Characteristics of cryptic/ectopic and contralateral scrotal testes in dogs between 1 and 2 years of age

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Abstract

Testicular malposition represents a common developmental genital defect in dogs and can affect one or both testes. In both humans and dogs, unilateral cryptorchism is more frequently detected and thought to be the expression of a genetic abnormality affecting both the undescended and scrotal testis. In the dog, there is evidence of degenerative processes affecting the maldescended testis. However, the histologic and functional changes that occur in the scrotal testis of unilateral cryptorchid or ectopic individuals remain a source of debate. Because the bilateral surgical removal of the testes leads to some undesirable side effects, the aim of this study was to evaluate the necessity for performing bilateral orchiectomy in young unilateral cryptorchid dogs. A morphologic study of both cryptic/ectopic and scrotal testes in young dogs affected by unilateral testicular maldescent was therefore conducted. The study was conducted on 10 dogs aged 1 to 2 yr and affected by unilateral testicular maldescent. We found that, in young dogs, even if no neoplastic lesions were observed, morphologic abnormalities are detectable between 1 and 2 yr of age in the maldescended testes with severity dependent on testicular position. In contrast, in the scrotal testes, the histologic and immunohistochemical exam failed to find signs of incorrect development or morphologic abnormalities. The results seem to suggest that, though the early removal of the undescended testis is recommended, continuous monitoring of the scrotal testis for the life of the dog is preferable to removing it considering the undesirable side effects related to castration.

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1. Introduction

In dogs, during the gestation and following birth, the male gonads move from the lumbar area where they originate to their final location in the bottom of the scrotum [1–3]. Testes must be palpable inside the scrotum by 6 to 8 wk of life [1]. This descent is necessary in dogs, as in most other mammals, to allow for the normal process of sperm cell production (spermatogenesis) and, therefore, male fertility [4,5]. Testes that remain inside the abdomen or the inguinal channel are defined as cryptic [1,2], whereas if they reach an abnormal location outside the inguinal ring, they are considered ectopic [5–7]. However, both abnormalities are often referred to in the literature as cryptorchidism, thus knowledge about the incidence of the specific conditions is lacking.

Testicular malposition represents a common developmental defect of the genitals in dogs [8]. In dogs, the incidence of cryptorchidism ranges between 0.8% and 15% [1,2,9–12], affecting pure breed dogs (77.5%)...
more often than mongrels (22.5%) [2]. High-risk breeds and an increased risk in small-sized dogs have been reported [2,13–15]. Similar to humans [7,16], in dogs, unilateral cryptorchidism is more frequently detected (75%) [1,2,17], and the right testis is twice as often involved in the condition [1]. Cryptorchidism is considered to be a sex-conditioned trait transmitted by one or more autosomal genes [1,2]. Congenital abnormalities, such as patellar subluxation, hip dysplasia, umbilical and inguinal herniation, and preputial and/or penis abnormalities have been associated with cryptorchidism in dogs [13,18].

In the dog, as in man, maldescended testes may affect fertility and predispose to neoplasm, spermatic cord torsion, and inguinal herniation [1,5,18]. Cryptorchid dogs show a 3.6- to 13.6-fold higher risk of developing a neoplasm compared with that of normal dogs [1,2,19,20], but in the dog, testicular neoplasms are to a lesser extent malignant compared with that in humans.

Despite broad evidence of degenerative processes affecting the maldescended testis, the histologic and functional changes that occur in the contralateral scrotal testis of unilaterally cryptorchid or ectopic individuals are still debated. Unilateral cryptorchidism is thought to be due to the expression of a genetic testicular abnormality affecting both the undescended and scrotal testis [5,21]. Several authors have shown that degenerative changes occur in the scrotal testis of unilaterally cryptorchid men [22–26] and rats [27], whereas others [28,29] have failed to detect abnormalities in the scrotal counterparts of unilateral cryptorchids. Codesal et al. [30] found in the scrotal testis tubular abnormalities and dysfunctions similar to those observed in the retained gonad of unilaterally cryptorchid children aged 4 to 14 years.

