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Pathological Evaluation of the Testes in the Casterated Dogs in Ahvaz District, Khuzestan Province

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ABSTRACT

Background: Despite extensive researches that have been done in the field of diseases of the reproductive system (testis and epididymis) in dogs; however, the actual amount of reproductive complications is not known exactly.

Objectives: The purpose of the present study was to evaluate the clinical, macroscopic and histopathological findings of testicular and epididymal tissues in dogs of Ahvaz district, Khuzestan province.

Methods: In the present study, seventy-seven casterated testes were evaluated in different animal ages (from seven months until twelve years). After macroscopic evaluation, the testicle and epididymis tissues were placed in 10% buffered formalin and processed.

Results: Sixty-eight cases of dogs had testicles with normal appearance. Four cases of dogs had cryptorchidism, which was determined during clinical examination. In five cases, the epididymis was swollen with dilated spaces. The microscopic study on the samples showed the presence of various lesions in 45 samples, equivalent to 58.44% of the samples. Four out of fourty five samples with lesions (8.89%) had unilateral cryptorchidism. Testicular degeneration was one of the most important cases that was observed in fourteen (31.11%). Twenty-two out of 45 samples with lesions (48.89%) had epididymitis. In these samples, severe hyperplasia of the mucous membrane was observed covering the epididymis. Epididymitis and degeneration were observed in twelve cases simultaneously. Two cases of chronic epididymitis were associated with bleeding. Another finding was the observation of intranuclear and intracytoplasmic inclusions (in twenty-nine cases). In twenty-one cases, epididymitis was also visible at the same time, which was determined by the presence of fibrosis around the ducts and mucous hyperplasia. Bleeding was seen in five cases (11.11%).

Conclusions: Considering the relatively high prevalence of testicular degeneration, epididymitis and the presence of inclusions in the studied dogs, castration in males and histopathological evaluations are very important for a definitive diagnose of complications; because they can lead to infertility, especially in purebred dogs that are kept in shelters (because of the chronic course of some infectious diseases).

Keywords: Cryptorchidism; Dog; Epididymis; Histopathology; Testicle

1. Introduction

Dogs with cryptorchidism are prone to develop Sertoli cell tumors and seminoma. Older dogs older than six years with cryptorchidism are more prone to tumor development. Castration plays an important role in controlling animal population and aggressive behavior in male dogs. Testicular tumors are caused by the uncontrolled growth of cells inside the testicular tissue. The origin of tumors is not precisely known, but a number of factors, including environmental factors, genetics and heredity play a role in this field. Certain breeds of dogs, such as Boxer, German Shepherd, Afghan Hound, Weimaraner, Shetland Sheepdog, Collie and Maltese, are more prone to developing testicular tumors (Sumner *et al*., 2021). Most dogs with testicular tumors do not have obvious clinical symptoms. Clinical symptoms may be limited to the presence of a mass (or masses) inside the affected testicle. Palpation of the scrotum with fingers may reveal nodular enlargement in the testicular tissue. Asymmetry in the size of the testicles and swelling of the scrotum are other signs. Sertoli cell tumor may produce estrogen and hyperestrogenism state is formed in the animal. In this situation, the male dog shows the behaviors of the female dog. Also, a series of symptoms such as enlargement of the mammary glands, hair loss and increased melanin pigment are seen in the skin in male dogs (Gould *et al*., 2007; Kudo *et al*., 2019).

Excessive secretion of estrogen can suppress the bone marrow, cause anemia (pale color of the mucous membranes) and lethargy in the animal. Behavioral changes have been reported such as urinating like a female dog and attracting attention to male dogs. In rare cases of malignant tumors, symptoms may be related to the involved organ where the tumor has metastasized (weight loss, anorexia, lethargy or vomiting). If the tumor has metastasized to the lymph nodes near the urinary tract or the prostate gland, symptoms include difficulty urinating or defecating. Testicular interstitial cell tumor metastasis to the skin has been reported also. Beside the neoplasias, the topics related to testicular tissue degeneration and epididymitis are very important; because if there is no control or treatment, it can lead to the infertility of the affected animal (Grieco *et al*., 2008; Nascimento *et al*., 2019).

In addition to the usual clinical findings, examination of the rectal area, blood test, urine test, radiography of the chest and if possible, ultrasound of the abdomen or CT- scan are necessary to ensure the involvement of lymph nodes or organs. If the lymph nodes are enlarged and appear abnormal on palpation, sampling is necessary to confirm the diagnosis. Immunohistochemical methods can be used in the diagnosis of neoplasms also. One of the most important definitive diagnosis methods is histopathologic evaluation on biopsy samples, using Hematoxylin and Eosin staining and in some cases by immunohistochemistry. Fine-needle aspiration (FNA) has also been reported for sampling. (Hohsteter *et al*., 2014; Sumner *et al*., 2021; Elahirad *et al.,* 2021). Since there had not been a comprehensive study on the pathology of testicular and epididymal tissues in dogs in Ahvaz, Khuzestan province; the purpose of this study was to evaluate the clinical, macroscopic and histopathological findings of testicular and epididymal tissue in dogs in this area. The results of the present study are useful and practical, especially for small animal clinicians.

2. Materials and Methods

To conduct this study, seventy seven samples of testicles of male dogs (both testicles from each dog) were taken which were referred by pet owners to castrate their dogs. Sampling from the population of dogs referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz (22 cases) and from the cases referred to the Veterinary Clinics of Ahvaz city (32 cases) and shelters (23 cases), in different ages (from 7 months to 12 years) and with an average age of 2.6±2.28 years. Breed of dogs were different (Native, Terrier, Shih Tzu, Spitz, Pomeranian, German shepherd, American Pit bull and Poodle). The number of small and large breeds were 21 and 56 cases respectively. This study was conducted in a course of 12 months (from January 1400 to January 1401). Ethical code was EE/1401.2.24.126557/scu.ac.ir.

Before castration, a detailed history of the studied dogs were taken such as age, breed, history of scrotal skin infections and administration of various drugs including corticosteroids. Clinical findings were taken and recorded by palpation. The condition of the skin and body hair, especially in terms of hyperpigmentation, such as hyperestrogenism syndrome was checked; as well as; the state of urine and faeces and, if necessary, examination of the rectal area through finger palpation.

The studied dogs were castrated under complete anesthesia and the testicles were removed together with the epididymis. Then, the macroscopic evaluation was done from the testicular and epididymal tissue in terms of the presence of cysts, their number and sizes, and other abnormal cases, then they were placed in 10% buffered formalin and stained H&E following tissue processing.

Statistical analysis

The current research is a descriptive analysis and the data are expressed as percentages. The statistical analyses were performed using SPSS (Version 22; SPSS Inc., IL, Chicago, USA).

3. Results

Macroscopic findings

In the present study, seventy seven testes and epididymis were examined in the studied dogs. Sixty-eight cases of dogs had testicles with normal appearance. Four cases of them had unilateral cryptorchidism, which were diagnosed during clinical examination. One of the testicles of the affected dogs was in the abdominal area and the other testicle in the scrotum. The affected testicle was visibly smaller and lighter than the testicle of the opposite side. In five cases, the epididymis was swollen with dilated spaces (Figure 1).

