DOI : 10.5958/2277-8934.2025.00004.7 *Vol 32 No 1, p 19-23*

SEASONALITY OF AQUAPORIN 5 IMMUNOREACTIVITY IN THE DROMEDARY CAMEL'S DUCTUS EPIDIDYMIS

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ABSTRACT

Intrinsic membrane proteins designated aquaporins promote the selective movement of water or other small, uncharged molecules down the osmotic gradient. The objective of this research was to use a light microscope to investigate the seasonal variations in the immunoreactivity of Aquaporin 5 (AQP-5) in the ductus epididymis of the local breed of Saudi dromedary camel. Samples were obtained from the head, body, and tail (caput, corpus, and cauda) of the ductus epididymis and processed using general histology and immunohistochemical methods. General histology and immunohistochemistry techniques were used to process samples taken from the head, body, and tail of the ductus epididymis. AQP-5 antibodies exhibited distinct and variable responses on the caput, corpus, and cauda of the ductus epididymis of the Saudi Arabian camels in both rutting and non-rutting seasons. The distribution of AQP-5 was strongly expressed in the non-rutting seasons. At the beginning of the rutting season, AQP-5 showed a strong response. However, in the rutting season in January, the excretion showed mild to moderate response in the caput, corpus, and cauda ductus epididymis. According to the present results, AQP-5 activity in Saudi dromedary male camels may play an essential role in fertility during rutting and non-rutting seasons at different levels.

Key words: Aquaporin 5, camel, epididymis, immunohistochemistry

The dromedary camels can live in various dry and semi-arid conditions due to their great environmental adaptation (Merkt *et al,* 1990; Tibary and El Allali, 2020). Seasonality in reproduction has been noted in various species, including male dromedary camels (Al-Bulushi *et al,* 2019). Camels have a longer breeding season than previously thought; dromedary camels are thought to breed seasonally (Eiwishy, 1987). Male camels breed periodically, and a noticeable increase in sexual activity (the rut) marks the start of the mating season (Marai *et al,* 2009). Nevertheless, at any time of year, by mating with an oestrous female, the male can still fertilise the oocyte (Marai *et al,* 2009).

The epididymis is a crucial reproductive organ that regulates sperm concentration and maturity, as well as storage, protection, motility, and fertilising capability (Flannigan and Goldstein, 2018; James *et al,* 2020).

The epididymis embryonic origin is the mesonephric ducts, a part of the intermediate mesoderm (McGeady *et al,* 2017). The epididymis is separated macroscopically into a head, body, and tail. Surrounding it is a dense, irregular connective tissue layer called the tunica albuginea, thick and coated in the visceral layer of the tunica vaginalis. A few smooth muscle cells can be seen sporadically throughout the dense connective tissue of the tunica albuginea in stallions (Eurell and Frappier, 2006).

The membrane protein channels known as aquaporins (AQPs) are essential for the quick movement of water through epithelium (An and Wang, 2016). Numerous human and other species tissues contain AQPs, which play a role in the bidirectional transmembrane transport of water and other tiny solutes. They regulate the fluid flow in tissues and cells, particularly the male reproductive organs, by facilitating the rapid passive passage of water (Verkman and Mitra, 2000; Kannan *et al,* 2020; Agre *et al,* 2002). From AQP0 to AQP12, in mammals, there are thirteen different AQP isoforms (Carrageta *et al,* 2020).

The morphology of the male camel epididymis was examined by numerous researchers, including Tingari and Moniem (1979); Alkafafy *et al* (2011); Purohit *et al* (2022); Saini *et al* (2023). There is a

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little investigation in the literature on the seasonal histological changes in the ductus epididymis, for example; that was described by Ibrahim and Singh (2014); Zayed *et al* (2012); Abdel-Maksoud *et al* (2019); Sary *et al* (2022;). Research on the immunoreactivity of aquaporins in dromedary camel is scarce (Al-Thnaian 2023 a and b, Marwa-babiker, 2024; Abdelhay, 2024; Elseory, 2024 a, b). The purpose of this research was to use histological and immunohistochemical methods to identify AQP-5 in the ductus epididymis.

Materials and Methods

Ethical approval: Each stage of the animal sample procedure was done according to the Saudi Arabian Ministry of Environment, Water, and Agriculture's ethical guidelines and procedures for slaughtering animals. The animal sampling was authorised by the King Faisal University Ethics Committee (KFU-REC-2023-NOV-ETHICS1545). In this study, twelve (12) Saudi Arabian male camels of the native breed (*Camelus dromedarius*) that were between the ages of four and eight years old and in good condition were used. The Al-Omran slaughterhouse in Al-Ahsa, Saudi Arabia, was the location of the animal slaughter. Six animals were in the December–February rutting season and six in the May- August non-rutting season.

