Development and validation of testicular biopsy for diagnosis of infertility in camels (*Camelus dromedarius*)

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Abstract

Male camel infertility is a heterogeneous disorder. A variety of factors may adversely impact sperm production and function and impair fertility. Twenty-six male dromedaries (4–14 years old) were used in this study during the rutting season. Four sexually mature male camels were used to develop and validate a procedure for testicular biopsy in dromedary camels. No undesirable side effects in testicular function were apparent after use of an automatic biopsy gun. In 22 infertile male dromedaries, an algorithmic approach based on information collected during a careful history, clinical examination, testicular evaluation, testicular ultrasonography, the results of the semen analyses, and testicular biopsy was used to diagnose infertility. In diagnosis of male camel infertility, the results of testicular ultrasonography and testicular biopsy were correlated identically. Testicular biopsy is a promising method that, along with carefully performed history, appropriate testicular ultrasonography procedure and semen analysis, can afford the veterinarian the opportunity for more precise diagnosis and treatment of male dromedary infertility disorders.

Keywords: Dromedary camels, infertility, testicular biopsy, testicular ultrasonography.

Introduction

The reproductive rate in Camelidae has been described as low.[1,2] Many theories have been advanced to explain this low reproduction rate based on physiological characteristics of these species.[3] Management may explain some of the reproductive failures but a substantial number of infertility problems are due to pathological processes in the male genitalia.

There are limited reports on the pathology of the male reproductive tract in Camelidae. Although the guidelines for breeding soundness examination of male camelids are in their infancy, there is evidence that thorough examination of males may help to identify many infertility problems.[1] It is very important that all studs be examined for at least the following parameters: testicular size, testicular consistency, ability to extend the penis and ability to ejaculate and produce viable semen. The average testicular length and width are 9.1 cm and 5.1 cm, respectively, for male dromedaries three years and older during the breeding season.[1] The testes should be resilient and non-painful. Each part of the genital tract can be the site of congenital or acquired lesions that need to be identified.[4] Scrotal and testicular pathology in the camelid can be acquired (trauma ,orchitis, hydrocele, degeneration) or congenital (hypoplasia, cryptorchidism).[1] Several other reproductive problems are reported in the Camelidae but their exact etiology is not known. Amongst the most commonly reported problems are absent or reduced libido, ejaculatory problems and unexplained sub-fertility or infertility.[3]

Testicular ultrasonography in camels is a very important technique for the evaluation of the testicular parenchyma as well as the surrounding tissues.[1] Moreover, testicular biopsies are useful procedures in the evaluation of testicular function in llamas.[5]

This study was designed to develop and validate testicular biopsy for assessment of the reproductive status of infertile male camels.

Materials and methods

A total of twenty-six male dromedary camels (4–14 years old) were used in this study during the rutting season (November to May).[6] Four dromedaries were used as controls for development and validation of testicular biopsy in male camels. These animals were housed at the Camel Research Center at King Faisal University. The animals were healthy, weighed 400–700 kg with a history of normal fertility. They were maintained under standard conditions of feeding and management. Each male camel was housed separately in a 12 x 6 m paddock and fed 3-4kg barley per day. Rhodes grass and water were provided ad libitum. Twenty-two infertile male camels were admitted to the

Veterinary Teaching Hospital, College of Veterinary Medicine and Animal Resources, King Faisal University and were subjected to the following examinations:

Reproductive history

A full reproductive history of the animal was recorded which included the breed, age, management, libido and the owner's complaint.

General physical examination

A general physical examination was conducted with emphasis on systemic problems or poor body condition score.

Testicular evaluation

Testicular size, mobility and consistency were examined carefully by palpation. Any morphological, congenital or pathological lesions of the testes were recorded. The length and width of each testis were measured using vernier calipers.

Testicular ultrasonography

Ultrasonographic examination of the scrotum and its contents was conducted subsequent to scrotal palpation. Ultrasonography of the testes was performed by direct manual manipulation of a convex multi-frequency transducer (1–15 MHz; Prosound SSD-3500SX, Aloka Co., Ltd; Tokyo, Japan). The testis was examined by placing the probe in a vertical position then in a horizontal position and sweeping it along the whole surface of the organ. Testicular ultrasonography used for the evaluation of the testicular parenchyma as well as the surrounding tissues.