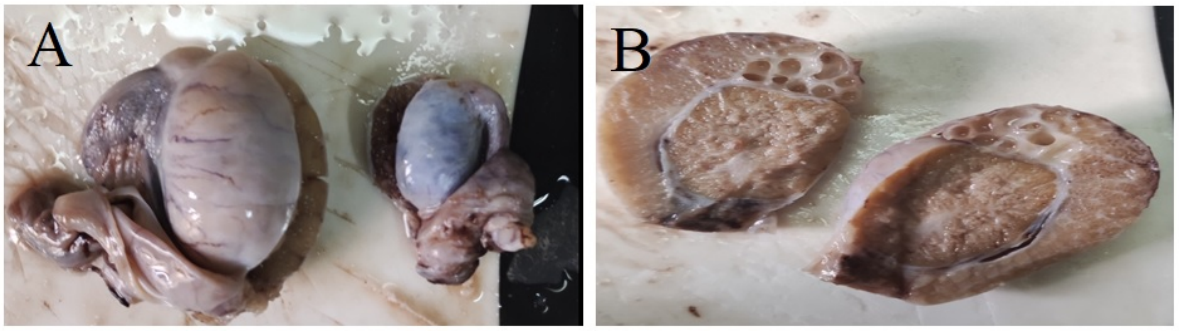

Figure 1: A: Cryptorchidism in the right testicle. Visible reduction in the size of the affected testicle compared to the opposite testicle. **B:** Testes and epididymis. The presence of multiple cysts inside the epididymis.

Microscopic findings

Immature testicles

In the microscopic examination, based on histological characteristics, the testicles were in three stages: immature, premature (maturing) and mature. In the testicular tissue of two dogs

of seven and eight months-old, the seminiferous tubules were covered by a row of cells, which were spermatogonia and nuclei of sertoli cells. Also, the twisted tubes of the epididymis were visible in a thin and small form (Figure 2).

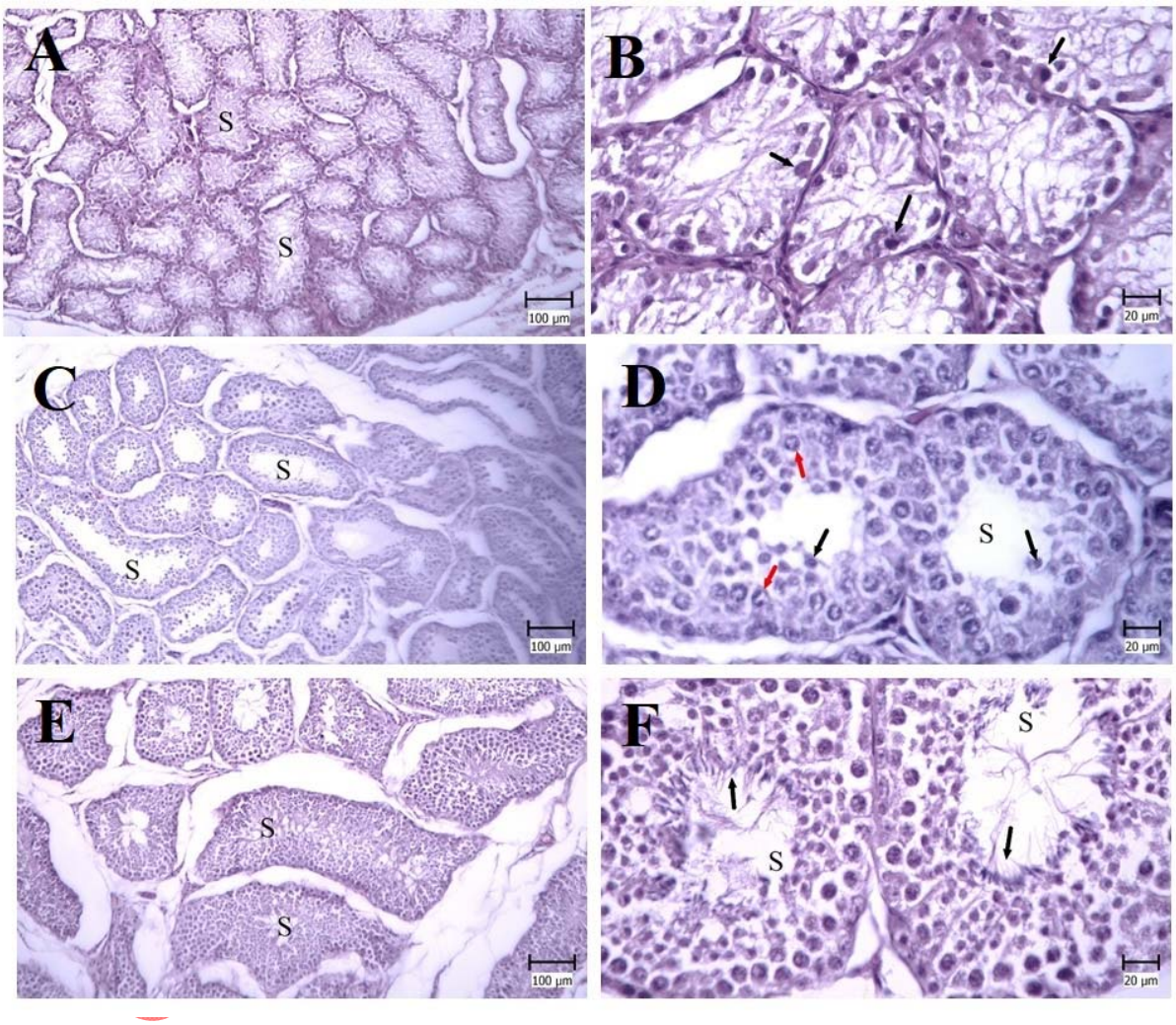

Figure 2: Immature testicle.

A: Presence of seminiferous tubules (S) covered by a row of cells (Hematoxylin and Eosin).

B: Part of image three with greater magnification. The presence of a single layer of seminiferous tubule mucus, spermatogonial cells (arrow) and sertoli cells (Hematoxylin and Eosin).

C: Seminiferous tubules (S) lined by the corresponding epithelium (Hematoxylin and Eosin).

D: Part of figure number seven with higher magnification. The presence of corresponding epithelial lining of the seminiferous tubules (S), which indicates the presence of primary spermatocyte cells (red arrow) and secondary spermatocyte (black arrow), (Hematoxylin and Eosin).

E: Seminiferous tubules (S) lined by the corresponding epithelium (Hematoxylin and Eosin).

F: Part of figure number eleven with greater magnification. Corresponding epithelial lining of seminiferous tubules (S) along with spermatozoa (arrow) (Hematoxylin and Eosin).

Premature testicles

The maturing testes were seen in the testicular tissue of eight dogs. Four cases were eight months-old and the other cases were nine, eleven, twelve and fourteen months-old. The mucus of the seminiferous tubules was multi-layered in these testicles. In these tubes, the beginning of the process of spermatogenesis was visible, and numerous cells of primary and secondary spermatocytes were visible, but spermatozoa had not formed yet. The ducts of the epididymis were also dilated and its mucus was visible in a semi-confluent form along with ciliated cells (Figure 3).

