Masood et al. /Journal of Camelid Science 2022, 15: 1-12
http://www.isocard.net/en/journal

Effects of natural seasonal environmental conditions on the reproductive histomorphometric dynamics of male dromedary camels

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Received: January 14, 2022; Accepted: February 20, 2022; Published: March 18, 2022

Abstract

Pakistan has faced catastrophic floods, droughts, and cyclones in recent years that have killed, and displaced thousands of animals, and damaged infrastructure. Climatologists forecasted that Pakistan is one of the countries most vulnerable to the effects of climate change. The effect is on the agriculture sector, and this inversely affects livestock, including camels. The present study was carried out to elucidate these effects on the morphology of the reproductive organs of 24 clinically healthy adult male dromedary camels (4-7 years old) raised in the city of Khairpur in southern Pakistan. The samples were collected from the local abattoir during four different seasons. Gross anatomical parameters, including weight, length, width, thickness, circumference, and volume of the testis, were measured. For histometric studies, tissues were prepared following a standard histological technique. The percentages of areas of interstitial tissue and seminiferous tubules were measured using AutoCAD®. Measurements of epithelium height, the muscular wall thickness (testes, epididymides, ductus deferentes) as well as the diameter of the secretory units of the bulbourethral and prostate were recorded. Significant statistical values were recorded in 16 out of 29 parameters during breeding seasons (winter and spring), except the weight of the prostate and bulbourethral glands which increased in summer and autumn. The data indicated that season has a marked influence on the histomorphometry dynamics of the male reproductive system of the one-humped camel which in turn affects reproduction in dromedaries. Therefore, care should be taken to optimize production or collect semen for artificial insemination during the relatively colder seasons.

Keywords: camel, male reproductive, morphometry, Pakistan

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Introduction

The weather extremes in Pakistan include high and low temperatures, heavy rainfall, and flooding. The highest temperature ever recorded in Pakistan is 53.7 °C (128.66 °F), in Turbat, Baluchistan, on 28 May 2017. It was also the second verified hottest temperature ever recorded in Asia and the fourth highest temperature ever recorded on earth (Haider, 2011). Each year before the onset of the monsoon, that is 15 April to 15 July, and after its withdrawal, that is 15 September to 15 December, there is always a distinct possibility that cyclonic storms will develop in the north Arabian Sea. Cyclones formed in the Arabian sea often result in strong winds and heavy rainfall in Pakistan's coastal areas only (Chaudhry, 2017).

Pakistan falls among the top 10 camel raising countries which possess more than one million head showing a regular growth trend (Faye, 2014). Since 1961 - the date of the first FAO statistics - the world camel population has more than doubled. The camel is capable of producing milk, meat, and wool under very harsh conditions, with a high added value for producers and interesting dietetic and nutritive properties for consumers. Camels are seasonal breeders. The breeding season is variable but generally coincides with the period of low temperatures, increased rainfall, and low humidity (Ali et al., 2017). While several researchers studied the effects of the natural environment on females (Ali et al., 2017), the male camel that is loose in a herd of females tends to come into season earlier and remain in a rut for a longer period than confined males. The geographical diversity of the breeding season in these animals suggests that local events play a role in the temporal pattern of reproduction. However, it remains unclear whether climatic, nutritional, management, or intrinsic factors are affecting reproduction and how strong the influence of each of them is. Understanding the basic histological changes during different seasons as well as better knowledge of the physiology of reproduction at the molecular and cellular levels will result in improvements in camel production.