In men with unilateral cryptorchidism, tumors can develop from the scrotal testis [31] in approximately 20% of the affected population [32]. Pinart et al. [33] reported that unilaterally cryptorchid boars exhibit a reduced number of seminal tubules associated with an increase of interstitial tissue in the scrotal testis. The same authors also found a prevalence of Stage I and II in the seminiferous epithelium cycle, suggesting abnormal and incomplete spermatid maturation in the scrotal testis. The interference of spermatid maturation can be attributed to the abnormal function of Sertoli cells [34]. In addition, the lumen of the seminiferous tubules is wider compared with that of normal testicles, whereas the lamina propria is similar to that of normal testes, as is the interstitial tissue, with normal Leydig cells, blood, and lymphatic vessels [35].

In the dog, the study of the characteristics of the scrotal testis in unilateral cryptorchids has received little attention. The condition is considered a heritable trait, and the affected dogs should therefore not be allowed to breed to permanently avoid reproduction. Therefore, one suggested method for management of unilateral cryptorchid dogs is bilateral orchiectomy independent of the condition of the scrotal testis [1,2,18]; however, this procedure should rely on scientific evidence for the necessity of a bilateral orchiectomy for the dog’s health and not merely to avoid breeding. In the past, dog owners referred unilaterally cryptorchid dogs to veterinarians only after the appearance of enlarged testes, or for neoplastic-related or non–neoplastic-related dysfunctions. In these clinical conditions, disease-associated effects on the scrotal testis could be easily suspected, leading to a real necessity for performing bilateral orchiectomy [4,31,32]. Nowadays, the information among dog owners allows for a prompt detection of suspected unilateral cryptorchid dogs within the first months of age. Thus, taking into account the strong differences that exist between humans and dogs in reproduction-related ethics, the real necessity for performing bilateral orchiectomy in young, unilaterally cryptorchid dogs should be investigated. It should be kept in mind that the bilateral, surgical removal of testes leads to some undesirable side effects, such as a higher risk of obesity and behavioral changes [36] or a higher occurrence of prostatic neoplasia, as well as transitional cell carcinoma of the urinary bladder, osteosarcoma, hemangiosarcoma, cranial cruciate ligament rupture, and diabetes mellitus [37]. The advantages and disadvantages of performing a bilateral orchiectomy should be deeply evaluated and discussed with the dog owners at the time of diagnosis.

To date, the specific condition of unilateral undescended testis and related consequences is not completely understood in humans or animal models, even less understood in the dog, and is still debated. In 1989, Mattheeuwes and Comhaire [38] reported the absence of histologic evidence of spermatogenesis in the cryptorchid testis and normal sperm cell production in the scrotal testis of unilaterally cryptorchid, mature, normal dogs. The effects of a cryptorchid testis on its counterpart have been investigated in beagle dogs with artificial unilateral cryptorchidism [39]. The authors found increased estrogen secretion by the cryptorchid testis that inhibited the endocrine and spermatogenic functions of its counterpart. Thus, the results of these studies seem to provide conflicting evidence regarding the condition of the scrotal testes in mature dogs affected by unilateral testicular maldescent.
Nevertheless, these few studies did not answer questions about the actual condition of the scrotal testis in young dogs with spontaneous unilateral cryptorchidism. For these reasons, the aim of the current study was to evaluate the morphologic characteristics of both the cryptic/ectopic and, especially, scrotal testes of young dogs affected by unilateral testicular maldescent to detect any morphologic abnormalities in either testis, therefore assessing the necessity for scrotal testicle removal at the time of the early orchietomy of the maldescended testicle. For these reasons, testicles have been histologically checked for presence of spermatozoa and/or complete spermatogenesis in the seminiferous tubules and evaluated by immunohistochemistry for presence of gonocytes (PLAP) and Sertoli cell maturity (vimentin, CK18, and \( \alpha \)-inhibin).