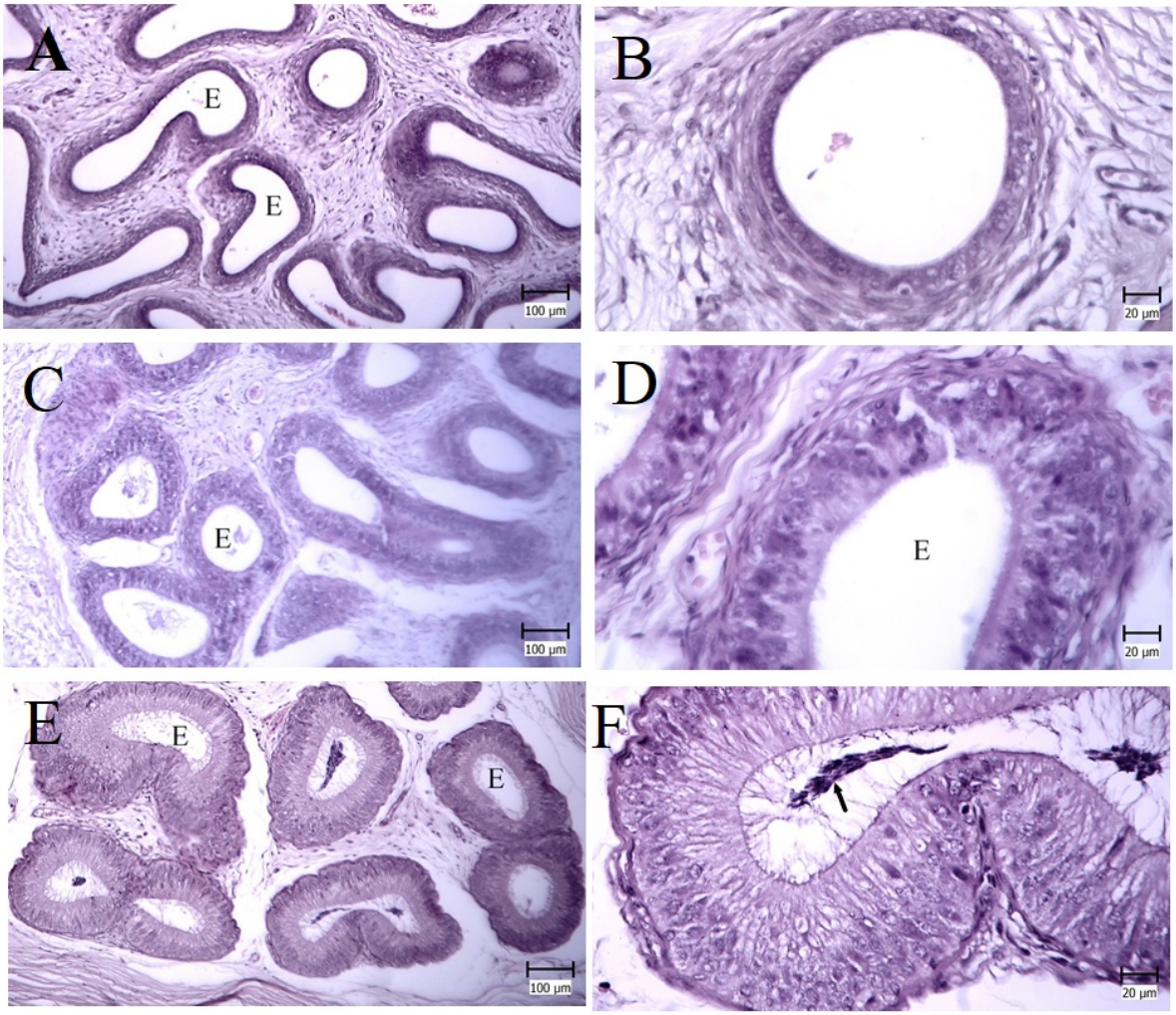

Figure 3: Premature testicle.

A: Epididymal tubes. The smallness of the tubes and their thin mucus (Hematoxylin and Eosin).

B: Part of figure number five with higher magnification. Thin mucus of the epididymal tube (Hematoxylin and Eosin).

C: Epididymal ducts (E) with matching pseudoepithelial tissue (Hematoxylin and Eosin).

D: Part of figure number nine with greater magnification. Epididymal ducts (E) with matching pseudo-epithelial tissue (Hematoxylin and Eosin).

E: Epididymal ducts (E) with pseudo-matched epithelial tissue, accumulations of spermatozoa cells inside them (Hematoxylin and Eosin).

F: Part of figure number thirteen with greater magnification. Epididymal duct with pseudo-matched epithelial tissue and a large number of spermatozoa inside them (Hematoxylin and Eosin).

Mature testicles

In sixty-seven cases of the testicles, different degrees of spermatogenesis were observed in the seminiferous tubules, which indicated the occurrence of puberty. The dogs were seven months-old (two cases), eight months-old (two cases), nine months-old (one case) respectively and the rest were one year-old or older. In these testicles, the process of spermatogenesis was fully visible and numerous spermatocyte cells were formed along with spermatozoa. The mucus of the epididymal ducts was also pseudo-confluent and spermatozoa were visible inside them.

Pathologic leasions

The microscopic evaluation of the samples showed the presence of various lesions in 45 samples, equivalent to 58.44% of the testicles (Table 1).

Table 1: The number of pathological lesions and their percentage among forty five testicles with lesions in dogs in Ahvaz district

Cryptorchidism	Degeneration	Epididymitis	Inclusion	Hemorrhage
Four (8.89%)	Fourteen (31.11%)	Twenty two (45.89 %)	Twenty two (45.89 %)	Five (11.11%)

Cryptorchidism

Out of 45 samples with lesions, four cases (8.89%) had unilateral cryptorchidism. In the microscopic evaluation of cryptorchidism samples, specific lesions were observed, which follow. The covering tissue of the seminiferous tubules in the mentioned testicles consisted only of sertoli cells, which had a red cytoplasm towards the center of the tube and the nucleus was present at the base of the cell. The affected dogs were in the age range of seven months until two years (Mean± SD: 15.25±7.36 months).

In the observations of testis in front of the seminiferous tubules, the complete cessation was also observed of spermatogenesis, and no germinal cells were seen inside the tubules, and the only present cells were sertoli cells. Epididymal ducts were small and covered by a thin mucus. Germinal cells were not seen inside the tubes. In one of the cases, numerous erythrocytes were observed in the interstitial tissue. In three dogs, spermatogenesis was observed in the opposite testis and spermatozoa cells were visible in the epididymal tubes also; however, in one dog, the testicle on the opposite side, underwent degeneration, and spermatozoa was not observed in the seminiferous tubules and epididymis (Figure 4).

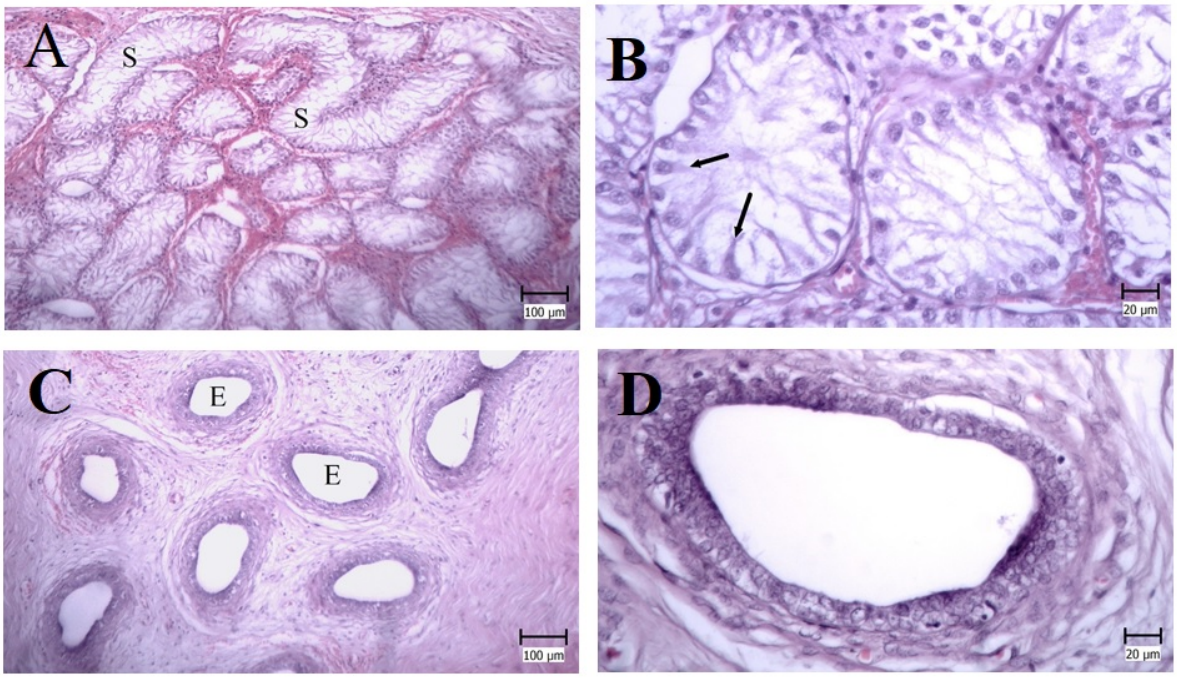

Figure 4: Cryptorchidism.

