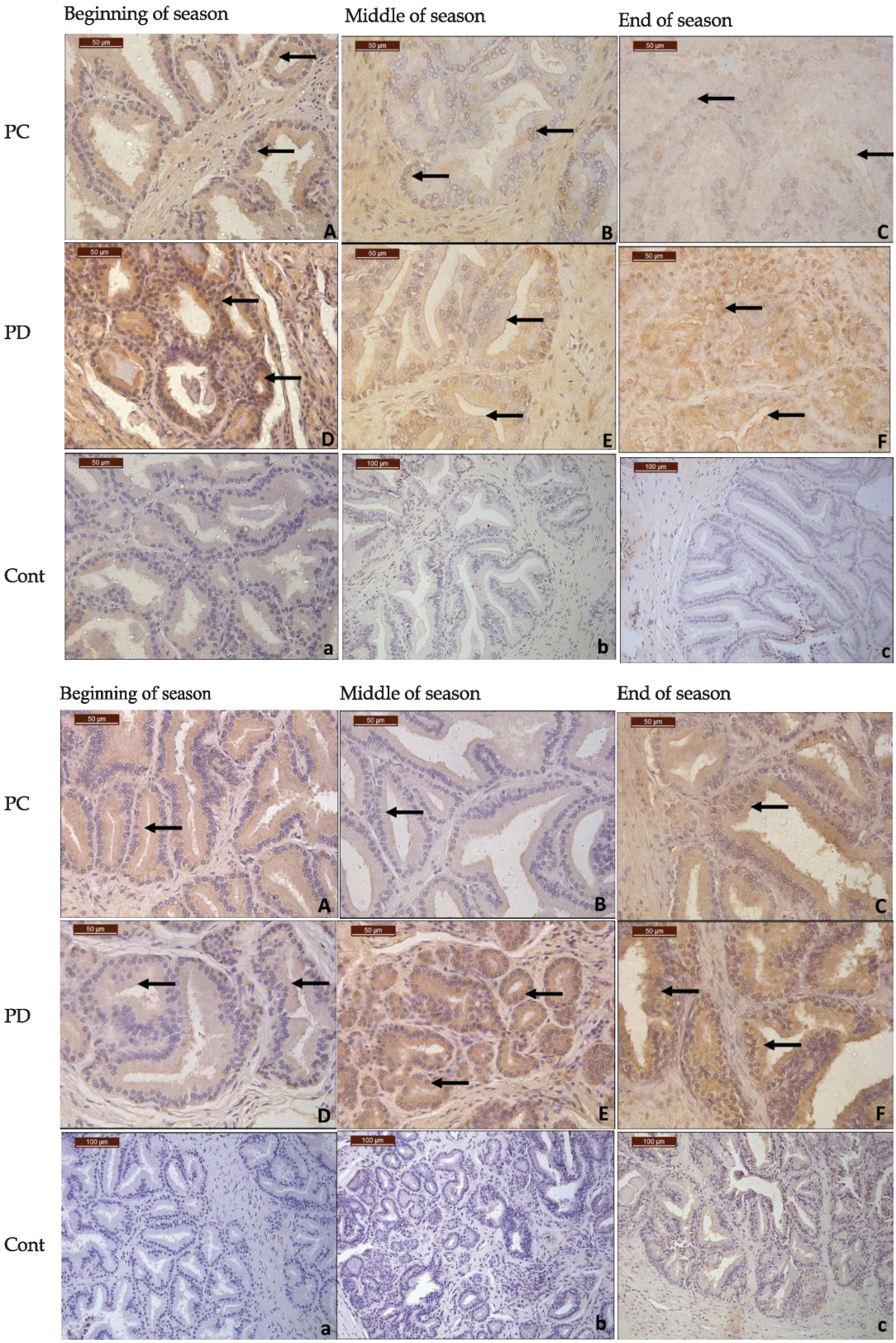
The prostate gland

In dromedary camels, AQP9 was slightly different throughout the rutting and non-rutting seasons in the corpus and disseminated parts of the prostate gland (Fig 1B). When exposed to AQP9 antibodies, the epithelial cells in both parts of the gland had mild to moderate immunoreaction (Figs 4, 5).