Relatively low reproductive performance has been a major obstacle to the population growth of dromedaries. The possible factors responsible for the poor reproductive performance of dromedary camels are limited breeding systems, delayed puberty, lengthy gestation period, a long calving interval, as well as the harsh environment. High fertility levels and better feeding are essential, not only for profitable production but also for selection purposes and genetic improvement. Despite the economic importance of camels in certain parts of the world, insufficient research has been conducted on their reproduction and related parameters. The literature describing the male reproductive tract is sparse compared to other domesticated animal species. For example, Ali et al. (1978) described the morphology of the ampulla ductus deferens in Sudanese camels and Ibrahim and Singh (2014) described the camel epididymis concerning the reproductive activity in Sudanese camels. Morphological studies are more available, but the majority are without correlations to seasons. Researchers agreed that season has an evident effect on the morphology of the testicular tissue with emphasis on Leydig cell shape and number linked to activity and testosterone in Egyptian camels (Abd-Elaziz et al., 2012), testes measurements, and seminiferous tubules in Pakistani camels (Pasha et al., 2011), and testes morphology and reproductive activity (Tingari et al., 1984), and Friedlander et al. (1984) focused on the ultrastructural analysis of Leydig cells differentiation during different seasons. Other researchers studied the seasonal effect on the anatomical changes of the testes of one-humped camels (Pasha et al., 2011) in Pakistan during four seasons, while others reported changes in three seasons only (Spring was excluded), including Zayed et al. (2013) in Egypt and Singh and Bharadwaj (1978a) in India. Therefore, the present study was undertaken to obtain a better knowledge of the gross measurements of male reproductive organs and the process of sperm production around the year in indigenous breeds of camels kept in the natural ecology of Pakistan (Figure1). This study is based on gross and light microscopic observations relative to the effect of seasonal variation in adult camels. The knowledge of reproductive morphology and physiology will contribute to the application of a more efficient timing of breeding in a certain optimal season. Following available literature recommendations as well as the findings of this study, a better economical and reliable camel production may be achieved through the timing of breeding in Pakistan and through using genetic evaluation which will facilitate the use of breeding technologies like artificial insemination and embryo transfer.

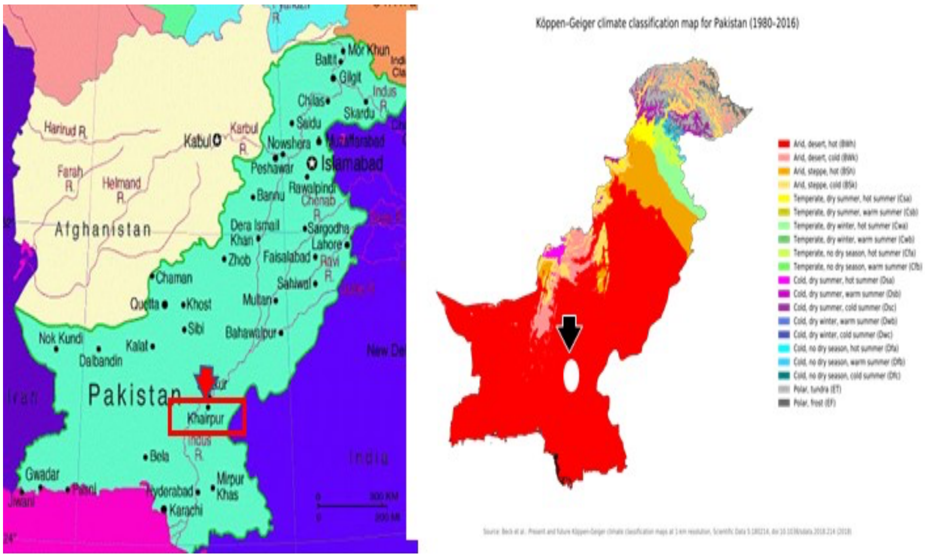

Figure 1. Map of Pakistan and the city where the research was performed. Notice classification map labeled the city as arid and hot (Khairpur).

Materials and methods

A total of 24 pairs (6 in each season) of testes with their corresponding epididymides, vas deferens, penis, and the accompanying prostate and bulbourethral glands, were collected from clinically healthy male one-humped camels (Camelus dromedarius), aged 4-7 years (estimated by dentition), in four seasons, namely winter, spring, summer, and autumn, over one year. Rectal temperature respiratory and pulse rates were recorded before slaughter to confirm the clinical status of the animals. The samples were collected immediately after slaughter from local abattoirs in Faisalabad and Lahore. Gross measurements were recorded on the spot and small pieces of tissues of 1 cm thickness from all organs mentioned above were fixed in Blouin's solution. Collected tissues were processed following standard histological techniques (Bancroft and Gamble, 2008). The metrological data of the study areas (Khairpur) during the entire period of study were collected from the climatology laboratory, University of Agriculture, Faisalabad, and the Meteorological Department, Government of Punjab, Lahore, Pakistan.