2. Materials and methods

2.1. Animals

The study was conducted on 10 dogs (Canis, C. lupus) aged 1 to 2 yr, belonging to several breeds (Table 1), and affected by unilateral testicular maldescent. In five dogs, the maldescended testicle was retained in the abdomen and, therefore, classified as cryptorchid, whereas the other five dogs had a maldescended testicle located extrabdominally and classified as ectopic. During clinical examination, particular attention was paid to recording the macroscopic qualitative features of the scrotal testis, as well as those of the ectopic testis. In addition, every congenital genital or extragenital malformation, detectable at the clinical exam, was recorded. The general condition of the dogs, as well as the presurgical findings from the routine check, showed that all were candidates for anesthesia and surgery. Thus, bilateral orchietomy was successfully performed by surgical techniques adapted to testicular position [40].

Immediately after testicle removal, the precise position of the abdominal testicles was recorded and a gross examination of both gonads performed to assess, by simple clinical estimation, the size and consistency of the testicles and to detect any evidence of lesions. Immediately after surgery, the removed testicles were longitudinally cut and fixed in 10% buffered formalin to allow for histologic and immunohistochemical examination.

In addition, to statistically compare the testicular histologic features of the dogs with testicular maldescent, 10 testicles from 5 normal dogs, of several breeds and aged 1 to 2 yr, were also fixed in 10% buffered formalin and included in the study as control.

2.2. Histology

A representative section from each testis was collected, dehydrated, and embedded in paraffin. Five-micrometer-thick serial sections were obtained from each paraffin block and stained with hematoxylin and eosin (H&E). Testicles were examined and histologically checked for the following morphologic features: seminiferous tubules with complete spermatogenesis (presence of spermatozoa); seminiferous tubules with incomplete spermatogenesis (presence of cells of the seminal line, but absence of spermatozoa); and SCO tubules, defined as seminiferous tubules completely devoid of germ cells and containing only Sertoli cells. Leydig cell hyperplasia was also recorded as being present or absent. Epididymides were checked for the presence of spermatozoa.

In addition, for each testicle, in the histologic section, 20 seminal tubules were randomly selected at \( \times 200 \) and checked for the presence of spermatozoa. The number of seminal tubules with spermatozoa were recorded, and the results obtained from normal control testes and scrotal counterpart obtained from cryp-

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Age (mo)</th>
<th>Testicular maldescent</th>
<th>Testicular position</th>
<th>Testicular size</th>
<th>Testicular consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Labrador retriever</td>
<td>12</td>
<td>Left cryptorchidism</td>
<td>Caudal to kidney</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>2</td>
<td>Poodle</td>
<td>12</td>
<td>Left cryptorchidism</td>
<td>Internal inguinal ring</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>3</td>
<td>German shepherd dog</td>
<td>16</td>
<td>Left cryptorchidism</td>
<td>Internal inguinal ring</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>4</td>
<td>Labrador retriever</td>
<td>16</td>
<td>Right cryptorchidism</td>
<td>Internal inguinal ring</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>5</td>
<td>Golden retriever</td>
<td>24</td>
<td>Right cryptorchidism</td>
<td>Internal inguinal ring</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>6</td>
<td>English bulldog</td>
<td>16</td>
<td>Left ectopia</td>
<td>Lateral to penis</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>German shepherd dog</td>
<td>24</td>
<td>Right ectopia</td>
<td>Lateral to penis</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>Miniature pinscher</td>
<td>16</td>
<td>Right ectopia</td>
<td>Lateral to penis</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>Mongrel</td>
<td>18</td>
<td>Right ectopia</td>
<td>Prescrotal</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>10</td>
<td>Miniature pinscher</td>
<td>12</td>
<td>Right ectopia</td>
<td>Prescrotal</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
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</table>
torchid/ectopic dogs were statistically compared by chi-square test, with significance set at $P < 0.05$.

