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Original Scientific Paper

CRYPTORCHIDISM IN DOGS

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Summary

Cryptorchidism is a disease that is manifested by the retardation of the testicles with the associated anatomical structures inside the abdomen or inguinal canal. In dogs, the appearance of cryptorchidism is of unclear etiology, but it is believed to have a genetic basis. The "gold standard" in the diagnosis of this disease is ultrasound diagnostics with a sensitivity of 95-100%.

The study was conducted on 10 dogs. The clinical examination of dogs suspected to suffer from cryptorchidism was initially carried out using adspection and palpation methods, after which an ultrasound examination was performed to identify and localize residual testicles. All dogs underwent surgical removal of both residual and physiologically descended testicles. A pathohistological analysis was performed on the removed residual testicles.

Bilateral cryptorchidism was found in 2 out of 10 dogs (20%), while unilateral cryptorchidism was found in 8 out of 10 dogs (80%). Right unilateral cryptorchidism was found in 5 out of 8 dogs (62.5%), while left unilateral cryptorchidism was found in 3 out of 8 dogs (37.5%). The predictive value of the comparison of ultrasound identification and localization of residual testicles with their surgical identification and localization in this study was 100%. The results of the pathohistological analysis showed the presence of tumorous changes in the type of seminoma on one testicle in one dog (unilateral inguinal cryptorchidism), while the diagnosis of testicular atrophy was made in the remaining 9 dogs with morphologically changed testicles.

Keywords: dog, cryptorchidism, ultrasound diagnosis, testicular tumors.

INTRODUCTION

Testes are paired male sex glands located in the scrotum. The role of the testes in animals is twofold: gametogenic, since they produce male gametes (sex cells - spermatozoa), and endocrine, since they synthesize and secrete male sex hormones (androgens) into the blood (Stančić, 2014). Unlike other mammals, the testicles in dogs do not descend through the inguinal canal for another 3-4 days after their birth and are in their final position in the scrotum on day 35 postnatally (Pretzer, 2008). They occupy their final scrotal position up to the sixth month of the animal's age (Spangenberg, 2021). Testicular descent is regulated by androgenic and non-androgenic factors, and is mediated by the action of the gubernaculum (Pretzer, 2008). In newborn dogs, the testicles are small, soft and can move between the scrotum and the inguinal canal, especially when the puppy is stressed or frightened.

Cryptorchidism in dogs, as in other species of animals, represents the retardation of the testes with the associated anatomical structures inside the abdomen or inguinal canal, i.e. the absence of descent of the mentioned structures into the scrotum (Spangenberg, 2021; Moon et al., 2014). Based on whether one or both testicles remain, cryptorchidism is divided into unilateral and bilateral, while based on the localization of the remaining testicle, cryptorchidism can be abdominal, inguinal and subcutaneous cryptorchidism (Felumlee et al., 2012; Mattos et al., 2000; Mialot, 1988). Unilateral cryptorchids can produce fertile sperm, while bilateral cryptorchids usually cannot and are sterile. Cryptorchid dogs have a higher risk of testicular neoplasia and spermatic cord torsion (Moon et al., 2014).

Suspicion of cryptorchidism is established by the general clinical diagnostics, primarily using adspection and palpation methods, while the "gold standard" in the diagnosis of cryptorchidism is the ultrasound examination with a sensitivity of 96.6% (Khan et al., 2018).

The only form of treatment for this disease is the surgical removal of the remaining testicle(s), and as it is a hereditary disease, castration of unilateral cryptorchids (removal of the descended testicle) is also recommended, because such dogs should not be used for further breeding (Gradil and McCarthy, 2012). After the surgical removal of the testicle, the remaining testicle must be sent for pathohistological analysis to determine whether it has undergone neoplastic changes.

The goals of this study are: determination of adequate diagnostic protocols for evaluation of residual testicles, selection of adequate surgical technique for removal of residual testicles and pathohistological analysis of testicles after their surgical removal.

MATERIALS AND METHODS

This study was conducted on 10 owner's dogs as part of clinical work at the University Veterinary Clinic of the Faculty of Agriculture in Novi Sad. After collecting detailed anamnestic data from the animal owners, the process of clinical diagnosis of cryptorchidism was initiated. Initially, with the methods of adspection and palpation, and then with the use of ultrasound diagnostics, testicular retardation was determined in all previously suspected individuals. The ultrasound examination was performed using an ultrasound machine BPU60 Vet (BMV, China), using a convex probe, frequency 6 MHz, in B mode in real time. Clinical diagnostic methods were used to determine the localization of residual testicles (Table 1).

