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IMMUNOREACTIVITY OF ALPHA SMOOTH MUSCLE ACTIN IN THE EPIDIDYMIS OF THE DROMEDARY CAMEL: IMPACT OF THE SEXUAL MATURITY AND THE BREEDING SEASONALITY

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ABSTRACT

This study was carried out to highlight the seasonal variation of the immunoreactivity of the protein alpha smooth muscle actin (α-SMA), within the epididymal duct of the male dromedaries. Immunostaining for detection of α-SMA was applied on paraffin-embedded sections taken from different regions of the epididymal duct from both juvenile and adult dromedaries, during the different seasons of the year. Immunoreactivity (IR) was mainly confined to the smooth muscle cells (SMCs) within the peritubular muscle coat and in the walls of the blood vessels. The intensity of IR displayed a remarkable seasonal variation in adults. The strongest IR has been recorded in all epididymal regions (head, body and tail), during the breeding season (corresponds to winter months). In the season of breeding inactivity (corresponds to summer months), the α-SMA-IR exhibited the lowest intensity. During the period of transition from activity to inactivity (corresponds to spring months) and from inactivity to activity (corresponds to autumn months), a moderate α-SMA-IR has been reported. On the other hand, a weak to moderate immunoreactivity for α-SMA appeared mainly in innermost layers of the peritubular cells surrounding the epididymal duct of the juvenile dromedaries throughout the year. In conclusion, both of the sexual maturity and the breeding seasonality demonstrated a clear impact on the immunostaining for α-SMA in male dromedaries. This may be relevant to the physiological alterations that are linked to hormonal control throughout the year.

Key words: Alpha smooth muscle actin, dromedary camel, epididymis, immunohistochemistry

There are many conflicts and contradictions on the breeding seasonality in dromedary camels (Al Eknah, 2000). Camels may retain their reproductive potency throughout the year. Though male dromedaries show a minimal reduction in the process of spermatogenesis during non-rut season, they still do it throughout the year. Thus, they may be recognised by many authors as atypical seasonal breeders (Zayed *et al,* 1995). Accordingly, the epididymis of the camel show minor seasonal differences both in morphometric and histological features (Zayed *et al,* 2012; Ibrahim and Abdel-Maksoud, 2019).

Actin isoforms are reliable differentiation markers (Skalli *et al,* 1986). α-SMA is principally expressed in contractile cells and is a characteristic isoform and a specific marker for both of SMCs and myoepithelial cells (Skalli *et al,* 1986; Moustafa, 2012). α-SMA is highly expressed in the SMCs in the walls of blood vessels (Skalli *et al,* 1989) and in the peritubular SMCs (Alkafafy and Sinowatz, 2012; Helal *et al,* 2013; Ibrahim *et al,* 2017; Marettova and Maretta, 2018). Immunohistochemical studies showed that the application of a monoclonal antibody, raised against α-SMA, has been reported to be a powerful probe in the study of SMCs differentiation (Francavilla *et al,* 1987).

In a continuing series of studies on the epididymal duct in male dromedaries, the present study has been conducted to use immunohistochemistry (IHC) to underline the impact of breeding seasonality and sexual maturity on the immunostaining for α-SMA within the different regions of the epididymal duct and to interpret their potential morpho-functional correlates.

Materials and Methods

Animals and tissues

Epididymal tissue specimens were obtained from both juvenile (age of 2 years; n =20) and adult (age of 5 years; n =20) clinically healthy, dromedary

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camels (*C. dromedarius*) slaughtered at the central abattoir of Cairo, Egypt. The specimens were collected from a total of 5 animals for each age group during each season, immediately after slaughter. Each epididymis was divided into three parts: head, body and tail.

Chemicals and methods

Specimens were fixed in Bouin's fluid, dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffine wax and sectioned at 5µm thickness. Tissue sections were mounted on positively charged and coated slides.

Immunohistological techniques

Dewaxed and rehydrated sections were subjected to inactivation of endogenous peroxidases by incubation in 1% hydrogen peroxide (H_2O_2) for 15 minutes. Antigen has been retrieved from sections placed in 0.01 mol/L citrate buffer (pH 6) by heating in a microwave oven (700 watt) for 10 minutes. The sections were blocked by phosphate buffered saline (PBS) containing 5% bovine serum albumin (BSA) for an hour, and then each section was incubated in humidified chamber with the mouse primary antibody (Dako, Hamburg, Germany) at a dilution rate of 1:200, for 1 hr, at room temperature. The sections were washed by PBS for 5 minutes 3 times and incubated with biotinylated rabbit anti-mouse secondary antibody (Dako, Hamburg, Germany) at a dilution rate of 1:300, for 30 minutes at room temperature. The sections were washed by PBS for 10 minutes. Then the secondary antibody was detected with Vectastain ABC kit (Vector Laboratories Inc., USA) firstly each section is covered with 100 x dilution of A and B reagent in PBS (1 µl reagent A+ 1 µl reagent B + 98 µl PBS), then washed by PBS for 10 minutes 3 times and the colour was developed using DAB reagent (Sigma-Aldrich, St. Louis, MO, USA). Sections were counterstained with hematoxylin for 30 seconds, washed in water, dehydrated through graded ethanol, cleared in xylene and mounted with DPX permanent mounting media.