Collection and evaluation of semen

Collection of semen from male camels was carried out using an electro-ejaculator (ElectroJac® 5; Ideal Instruments, Neogen Co., Lansing, MI) as described in an earlier study.[7] Immediately after collection, semen samples were evaluated for physical, morphological and physiological characteristics.

Testicular biopsy

Development of the procedure. Testicular biopsy was developed in two stages. In the first stage, the suitability of the instruments and the size of the biopsy specimens for histopathological study were tested. To achieve this goal, biopsies were performed on ten camel testes in the laboratory using a disposable automatic gun (14, 16, and 18 gauge; Speedy-Ram®, RI.MOS, Mirandola, Italy). The biopsy tissues were placed in 10% formaldehyde solution and processed for histopathological examination.

In second stage, potential damage to testicular function was assessed. Four sexually mature male camels with a history of normal fertility were used. Results of the physical examination and testicular ultrasonography were within normal limits and semen was of acceptable quality (Figure 1; Tables 1 and 2). Prior to surgery, camels were sedated with a mixture of xylazine (0.15 mg/kg) and ketamine hydrochloride (2.5 mg/kg) administered intravenously[8] while restrained in sternal recumbency. The scrotal surface was prepared for aseptic surgery and painted with a local anesthetic gel (Lidocaine 5%, Julphar, U.A.E.). The area for puncture was covered with a sterile towel with a 12 cm x 6 cm fenestration. The testis was held firmly in the scrotum so as to tighten the scrotal skin over the testis. The biopsy sample was obtained using the 14 and 16 gauge automatic gun at the midpoint of the posterior border of the testis. The tissues were placed in 10% formaldehyde solution and processed for histopathological examination (Figure 5). The animals were observed for seven days after surgery to detect any detrimental effects of the procedure. Seventy-eight days after the biopsy, physical examinations, testicular ultrasonography and semen analysis were performed.

Evaluation of reproductive dysfunction. Testicular biopsies were performed in the infertile male dromedaries with a 16-gauge needle when the results of the physical and ultrasonographic examinations did not yield a clear diagnosis of the cause of infertility or in cases of unacceptable semen quality.

Statistical analysis

Data are presented as means ± SEM and the analysis was conducted using Stastical Package for Social Sciences, version 16.0.[9]

Results

During development of the biopsy procedure it was found that the use of the 18-gauge needle did not yield a tissue sample containing a sufficient number of tubules to permit a histopathologic diagnosis. When biopsies were performed in the control animals, a small amount of hemorrhage was observed immediately after the samples were obtained in three of four camels (six of eight testes; 75%). Hemorrhage persisted for three minutes after the sample was obtained followed by slight testicular swelling for five days in one testis (one of eight; 12.5%). No other clinical changes were observed in any of the control animals. When physical and ultrasonographic examinations and semen evaluation were performed 78 days after the biopsies were performed, abnormalities were not observed and semen quality was acceptable (Table 2). Results of examination of the testes of the infertile animals are shown in Table 3.

Testicular texture

The texture of testes was palpably normal in four cases (18.2%), soft in 14 cases (63.6%) and hard in four cases (18.2%).

Testicular size

Testicular length and width (means ± S.E.M.) of the control and infertile dromedaries were 9.6 ± 0.2 and 4.8 ± 0.1 cm; and 9.3 ± 1.1 and 4.7 ± 0.6 cm, respectively. However, in cases of testicular hypoplasia, these measurements were 5.5 ± 1.5 and 3.1 ± 0.7 cm, respectively (Table 1).

Testicular ultrasonography

The image obtained from normal testes showed a peripheral area of homogenous tissue corresponding to the testicular parenchyma and a central echogenic area corresponding to the fibrous rete testis (Figure 1).[1] In the majority of the infertile animals (19 of 22; 86.4%), mild to severe degeneration of the testicular parenchyma characterized by loss of its homogeneous appearance due to the presence of localized lesions such as cysts or fibrotic nests was observd during the ultrasonographic examination (Figure 2) Testicular hypoplasia was diagnosed in three (13.6%) infertile animals on the basis of measurements obtained with electric calipers (Table 3; Figure 3).[1] Testicular fibrosis was characterized by hyper-echoic areas in the testicular parenchyma (Figure 4).