Morphometric analysis

The diameter of the seminiferous tubules of each animal was determined at 400X with the help of a micrometer. The diameter was measured from two different planes of each tubule (largest and smallest diameters). The average of the two measurements was recorded. Only rounded or partially convoluted tubules of the clear transverse section were measured. A total of six seminiferous tubules per testis was measured to minimize the chances of error. The percentages of cross-sectional areas of interstitial tissue and seminiferous tubules were obtained by using AutoCAD®. These areas were measured from the photomicrographs of the testicular sections taken by Nikon Optiphot 2 microscopes at 100X magnification. Five slides were prepared from each testis and six random fields were selected in each section. The rounded tubules from different regions (head, body, and tail) of epididymis were measured for the height of epithelia, the thickness of the muscular wall, and the lumen diameter. Six tubules were measured from each section. The height of the epithelium of the ductus deferens was measured. All measurements were performed at 400X magnification with the help of an ocular micrometer. Accessory sex glands (prostate and bulbourethral glands) were examined for their different layers and type of epithelium. The epithelium height and diameter of the secretory units were measured for each gland at 100X magnification.

Statistical Analysis

Means, standard errors, and ranges were calculated for each parameter under study (Microsoft Excel®). The means of different parameters were compared using one-way analysis of variance (ANOVA) (Minitab, Mtb 13®) and significantly different means were considered at $P<0.05$ (Duncan's multiple ranges, DMR test).

Results

Twenty-four sets of reproductive organs, six in each season, were studied to ascertain the structural and functional correlations between different segments of the male dromedary genital system. The testes of the camel were ovoid, the parenchyma was brown, and surrounded by a tough connective tissue (tunica albuginea). The testes were inside the scrotum located in the perineal region (Figure 2).

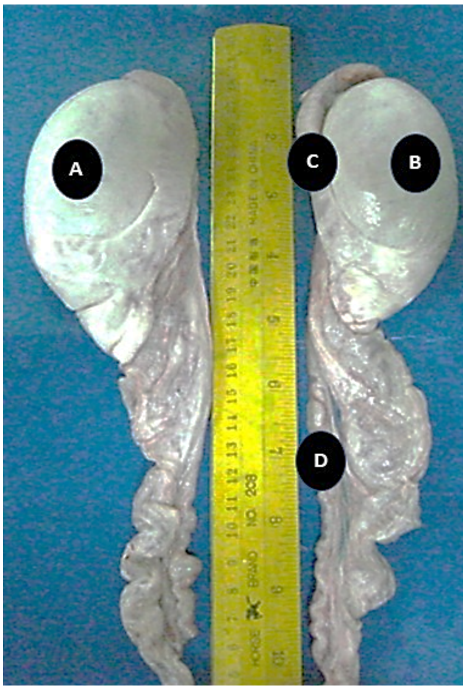

Figure 2. Comparative gross view of the right and Left testes, epididymis and ductus deferens of one-humped camel (*Camelus dromedarius*). A: left testis. B: Right testis; C: Epididymis and D: Ductus deferens.

All measurements were reported as means (±SEM). Gross and microscopic anatomical parameters of the tests showed that the highest recorded values were during winter, followed by spring, autumn, and summer (Tables 1 and 2).

The testes, weight, thickness, circumference, the diameter of seminiferous tubules, and percentage areas of interstitial tissue were statistically significantly different among seasons ($P<0.05$). Results showed clearly that testicular parameters were high during the winter and spring seasons and low during summer and autumn (Figure3).

The epididymis is lined by ciliated pseudostratified columnar epithelium. Values of the epididymal weight, length (head, body, and tail), epithelial height, and luminal diameter are presented in Tables 1 and 2 (Figure 4).