After a detailed clinical examination of all individuals, preoperative preparation of the patient was performed, which consisted of several procedures. Blood was taken from all patients to perform the following laboratory analyses: complete blood count with differential leukocyte formula, biochemical parameters (creatinine, AST, ALT, total bilirubin, albumin, phosphorus, calcium, glucose, globulins, total proteins, urea, alpha-amylase, magnesium, triglycerides, lipase, ALP, GGT). Complete blood count with differential leukocyte formula was analyzed on the MEK-6550 machine (Nihon KOHDEN CORPORATION, Japan). Biochemical analysis of blood serum parameters was performed on a Chemray apparatus (Rayto Life and Analytical Sciences Co., China). All dogs were tested for vector-borne diseases - CaniV-4 Rapid Diagnostic Test (Bionote, Korea) as part of the preoperative preparation of the patient.

The preparation of the dogs for the operation started when, on the basis of all the above-mentioned analyses, it was determined that the dogs are suitable for the surgical procedure. Surgical procedures in dogs were performed under general inhalation anesthesia, according to all principles of good veterinary and surgical practice. First, premedication for general anesthesia was performed, which involved the application of a sedative to the animal. In all 10 individuals, sedation was performed using xylazine (XYLASED, Bioveta, Czech Republic), intravenously administered at a dose of 1 mg/kg of the animal's body weight. After obtaining the premedication procedure, the preparation of the surgical field started. The hair was removed from the area of the entire abdomen, prepuce and scrotum (Figure 1). At first, the skin was washed with neutral soaps, and then

antisepsis was performed using a 70% ethyl alcohol solution and a 10% povidone iodine solution. Induction of general anesthesia in all animals was performed by intravenous administration of ketamine (Ketamidor 10%, RICHTER PHARMA AG, Austria) in a dose of 3 mg/kg of the animal's body weight. Maintenance of general anesthesia was performed using sevoflurane (Sevorane 100%, AESICA QUEENBOROUGH LIMITED, Great Britain) with a minimum alveolar concentration of 2.3%.

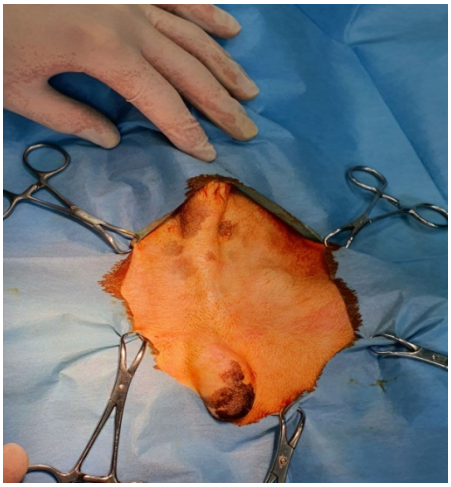


Figure 1 Surgical field preparation (Photography: Ivan Galić, 2021)

In dogs diagnosed with inguinal cryptorchidism, the incision was made directly through the inguinal canal - through the skin and then the subcutaneous tissue (Figure 2). After evaluating the suspect testicle, a double ligation of the spermatic cord was performed. The spermatic cord was ligated with absorbable multifilament suture PGA, USP 0 or 2/0 (Yavo, Poland). After ligation and removal of the suspected testicle, suturing of the subcutaneous tissue was performed with absorbable monofilament thread MONOSORB, USP 0 or 2/0 (Yavo, Poland) with continues suture. The skin was sutured with nonabsorbable monofilament NYLON, USP 0 or 2/0 (Yavo, Poland), by single knotted suture (Figure 3).