Positive and negative controls

Immunohistochemical negative controls, where each primary or secondary antiserum or the ABC reagent was omitted, gave no positive staining. Positive controls were used according to the instructions provided by the manufacturers of the primary antibodies.

Labelling assessment and photomicrography

The intensity of immunolabelling has been evaluated using a semi-quantitative subjective scoring by three independent observers. A digital imaging system (DM LB light microscope and EC3 digital camera, Leica Microsystems, Wetzlar, Germany) has been used to capture the photomicrographs.

Results

Effect of sexual maturity

In the present work the cytoplasm both of peritubular and vascular SMCs in the epididymal sections from adult dromedaries (Fig 1) showed a distinct α-SMA-immunoreaction when compared to those from juvenile dromedaries (Fig 2). Yet, this immunoreactivity is season-dependent in adults. On the other hand, a weak to moderate immunoreactivity for α-SMA appeared in innermost layers of the peritubular cells surrounding the epididymal duct of the juvenile dromedaries. The peripheral layers of the peritubular SMCs in the same sections displayed a negative to weak immunoreaction. The immunoreactivity was distinct both in head and tail regions but less distinct in the body region. The vascular SMCs were almost strongly reactive especially in the larger vessels.

Effect of the season

The cytoplasm in both peritubular and vascular SMCs in the epididymal sections from adult dromedaries showed a strong positive α-SMA-immunoreaction in the winter (Fig 1 A-C). This reactivity has been markedly declined in the epididymal sections from adult dromedaries in the summer (Fig 1 G-I). The sections taken during spring (Fig 1 D-F) and autumn (Fig 1 J-L) displayed a weak to moderate immunoreactivity. In general, the immunoreactivity was more distinct both in the head and tail regions than in the body region throughout the year. In the juvenile dromedaries, the influence of the season either on the intensity or on the pattern of immunostaining was insignificant (Fig 2). The vascular SMCs were almost strongly reactive especially in the larger vessels throughout the year.

Discussion

A distinct immunostaining for α-SMA has been reported within the cytoplasm of the SMCs both in epididymal tubules and in the walls of the blood vessels in epididymal sections from adult dromedaries during the breeding months. This is in agreement with the findings reported in the testis

