Pathology – Research and Practice 211 (2015) 772–775

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Histopathological pattern of gonads in cases of sex abnormalities in dogs: An attempt of morphological evaluation involving potential for neoplasia

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ARTICLE INFO

Article history:
Received 13 April 2014
Received in revised form 9 July 2015
Accepted 10 July 2015

Keywords:
Disturbances in sex differentiation
Dog
Dysgenetic gonads
Tumors

ABSTRACT

Disturbances in sex differentiation (DSD – disorder of sexual development) may result from disturbances in sex chromosomes or a disturbed development of gonads, or from genotypic disturbances. The objective of this article is to describe the histological structure of gonads in dogs showing sexual disturbances and a case of a cancer resembling gonadoblastoma in one of the animals. Among the 10 examined dogs with disturbances of sex development only a single case of a gonadoblastoma was observed. In animals with sex disturbances, similarly to humans, there exists a potential tendency for neoplastic lesions in dysgenetic gonads. As a rule, its frequency in population is confined due to the early procedure of castration of non-breeding dogs.

In the present study dogs demonstrated phenotypical traits of bitches with developmental anomalies such as hyperplastic clitoris with vestigial os penis (baculum), or abnormalities in the location and structure of the vulva. The material for the study included canine gonads of various breeds, sampled from phenotypical bitches, aged 7 months to 4 years – patients of the Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, University of Environmental and Life Sciences in Wroclaw (Poland) in years 2006–2013. The organs were surgically removed from the abdomen and sent for histopathological examination for the purpose of determining their histological structure. The 10 examined cases of altered gonads included 6 bilateral cases of testes (60%), 2 cases of bilateral ovotestis (20%), one case of co-manifestation of testis and ovotestis (10%), and a single case of a testis and a neoplastically altered gonad (gonadoblastoma) (10%).

1. Introduction

Disturbances in sex differentiation (DSD – disorders of sexual development) earlier referred to as intersexualism, may result from disturbances in sex chromosomes, genotype or gonadal development. Within the group, a true hermaphroditism and pseudohermaphroditism can be distinguished. The true hermaphroditism involves bilateral manifestation of ovotestes, unilateral ovotestis with ovary or testis on the contralateral side or parallel manifestation of ovary and testis. Pseudohermaphroditism involves the presence of normal gonads and an incompatible phenotype. DSD are frequently observed in the American cocker spaniels; similarly to the anomalies recognized in children. Therefore the dogs represent a good model for studies of disturbances observed in humans [13]. The anomaly in dogs is thought to reflect a recessive autosomal mutation, which has not been unequivocally identified [14,22]. Similar disturbances, involving a disturbed sexual development in humans, apart from sexual investigations, hormonal dysfunction and generative potential, are evaluated in the aspect of promotion of neoplasia [2–5,11,16–18]. Classical causes of such anomalies in humans involve mutations within the *SRY* gene, responsible for the differentiation of testes. These mutations are encountered in around 20% of patients with pure dysgenesis of gonads. Other cases (around 1/3 of the cases) reflect an abnormal exchange between homologous *PRKY* and *PRKX* protein kinase genes within the pseudoautosomal region of X and Y chromosomes [21]. Also the most frequent disturbance of sex development in dogs, identified in over 30 breeds, involves the syndrome of sex reversal. Such genotypical female individuals, manifesting the karyotype 78XX, have external sexual organs with their structure disturbed to variable degree (as a rule they show enlarged clitoris, frequently with a vestigial

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http://dx.doi.org/10.1016/j.prp.2015.07.010
0344-0338/

os penis (baculum) and vulva structurally resembling a prepuce) and, despite the absence of *SRY* gene, gonads in the form of testes or ovotestes containing no spermatogonia [13,14,18]. In humans with sexual disturbances the carcinogenic process frequently starts within gonads [3,5,9,15]. In animals such a phenomenon is very rare due to frequently performed castrations.

The objective of the article is to describe the histological structure of gonads in dogs showing sexual disturbances and a case of a cancer resembling gonadoblastoma in one of the animals. This study is the first one in Poland to analyse a case of gonadal tumor in a dog with disturbed sexual development.

2. Materials and methods

The study was conducted on 20 canine gonads from 10 dogs (Table 1). The lesions were detected in animals of various breeds and ages (between 7 months and 4 years), phenotypical bitches, patients of the Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, University of Environmental and Life Sciences in Wroclaw (Poland) in years 2006–2013. The gonads in each case were located in the abdomen. The laparotomy showed tubular structures resembling uterine horns and atypical gonads with tubular structures similar to epididymis, which were surgically removed and sent for histopathological examination.

The material was fixed in 10% buffered formalin, dehydrated in alcohol, made translucent in xylene, embedded in paraffin blocks and serially sectioned to 5 μm thick sections, which were routinely stained with hematoxylin and eosin. The preparations were examined under an Olympus CX-41 light microscope coupled with an Olympus DP-20 photographic camera.