A: Note the presence of seminiferous tubules (S) furnished with a row of cells (Hematoxylin and Eosin).

B: Part of figure number fifteen with greater magnification. Seminiferous tubules can be seen lined with a row of sertoli cells (arrow). Pay attention to the location of the cells' nuclei and their pink and elongated cytoplasm (Hematoxylin and Eosin).

C: Epididymal tubes (E). Pay attention to the smallness of the tubes and their thin mucus (Epididymis) (Hematoxylin and Eosin).

D: Epididymal tubes (E). Part of figure number seventeen with higher magnification. Pay attention to the smallness of the tubes and their thin mucus (Epididymis) (Hematoxylin and Eosin).

Testicular degeneration

The frequency of testicular degeneration was fourteen testicles out of 77 (18.18%) and 31.11% out of 45 testicles in the examined samples. In these samples, the cessation of spermatogenesis could be seen in the form of absence stages of spermatogenesis in the covering tissue of the tubes. Also, the cytoplasm of sertoli cells was vacuolated, and inside some tubes were visible giant spermatid cells. These cells had many nuclei with a cytoplasm. Local fibrosis of the interstitial tissue was also seen along with the formation of cysts and thickening of the basal membrane in some samples of tubes (Figure 5).

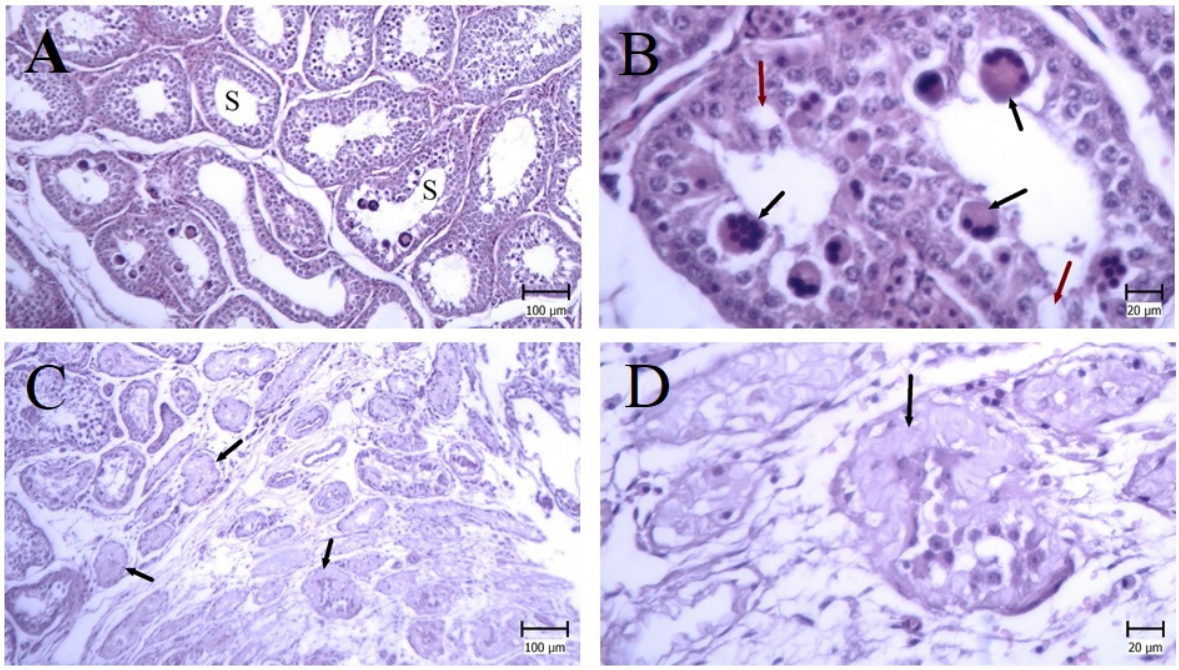

Figure 5: Testicular degeneration.

A: Absence of spermatogenesis in the seminiferous tubules (S) and thinning of the lining of tubules (Hematoxylin and Eosin).

B: Absence of spermatogenesis in the seminiferous tubules, thinning of the mucus covering these tubes, numerous vacuoles inside the mucus (red arrow) and giant cells (black arrow) (Hematoxylin and Eosin).

C: An increase in the thickness of the basal membrane of the seminiferous tubules and hyalinization (arrow) (Hematoxylin and Eosin).

D: Part of figure number twenty one with higher magnification. An increase in the thickness of the basal membrane of the seminiferous tubules and hyalinization (arrow) (Hematoxylin and Eosin).

Epididymitis

Out of 45 samples with lesions, twenty-two cases (48.89%) had epididymitis. The frequency of the total lesion was 28.57% in the samples. Dogs with epididymitis were of different ages. Severe hyperplasia of the mucous membrane covering the epididymis was observed in these samples. Proliferated cells could be seen in the form of curtains protruding from the surface of the mucosa, and in some cases, cysts were formed inside the mucosa. Also, accumulations of mononuclear inflammatory cells and severe fibrosis were seen around the epididymal tubes. Regarding fibrosis, there were several layers of connective tissue around the tubes, and in some cases, the thickness of this layer was very high. In twelve cases, epididymitis and degeneration were observed simultaneously. In two cases of chronic epididymitis, it was simultaneously accompanied by bleeding, which was determined by the presence of a large number of erythrocytes inside the epididymal and seminiferous tubes, and between the seminiferous tubules unilaterally (Figure 6).

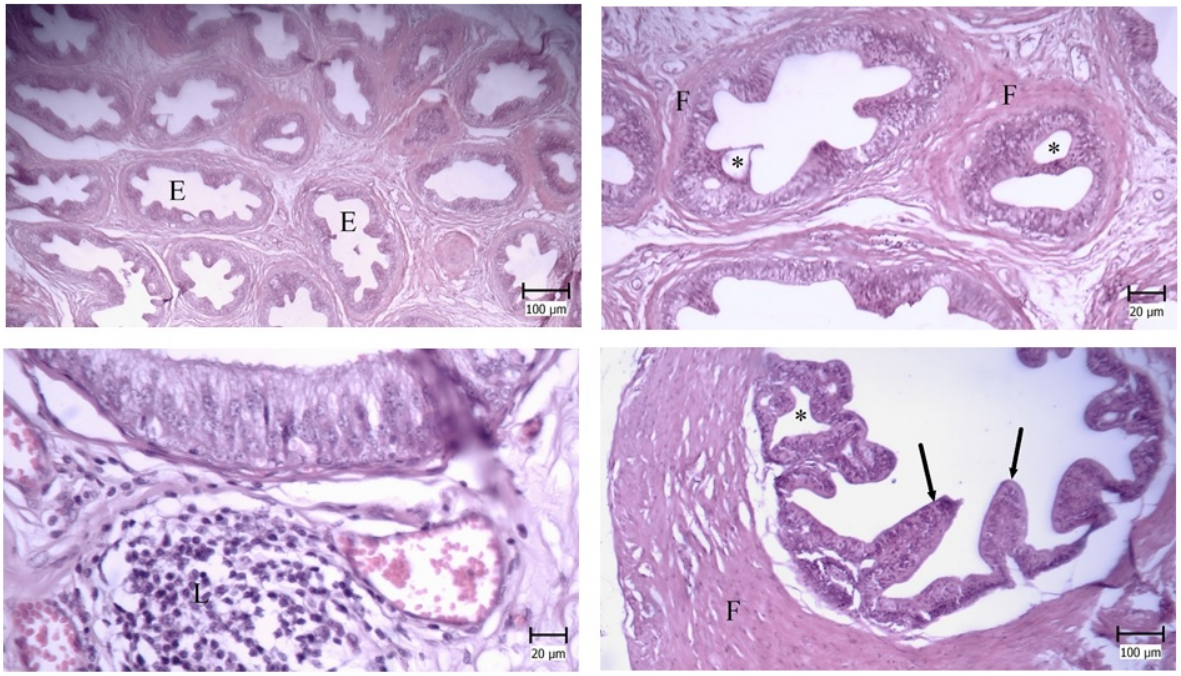

Figure 6: Epididymitis.