2.3. Immunohistochemistry

Serial sections (5 μm) from the paraffin blocks of the 10 pairs of testicles of dogs with testicular maldescent were obtained and processed by the avidin-biotin-peroxidase (ABC) method [41] using a commercial immunoperoxidase kit (Vectastain Standard Elite; Vector Laboratories, Burlingame, CA, USA). After deparaffinization, sections were incubated with 0.5% hydrogen peroxide in methanol for 20 min to quench endogenous peroxidase activity and then rehydrated. The primary monoclonal antibodies used in this study and the relative target cells were placental alkaline phosphatase (PLAP; DAKO Corporation, Carpinteria, CA, USA) diluted 1:25 to recognize gonocytes; vimentin (DAKO Corporation) diluted 1:1000 to recognize mature Sertoli cells; $α$-inhibin (AbD Serotec, Oxford, UK) diluted 1:50; and CK 18 (Zymed Laboratories, San Francisco, CA, USA) diluted 1:300 to recognize immature Sertoli cells. For all the antibodies employed, the cross reaction with canine antigens was already demonstrated in previous reports [42,43]. The unmasking procedure was performed before the application of CK 18, PLAP, and $α$-inhibin. For CK 18, sections were incubated for 10 min at 37°C in pepsin solution (Zymed Laboratories). For PLAP and $α$-inhibin, sections were microwaved for 8 min at 750 W in ethylenediamine tetraacetic acid (EDTA) buffer (pH 8) and citrate buffer (pH 6), respectively.

Sections were then incubated overnight at 4°C with both of the primary antibodies diluted in Tris buffer containing 0.6% normal horse serum. After washing three times in Tris buffer, the sections were incubated with horse anti-mouse biotinylated immunoglobulin for 30 min at a 1:200 dilution in Tris buffer (Vector Laboratories). The sections were then incubated with the ABC complex (Vectastain Elite; Vector Laboratories) at a 1:100 dilution for 30 min at room temperature and developed with diaminobenzidine chromogen (DAB) or 3-amino-9-ethyl carbazole (AEC; Vector Laboratories). The sections were then counterstained with Mayer’s hematoxylin.

Negative control slides were incubated only with secondary antibody; the primary antibody was replaced with normal horse serum. The positive controls were interstitial fibroblasts for vimentin, epithelial cells from rete testis for CK 18, myoid peritubular cells for PLAP, and Leydig cells for $α$-inhibin.

3. Results

3.1. Clinical findings

Data regarding the age, breed, type of testicular maldescent (cryptorchidism or ectopia), and the maldescended testicle side and position, size, and consistency are reported in Table 1. Upon clinical examination of the 10 dogs, no other genital or extragenital abnormalities were detected, except one dog that was affected by bilateral patellar subluxation.

Immediately after surgical removal, all scrotal and maldescended testicles were submitted first to a gross evaluation and then processed for histology and immunohistochemistry. There was no evidence of lesions in either the cryptic/ectopic or scrotal testicles at gross examination. However, at the simply qualitative assessment, the size of the cryptic/ectopic testicles appeared to be reduced in all 10 dogs and testicular consistency reduced in 7 of the 10 maldescended testicles compared with that of their scrotal counterparts.

3.2. Histology

In all testicle examined, inflammatory and neoplastic lesion were completely absent. Results for the cryptic/ectopic and scrotal testes are presented in Tables 2 and 3, respectively. In the sections of both scrotal and normal control testes, complete seminal line was present in all cases, and spermatozoa were also present in the epididymis. Of the 10 maldescended testicles, five were cryptic and five ectopic. Seven of the 10 maldescended testicles (five cryptic and two ectopic testicles) were composed of SCO tubules (Fig. 1). In only two of these seven cases (one cryptic and one ectopic testicle), intermingled with Sertoli cells, rare large cells with abundant and strongly eosinophilic cytoplasm and round vesicular basophilic nuclei were recognizable in few tubules. Because of their morphology, these cells were interpreted as gonocytes. In the remnant three ectopic testes, SCO tubules were absent, and in the seminiferous tubules, an incomplete seminal line was always evident.