Semen evaluation

Evaluation of semen samples from the infertile male dromedaries showed seven cases (31.8%) of oligoasthenozoospermia, six cases (27.3%) of athenozoospermia, six cases (27.3%) of azoospermia, two cases (9.1%) of oligozoospermia and one (4.5%) case of teratozoospermia (Table 3).

Histopathology

Histopathologic examination of the testicular biopsy specimens from infertile camels showed mildly degenerated semineferous tubules containing multiple cell layers of scattered spermatocytic cells with pyknotic nuclei in addition to few spermatids and surrounded by excess numbers of Leydig cells (Figure 6). Severely degenerated seminiferous tubules were lined by only one layer of Sertoli cells and contained primary spermatocytic cells with pyknotic nuclei (Figure 7). Some testicular biopsy specimens from infertile dromedaries contained interstitial fibrosis accompanied by mildly degenerated seminiferous tubules that contained vacuolated Sertoli cells and few spermatids (Figure 8). Histopathologic findings were compared to the abnormalities observed with ultrasonography and were found to be correlated in all cases of infertility (Table 3).

Discussion

Under the conditions of this study, the disposable automatic gun with an 18-gauge needle was found to yield tissue samples with an insufficient number of seminiferous tubules. Biopsy

instruments used in stallions,[10] llamas[5] and bulls[11] utilized a 14-gauge needle and resulted in no difference between the biopsied and the non-biopsied testis. Other investigators have used a 12-gauge split needle biopsy needle in boars, rams, bulls and pony stallions with no deleterious effects.[12]

In the present experiment, short-term changes appeared in the biopsied testes. Similar findings were reported in stallions[13] and bulls.[11] No untoward effects were observed in the testicular parenchyma or semen characteristics when control animals were examined 78 days after tissue samples were obtained. The mean number of progressively motile and morphologically normal sperm was not significantly different before and after the biopsy procedure. Similarly, there was no reduction in the number of normal seminiferous tubules seen in histologic sections obtained from llamas six weeks after a needle biopsy[5] and there was no evidence of testicular atrophy in men following a testicular biopsy.[14] The safety of needle biopsies has been demonstrated in dogs,[15] humans[16] and stallions.[17]

The testicular length and width in infertile dromedaries were similar to those reported for normal adult (three years and older) dromedary camels[1] which indicates that no changes in testicular measurements occur in the presence of testicular degeneration and fibrosis.

Ultrasonograpic imaging systems have provided new methods for diagnostic examinations of pathological conditions of the genital system of domestic animals.[18,19] The similarity found between the results of testicular biopsies and testicular ultrasonography supports the use of biopsies as a diagnostic tool in the evaluation of testicular function. Biopsies could provide a clinically useful tool in the evaluation of potential breeding ability in dromedaries as well as aid in the investigation of infertility as has been described in other animals.[12,20,21] Taken together, semen analyses and either testicular ultrasonography or testicular biopsy could be used for predicting infertility in male dromedaries.

Conclusion

Testicular biopsy should probably be reserved for cases where a fertility problem is suspected but cannot be confirmed through more conventional means of fertility evaluation, or in cases where a problem has been identified and a definitive diagnosis is desired.

Acknowledgements

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Table 1: Testicular length and width (mean ± SEM) in male dromedaries.

Testicular state	Length (cm)	Range	Width (cm)	Range
Control (normal testes)	9.6^{a} ± 0.2 (n=8)	8.4 – 10.4	4.8^{a} ± 0.1 (n=8)	4.4 – 5.2
Degeneration and fibrosis	9.3^{a} ± 1.1 (n=37)	7.4 – 12.0	4.7^{a} ± 0.6 (n=37)	3.8 – 6.2
Hypoplasia	5.5^{b} ± 1.5 (n=6)	3.5 – 7.4	3.1^{a} ± 0.7 (n=6)	2.3 – 4.1

Means with dissimilar superscripts in the same column are significantly different at $P<0.05$

Table 2: Semen analysis of the control dromedary camels before and 78 days after testicular biopsy (mean ± SEM).

Semen parameters	Before testicular biopsy	78 days after testicular biopsy
Motility %	77.5 ± 6.23	73.50 ± 8.29
Sperm concentration ($x10^{6}$/ml)	364.75 ± 58.08	350.00 ± 79.06
sperm abnormalities %	18.75 ± 7.14	16.25 ± 10.78

Table 3: Testicular examination of the infertile male dromedaries.