Figure 2 Access to the inguinal residual testis (Photography: Ivan Galić, 2021)

Figure 3 The appearance of the surgical wound after suturing the skin in inguinal cryptorchidism (Photography: Ivan Galić, 2021)

In the case of bilateral cryptorchidism, the incision was placed medially. In this case, the foreskin was first removed from the abdominal wall and moved laterally, after which an incision was made along the white line of the abdominal wall. After visualization of the suspicious testicle, a double ligation of the spermatic cord and removal of the remaining testicle was performed. The spermatic cord was ligated with absorbable multifilament suture PGA, USP 0 or 2/0 (Yavo,

Poland). Then the abdominal wall was closed. Abdominal wall suturing was performed with absorbable monofilament thread MONOSORB, USP 0 or 2/0 (Yavo, Poland) with continues suture. Suturing of the subcutaneous connective tissue was performed with absorbable monofilament thread MONOSORB, USP 0 or 2/0 (Yavo, Poland) with continues suture. The skin was sutured with nonabsorbable monofilament NYLON, USP 0 or 2/0 (Yavo, Poland), by single knotted suture.

After removing the suspect testicle, the testicles were placed in formalin and sent for pathohistological analysis. Pathohistological analysis was performed in the pathology laboratory of the Faculty of Agriculture, University of Novi Sad.
As part of the postoperative therapeutic protocol, non-steroidal anti-inflammatory drugs and antibiotics were administered to all dogs. In pain therapy, metamizole sodium (Noramin, Evrolek Pharmacija d.o.o., R. Serbia) was used, administered intravenously, in a dose of 25 mg metamizole/kg animal body weight. Pain therapy in all dogs was carried out continuously for 3 days. As part of the antibiotic protection, all dogs were once given a combination of penicillin and streptomycin in a dose of 0.5 ml of suspension per 5 kg of the dog's body weight - Penstrep (Dopharma, the Netherlands), and then twice, every 72 hours, a long-acting antibiotic, a combination of penicillin and streptomycin - Shotapen, in a dose of 1 ml of suspension per 20 kg of the dog's body weight, (Virbac S.A., France). Sutures were removed in all dogs on the 14^{th} day after surgery.

RESULTS

In this study, using methods of general clinical examination and ultrasound diagnostics, unilateral cryptorchidism was diagnosed in 8/10 dogs (80%), while bilateral cryptorchidism was diagnosed in 2/10 dogs (20%). Right unilateral cryptorchidism was found in 5/8 dogs (62.5%). A left testicle was found in 3/8 unilateral cryptorchids (37.5%). In 7/8 unilateral cryptorchids, the localization of the testes was determined in the inguinal region (87.5%), while in one case, the localization of the testes was determined in the abdominal cavity (12.5%). Bilateral cryptorchidism was confirmed in 2/10 dogs. In one bilateral cryptorchid, one testicle was localized in the inguinal canal, while the other was located in the abdominal cavity. In the second bilateral cryptorchid, both testicles were localized in the abdomen (Table 1).

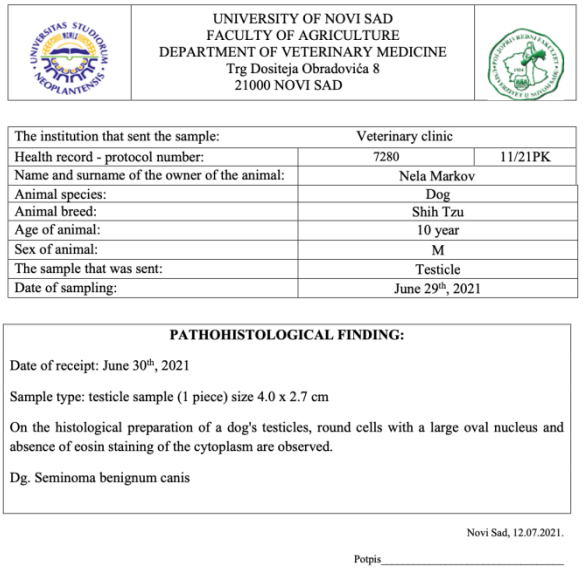
Table 1 Types of cryptorchidism in clinically examined dogs

Dog	Race	Age	Weight	Cryptorchism type	Body side
1	Shih Tzu	10 years	6 kg	Inguinal – unilateral	Left side
2	Bearded Collie	2 years	25 kg	Inguinal - unilateral	Right side
3	Siberian Husky	2 years	22 kg	Inguinal - unilateral	Right side
4	Border Collie	3 years	25 kg	Inguinal - unilateral	Right side
5	Cavalier of Prince Charles	1 year	8 kg	Inguinal - unilateral	Left side
6	French Bulldog	3 years	9 kg	Inguinal - unilateral	Right side
7	Miniature Pinscher	6 years	5 kg	Inguinal - unilateral	Left side
8	Miniature Poodle	2 years	4 kg	Abdominal unilateral	Right side
9	Yorkshire Terrier	2 years	5 kg	Abdominal / inguinal bilateral	Bilateral
10	Dachshund	4 years	8 kg	Abdominal - bilateral	Bilateral