In the interstitium, focal fibrosis was observed in two ectopic testes.

In all maldescended testicles, the epididymis was constantly empty but morphologically well developed.

A mean number of 17 of 20 counted tubules contained spermatozoa in the normal testes used as “controls” compared with a mean of 16 of 20 tubules in the scrotal counterparts of the studied dogs. The chi-square test evidenced that the number of tubules with
spermatozoa was not different between normal control testes and scrotal testes from cryptorchid/ectopic dogs. Thus, scrotal counterpart obtained from cryptic/ectopic dogs could be considered comparable with that of normal control testes.

3.3. Immunohistochemistry

3.3.1. Cryptic/ectopic testicles

All testes examined immunohistochemically were reactive to all the antibodies employed, and internal positive control structures were always clearly recognizable in all examined sections. Immunohistochemical results for the cryptic/ectopic testicles are summarized in Table 2.

Large cells with abundant and strongly eosinophilic cytoplasm recognizable in a few tubules (of one cryptic and one ectopic testicle) showed a strong immunostaining for PLAP, demonstrating to be gonocytes (Fig. 2). Sertoli cells in all 10 cases were positive for vimentin and negative for both CK 18 and α-inhibin (Fig. 3). In only three cryptic and two ectopic testicles, rare Sertoli cells faintly positive for α-inhibin were occasionally observed.

In all cases, in the interstitium, fibroblasts exhibited normal vimentin expression, and Leydig cells, easily recognizable in all cases, were normally positive for α-inhibin.
All the epithelial cells of the epididymides were normally reactive for CK 18.

3.3.2. Scrotal testicles

Immunohistochemical results for scrotal testes are summarized in Table 3. A complete seminal line with spermatozoa was observed in all scrotal counterpart testicles (Fig. 4), and spermatozoa were always present in the epididymis. No results were obtained with PLAP, confirming the total absence of gonocytes. Sertoli cells, as normal mature Sertoli cells, were always positive for vimentin and negative for both CK 18 and α-inhibin (Fig. 5). In the interstitium, fibroblasts were normally positive for vimentin, and Leydig cells showed the usual expression of α-inhibin.

4. Discussion

The aim of the current study was to examine the morphology of the testicles in 1- to 2-yr-old dogs affected by unilateral cryptorchidism/ectopia to assess the real necessity of a bilateral removal of the gonads at a young age. Because of the small number of subjects, it is not our goal to draw real epidemiologic considerations. However, it is interesting to note that, even with a small number of dogs, most were representative of the breed considered at high risk for testicular maldescent [2,13–15]. In all dogs, except for a miniature pinscher affected by bilateral patellar subluxation, no other developmental diseases reported to be associated with testicular maldescent were
found. Patellar subluxation is recognized as a testicular maldescent–associated disease [13]. Cryptorchidism and ectopia occurred with equal incidence, and the right testes were more frequently involved, which agrees with data reported for the dog [1,2] and man [7]. In most cases, the intra-abdominal testes laid on the internal inguinal ring; only in one dog was the gonad found caudal to the kidney. This finding seems to suggest that, in the dogs of the current study, the process of testicular descent was normal during the first phase of intra-abdominal migration with an imbalance in the second phase of inguinal descent. The ectopic testicles were located subcutaneously, lateral to the penis in three cases and immediately before the scrotal pouch in the other two (prescrotal position). Therefore, for these five dogs with testicular ectopia, during testicular descent the second phase of migration was also impaired. At gross examination, all of the maldescended testicles were reduced in size and, in most cases, in consistency.

For the results from the histologic and immunohistochemical examination, the findings from the scrotal testicles should be discussed separately from those for cryptic/ectopic testicles.