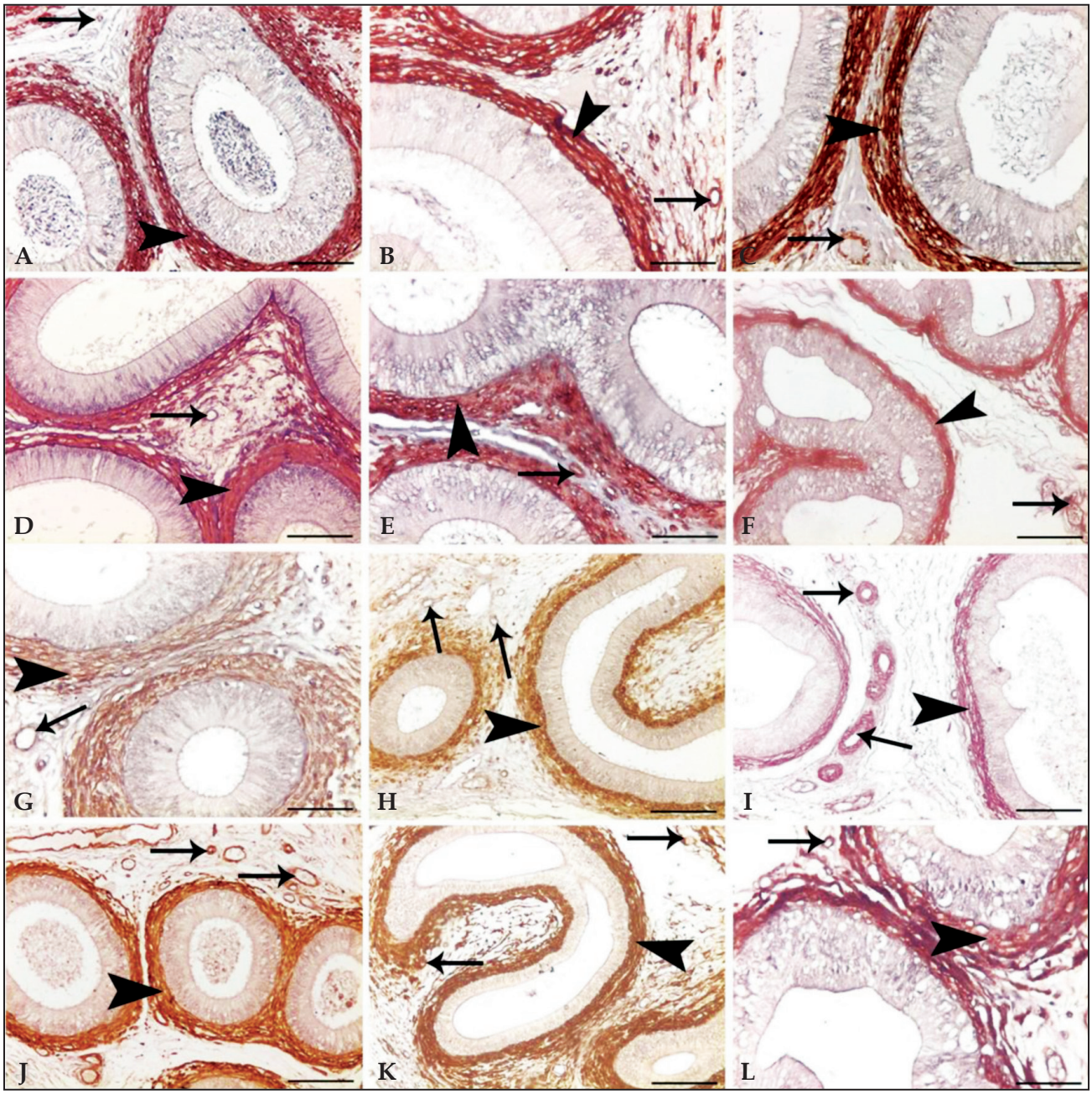

Fig 1. Alpha smooth muscle actin-immunostained adult camel epididymal sections showing Head, body and tail (arranged from the left to the right, respectively); during winter (A, B and C), spring (D, E and F), Summer (G, H and I) and autumn (J, K and L).. The intensity of immunoreaction was season-dependent as shown in the periductal (arrowheads) and the vascular (arrows) SMCs. The strongest intensity was seen in sections during winter and the weakest one was in sections during summer. Scale bars: 100 µm (A, B, C, E, G, I and L) and 200 µm (D, H, J and K).

(Schlatt *et al,* 1993; Moustafa, 2012; El-Azab and El-Mahalaway, 2019), efferent ductules (Alkafafy and Sinowatz, 2012; Ibrahim, 2015); epididymis (Abd-Elmaksoud, 2009; Alkafafy, 2009; Alkafafy *et al,* 2011; Alkafafy and Sinowatz, 2012; Ibrahim *et al,* 2017), ductus deferens (Alkafafy *et al,* 2010; Marettova and Maretta, 2018) and mammary gland (Helal *et al,* 2013) from different animal species.

The differentiation of SMCs has been previously studied using immunolocalisation of α-SMA both in normal and disease conditions (Skalli *et al,* 1989). The cellular differentiation of the peritubular SMCs is related to the emergence of contractile filaments within their cytoplasm. This synchronises with the progressive increase of α-SMA-immunoreaction (Francavilla *et al,* 1987). Our findings in the juvenile