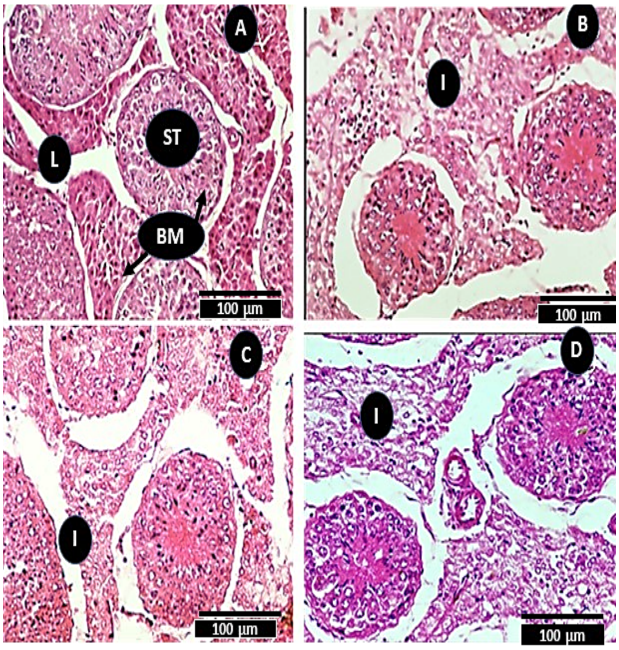

Figure 3. Microscopic images of the testes of one-humped camel (*Camelus dromedarius*) spring (A), winter (B), autumn (C), and summer (D). ST: Seminiferous tubule; BM: basement membrane; I: interstitial tissue. L: Leydig cells within the interstitial tissue. White spaces around the seminiferous tubules are fixation artifacts due to the shrinkage of the tissue in the fixative solution. H.&E. stain.

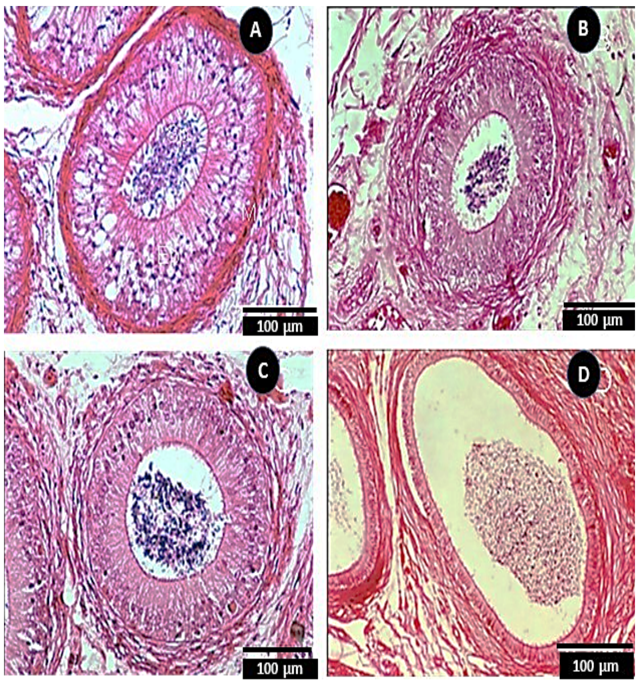

Figure 4. Microscopic images of the epididymis of one-humped camel (*Camelus dromedarius*) spring (A), winter (B), autumn (C), and summer (D). Notice the height of the epithelium during the hot summer season. H.&E. stain.

The ductus deferens is lined with simple columnar epithelium. The right ductus deferens' length is 411.44 ± 8.61 while the left is 413.56 ± 8.61 mm. The epithelium height of right and left ductus deferens was measured as 62.62±1.67 and 62.92±1.69 µm, respectively. The thickness of the muscular wall was recorded as 397.27±2.85 and 380.05±2.88 µm in right and left ductus deferentes, respectively (Figure 5).