Camel number	External testicular evaluation	Testicular Length x width (cm)	Testicular ultrasonography	Semen analysis	Histopathologic diagnosis
1	Soft texture	Left: 6.2 x 3 Right: 8.4 x 4.4	Testicular hypoplasia, Sever degeneration	Teratozoospermia Mot[1]. 0 Conc[2]. 6 Abnorm[3]. 92	Testicular hypoplasia, Sever degeneration
2	Hard texture	Left: 9.6 x 4.4 Right: 10.1 x 5.1	Fibrosis and degeneration	Azoospermia	Fibrosis and degeneration
3	Slightly soft	Left: 8.0 x 4.0 Right: 8.7 x 4.9	Mild degeneration	Asthenozoospermia Mot[1]. 30 Conc[2]. 420 Abnorm[3]. 57	Mild degeneration
4	Soft texture	Left: 7.6 x 4.1 Right: 9.0 x 4.3	Mild - severe degeneration	Asthenozoospermia Mot[1]. 25 Conc[2]. 214 Abnorm[3]. 43	Mild - severe degeneration
5	Soft texture	Left: 9.8 x 4.1 Right: 10.1 x 5.9	Moderate degeneration	Azoospermia	Moderate degeneration
6	Soft texture	Left: 7.4 x 4.1 Right: 7.0 x 4.1	Testicular hypoplasia and degeneration	Oligo- asthenozoospermia Mot[1]. 10 Conc[2]. 26 Abnorm[3]. 48	Testicular hypoplasia and degeneration
7	Soft texture	Left: 10.5 x 5.1 Right: 9.3 x 5.0	Moderate degeneration	Asthenozoospermia Mot[1]. 20 Conc[2]. 18 Abnorm[3]. 65	Moderate degeneration
8	Small and hard	Left: 3.5 x 2.3 Right: 4.3 x 2.4	Testicular hypoplasia	Azoospermia	Severe testicular hypoplasia
9	Soft texture	Left: 8.2 x 4.0 Right: 10.0 x 4.9	Mild - moderate degeneration	Azoospermia	Mild - moderate degeneration
10	Normal firm texture	Left: 9.2 x 4.3 Right: 9.2 x 4.5	Mild - moderate degeneration	Asthenozoospermia Mot[1]. 5 Conc[2]. 394 Abnorm[3]. 75	Mild - moderate degeneration
11	Soft texture	Left: 12.0 x 6.2 Right: 11.5 x 5.6	Mild - moderate degeneration	Oligo- asthenozoospermia Mot[1]. 20 Conc[2]. 10 Abnorm[3]. 25	Mild - moderate degeneration
12	Slightly soft	Left: 8.0 x 3.8 Right: 7.5 x 4.4	Moderate degeneration	Oligo- asthenozoospermia Mot[1]. 10 Conc[2]. 34 Abnorm[3]. 62	Moderate degeneration
13	Unilateral aplasia, soft texture	Left: 9.9 x 4.7 Right: aplasia	Moderate degeneration	Oligo- asthenozoospermia Mot[1]. 0 Conc[2].53 Abnorm[3]. 57	Moderate degeneration
14	Normal firm texture	Left: 7.6 x 4.0 Right: 7.8 x 4.9	Mild degeneration	Asthenozoospermia Mot[1]. 55 Conc[2]. 47 Abnorm[3]. 39	Mild degeneration
15	Normal firm texture	Left: 8.5 x 4.4 Right: 10.0 x 4.8	Mild degeneration	Azoospermia	Mild degeneration
16	Slightly soft	Left: 7.4 x 4.1 Right: 8.7 x 3.9	Mild degeneration	Oligo- asthenozoospermia Mot[1]. 10 Conc[2]. 12 Abnorm[3]. 68	Mild degeneration
17	Slightly soft	Left: 9.9 x 4.7 Right: 10.4 x 5.5	Mild degeneration	Oligo- asthenozoospermia Mot[1]. 30 Conc[2]. 26 Abnorm[3]. 16	Mild degeneration
18	Hard texture	Left: 9.5 x 5.3 Right: 9.4 x 4.6	Mild degeneration	Oligozoospermia Mot[1]. 10 Conc[2]. 12 Abnorm[3]. 70	Mild degeneration
19	Slightly soft	Left: 9.6 x 3.9 Right: 9.7 x 4.3	Moderate - severe degeneration	Severe oligozoospermia Mot[1]. 0 Conc[2]. 9 Abnorm[3]. 74	Moderate - severe degeneration
20	Unilateral hypoplasia, slightly hard texture	Left: 4.4. x 2.9 Right: 10.7 x 5.8	Mild - moderate degeneration	Asthenozoospermia Mot[1]. 15 Conc[2]. 18 Abnorm[3]. 66	Mild - moderate degeneration
21	Slightly soft	Left: 8.5 x 5.1 Right: 8.6 x 5.0	Mild degeneration	Azoospermia	Mild degeneration
22	Slightly soft	Left: 10.0 x 4.3 Right: 9.9 x 5.4	Moderate degeneration	Oligo- asthenozoospermia Mot[1]. 10 Conc[2]. 24 Abnorm[3]. 38	Moderate degeneration