The specimens were taken from the epididymal head, body, and tail (the caput, corpus, and cauda regions). They were used immediately after animal slaughter and fixed with 10% neutral formalin. Then, specimens were dehydrated in an ascending series of ethanol cleared in xylene and embedded in paraffin wax. A rotatory microtome was used for cutting 5µm thick tissue sections. The sections were stained with Hematoxylin and Eosin (H&E) according to Culling (1974) and Suvarna *et al* (2019).

Each animal group was dewaxed in xylene and rehydrated in decreasing amounts of ethyl alcohol for the immunohistochemical analysis. PBS (phosphate-buffered saline) was used to clean the sections. Tissue sections were rehydrated in PBS after being deparaffinised in xylene and ethanol alcohol. For fifteen minutes, antigen retrieval was carried out in a microwave oven using 0.01M PBS (pH 7.4). The parts were then allowed to cool at room temperature before being cleaned in PBS again. Endogenous peroxidase was blocked by using 3% hydrogen peroxide for 30 min. After washing in PBS three times, the goat serum (10%) was used for 20 minutes to avoid non-specific reactions. Then, the primary antibody, polyclonal rabbit anti-AQP5 was applied (Abcam, Cambridge, UK dilution 1:200) according to the manufacturer's instructions. The sections were then kept in a moist chamber for overnight. Sections were treated with avidin-HRP third antibodies and secondary antibodies labeled with biotin. To find the positive staining DAB was utilised. Section counter-staining was done using hematoxylin stain. Negative control sections have the same procedure except for skipping the primary antibody (Suvarna *et al,* 2019).

Results and Discussion

The epididymis was found divided macroscopically into a caput, corpus, and cauda (Figs 1 and 2). The ductus epididymis was highly convoluted, it had circular smooth muscle fibres and a tiny amount of loose connective tissue around it. It was lined by a high-pseudostratified columnar epithelium with stereocilia (Figs 1 and 2). The spermatozoa were present in the lumen of the different parts of the epididymis in both rutting and non-rutting seasons (Figs 1 and 2).

AQP-5 antibodies in Saudi dromedary camels during rutting and non-rutting seasons exhibited different levels of response on the caput, corpus, and cauda of the epididymis (Figs 1 and 2, Table 1).

The immune reactivity of AQP-5 at the beginning of the rutting season (October) strong expressions were observed in the caput, corpus, and cauda of the epididymis in the lining epithelium and muscular coat and connective tissue (Fig 1, a, b and c, respectively). However, in January there was mild immunological expression in the caput (Fig 1 d). The

Table 1. AQP-5 seasonal immunoreactivity in the epididymis of Saudi Arabian dromedary camels.

Season	Ca			Co			Cu		
	LE	MC	CT	LE	MC	CT	LE	MC	CT
Beginning of Rutting	+++	+++	+++	+++	+++	+++	+++	+++	+++
Rutting	+	+	+	++	++	++	++	++	++
Beginning of non-rutting	+++	++	++	+++	++	++	+++	++	++
Non-rutting	+++	++	+++	+++	++	+++	+	+	+

Abbreviations: + mild, ++ moderate, +++strong, Ca caput, Co corpus, Cu cauda, LE lining epithelium, MC muscular coat, CT connective tissue.