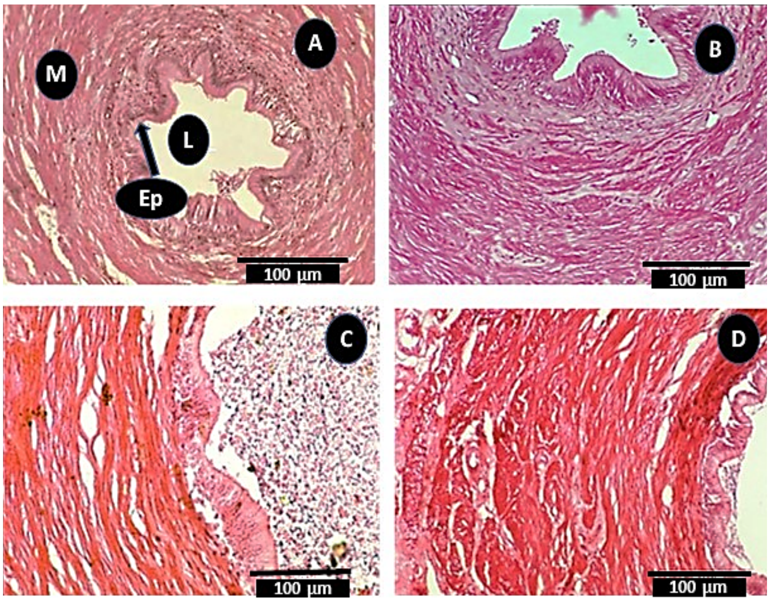

Figure 5. Microscopic views of the ductus deferens of one-humped camel (Camelus dromedarius) spring (A), winter (B), autumn (C), and summer (D) Ep: Epithelium; L: Lumen; M: Tunica muscularis. H.&E. stain.

The total length of the penis was recorded as 482.96 ± 11.25 mm, while the length of the urethral process of the penis was measured as 3.41 ± 6.09 mm. Seasonal variation did not influence the length of the penis or the urethral process. Both accessory sex glands' weight, width, epithelial height, and the diameter of secretory end pieces of the prostate gland were statistically significantly different among different seasons (P<0.05) (Tables 1 and 2) (Figure 6). However, the length of both glands, as well as the thickness of the bulbourethral gland showed no statistical differences.

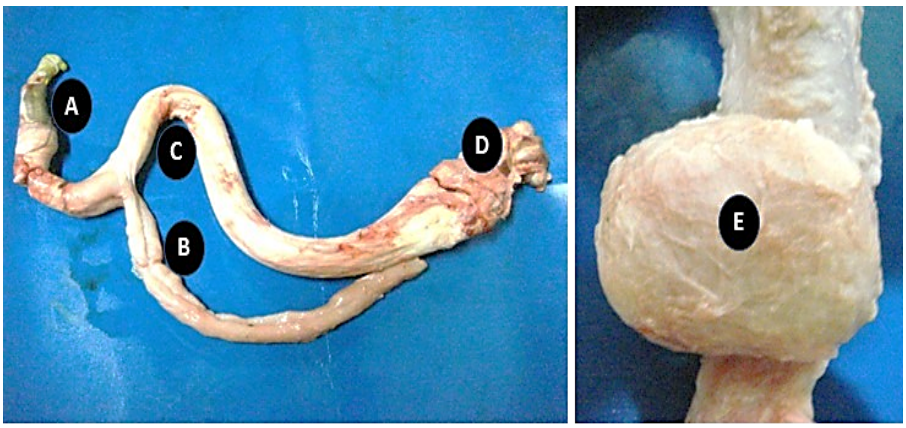

Figure 6. Camelus dromedarius Penis (Left) and Prostate gland (Right). A: Curved tip of the penis; B: Retractor penis muscle; C: Sigmoid flexure. D: Root of the penis; E. Prostate gland.

In summary, statistical analysis revealed that most of the studied parameters showed significantly higher values in the winter and spring seasons. However, the weight of the prostate and bulbourethral glands increased during summer and autumn.

Discussion

Camels are seasonal breeders. Male camels show breeding activity during relatively cold months of the year, including enlarged testes and accessory sex glands. In the present study, the gross and microscopic measurements were carried out on the male reproductive system of 24 adult one-humped camels (Camelus dromedarius) (6 in each season) in four seasons (winter, spring, summer, and autumn) in southern Pakistan. The testes of the Camel are ovoid, located in the perineal region for protection from the heated sand during the summer season when the animal sits. The parenchyma is brown and surrounded by a tough white connective tissue (tunica albuginea). The tunica albuginea limits the irregularities in the shape of the testes between different seasons when testes change size. These findings are in line with those described by Pasha et al. (2013), Degen and Lee (1982), and Hafez and Hafez (2001) who studied both dromedary and Bactrian camels.

