Animal Reproduction Science 150 (2014) 56–61

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Histological comparison of the smooth uterine muscle of healthy golden retriever bitches, carriers of the progressive muscular dystrophy (GRMD) gene, and GRMD-affected bitches

M.P. Brolio[a,*], D.S. Cima[b], M.A. Miglino[a], C.E. Ambrósio[c]

[a] Department of Surgery, Faculty of Veterinary Medicine and Animal Science of the University of São Paulo – FMVZ-USP, Orlando Marques de Paiva, 87 street, Sao Paulo, SP 05508-270, Brazil
[b] Paulista University – UNIP, Tenente Júlio Prado Neves, 965 street, São Paulo, SP 02370-000, Brazil
[c] Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering of the University of São Paulo – FZEA–USP, Duque de Caxias Norte, 225, Pirassununga, SP 13635-900, Brazil

ARTICLE INFO

Article history:
Received 14 December 2013
Received in revised form 25 July 2014
Accepted 15 August 2014
Available online 27 August 2014

Keywords:
Muscular dystrophy
Animal model
Dog
Histology
Uterus

ABSTRACT

There is evidence to suggest that weakness of the pelvic and/or uterine musculature may negatively affect the obstetric performance of women who carry the gene for Duchenne muscular dystrophy (DMD). The golden retriever dog is the ideal animal model for preclinical studies of progressive muscular dystrophy, and this model is referred to as "golden retriever muscular dystrophy (GRMD)". This study evaluated and compared the histopathological aspects of the uterine muscle of eleven dogs: health, $n = 4$; carriers of GRMD gene, $n = 5$; and affected females, $n = 2$. The obtained results showed that the uterine muscle of healthy dogs was exclusively composed of type III collagen, while a predominance of type I collagen and small amounts of type III were observed in the uterine muscle of the carriers. The myometrium of the affected bitches showed small quantities of both collagen types. The differences noted in the three evaluated groups suggest that female carrier and those individuals affected by muscular dystrophy had collagen alteration and muscle fiber commitment in the uterine muscle, a deficiency which could directly influence the composition and function of this tissue. In addition, this information is highly relevant to the reproductive management of these animals. This data open important venues for translate reproductive protocols for women, who carry the dystrophin gene.

1. Introduction

Duchenne muscular dystrophy (DMD) is a fatal neuromuscular disease (Willmann et al., 2009) and is the most common form of muscular dystrophy (Banks and Chamberlain, 2008). DMD is caused by the absence of a protein called dystrophin, which is an important component of the dystrophin–glycoprotein complex found in the basal lamina and cytoskeleton (Wang et al., 2009). The dystrophin–glycoprotein complex is crucial for the function of smooth muscle cells, as well as for the cardiac and skeletal stabilization of these cells during contraction and relaxation. Because DMD is a genetic X-linked disease, it primarily affects males, although rare cases of affected

* Corresponding author. Tel.: +55 11 30917690; fax: +55 11 30917690.
E-mail addresses: mpbrolio@usp.br, mpbrolio@gmail.com (M.P. Brolio), dsantilli3@gmail.com (D.S. Cima), miglino@usp.br (M.A. Miglino), ceambrosio@usp.br (C.E. Ambrósio).

http://dx.doi.org/10.1016/j.anireprosci.2014.08.005
0378-4320/

females have also been reported. Individuals affected by DMD show significant changes in body composition, compared with the normal population (Palmieri et al., 1996).

Dystrophin is encoded by the DMD gene located on the short arm of the X chromosome. Deletions (65% of cases), point mutations (30% of cases), and duplications (5% of cases) in the gene cause dystrophin deficiency in all types of muscle cells (Bieber and Hoffman, 1990). Dystrophin-specific antibodies have been used to identify carriers via immunohistochemistry. Typically, a variable proportion of dystrophin-deficient fibers is observed in asymptomatic carriers, consistent with the random inactivation of X chromosomes in females (Bonilla et al., 1988). In addition, compared to other types of muscle, overall muscle weakness, cardiomyopathy has also been described as a symptom in DMD carriers (Mirabella et al., 1993) and GRMD (Chetboul et al., 2004; Kane et al., 2013). And the presence of dystrophin has been also documented in the human uterus (Medioni et al., 1991).