Mot[1] = Sperm motility % Conc[2] = Sperm cell concentration ($x10^6$ /ml)

Abnorm[3] = Sperm abnormalities %

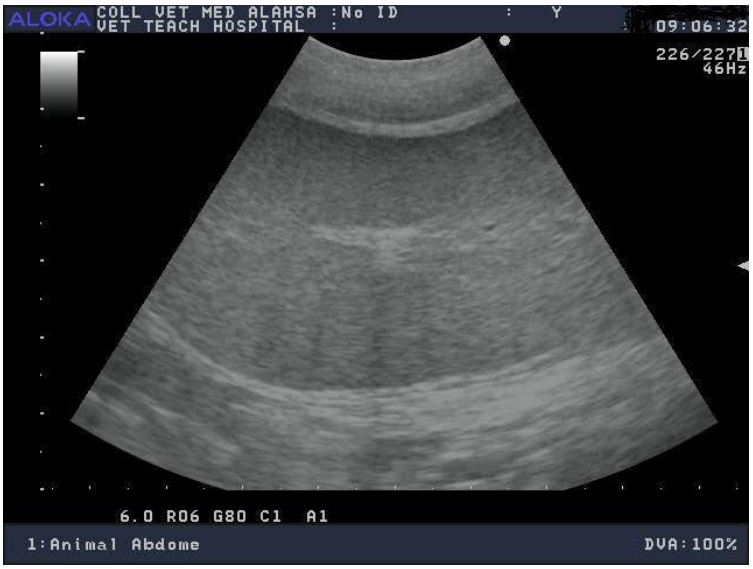

Figure 1: Ultrasonography of a testis in a control dromedary showing a homogenous peripheral area corresponding to the testicular parenchyma and a central more echogenic area corresponding to the fibrous rete testis.

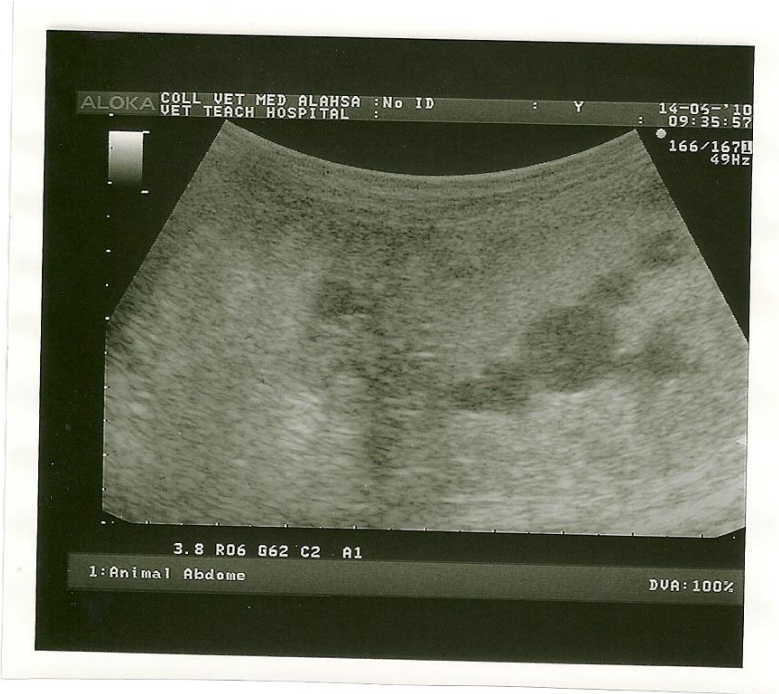

Figure 2: Testicular degeneration in a camel (hypo-echoic areas in the testicular parenchyma).