Since the only method of treating cryptorchidism in dogs is surgical removal of the residual testicle, surgical procedures and removal of the residual testicle were performed. Thereafter, castration, or surgical removal of the remaining testicle, was performed, considering cryptorchidism is a hereditary disease.

After removal of residual testicles, each removed testicle was sent for pathohistological examination. Out of a total of ten dogs, one dog was diagnosed with a residual testicular tumor in the seminoma type (unilateral inguinal cryptorchid) (Figure 5, 6), while the others were diagnosed with testicular atrophy (Figure 7).

Figure 5 Morphologically altered testicle-above; Morphologically unchanged testicle-down (Photography: Jovan Spasojević, 2021)



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DEPARTMENT OF VETERINARY MEDICINE
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21000 NOVI SAD

The institution that sent the sample:	Veterinary clinic	
Health record - protocol number:	7280	11/21PK
Name and surname of the owner of the animal:	Nela Markov	
Animal species:	Dog	
Animal breed:	Shih Tzu	
Age of animal:	10 year	
Sex of animal:	M	
The sample that was sent:	Testicle	
Date of sampling:	June 29th, 2021	

PATHOHISTOLOGICAL FINDING:

Date of receipt: June 30th, 2021

Sample type: testicle sample (1 piece) size 4.0 x 2.7 cm

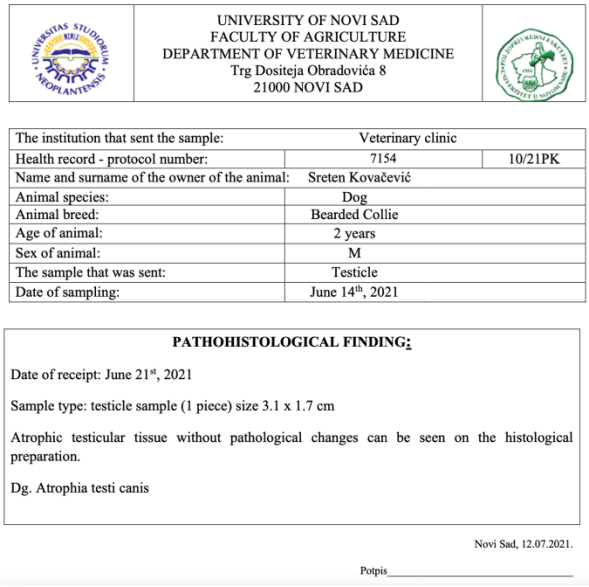
On the histological preparation of a dog's testicles, round cells with a large oval nucleus and absence of eosin staining of the cytoplasm are observed.

Dg. Seminoma benignum canis

Novi Sad, 12.07.2021.

Potpis_____

Figure 6 Pathohistological finding and confirmation of tumorous changes



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The institution that sent the sample:	Veterinary clinic	
Health record - protocol number:	7154	10/21PK
Name and surname of the owner of the animal:	Sreten Kovačević	
Animal species:	Dog	
Animal breed:	Bearded Collie	
Age of animal:	2 years	
Sex of animal:	M	
The sample that was sent:	Testicle	
Date of sampling:	June 14th, 2021	

PATHOHISTOLOGICAL FINDING:

Date of receipt: June 21st, 2021

Sample type: testicle sample (1 piece) size 3.1 x 1.7 cm

Atrophic testicular tissue without pathological changes can be seen on the histological preparation.

Dg. Atrophia testi canis

Novi Sad, 12.07.2021.