Although there is limited information on the effects of the lack of dystrophin in the smooth muscle, some reports of delayed gastric emptying (Barohn et al., 1988) and fatal gastric distension (Robin and de Falewski, 1963) in patients with DMD were found. Intestinal obstruction and impaired bladder function (Huvos and Pruzanski, 1967) have also been described. Dystrophin is also expressed in the smooth muscles of the arteries (Hoffman et al., 1987; Lees et al., 1994), particularly in the tunica media of blood vessels, larger than arterioles, such as muscular arteries that have thick layer of smooth muscle (Miyatake et al., 1989). The smooth muscle of dystrophin deficient contributes to vascular and dystrophic phenotype of DMD (Ito et al., 2007).

And just one article, from 1991, Ville et al. (1991) described a case report in pregnant limb-girdle patient showing microscopically analyses and immunostain of myometrium without involvement of the muscle disease.

Golden retriever dogs with muscular dystrophy (GRMD) represent the best animal model for therapeutic trials to identify potential DMD treatments (Kerkis et al., 2008; Bish et al., 2012; Araujo et al., 2013; Gaiad et al., 2014). These animals are essential for studying the development of this disease, because GRMD dogs display genes and clinical symptoms homologous to human patients (Shelton and Engvall, 2002).

A comparative evaluation of the smooth muscle functioning in the tubular organs of healthy dogs, carriers, and GRMD-affected dogs is important to identify significant changes in the function of these organs. While skeletal muscle has been extensively studied, there is a lack of information regarding the functioning of the smooth muscle of dogs affected by this disease.

Female carriers of GRMD would be expected to have, on average, half of the normal amount of dystrophin in both their uterus as in global musculature; thus, we hypothesized that this lack of dystrophin would result in detectable clinical and morphological consequences.

To investigate this hypothesis, we analyzed fragments of uterine muscle from healthy, GRMD carrier and GRMD-affected dogs for potential changes in histological composition.

2. Materials and methods

This research was performed in accordance with the ethical principles of animal research adopted by the "Ethic committee in the use of animals" of the School of Veterinary Medicine and Animal Science of the University of São Paulo" and is registered under protocol number 2282/2011.

2.1. Sample evaluation

Eleven uteri from golden retriever dogs were used. Four of these samples came from healthy bitches between one and nine years of age (animals 1H, 2H, 3H, and 4H). Five of the uteri were from GRMD carriers between the ages of three and six years (animals 1C, 2C, 3C, 4C, and 5C). The remaining two uteri were from GRMD-affected female dogs, which were nine and ten months of age (animals 1A and 2A). All of the samples were obtained from the GRMD-Brazil Kennel of the Department of Surgery, FMVZ-USP.

The uteri of healthy and carrier bitches were obtained by ovariohysterectomy, and the samples from affected females were collected during the necropsy of the animals, whose deaths occurred naturally due to the progression of muscular dystrophy. Necropsies were performed approximately 40–60 min after the death of the animals. Just the time required for preparation of the autopsy room and availability of the pathologist responsible for the procedure.

2.2. Histological processing of samples

All histological processing of the samples prior to light microscopy, as well as the hematoxylin–eosin, Masson's trichrome, and Picrosirius – Van Guienson staining protocols, were performed according to previously described methods (Gerger et al., 2010). An OLYMPUS BX60 microscope and ZEISS camera, model AxioCam Hcr, were used. Photomicrographs were performed using the KS400, 3.4, ZEISS brand, 2000 program.

3. Results and discussion

The basic morphological aspects of the uteri of healthy female dogs and the characteristics of their subdivisions, including the perimetrium, myometrium and endometrium, have been analyzed and discussed in previous studies (Ambrósio et al., 2011; Samuelson, 2007) and are therefore not addressed here. Staining via the hematoxylin–eosin, Masson's trichrome, and Picrosirius – Van Guienson methods were performed, and no differences were observed between the tissues of the three groups evaluated (Fig. 1).

Collagen is very important in maintaining the integrity, stability and elasticity of tissues and organs and also plays an essential role in the remodeling of uterine tissue during pregnancy and the subsequent involution of this tissue. The resistance to traction and the elastic resistance, both depend on the composition, arrangement, size and diameter of the collagen fibers.

Picrosirius red staining followed by polarized microscopy was used to observe differences in the