Discussion

The epithelial cells of the male reproductive tract carry fluid and electrolytes, drastically altering the luminal environment where spermatozoa form, store up and are passed. These activities occur in different parts of the male reproductive system (Clulow *et al*, 1998). According to Pastor-Soler *et al* (2001), the AQP9 is a water channel that permits the passage of neutral solutes in addition to water.

The present study is the demonstration of the seasonal variations of AQP9's dispersion in the



Figs 4 & 5. AQP9 immunolocalisation in the prostate gland of dromedary camels throughout both the rutting and non-rutting seasons in the corpus and disseminated parts of the gland. The epithelial cells (arrow) in both parts exhibit weak to moderate AQP9 positivity. The expression of the protein in the gland does not significantly change throughout the year. X40. (a), (b) and (c) negative control 20X. Pc, corpus prostate; PD, disseminated prostate; Cont, negative control.

dromedary camel's vas deferens and prostate gland over one year. In the early period of the rutting season, which is reported to start in October and end in April (El-Shoukary *et al,* 2020; Tibary and El Allali, 2020), the lining epithelium of the vas deferens' initial and ampullary parts are moderately immunoreactive to AQP9 antibodies, while the middle region's luminal spermatozoon and epithelial cells are expressed weakly. All these parts showed mild reactivity throughout the middle meeting period. On the other hand, during the last period of this season, AQP9 protein clarified strong reactivity across all parts, marking the season's peak. During the non-rutting period, the vas deferens protein displayed significant immunoreactivity for AQP9 in the first three periods for the remainder of the year. The second third displayed inconsistent patterns, while the last had a modest response to antibodies.

These findings in the vas deferens of dromedary camels corroborated the discovery of AQP9 in the vas deferens of animals by Pastor-Soler *et al* (2001) in rats, Domeniconi *et al* (2007) in dogs and Oberska *et al* (2024) in bovines. Additionally, it validated an earlier theory that said the vas deferens appears to do much more than transport spermatozoa out of the epididymis; in humans, it enhances sperm survival by protecting them from complement, proteases and reactive oxygen species (Ezer and Robaire, 2002). However, in monkeys, sperm are retained until fully charged and can travel at high speeds (Horst *et al,* 1999).

Furthermore, as noted by Stevens *et al* (2000), camels exhibit regional variations in the tissular distribution and cellular-specific location of the AQP9, as well as in the structure and functioning of the vas deferens.

However, it is essential to note that the mechanism underlying the transepithelial fluid in the vas deferens remains unknown. This intriguing area requires further research and understanding, presenting an exciting opportunity for future discoveries.

In the current study, dromedary camels' AQP9 protein in the corpus and disseminated parts of the prostate gland oscillated very little during both rutting and non-rutting seasons. The detection of AQP9 in the mammalian prostate gland (Pastor-Soler *et al,* 2001; Domeniconi *et al,* 2007; Jian-bo *et al,* 2008) was in consonance to the results of this study.

The limited distribution and seasonal variations observed in camel AQP9 may be related to early findings that testosterone significantly upregulates this protein in the prostate, where its overexpression indicates problems in the gland's cells. This reaffirms the potential of AQP9 as a unique therapeutic target for various malignancies, underscoring the importance and impact of our work in this field (Chen *et al,* 2016; Bründl *et al,* 2018; Kushwaha *et al,* 2023).

Conclusion

The present study concluded that the AQP9 expression varies during the non-rutting season and increases at the end of the rutting season. It also exhibits varying responsiveness in distinct regions of the vas deferens. In contrast, the prostate gland has no significant distribution or seasonal variation. The results indicated the significance of this protein, particularly for sperm via luminal immigration in the vas deferens. Furthermore, the consistent low protein expression in the prostate gland throughout the year was a positive, healthy sign, reassuring the health of the gland.

Conflict of interest

None declared

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