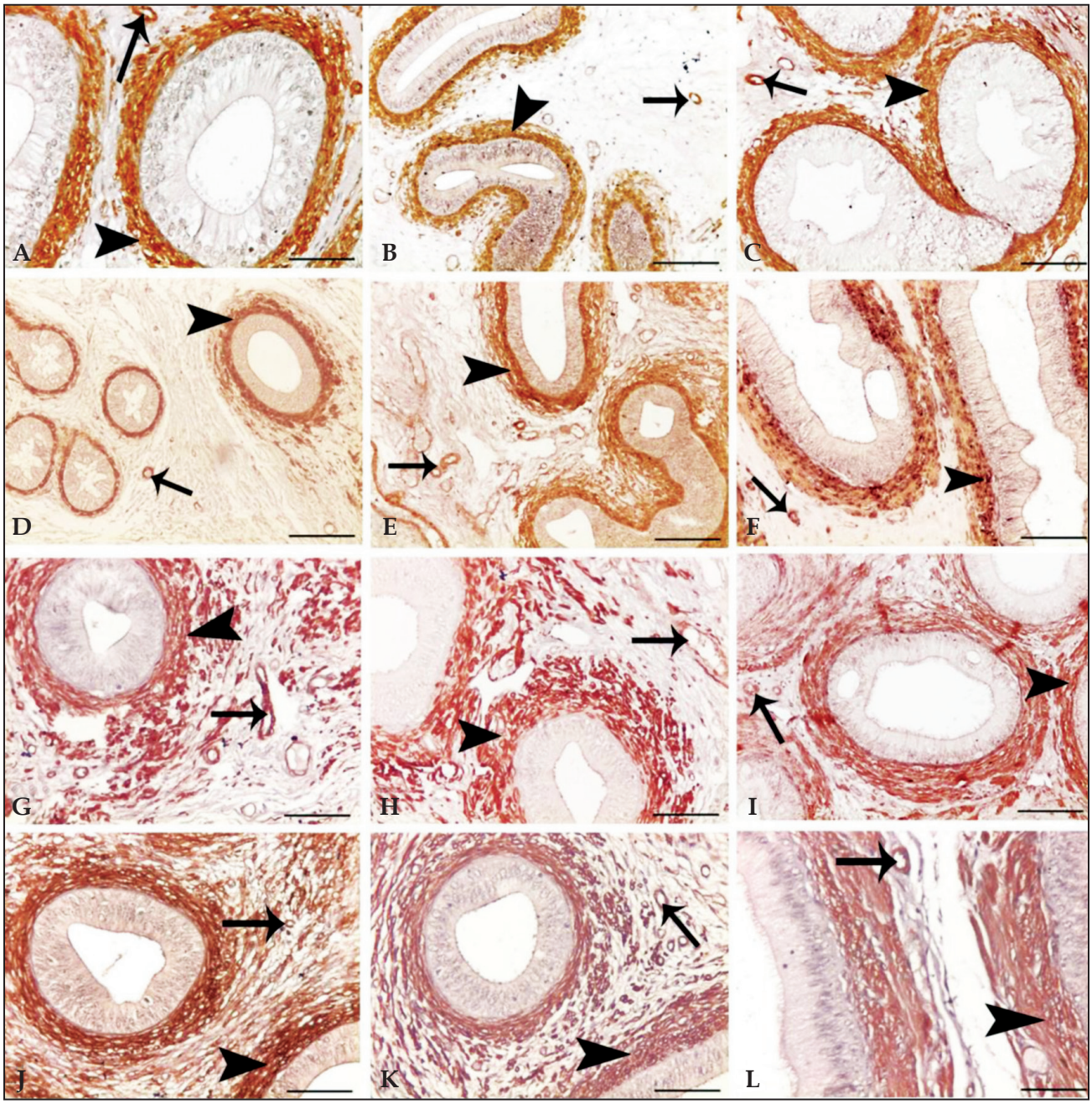

Fig 2. Alpha smooth muscle actin-immunostained juvenile camel epididymal sections showing head, body and tail (arranged from the left to the right, respectively); during winter (A, B and C), spring (D, E and F), Summer (G, H and I) and autumn (J, K and L). The intensity of immunoreaction displayed in the periductal (arrowheads) and the vascular (arrows) SMCs, was weak to moderate during all seasons with minimal season-dependent variations. Scale bars: 50 µm (L), 100 µm (A, G, H, I, J and K) and 200 µm (B, C, D, E and F).

dromedaries go in line with those reported prenatally in the epididymal sections from the bovine foetus (Alkafafy and Sinowatz, 2012) and postnatally in the epididymal sections from new-born rats (Francavilla *et al,* 1987). Additionally, this differentiation may subject to variations during developmental stages (Schlatt *et al,* 1993; Alkafafy and Sinowatz, 2012), breeding seasonality (Ibrahim, 2015; Ibrahim *et al,* 2020) or cyclic functional activity (Helal *et al,* 2013). Thus, the differentiation of the peritubular cells (testis, efferent ductules, epididymis and ductus deferens) during sexual development is a hormone-dependent process and is mainly regulated by androgens (Schlatt *et al,* 1993; Ibrahim, 2015). A similar notion has been suggested in cases of perialveolar and periductal myoepithelial cells in the mammary gland (Helal *et*

al, 2013) and in the poll gland (Ibrahim *et al*, 2020) glands in female and male camels, respectively. The distinct α-SMA-immunoreactivity in the camel epididymis during breeding season may correlate to the propulsive capacity of the epididymal duct, which is mainly derived from the contractility of the peritubular SMCs (Hinton, 2010).