The testicular weight of this study fell in the ranges reported by Hafez and Hafez (2001) in dromedary camels and Degen and Lee (1982) who described a range of 80-120 g and 86-178 g for each testis of the camel, respectively. However, these are higher than those reported (80-100 g) by Skidmore and Adams (2000) in United Arab Emirate camels. The reported differences could be attributed to different environmental conditions, older ages, and better nutritional regimens in the present study. This study confirmed that seasonal changes in the testes of examined camels were different during different seasons. We are reporting that the highest mean value of testicular weight was recorded during the coldest months of the year, i.e., November to March. Pasha et al. (2011) reported testis changes during different seasons. Owaida (1973) studied seasonal changes in the age of the camel. Other researchers reported testosterone changes and linked them to dehydration in male camels (Charnot, 1965), while Deen (2008) described the relationship between libido and the testosterone hormone level in the blood. Tingari et al. (1984) correlated the shape of the testis with reproductive activity by following the changes during one entire year. Monaco et al. (2015) studied the effect of GnRH on Tunisian camels by measuring testosterone levels, libido, and semen. This coincides with a pronounced rise in sexual activity and increased testosterone levels in male dromedaries during cold months of the year.

The highest mean value of testicular volume was recorded during winter, followed by spring, summer, and autumn. The mean testicular volume fell in the range previously reported by Pasha et al. (2011) and Degen and Lee (1982), who studied the macroscopic anatomy of male reproductive systems of the camel in three different age groups. The reported values are comparatively higher than those reported by Zaghloul et al. (1988). This variation might be due to the difference in the age range of dromedaries, breeds, and the total number of animals used in the studies.

The present study revealed that there is an increase in testicular size (i.e., length, width, thickness, and circumference) of camel testes during winter and spring. These findings are partly in line with those of Djang et al. (1988) who studied the seasonal influence in length and width of camel testes. Increased testicular size has been correlated with an increase in several Leydig cells and a rise in blood testosterone levels (Preston et al., 2012) which ultimately causes enhanced sexual activity during cold months in sheep. In another study, the left and right testicular weights of one-humped camel bulls in Nigeria were reported to be 83.50 ± 11.07 g and 83.50± 6.33 g (Mahmoud et al., 2015), while in this study, the weight was recorded in all four seasons and the overall average was right testis 110.59±5.89 g. and left testis 118.28±6.07 g.

The mean values for the diameter of the seminiferous tubule of the present study fell in the ranges previously described by Singh and Bharadwaj (1978a) who gave a range of 113-250 µm in the camels of 4-20 years of age. The highest mean values of seminiferous tubule diameter were recorded during spring followed by those of winter, autumn, and summer, respectively. However, somewhat lower values have been reported in previous studies (Pasha et al., 2011). This difference could be due to differences in the age range of the dromedaries used in their studies.

The mean values for the percentage area of interstitial tissue fell in the ranges previously described by Singh and Bharadwaj (1978a) who gave a range of 27-70% in the camels of 4-20 years of age. The highest percentages of interstitial (intertubular) tissue were observed during winter and spring and were significantly higher than those of summer and autumn. These findings are following those of Pasha et al. (2011), Tingari et al. (1984), and Singh and Bharadwaj (1978a). However, Zayed et al. (1995) have reported lower values for interstitial connective tissue during different seasons, ranging from 24% in autumn to about 39% in spring. A discrepancy in findings could be due to the different climatic conditions of Egypt as compared to Pakistan. Since interstitial connective tissue consists of Leydig cells, collagen, reticular fibers, and increased interstitial connective tissue may be interpreted as increased Leydig cell and increased testosterone secretion. This is not always true as researchers need to be very careful when estimating numbers of Leydig cells within the testicular tissues when looking at the overall tissue within the testis.

In the present study, the total weight of the epididymis average values is in line with those previously described by Singh and Bharadwaj (1978b) who reported a range of 10-40 g. Though, Ali et al. (2012) reported lower weight for the right (16.71± 1.41 g) and left (16.79± 1.34 g) epididymides, respectively. He did not mention the season, so the difference could be attributed to climatic conditions, and the size and age of the animals used. The mean length of epididymis reported in the present study was in line with those reported by Mahmoud et al. (2015) and Pasha et al. (2013). However, it was slightly greater than those previously reported by Degen and Lee (1982) in Israel. This variation could be due to the variation of local breeds of the study area and the number of camels used in the study. No reference was available to compare the lengths of different segments of the epididymis (head, body, and tail).