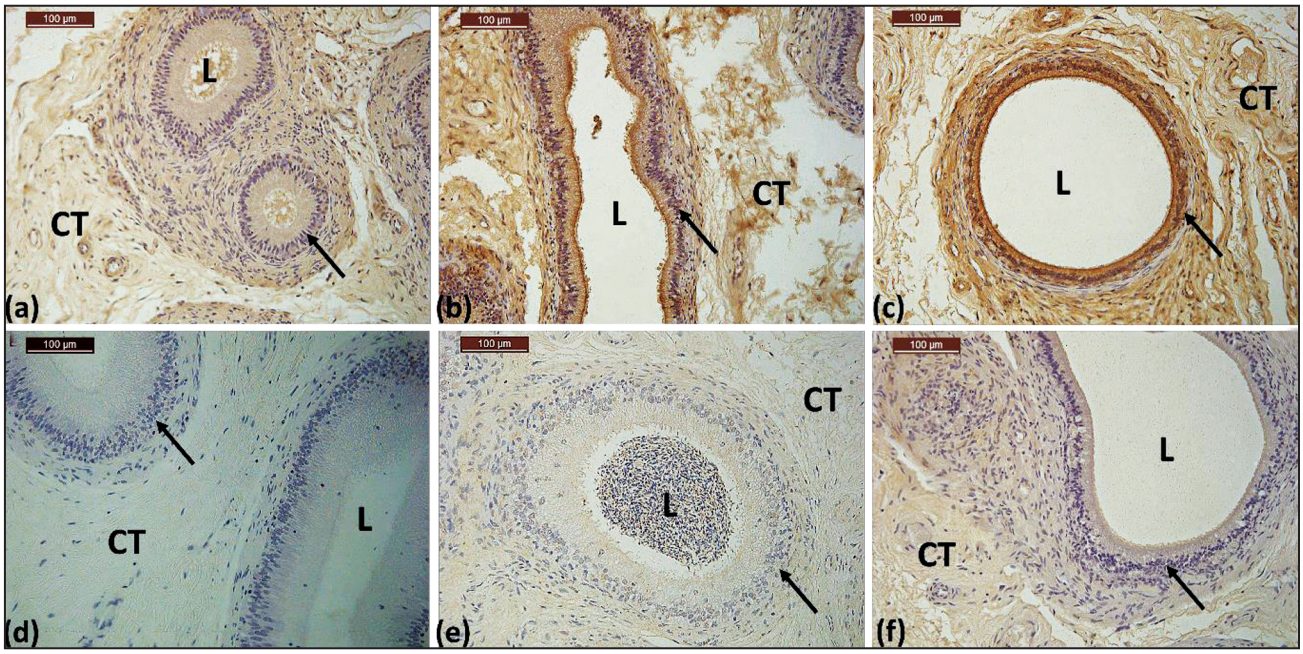

Fig 1. (a), (b) and (c): the photomicrographs of the caput corpus and cauda of the ductus epididymis of Saudi Arabian dromedary camel at the beginning of the rutting season in October showing a strong immune response in the lining epithelium (arrow), 20X,20X,20X, respectively. (d), (e), and (f): the photomicrographs of the caput, corpus, and cauda of the ductus epididymis of Saudi Arabian dromedary camel in the rutting season in January showing mild (caput) to moderate (corpus and cauda) immunoreactivity of AQP-5. Epithelium and stereocilia (arrows) and smooth muscle (S). Spermatozoa in the lumen (L), lining epithelium (arrows), the connective tissue (CT). 20X, 20X, 20X, respectively.