Potpis_____

Figure 7 Pathohistological findings of testicular atrophy

DISCUSSION

Cryptorchidism is a disease of unexplained etiology that is believed to have a genetic basis. Numerous studies have shown that the incidence rate of this disease in dogs ranges from 0.8% to 9.7% (Ruble and Hird, 1993; Priester et al., 1970; Reif, 1969). Breeds of dogs in which cryptorchidism occurs most often are: Maltese dogs, Chihuahuas, German boxers, English bulldogs, Toy poodles, Yorkshire terriers, Dachshunds, Pomeranian spitz, Pekingese, Siberian huskies (Johnston, 2001). In this study, the rate of incidence of cryptorchidism in dogs was not investigated, but from Table 1 it can be concluded that the results of this study are in accordance with others, when it comes to breeds that are considered predisposed to this disease.

Clinical diagnosis of cryptorchidism in dogs is based on the use of general and special clinical diagnostic methods, and ultrasound diagnostics can be considered the "gold standard" in cryptorchidism diagnosis. Ultrasound diagnostics, with a sensitivity of 96.6%, is used to detect abdominal cryptorchids, and with a sensitivity of even 100% to detect inguinal cryptorchids (Khan et al., 2018). Research conducted by Felumlee et al. (2012) on dogs, indicates that there was a 100% positive predictive value for a total of 42 out of 43 dogs, of which 28 were abdominal and 14 were inguinal cryptorchids. In the mentioned dogs, the ultrasound findings and position of the testicles are identical to their surgical localization. In one of the 43 cryptorchid dogs, an abdominally retained testis could not be identified using ultrasound diagnostic and was later found in the abdomen during surgery. Surgical and ultrasound findings were the same for 42/43 (97.7%) residual testes. The sensitivity of ultrasound diagnostic was 96.6% for abdominal and 100% for inguinal positioned testicles. In our study, the methods of general clinical diagnostics (aspection and palpation) in combination with ultrasound diagnostics were used as diagnostic methods for detecting cryptorchid dogs. In all dogs, the location of the residual testicle was detected using ultrasound diagnostics, which was later confirmed during surgery. Based on this, it can be concluded that in our study, ultrasound diagnostics was used with a degree of sensitivity of 100%, which coincides with the results of the research done by Felumlee et al. (2012).

In our study, based on the results of the clinical examination, unilateral cryptorchidism was diagnosed in 80% of dogs (8/10), while bilateral cryptorchidism was diagnosed in 20% of dogs (2/10). Right unilateral cryptorchidism was found in 5 out of 8 dogs (62.5%). Left unilateral cryptorchidism was found in 3 out of 8 dogs (37.5%). In 7 out of 8 dogs with unilateral cryptorchidism, the localization of the testicles was determined in the inguinal region (87.5%), while in one case, the localization of the testicles was

determined in the abdominal cavity (12.5%). In the research conducted by Tannouz et al. (2019), the presence of unilateral cryptorchidism was found in 70% of cases, and bilateral cryptorchidism in 30% of cases. The same authors stated that the highest prevalence of cryptorchidism was detected in the right inguinal region (36.2%), followed by the left inguinal region (25.4%), than within the right side of the abdominal cavity (23.3%). The prevalence of cryptorchidism within left side of abdomen was 15.1%.

The results obtained in our study partially coincide with the results obtained by Tannouz et al. (2019), and the reason for the partial coincidence lies in the different number of examined patients.

Ultrasound diagnostics is also considered the primary diagnostic method in the diagnosis of cryptorchidism in other animal species, as well as in humans. Ultrasound diagnostic is a sensitive and highly specific method for the detection and localization of a residual testicle in horses (Felumlee et al., 2012; Ras et al., 2010; Schambourg et al., 2006). Research conducted by Schambourg et al. (2006) on 38 horses, of which unilateral cryptorchidism was detected in 32 cases, and bilateral in 6 cases, indicates that in 97.5% of cases the residual testicle was successfully located and that it is possible, using ultrasonography, to give an accurate diagnosis of abdominal cryptorchidism on the field without additional hormonal analyzes or rectal examination. In the same study, it is also stated that no other abdominal structure can be mistaken for an abdominally retained testicle, provided that the hyperechoic *tunica albuginea* is visible and that the central vein or the epididymis are easily visible, which makes transabdominal ultrasonography a reliable diagnostic method.