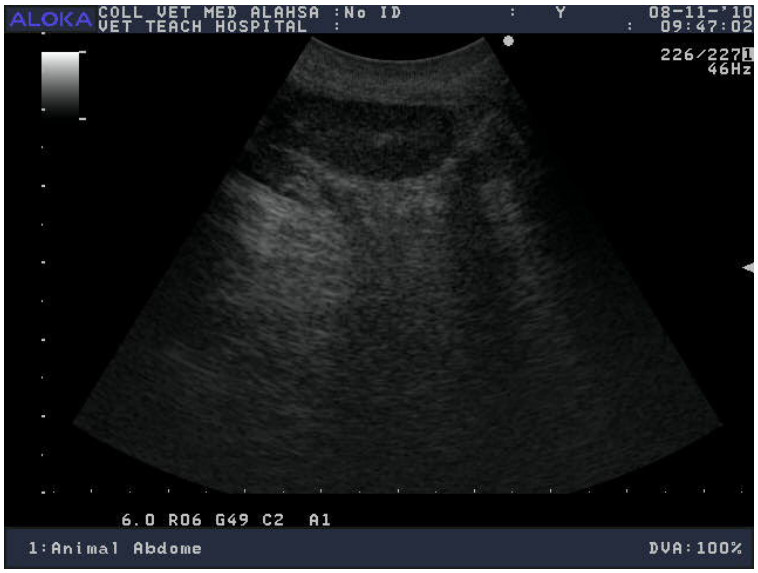

Figure 3: Testicular hypoplasia in a camel (hypo-echoic parenchyma)

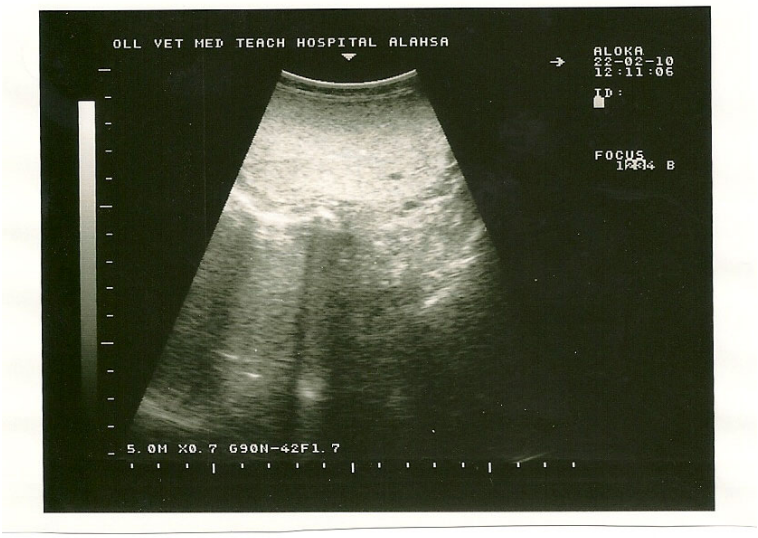

Figure 4: Testicular fibrosis in a camel (hyper-echoic parenchyma)