3. Results

The animals under study exhibited phenotypic traits of bitches, with presence of developmental anomalies such as hyperplastic clitoris with vestigial os penis (baculum), or abnormalities in the location and structure of vulva. In these patients non-palpable testicles or undeveloped scrotum were observed. The 10 examined cases of altered gonads included 6 cases of bilateral testicles (60%), 2 cases of bilateral ovotestes (20%), one case of co-manifestation of testis and ovotestis (10%) and a single case of a testis with a neoplastically altered gonad (10%).

In cases of bilateral testes ($n = 6$) the testicles manifested a regular structure of seminiferous tubules with evident tunica propria. Numerous supportive Sertoli cells partially filled the lumen of the tubules. No spermatogonia or spermatozoa were detected.

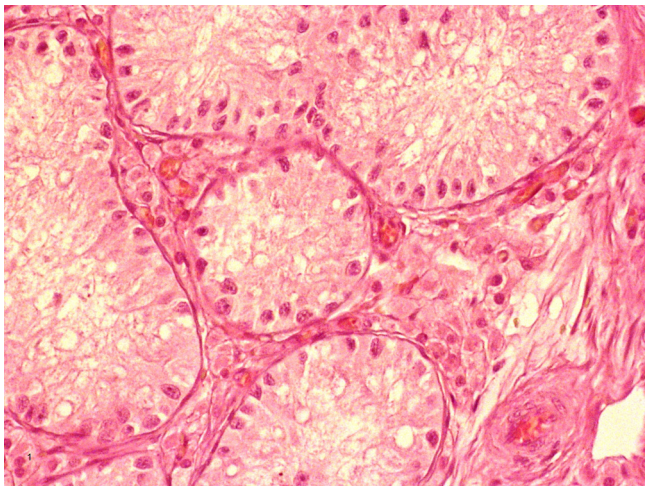

Fig. 1. Numerous seminiferous tubules devoid of spermatogonia and Leydig cells in the sublayer of connective tissue, case No.8 HE staining, magnification of 200×.

The delicate and well blood-supplied interstitial tissue contained Leydig cells (Fig. 1). Epididymes and ductuli efferentes showed a normal histological structure, lined with a normal unilayered columnar epithelium; their lumina were empty.

The structure of all examined ovotestes was similar; they varied in the dominance of testis or ovarian components. One of the gonads sampled from the Bernese mountain dog (case no. 3) was found to contain traits corresponding to testis structures, i.e. typical seminiferous tubules consisting of supporting cells, with no spermatogonia encountered. The sublayer of the connective tissue contained interstitial cells. Samples of the second gonad contained the previously described structures, i.e. a testis with no spermatogonia next to a normal ovary, in which large corpus luteum prevailed next to a single ovarian follicle.

The histological structure of the ovotestis in the Leonberger (case no. 4) contained a scanty sublayer of the connective tissue and regular seminiferous tubuli of a round cross-section, consisting of Sertoli cells and devoid of spermatogonia. Next to the structures of a male gonad, normal ovarian structures were encountered, i.e. relatively numerous normal ovarian follicles at various stages of development (mainly growing and maturing ones). Next to them, cyst-like structures were observed, filled with a protein-like substance with lutein cells present at the periphery, i.e. developing corpora lutea.

In samples of both gonads of Akita-inu (case no. 5) a few peripherally located seminiferous tubuli were noted, consisting of a few

Table 1
Summary of cases involving sex disturbances – external genitalia and gonads histopathology.

No.	Breed and age of the dog	External genitalia	Histopathological diagnosis
1	Amstaff, Age n/d	Female type, enlarged clitoris with bone	Bilateral testes without spermatogonia
2	Pug, 7 months	Female type, enlarged clitoris	Bilateral testes without spermatogonia
3	Bernese Mountain Dog, 2 years	Female type, enlarged clitoris	Testis (without spermatogonia) and ovotestis (with primary follicle and corpus luteum)
4	Leonberger, 15 months	Female type, enlarged clitoris with a bone	Bilateral ovotestes (with primary follicle and corpus luteum)
5	Akita inu, 1 year	Female type, enlarged clitoris	Bilateral ovotestes (with primary follicle and corpus luteum)
6	Mixed breed, 2 years	Female type, enlarged clitoris	Bilateral testes without spermatogonia
7	French bulldog, 10 months	Male type i.e. praeputium, wide external orifice, penis present, no scrotum, no visible palpable testicles,	Bilateral testes without spermatogonia
8	Pug, 1 year	Female type, enlarged clitoris	Bilateral testes without spermatogonia
9	Pug, 1 year	Female type, enlarged clitoris	Bilateral testes without spermatogonia
10	French bulldog, 4 years	Female type, enlarged clitoris with a bone	Testis with a disturbed structure without spermatogonia, neoplastically transformed: gonadoblastoma