In humans, ultrasound diagnostics have also been used to detect residual testicles, but with less satisfactory results in some studies (Felumlee et al. 2012; Pekkafali et al., 2003; Elder, 2002; Malone and Guiney, 1985). In children, ultrasound diagnostic is important because it is a non-invasive technique that does not use ionizing radiation and facilitates planned surgical intervention (Felumlee et al., 2012; Nijs et al., 2007; Cain et al., 1996; Weiss et al., 1986; Kullendorff and et al., 1985; Wolverson et al., 1983). When comparing ultrasound findings with surgical locations of non-palpable testicles in children, ultrasound diagnostic was successful in locating of 103 out of 152 (68.0%) non-palpable testicles: 16 in the abdomen and 87 in the inguinal canals (Nijs et al., 2007).

Gharagozlou et al. (2014) investigated the use of anti-Müllerian hormone as a potential diagnostic marker in dogs. The research was conducted on 10 bilateral cryptorchids, 7 intact dogs and 7 neutered dogs over 6 months of age of Toy breeds. It was found that serum concentrations of anti-Müllerian hormone are significantly higher in intact and neutered dogs than in bilateral cryptorchids,

which can be used in the differential diagnosis of neutered and cryptorchid dogs (Gharagozlou et al., 2014).

Dogs diagnosed with cryptorchidism have a higher risk of developing testicular neoplasia. The most common are tumors of the interstitium, i.e. *Leydig* cells, tumors of the testicular germinal epithelium-seminomas, and *Sertoli* cell tumors (North et al., 2009). Testicular tumors represent more than 90% of all tumors of the genital organs in dogs and dogs have the highest incidence of all animal species (North et al., 2009). The higher incidence of tumors in the seminoma type can be attributed to the fact that these types of tumors result in clear clinical signs, i.e. testicular enlargement (Grieco et al., 2008; Marinković, 2006). This finding was also in our case, which was clearly observed after the orchidectomy of both testicles (Figure 5) in a dog in which, based on the pathohistological findings, a tumor of the seminoma type was determined. Lalošević et al. (2008) reported two seminomas out of thirteen testicular tumors found in dogs, one of which, as in our study, was a unilateral cryptorchid. Also, the research conducted by Liao et al. (2009) indicates that the right testicle is more often affected by the tumor, due to the fact that it often lags behind in the abdomen, that is, the inguinal canal, as was also the case in our study.

In the analysis presented by Marinković et al. (2006), the seminoma type tumor appeared at the earliest at the age of nine, and at the latest at the age of fifteen, with an average age of dogs of 10.8 years, which coincides with the age of our dog, which was ten years old. In the same study, when it comes to the incidence of testicular tumors in dogs that did not show any symptomatology characteristic for the presence of tumors, the following results were obtained on a total of 232 dogs examined: the study included males of different breeds, of different ages, and both testicles were taken for pathological-histological analysis. It was found that 27% of the dogs had a testicular tumor, of which 50% had a *Leydig* cell tumor, 42% had a seminoma, and only 8% had a *Seroli* cell tumor. Also, 31% of the total number of patients had combinations of these tumors. *Leydig* cell tumors and seminoma appeared together most often, followed by *Leydig* cell tumor and *Sertoli* cell tumor, while the combination of seminoma and *Sertoli* cell tumor was recorded in a small number of cases.

Orchidectomy is the recommended method of removing a testicle affected by a tumor, but as there is a possibility of tumor metastasis, chemotherapy represents an additional therapeutic protocol to orchidectomy (Botelho Soares de Brito et al., 2014). Although metastases are rare, they can occur, and the prognosis will depend on the size and localization of the metastases. Seminomas in dogs rarely metastasize, unlike seminomas in humans. They metastasize only in 6 to 11% of

cases to inguinal, iliac and sublumbar lymph nodes, lungs and visceral organs (McEntee, 2002; Moulton, 1990).

CONCLUSION

The final diagnosis of the presence of cryptorchidism in dogs can be made from the age of 6 months. Ultrasound diagnostics is considered the primary diagnostic method and the "gold standard" in the diagnosis of cryptorchidism in dogs, with a sensitivity of 90% to 100% depending on the type of cryptorchidism. Surgical therapy, i.e. removal of residual testicles, is the only optional solution in the treatment of cryptorchidism. Also, it is necessary to neuter the animal at the same time, i.e. remove the physiologically descended testicle. Considering the pathomorphological changes of all residual testicles, pathohistological analysis should be introduced as a standard within diagnostic and therapeutic protocols in the treatment of cryptorchidism.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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