A: Pay attention to the presence of multiple membranes in the mucus of the epididymal tubes (E) (Hematoxylin and Eosin).

B: Pay attention to the presence of numerous cysts (asterisks) in the epididymal mucosa, which were caused by the hyperplasia of the cells covering the tubes. Also, several rows of connective tissue (F) could be seen around the epididymal tubes (Hematoxylin and Eosin).

C: Pay attention to the accumulation of mononuclear inflammatory cells (L) in the interstitial tissue of the epididymal tubes (Hematoxylin and Eosin).

D: Pay attention to the presence of multiple membranes formed (arrow) in the epididymal mucosa, which was caused by the hyperplasia of the cells covering the tubes. Also, a thick layer of connective tissue (F) could be seen around the epididymal tubes (Hematoxylin and Eosin).

Another finding that was seen in this research, was the observation of intranuclear and cytoplasmic inclusions bodies. These inclusions were associated with chromatin bordering in the nucleus and vacuole formation in the cytoplasm. They were eosinophilic. This microscopic finding was diagnosed in twenty-nine dogs. In twenty-one cases, epididymitis was also observed at the same time, which was determined by the presence of fibrosis around the ducts and mucous hyperplasia. In two cases of dogs that were less than one year old, chronic epididymitis was also observed, in addition to intranuclear and intracytoplasmic inclusions. In twelve cases of testes with degeneration, nuclear and cytoplasmic inclusions were observed also. In one of the five year-old dogs, the amount of inclusions was very high, inside the epididymal cells and the seminiferous tubules cells (primary and secondary spermatocytes). In this sample, the cessation of spermatogenesis was also evident.

In a four year-old dog, the presence of inclusion was accompanied by severe destruction of epididymal cells. In this sample, lymphocytic vasculitis was observed, which was accompanied by the presence of many lymphocytes in the arteriole wall and its surroundings. Also, intranuclear and intracytoplasmic inclusions were observed in the cells inside the vessel wall (Figure 7).

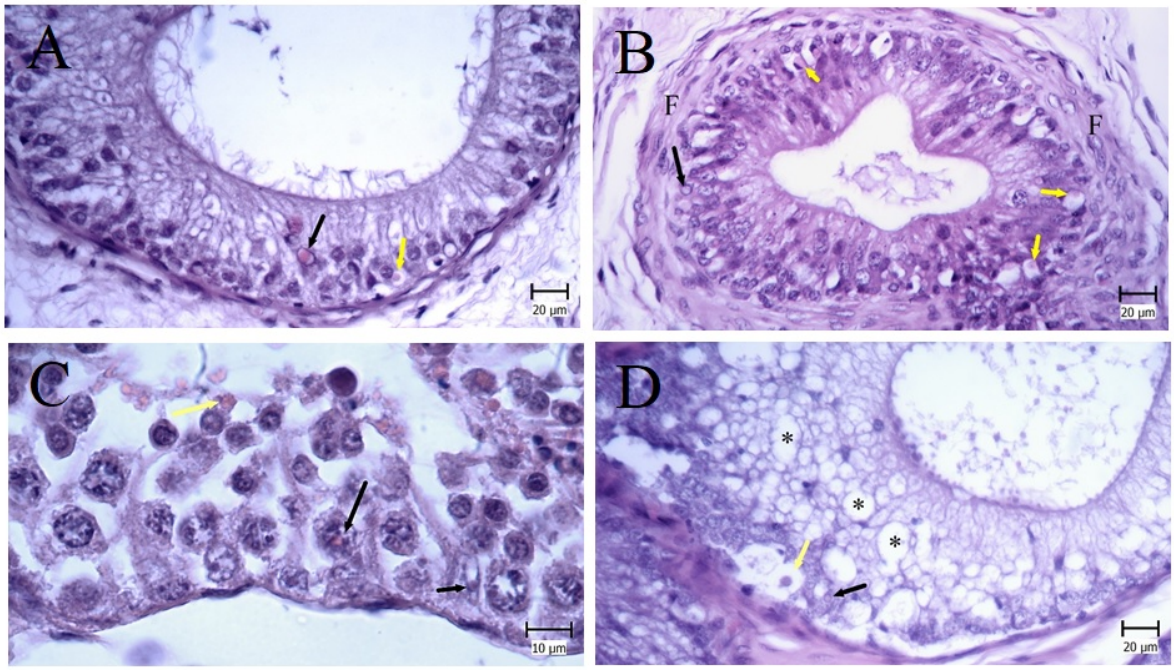

Figure 7: Intranuclear and cytoplasmic inclusion bodies.

A: Eosinophilic inclusion bodies inside the nucleus (black arrow) and the cytoplasm (yellow arrow) in the cells covering the mucous membrane of the epididymal ducts (Hematoxylin and Eosin).

B: Part of figure number 27 with higher magnification. Intranuclear (black arrow) and intracytoplasmic (yellow arrow) eosinophilic inclusions are seen in the cells lining the epididymal ducts with fibrosis (F) around the epididymal ducts (Hematoxylin and Eosin).

C: Eosinophilic inclusions are seen in the nucleus (black arrow) and cytoplasm (yellow arrow) in primary and secondary spermatocyte cells (Hematoxylin and Eosin).

D: Eosinophilic inclusions are seen in the nucleus (black arrow) and in the cytoplasm (yellow arrow) in the cells lining the epididymal ducts. Pay attention to the presence of numerous and large vacuoles in

the mucus along with the destruction of the cells covering the epididymal mucus (Hematoxylin and Eosin).

Hemorrhage

Hemorrhage was seen in five cases (11.11%) out of 45 testicles with lesions. A large number of erythrocytes were observed between and inside the seminiferous tubules.

4. Discussion

In the present study, testicular tissue damage and epididymis were investigated on seventy-seven dogs. Testicular tissue degeneration was one of the most important cases, which was observed in 18.1% of cases. In these samples, the cessation of spermatogenesis was evident in the form of the absence of spermatogenesis stages in the covering tissue of the tubes, as well as, the cytoplasm of sertoli cells was vacuolated and inside some tubes were visible giant spermatid cells.

Various factors can affect testicular hemodynamics in animals, such as environmental (thermal and seasonal effects) and physiological factors (species, breed, age, size, body weight, and maturity) (Samir *et al*., 2022). Since the infectious agents such as *Canine Distemper Virus* (CDV), mycoplasmas, blastomycosis, *Brucella canis*, *Leishmania infantum*, rickettsial agents such as *Ehrlichia* and *Rocky Mountain spotted fever* are involved in creating chronic orchitis and epididymitis, attention should be paid to important factors during examination. The blood-testis barrier plays an important role in the immune response to spermatoside antigens. When this barrier is damaged, orchitis occurs, which is caused by the invasion of lymphocytes into the testicular tissue, and sometimes causes infertility in animals. It should be noted that in some animals with diseases of the male reproductive system (testicles), no clinical symptoms may be seen by the owner or even the veterinarian, and the only reason for referral is the infertility of the

animal (Ettinger & Feldman, 2015; Manna *et al*., 2012; Egloff *et al*., 2018; Groch *et al*., 2020; Jameie et al., 2020; Camargo-Castaneda *et al*., 2021).