The mean epithelial height of the body of epididymis in different seasons was like the findings of Ibrahim and Singh (2014), who observed a similar trend in the height of epithelium in the various seasons of the year. However, it was higher in the breeding season and decreased during the non-breeding season. This is in line with the findings of Ibrahim and Singh (2014) who reported that the height of epididymal epithelium (cauda epididymis) does change significantly in breeding and non-breeding seasons. In the tropical lizard, the epididymal epithelium has been reported to be well developed and secrete more during the breeding season (Ferreira et al., 2009). Secretions produced by epididymal epithelium help in the maturation of spermatozoa (Jones, 1999; Ferreira et al., 2009).

The luminal diameter of the epididymis was significantly higher in the non-breeding season (summer and autumn) than in the breeding season (winter and spring). Our values are lower than those reported by Ibrahim and Singh (2014). However, they have also reported that luminal diameter increases during winter and decreases during the summer season. An increased luminal diameter facilitates the easy passage of spermatozoa. However, this coincides with a decreased size of seminiferous tubules and the rate of spermatogenesis. Similar findings have been reported by Alkafafy et al. (2011) in the epididymis and Zayed et al. (2013) in the efferent ductules of the camel.

The values of ductus deferens length were a little lower than those previously recorded by Skidmore and Adams (2000). They reported the total length of the ductus deferens as 450 -500 mm. The difference could be due to non-specific age and breeds of camels involved in their study. The season did not influence the length of the ductus deferens. No literature was found to compare the seasonal influence on ductus deferens length in camels. Epithelium height of the ductus deferens was higher than those reported by Ali et al. (1978) which may be due to different age groups used in the study. No literature was found to compare the seasonal influence on the epithelial height of the ductus deferens and the thickness of its tunica muscularis.

The mean length of the penis in the present study was a little lower than previously described by Mobarak et al. (1972) who mentioned 590-680 mm. The difference might be due to the large body size of animals, or the non-specific age of camels involved in their study. No literature was found to compare the value of the urethral process reported in the present study.

Prostate gland weights are comparable with those reported by Degen and Lee (1982) and Abou et al. (1988) in different age groups. The prostate glands were heavier during the breeding season than the non-breeding season. Values of dimensions (length, width, and thickness) of the prostate gland were in line with the ranges described by Degen and Lee (1982).

In the present study, the season had a significant influence on the width and thickness of the prostate gland. The highest mean value of width and thickness of the prostate gland was recorded during spring. No literature was found to compare the effect of season on the width and thickness of the prostate gland of the camel.

The Epithelial height of the prostate gland was as reported by Ali et al. (1978). The mean values obtained during the spring season were significantly higher than those of summer and autumn. No literature was found to compare the effect of season on the diameter of the secretory end pieces of the camel prostate gland.

The mean weight of the right and left bulbourethral glands coincided with the range previously described by Degen and Lee (1982) and Abou et al. (1988). However, Derar et al. (2012) reported lower values of bulbourethral gland weight, but they did not consider the season. The highest mean values of weight were recorded during spring, which was significantly different from those recorded during autumn and summer. These findings are in line with those of Abou et al. (1988) who recorded the maximum weight of the bulbourethral gland during spring and the lowest during summer. Results of the present study partly agree with the findings of Ali et al. (1978), who noticed that the bulbourethral glands of the camel were enlarged, and their epithelial cells were tall between February and April. The mean length, width, and thickness of bulbourethral glands are in the ranges described by Degen and Lee (1982). The mean values of the left bulbourethral gland width were significantly higher in winter and spring than during autumn. No literature was found to compare the effect of season on the width of the bulbourethral gland of the camel.

The height of epithelium increased during spring and winter while it reduces to a minimum during summer and autumn. The mean height of the epithelium was a little lower than those previously described by Ali et al. (1978) who gave an average value of 21 μm. No literature was found to compare the influence of season on the parameters of bulbourethral glands. Likewise, the diameter of the secretory end pieces of bulbourethral glands was significantly higher ($P > 0.05$) during the breeding season than in the non-breeding season. Thus, enhanced size, weight, dimensions, and diameter of secretory end pieces of prostate and bulbourethral glands contribute to increased sexual activity shown by male camels during breeding/rutting season.

In conclusion, based on the above findings, we may be able to improve reproductive efficiency if special care is provided to camels during the winter and spring seasons. Knowing the morphological changes and the physiology associated with them, the findings of this study may also help camel theriogenologists and breeders to improve the productive and reproductive potential of this species.

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