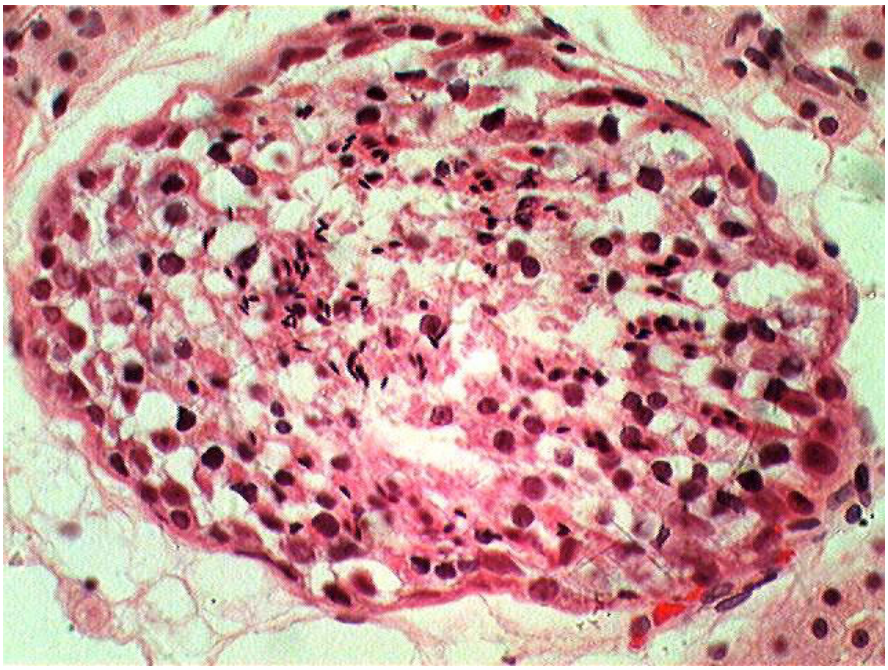

Figure 5: Testicular biopsy of a camel showing a normal seminiferous tubule that contains multiple cell layers of spermatocytic cells in addition to spermatids and surrounded by Leydig cells. H and E X=400.

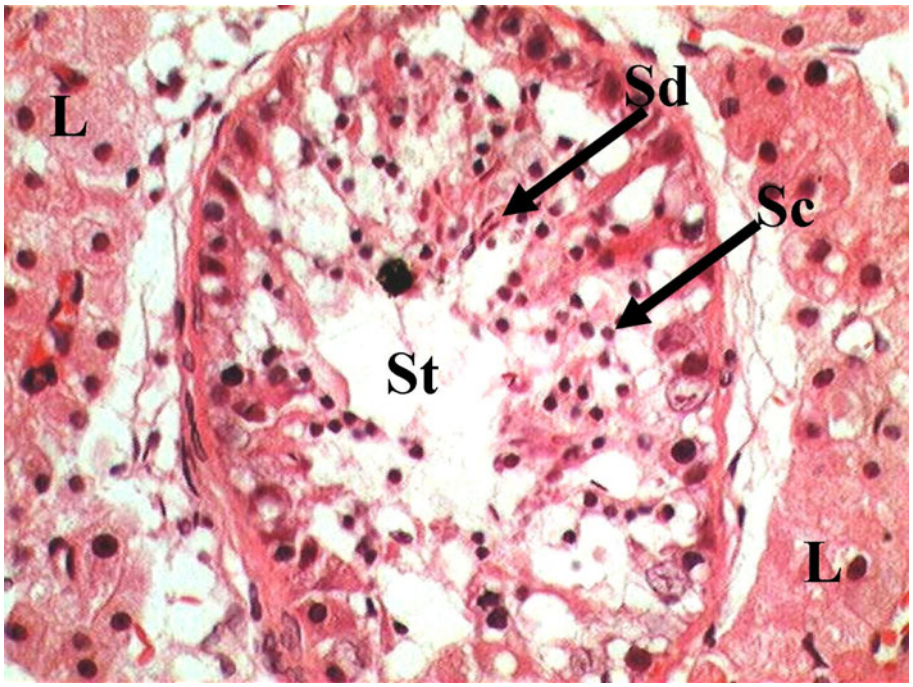

Figure 6: Testicular biopsy of a camel showing a mildly degenerated seminiferous tubule (St) that contains multiple cell layers of scattered spermatocytic cells with pyknotic nuclei (Sc), in addition to few numbers of spermatids (Sd) and surrounded by excess numbers of Leydig cells (L). H and E X=400.