Temperature changes and heat stress are another factors that cause testicular degeneration. In a study on 138 dogs that lived in tropical areas were sampled and based on the microscopic results, testicular degeneration was the most common tissue damage (64.8%) (Ortega-Pacheco *et al*., 2006). In order to investigate the effects of geographical regions on testicular morphology, sampling was done from dogs living in the UK, Finland, and Denmark. According to their reports, testicular degeneration was significantly higher in dogs living in Finland, which indicates the influence of geography and environmental factors on testicular function. Testicular degeneration may be caused by factors such as seasonal performance, light effects, environment, toxins, ageing, and infectious agents (Sumner *et al*., 2021). The present study was performed in Ahvaz region (South-West of Iran) that which is located in a climate of warm and wet. The humidity level reaches its peak, in the summer season.

Several studies have shown that increasing the temperature of the environment can interfere with the temperature regulation mechanism of the testicles, disturb the evaporative heat loss from the surface of the scrotum and lead to an increase in the temperature inside the testicle, which increases the heat The testicle increases the metabolism of the testicle and the need for oxygen, resulting in hypoxia and the formation of reactive oxygen species, which has a significant effect on the reduction of sperm production and damage to the testicular tissue (Adwell *et al*., 2018). In various studies, it has been shown that there are chemicals in dog testicle tissue and seminal plasma in concentrations that can inhibit sperm movement in laboratory conditions (Lea *et al*., 2016; Sumner *et al*., 2020). In the present study, another important finding that was detected was intranuclear and intracytoplasmic inclusions in the mucous cells covering the epididymal tubules. These inclusions, according to the color and type, are caused by paramyxovirus infection.

Aging is one of the most common causes of testicular degeneration. In this research, nine out of 14 dogs with degeneration were over five years old. Aging in dogs is associated with a

decline in epididymal sperm quality, and an age-related increase in the incidence of poor epididymal sperm quality may cause infertility, especially in older dogs (Bhanmeechao *et al*., 2018a). In a study on seventy-eight dogs, affected to mammary gland tumors, intraductal papillary carcinoma and complex carcinoma, had the highest incidence. The researchers announced that the risk of malignant tumors increased in intact purbred females as the individual ages (Golchin *et al.,* 2023). Tumors are generally formed in old ages. Chondrosarcoma was recognized in a nine-year-old cat (Shokrpoor *et al.,* 2021). In another study, age-related changes with interstitial fibrosis and degeneration of testicular germ cells on fifty five dogs showed a direct relationship between the degeneration of spermatogenic tubes and the reduction of spermatogenesis and interstitial fibrosis in old dogs (Bhanmeechao *et al*., 2018b). In a study, testicular degeneration was the most common microscopic injury. According to researchers, testicular degeneration reduces the attention of healthy sperm, which can have a living effect on sperm count and reproductive performance of the animal (Camara *et al*., 2014). In another study, that was conducted on 100 male dogs, the most severe lesions were degeneration of the seminiferous tubules along with hyalinization of the basal membrane of these tubules and fibrosis (Latifi *et al*., 2021). The existence of viral inclusion within the epididymis has also been reported by other researchers (Foster, 2016). In a study conducted on the fishing otter infected with CDV, intranuclear and cytoplasmic inclusions were reported in the epithelium of the bladder and epididymis (Keller *et al*., 2012).

Considering the many causes of intranuclear and intracytoplasmic inclusions, including CDV, it is emphasized that purebred dogs that are intended to be used for breeding should not be kept in kennels and shelters. In the present study, 28.57% of the samples had epididymitis. In these samples, severe hyperplasia of the mucous membrane covering the epididymis was observed. Proliferated cells could be seen in the form of curtains protruding from the surface of the mucosa, and in some cases, cysts were formed inside the mucosa. Epididymitis and degeneration were observed simultaneously in twelve of the samples. Two cases of chronic epididymitis were associated with bleeding. Another finding was the observation of intranuclear and intracytoplasmic inclusions, which were seen in 29 cases. In 21 cases, epididymitis was also

visible at the same time, which was determined by the presence of fibrosis around the ducts and mucous hyperplasia. Bleeding was seen in 6.4% of the samples. The simultaneous occurrence of epididymitis and intranuclear and intracytoplasmic inclusions in a large number of dogs can indicate that epididymitis occurs as a result of the damage caused by virus infection and the destruction of duct lining cells. to give Mucous hyperplasia and fibrosis around the ducts can be a compensatory response against the damage caused by the virus. Another histopathological injury that was diagnosed simultaneously with inclusions was testicular degeneration. This damage may also have occurred as a result of virus contamination (mainly CDV) and the virus has destroyed spermatocytes or sertoli cells, which stops spermatogenesis (Ettinger & Feldman, 2015).

In another study with the aim of determining the feasibility of laparoscopic cryptorchidism in the treatment of cases of simple cryptorchidism and neoplastic testicles in 15 dogs, unilateral cryptorchidism was found in twelve cases, of which nine had right sided cryptorchidism (Lew *et al*., 2005). In a study that surgically induced cryptorchidism lesions in dog testicles, histopathological data showed that the testicular spermatogenic tubes and epididymis contained fewer germ cells and severe atrophy (Jhun *et al*., 2018).

In the present study, the microscopic evaluation of the samples showed the presence of various lesions in 58.44% of the cases. In 89.8% of the affected dogs, they had unilateral scrotum. The covering tissue of the seminiferous tubules, in the affected testes, consisted of only sertoli cells, which had a red cytoplasm towards the center of the tube and the nucleus was present at the base of the cell. In the present study, 68 cases of dogs had testicles with normal appearance and four cases of dogs were suffering from cryptorchidism. In five cases, the epididymis was swollen and with dilated spaces. It was investigated the clinical and histopathological aspects of cryptorchidism in dogs and cats. The researchers' results showed that among 98 dog and cat, 11 (11.22%) had cryptorchidism. In their study, most dogs of the Maltese breed, Anatolian Shepherd, Terrier, Persian, Turkish Angora, and domestic short-haired breeds were more affected. The location of hidden testicles varied from the inguinal region to the intra-abdominal region, and they were unilateral or bilateral (Othman *et al*., 2022).

In the present study, in the microscopic examination, based on the histological characteristics, the testes were seen in three stages: immature, maturing and mature. In the immature stage, the testicular tissue of two seven- and eight-month-old dogs, the seminiferous tubules were covered by a row of cells, which were spermatogonia and nuclei of sertoli cells. The maturing testes was seen in the testicular tissue of eight collared dogs. In the maturation stage, different degrees of spermatogenesis were observed in the seminiferous tubules (in sixty-seven cases), which indicated the occurrence of puberty. In another study on 80 beagle dogs, it was showed that hypospermatogenesis inside the seminiferous tubules was observed in 75% of 6-7 month old dogs; While this ratio was 10% in dogs older than 11 months. Hypoplasia or atrophy of the seminiferous tubules was seen in 25-40 of the dogs under 12 months; while this ratio decreased to 14 to 17 percent in dogs between 12- 36 months. The researchers announced that dogs at least 10 months old can be used for microscopic evaluation of the testicles; of course, in order to minimize random findings, it is better for dogs to be over 12 months old (Goedken *et al*., 2008).