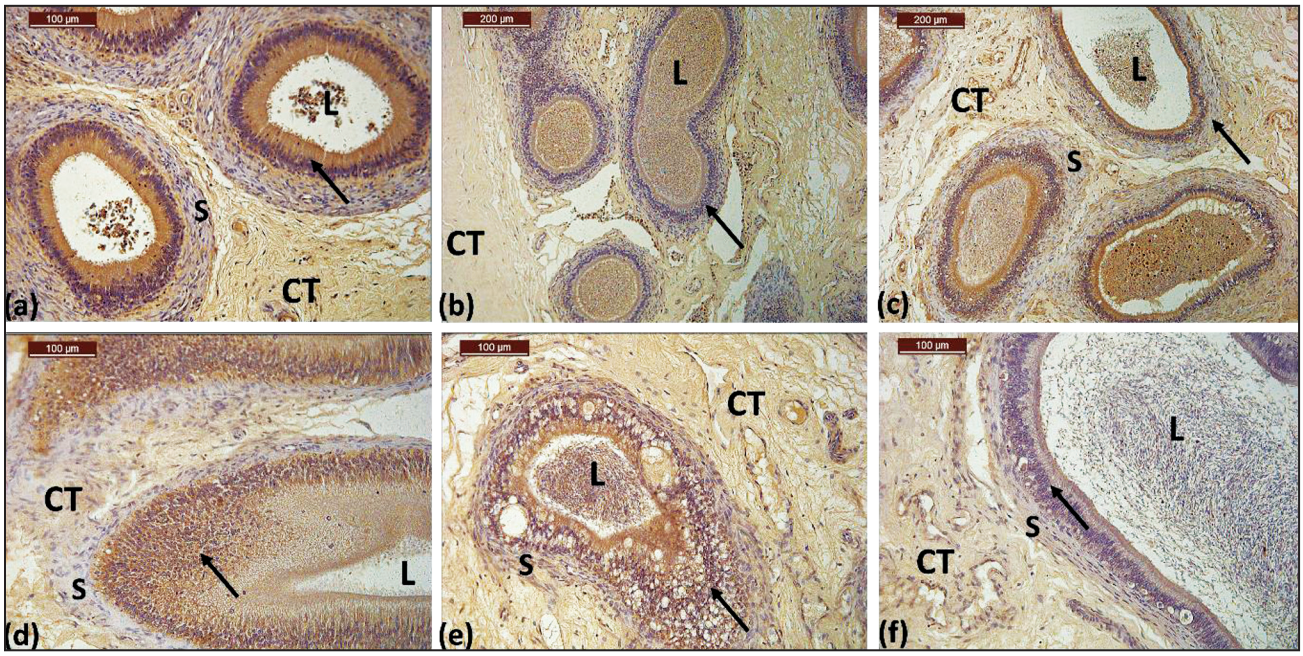

Fig 2. (a), (b), and (c): the photomicrographs showing the caput, corpus, and cauda of the ductus epididymis of Saudi Arabian dromedary camel in the beginning of non-rutting season (May), the lining epithelium (arrows), smooth muscle (S), connective tissue (CT) and lumen (L). 20X, 10X, 10X, respectively. (d), (e) and (f): the photomicrographs showing the caput, corpus, and cauda of epididymis immunoreactivity of AQP-5 in the non-rutting season (August). Epithelium and stereocilia in the caput, corpus, and cauda (arrows), Smooth muscle (S), Connective tissue (CT) Lumen (L). 20X, 20X, 20X, respectively.

corpus and cauda of epididymis showed moderate expression of AQP-5 in the spermatozoa in the lumen, the lining epithelium, and the connective tissue (Fig 1 e and f).

The immunoreactivity of AQP-5 at the beginning of the non-rutting season (May) in the Saudi dromedary camel's ductus epididymis caput, corpus, and cauda displayed a strong response

to AQP-5 in the epithelium and stereocilia. The muscular coat and connective tissue had a moderate response (Fig 2 a, b, and c). In addition, in August the caput and corpus displayed a significantly strong immunological expression of AQP-5 in the lining epithelium and connective tissue, however, the muscular coat had a moderate immune expresssion of AQP-5 (Fig 2 d and e, respectively). Nevertheless, in August the cauda ductus epididymis had a mild response (Fig 2 f).

The main study's findings showed that the ductus epididymis of Saudi Arabian dromedary camels had positive response of AQP-5, which includes the caput, corpus, and cauda.

The ductus epididymis was separated histologically into three distinct regions: the head, body, and tail. The results of Axner *et al* (1999), who claimed that domestic cats epididymis is divided into six different sections, are in conflict with the current study. The present observations demonstrated that the ductus epididymis of the Saudi Arabian dromedary camel was lined with high-pseudostratified columnar epithelium with stereocilia. It was also encircled by circular smooth muscle fibres and a small amount of loose connective tissue. These findings support the result of Eurell and Frappier (2006) in domestic mammals and Saini *et al* (2023) in dromedary camel.

The results of this experiment showed that throughout the rutting and non-rutting seasons (winter and summer, respectively), AQP-5 was expressed in differing degrees in the caput, corpus, and cauda of the ductus epididymis. The corpus's epithelium demonstrated a notable immunological response to the AQP-5 at the beginning of the rutting season. This finding corroborates with Al-Thnaian (2023b) and Abdelhay (2024) who discovered those of AQP-1 and 9, respectively in dromedary camels. On the other hand, this research revealed a mild reaction of AQP-5 in the epithelium, muscle coat, and connective tissue of the caput epididymis in the rutting season (January).

Al-Thnaian (2023 a and b) and Abdelhay (2024) showed that the dromedary camel's epididymis consisted of aquaporin 1, 7 and 9, respectively.

These results may provide credence to the idea that AQP-5 is important for spermatozoa differentiation and maturation in dromedary camels during rutting and non-rutting seasons.

Conclusion

Based on these results AQP-5 may play the main factor influencing male dromedary camel fertility during the rutting and non-rutting seasons. Further future studies should consider the physiological roles of AQP-5 in the transportation of lipids, energy, and water in the camel male reproductive system.

Acknowledgements

The authors would like to thank Dr. Abdelhay M. Ali and Dr. Althnaian T.A. for their help with this study.

Conflict of interest

There is no conflict of interests of any sort between authors or elsewhere.

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