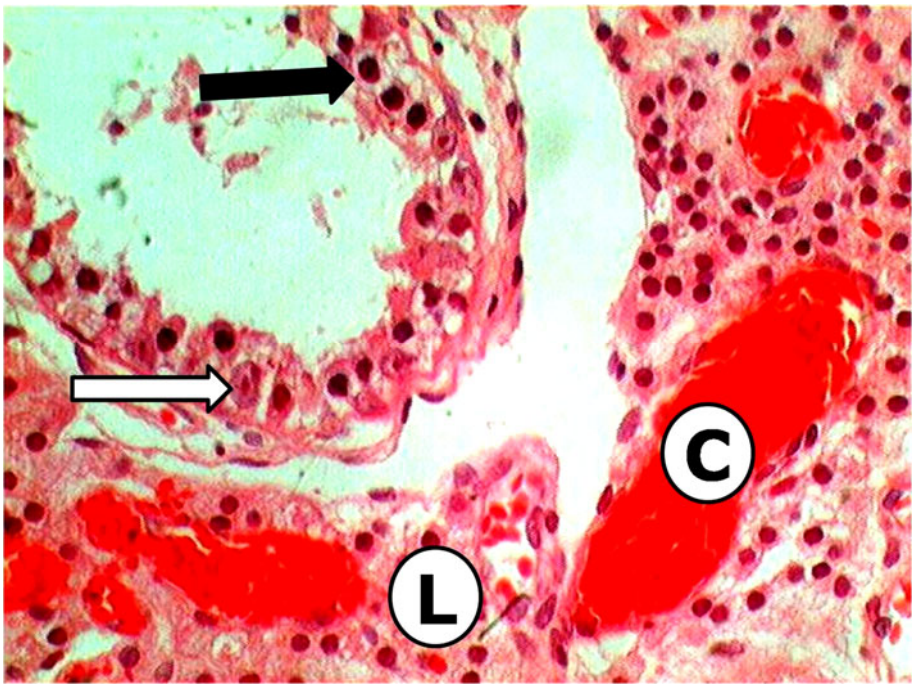

Figure 7: Testicular biopsy of a camel showing a severely degenerated seminiferous tubule lined by only one layer of Sertoli cells (white arrow) that contains primary spermatocytic cells with pyknotic nuclei (black arrow) and associated with hyperplastic interstitial Leydig cells (L) and congested capillaries (C). H and E X=400.

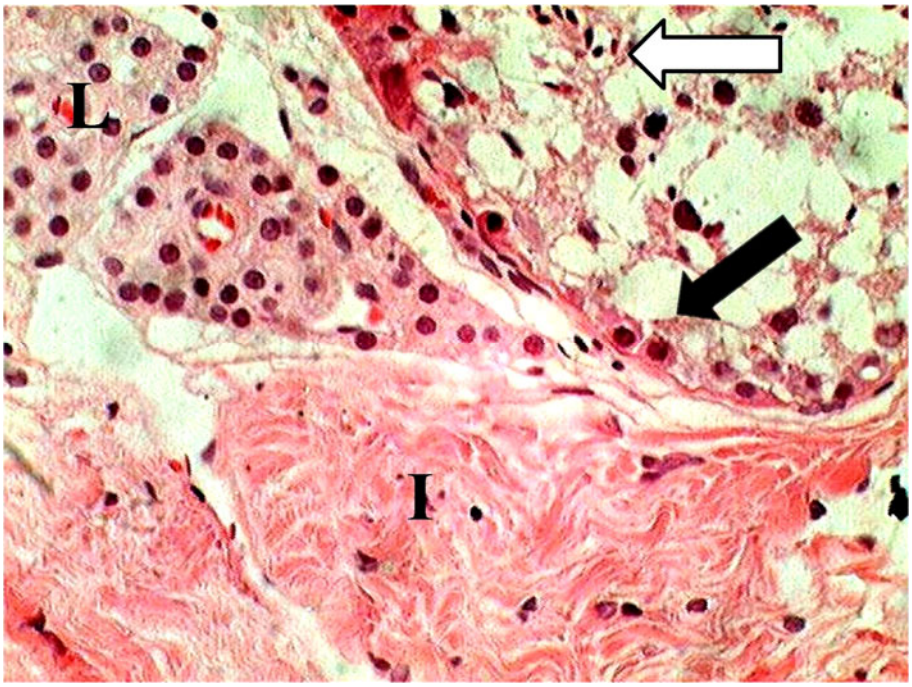

Figure 8: Testicular biopsy of a camel showing a mildly degenerated seminiferous tubule that contains vacuolated Sertoli cells (black arrow) and few spermatids (white arrow), in addition to an excess of interstitial Leydig cells (L) and interstitial fibrosis (I). H and E X=400.