The most common testicular tumors are included seminoma, Leydig cell tumor, sertoli cell tumor and mixed tumors. The age of dogs with testicular tumors is between 2 and 19 years. Testicular neoplasias are usually observed in old dogs (Dzimira *et al*., 2017). A case of cryptorchidism was described within the left inguinal canal in a collared dog. The testicle affected by cryptorchidism had a Leydig cell tumor and at the same time, seminoma was observed in the healthy testicle (Bigham *et al*., 2009). Some other researchers, aimed at investigating testicular precancerous lesions such as immaturity and atrophy and comparing them with immunohistochemistry in the testicles of twenty-six male dogs of different ages and breeds suffering from cryptorchidism, and showed the amount of atrophy and degenerative changes in affected testicles (Pecile *et al*., 2021).

Cytological evaluation of the testicular tissue plays a role in the diagnosis of infertility in dogs, but there are relatively few reports on the normal cytological features of the testis, and this is in contrast to human medicine, where the normal cytology of the testis is fully described. Santos *et al*. (2010) evaluated the cytology of normal testicular tissue in 6 male dogs without

testicular pathology and aged 3 to 8 years. The samples showed low blood contamination and a heterogeneous population of isolated or packed cells with high pleomorphism. The results of their study showed that the obtained cells are similar to men's cells, although there are some characteristics such as the much larger size of sertoli cells and the very vacuolated appearance of Leydig cells in dogs (Santos *et al*., 2010).

In conclusion, considering the relatively high prevalence of testicular degeneration, epididymitis and the presence of inclusions in the studied dogs, castration in males and histopathological evaluations are very important for a definitive diagnose of complications; because they can lead to infertility, especially in purebred dogs that are kept in shelters and are usually exposed to pathogens (including *Canine distemper* and *Brucella canis*).

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References

Adwell, C.B., Brito, L.F., Oba, E., Wilde, R.E., Rizzoto, G., & Thundathil, J.C., *et al.* (2018). Arterial blood flow is the main source of testicular heat in bulls and higher ambient temperatures significantly increase testicular blood flow. *Theriogenology*, *116*(1), 12-16. [DOI:10.1016/j.theriogenology.2018.04.022]

Bhanmeechao, C., Srisuwatanasagul, S., Prapaiwan, N., & Ponglowhapan, S. (2018[a]). Reproductive aging in male dogs: the epididymal sperm defects and expression of androgen receptor in reproductive tissues. *Theriogenology*, *108*(1), 74-80. [DOI:10.1016/j.theriogenology.2017.11.011] PMID: 29197295.

Bhanmeechao, C., Srisuwatanasagul, S., & Ponglowhapan, S. (2018[b]). Age-related changes in interstitial fibrosis and germ cell degeneration of the canine testis. *Reproduction Domestic Animals*, 53(s3), 37-43. [DOI:10.1111/rda.13354 PMID: 30474336]

Bigham, A.S., Karimi, I., Shadkhast, M., Imani, H., & Khaghani, A. (2009). Left intrainguinal canal cryptorchidism concurrent with two pathological findings in a mixed-breed aged dog. *Comparative Clinical Pathology*, *18*, 463–466. [DOI:10.1007/S00580-009-0820-0]

Camara, L.B.R.M., Camara, D.R., Maiorino, F.C., Silva Júnior, V.A., & Guerra, M.M.P. (2014). Canine testicular disorders and their influence on sperm morphology. *Animal Reproduction*, *11*(1), 32-36.

Camargo-Castaneda, A.M., Stranahan, L.W., Edwards, J.F., Garcia-Gonzalez, D.G., Roa, L., & Avila-Granados, L.M. (2012). Characterization of epididymal and testicular histologic lesions and use of immunohistochemistry and PCR on formalin-fixed tissues to detect Brucella canis in male dogs. *Journal of Veterinary Diagnostic Investigation*, *33*(2), 352-356. [DOI:10.1177/1040638720986883 PMID: 33468036]

Egloff, S., Schneeberger, M., Gobeli, S., Krudewig, C., Schmitt, S., & Reichler, M. (2018). Peterhans S. *Brucella canis* infection in a young dog with epididymitis and orchitis. *Schweizer Archiv fur Tierheilkunde*, *160*(12), 743-748. [DOI:10.17236/sat00190 PMID: 30516477]

Elahirad, E., Sasani, F., Gharagozlou, M.J., Khosravi, A., & Khanbarari, F. (2021). Evaluation of cytokeratin-7 expression in different dammary gland neoplasms. *Iranian Journal of Veterinary Medicine*, *15*(1), 56-66. [DOI:10.22059/ 295956.1005052]

Ettinger, S.J., & Feldman, E.C. (2015). *Textbook of Veterinary Internal Medicine, Diseases of the Dog and Cat*. Vol. 2. 7th ed. p. 1646-1690. WB. Saunders Elsevier, St. Louis, issouri. USA. ISBN 1-4160-0110-7.

Foster, R.A. (2016). Male genital system. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals, 3*, 465-510. [DOI:10.1016/B978-0-7020-5319-1.00016-5]

Goedken, M.J., Kerlin, R.L., & Morton D. (2008). Spontaneous and age-related testicular findings in Beagle dogs. *Toxicologic Pathology*, *36*(3), 465-471. [DOI:10.1177/ 0192623308315670]

Golchin, D., Sasani, F., Pedram, M.S., & Khaki, Z. (2023). Clinicopathological diversity and epidemiological aspects of Canine and Feline mammary gland tumors in Tehran: A survey (2020-2022). *Iranian Journal of Veterinary Medicine*, *17*(3), 231-242. [DOI:10.1177/0192623308315670]

Gould, M.K., Fletcher, J., Iannettoni, M.D., Lynch, W.R., Midthun, D.E., & Naidich, D.P. (2007). Evaluation of patients with pulmonary nodules: when is it lung cancer? ACCP evidence-based clinical practice guidelines. *Chest*, *132*(3), 108-130. [DOI:10.1378/chest.07-1353]

Grieco, V., Riccardi, E., Greppi, G.F., Teruzzi, F., Lermano, V., & Finazzi, M. (2008). Canine testicular tumours: A study on 232 Dogs. *Journal of Comparative Pathology*, *138*(2-3), 86-89. [DOI:10.1016/j.jcpa.2007.11.002]

Groch, K.R., Díaz-Delgado, J., Santos-Neto, E.B., Ikeda, J.M., Carvalho, R.R., & Oliveira, R.B. (2020). The pathology of cetacean morbillivirus infection and comorbidities in Guiana Dolphins during an unusual mortality event (Brazil, 2017–2018). *Veterinary Pathology*, *57*(6), 845-857. [DOI:10.1177/0300985820954550 PMID: 32964811]

Hohsteter, M., Artukovic, B., Severin, K., Kurilj, A.G., Beck, A., & Sostaric-Zuckermann, I.C. (2014). Grabarevic Z. Canine testicular tumors: two types of seminomas can be differentiated by immunohistochemistry. *BMC Veterinary Research*, *10*(169), 1-9. [DOI: 10.1186/s12917-014-0169-8 PMID: 25096628]

Jameie, A., Dalimi, M., & Pirestani, M. (2020). Mohebali. Detection of *Leishmania infantum* infection in reservoir dogs using a multiepitope recombinant protein (PQ_{10}) *Archives of Razi Institute journal*. *75*(3), 327-338. [DOI:10.22092/ARI.2019.126524.1346]

Jhun, H., Park, H.J., Lee, R., Song, H., Hur, T.Y., & Lee. S. (2018). Germ cell-specific apoptosis by extracellular clusterin in cryptorchid dog testes. *Animal Reproduction Sciences*, *193*(1), 158-164. [DOI:10.1016/j.anireprosci.2018.04.064]