First, scrotal testes showed a number of tubules with spermatozoa not statistically different from that of control testes and demonstrated to be histologically similar to the testes of normal dogs of the same age. The marked differences in the seminal lines observed in cryptic compared with ectopic testicles could be explained, taking into proper account the small number of examined dogs, by the young age of the dogs. In these cryptorchid animals, in fact, it is possible that the intra-abdominal position of the gonad impaired the normal development of the seminal line and that the abnormal position of the testis causes the quick atrophy of immature seminal cells. In the ectopic testicles, differences have been detected depending on the final position of the testis: the seminal line was better developed in prescrotal testicles than in testicles located lateral to the penis. In a study on a large number of human testicular biopsies, no significant statistical histologic differences were found between cryptic and ectopic testes [44]. Nevertheless, as in that study intra-abdominal testes numbered 389 and inguinal testes numbered only 4, statistical evaluation could be biased by the great difference between sample numbers. On the other hand, in a more recent study [45], where the numbers of apoptotic seminal cells in intra-abdominal and inguinal testes were compared, inguinal testes showed a lower number of apoptotic cells than that of intra-abdominal testes [45]. Human findings together with the results obtained in the current study suggest further studies totally devoted on the possible histologic differences existing between cryptic and ectopic testicles. Taken together, cryptic and ectopic testicles always demonstrated marked Sertoli cell immunolabeling for vimentin, whereas they were consistently negative for CK 18. Occasionally, rare Sertoli cells exhibited faint immunolabeling for α-inhibin. Vimentin positivity and CK 18 negativity is interpreted in man as evidence of maturity of the Sertoli cells [46], and this suggests that, in the current study, Sertoli cells of cryptic and ectopic testes were always mature. In addition, the rare α-inhibin positivity seems to suggest that just a few Sertoli cells had not completed the maturational process. Nevertheless, because these latter cells also expressed vimentin, it is reasonable to conclude that the maturation process was very close to finishing.

As expected because of the young age of the dogs, neither in cryptic/ectopic testes nor in scrotal counterparts were tumors detected. In the scrotal testicles, morphologic abnormalities were not detected. In all cases, the seminal line was present, and spermatozoa were observed in the epididymis, similar to what is usually found in normal testicles. Immunohistochemistry demonstrated mature Sertoli cells positive for vimentin and consistently negative for α-inhibin.

The results obtained in the current study seem to confirm that, even when no neoplastic lesions have been observed, morphologic abnormalities in young dogs are detectable early in the maldescended testes, with severity depending on testicular position. This finding seems to confirm the suggestion for the surgical removal of the maldescended testis, previously reported by several authors. In fact, because the actual risk for testicular neoplasm in the maldescended testis in aged dogs is well recognized [1,2,19,20], the removal of the maldescended testis in young patients could prevent the development of testicular neoplasms and, in the case of intra-abdominal testicles, spermatic cord torsion [1,2,8,18]. The surgical removal of the maldescended testis should be suggested as soon as the dog can undergo anesthesia and surgery. On the other hand, in the scrotal testes, the histologic and immunohistochemical exam failed to find signs of incorrect development or morphologic abnormalities. On the contrary, both the germinal and Sertoli cells exhibited a normal degree of maturity and a normal number of sperm cells in the epididymis. These observations in young dogs are in contrast with findings reported by Codesal et al. [30] in children. Taken together, these findings seem to again raise the question of the real
necessity for scrotal testicle removal for safety reasons in young dogs affected by unilateral testicular undescend as distinguished from the reason to prevent the breeding of affected dogs. The results seem to suggest that, whereas the early removal of the undescended testis is recommended, the continuous monitoring of the scrotal testis for the life of the dog is preferable to its removal considering the undesirable side effects related to castration [37]. However, for a better evaluation of the real condition of the scrotal counterparts in dogs affected by unilateral cryptorchidism/ectopia, further investigation in older dogs is necessary to evaluate if the scrotal testis, with the increasing age of the animal, could also develop morphologic abnormalities that require surgical removal of the gonad for the dog’s safety.

References