In conclusion, the spatial distribution of α-SMA was dependent both on season and sexual maturity. Distinct binding sites to α-SMA were consistently evident in the peritubular SMCs throughout the whole length of the duct in adult camels, especially during the months of breeding season. This seasonal variation may be relevant to the hormone-dependent physiological alterations throughout the year.

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References

Abd-Elmaksoud A (2009). Comparative expression of laminin and smooth muscle actin in the testis and epididymis of poultry and rabbit. Journal of Molecular Histology 40(5-6):407-416.

Al Eknah MM (2000). Reproduction in old world camels. Animal Reproduction Science 60-61:583-592.

Alkafafy M (2009). Some immunohistochemical studies on the epididymal duct in the donkey (*Equus asinus*). Journal of Veterinary Anatomy 2:23-40.

Alkafafy M, Elnasharty M, Sayed-Ahmed A, Abdrabou M (2011). Immunohistochemical studies of the epididymal duct in Egyptian water buffalo (*Bubalus bubalis*). Acta Histochemica 113:96-102.

Alkafafy M and Sinowatz F (2012). Prenatal development of the bovine epididymis: Light microscopical, glycohistochemical and immunohistochemical studies. Acta Histochemica 114(7):682-694.

El-Azab NE and El-Mahalaway AM (2019). A Histological and Immunohistochemical Study on Testicular Changes Induced by Sliver Nanoparticles in Adult Rats and the Possible Protective Role of Camel Milk. The Egyptian Journal of Histology 42(4):1044-1058.

Francavilla S, Moscardelli S, Properzi G, de Matteis MA, Barcellona P Scorza, Natali PG and de Martino C (1987). A correlation between the ultrastructure of epithelium and tubule wall, and the fluorescence-microscopic distribution of actin, myosin, fibronectin, and basement membrane. Cell and Tissue Research 249:257-265.

Helal A, Emara S and Alkafafy M (2013). Immunohistochemical studies on the dromedary camel (*Camelus dromedarius*) mammary gland during lactation and non-lactation periods. Journal of Camel Practice and Research 20(2):175-182.

Hinton BT (2010). What does the epididymis do and how it do it? In Handbook of Andrology, edited by Robaire B and P. Chan. Lawrence, KS: Allen Press, Inc. 2010.

Ibrahim D and Abdel-Maksoud FM (2019). Immunohistochemical and ultrastructural features of the seasonal changes in the epididymal epithelium of camel (*Camelus dromedarius*). Microscopy and Microanalysis 25(5):1273-1282.

Ibrahim ZH, Joshi D and Singh SK (2017). Seasonal immunohistochemical reactivity of S-100 and α-smooth muscle actin proteins in the epididymis of dromedary camel (*Camelus dromedarius*). Andrologia 49(6):e12667

Ibrahim ZH (2015). Morphometric and Immunohistochemical studies on Camel Efferent Ductules in Relation to Reproductive Activity. Nova Journal of Medical and Biological Sciences 4(4):1-7.

Ibrahim ZH, Al-Kheraije KA and El-Tigani-Asil EA (2020). Seasonal studies on morphology and immunohistochemical localisation of s-100 and alpha smooth muscle actin proteins in poll glands of dromedary camel. Journal of Camel Practice and Research 27(1):39-47

Marettova E and Maretta M (2018). Immunohistochemical study of the goat ductus deferens. Folia Veterinaria 62(1):11-17.

Moustafa AM (2012). Age-related changes in the immunohistochemical localisation pattern of α-smooth muscle actin and vimentin in rat testis. The Egyptian Journal of Histology 35:412-423.

Schlatt S, Weinbauer GF, Arslan M and Nieschlag E (1993). Appearance of α-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. Journal of Andrology 14(5):340-350.

Skalli O, Pelte MF, Peclet MC, Gabbiani G, Gugliotta P, Bussolati G, Ravazzola M, and Orci L (1989). Alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, is present in microfilamentous bundles of pericytes. Journal of Histochemistry and Cytochemistry 37:315-321.

Zayed AE, Ismail KA, Ibrahim AA and EL-Maksoud FMA (2012). Morphological studies on the seasonal changes in the epididymal duct of the one-humped camel (*Camelus dromedarius*). Veterinary Science Development 2:7-14.

Zayed AE, Hifny A, Abou-Elmagd A and Wrobel KH (1995). Seasonal changes in the intertubular tissue of the camel testis (*Camelus dromedarius*). Annals of Anatomy 177: 199-212.