Keller, S.M., Gabriel. M., Terio. K.A., Dubovi. E.J., VanWormer. E., & Sweitzer, R. (2012). Canine distemper in an isolated population of fishers (Martes pennanti) from California. *Journal of wildlife diseases*, *48*(4), 1035-1041. [DOI:10.7589/2011-12-350]

Kudo, T., Kamiie, J., Aihara, N., Doi, M., Sumi, A., & Omachi, T. (2019). Malignant Leydig cell tumor in dogs: two cases and a review of the literature. *Journal of Veterinary Diagnostic Investigation*, *31*(4), 557-561. [DOI:10.1177/1040638719854791]

Latifi, F., Baruti-Gafurri, Z., Haziri, I., & Manxhuka-Kerliu, S. (2021). Hormonal and histopathological fertility assessments of male dogs in Kosovo. *Indian Journal of Animal Research*, *55*(4), 433-438. [DOI:10.18805/ijar.B-1267]

Lea, R.G., Byers, A.S., Sumner, R.N., Rhind, S.M., Zhang, Z., & Freeman, S.L. (2016). Environmental chemicals impact dog semen quality in vitro and may be associated with a temporal decline in sperm motility and increased cryptorchidism. *Scientific Reports*, *6*(1), 1-4. [DOI:10.1038/srep31281]

Lew, M., Jałynski, M., & Brzeski, W. (2005). Laparoscopic removal of gastric foreign bodies in dogs- comparison of manual suturing and stapling viscerosynthesis. *Pol Journal of Veterinary Science*, *8*(2), 147-153. PMID: 15989135.

Manna, L., Paciello, O., Morte, R.D., & Gravino, A.E. (2012). Detection of Leishmania parasites in the testis of a dog affected by orchitis: case report. *Parasites & Vectors*, *5*, 216. [DOI:creativecommons.org/licenses/by/20]

Nascimento, L.H.H., Tochetto, C., Lucena, B.R., & Kommers, G.D. (2019).Testicular Interstitial Cell Tumor with Disseminated Cutaneous Metastasis in a Dog. *Acta Science Veterinary*, *47*(supl.1), 434. [DOI:10.22456/1679-9216.96391]

Othman, J.A., Talib, G.M.A., Goran, M.R., & Dana, O.I. (2022). Clinical and histological aspects of cryptorchidism in dogs and cats. *Al-Anbar Journal of Veterinary Sciences*, *15*(1), 34-42. [DOI:10.37940/AJVS.2022.15.1.5]

Pecile, A., Groppetti, D., Pizzi, G., Banco, B., Bronzo, V., & Giudice, C. (2021). Immunohistochemical insights into a hidden pathology: Canine cryptorchidism. *Theriogenology*, *176*(1), 43-53.[DOI:10.1016/j.theriogenology.2021.09.011]

Samir, H., ElSayed, M.I., Radwan, F., Hedia, M., Hendawy, H., & Hendawy, A.O. (2022). An updated insight on testicular hemodynamics: Environmental, physiological, and technical

perspectives in farm and companion animals. *Veterinary Research Communication*, *18*, 1-23. [DOI:10.1007/s11259-022-10022-9]

Santos, M., Marcos, R., & Caniatti, M. (2010). Cytologic study of normal canine testis. *Theriogenology*, *73*(2), 208-214. .[DOI:/0.1016/j.theriogenology.2009.07.030]

Shokrpoor, S., Pourreza, B., Khodadadi, M., Moradi Ozarlou, M., Khoshvaghti, R., & Fazel, B. (2021). Clinical, Radiological and Microscopical Findings of Chondrosarcoma in a Cat. *Iranian Journal of Veterinary Medicine*, *15*(4), 452-455. [DOI:10.22059/ 318789.1005158]

Sumner, R.N., Byers, A., Zhang, Z., Agerholm, J.S., Lindh, L., & England, G.C.W. (2021). Environmental chemicals in dog testes reflect their geographical source and may be associated with altered pathology. *Scientific Reports*, *11*(73), 1-11. .[DOI:10.1038/ s41598-021-86805-y]

ارزیابی پاتولوژیکی بافت بیضه در سگ‌های اخته شده در شهرستان اهواز، استان خوزستان

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چکیده فارسی

زمینه مطالعه: علیرغم تحقیقات زیادی که در زمینه بیماری‌های دستگاه تناسلی (بیضه و اپیدیدیم) در سگ‌ها انجام شده است؛ اما هنوز میزان دقیق عوارض تولید مثلی، مشخص نیست.

هدف: هدف از انجام مطالعه حاضر، ارزیابی یافته‌های بالینی، ماکروسکوپی و هیستوپاتولوژی بافت بیضه و اپیدیدیم در سگ‌های منطقه اهواز، استان خوزستان بود.

روش کار: در مطالعه حاضر، 77 نمونه از بیضه سگ‌های نر، در سنین مختلف (از 7 ماه تا 12 سال)، مورد ارزیابی قرار گرفتند. پس از ارزیابی ماکروسکوپی، بافت‌های بیضه و اپیدیدیم در ظرف حاوی فرمالین بافر 10 درصد قرار گرفتند و مراحل رنگ‌آمیزی دنبال شدند.

نتایج: شصت و هشت مورد از سگ‌ها، دارای بیضه‌هایی با ظاهر طبیعی بودند. 4 مورد از سگ‌ها مبتلا به کریپتورکیدیسم بودند که در معاینه بالینی مشخص گردید. در 5 مورد، اپیدیدیم متورم و با وجود فضاهای متسع مشخص گردید. مطالعه میکروسکوپی صورت گرفته بر روی نمونه‌ها، نشان-دهنده وجود ضایعات متنوعی در 45 نمونه، معادل 58/44 درصد از نمونه‌ها بود. از 45 نمونه ضایعه‌دار، 4 مورد (8/89 درصد)، نهان‌بیضگی یک

طرفه داشتند. دژنرسانس بافت بیضه یکی از مهمترین مواردی بود که در 14 مورد (31/11 درصد) مشاهده گردید. از 45 نمونه ضایعه‌دار، 22 مورد (48/89 درصد) مبتلا به اپی‌دیدیمیت بودند. در این نمونه‌ها، هیپرپلازی شدید مخاط پوشاننده اپیدیدیم مشاهده گردید. در 12 مورد از نمونه ها، به شکل همزمان اپی‌دیدیمیت و دژنرسانس مشاهده گردید. دو مورد از اپیدیدیمیت‌های مزمن همراه با خونریزی بودند. یافته دیگر، مشاهده گنجیدگی‌های درون هسته‌ای و داخل سیتوپلاسمی بود (در 29 مورد). در 21 مورد، به طور همزمان اپیدیدمیت نیز قابل مشاهده بود که با وجود فیبروز اطراف مجاری و هیپرپلازی مخاط مشخص گردید. خونریزی در 5 مورد (11/11 درصد)، دیده شد.

نتیجه‌گیری نهایی: با توجه به شیوع نسبتا بالای دژنرسانس بیضه، اپی‌دیدیمیت و حضور گنجیدگی‌های داخل هسته‌ای و داخل سیتوپلاسمی، اهمیت اخته‌کردن سگ‌ها در سنین جوانی و ارزیابی‌های هیستوپاتولوژیک بسیار حائز اهمیت است؛ چرا که این عوارض، می‌توانند منجر به ناباروری، بویژه در سگ‌های اصیل که در پناهگاه‌ها نگهداری می‌شوند، گردد (به‌دلیل روند مزمن برخی از بیماری‌های عفونی).

کلمات کلیدی: کریپتورکیدیسم؛ سگ؛ اپیدیدیم؛ هیستوپاتولوژی؛ بیضه