

Sonographic abnormalities in augmented bladder using porcine intestinal submucosa (SIS)

[Anormalidades ultrassonográficas em bexigas submetidas à cistoplastia utilizando submucosa intestinal suína (SIS)]

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ABSTRACT

Among the different materials for bladder augmentation, porcine intestinal submucosa (SIS) is the most widely investigated and stands out for its ability as a cell scaffold. In this context, the ultrasound examination allows the detection of changes from the surgical procedure, enabling the early verification of potential complications and evaluation of patient outcomes. The aim of this paper is to describe the main sonographic findings in dogs submitted to cystoplasty using acellular SIS and seeded with homologous smooth muscle cells at 30 (M30) and 60 (M60) days postoperatively. Sonographic changes included irregularities and thickening of bladder wall especially at M30. Additionally, were visualized urinary sediment and uroliths in animals submitted to acellular SIS cystoplasty. Abdominal ultrasonography was useful in the postoperative evaluation of animals undergoing cystoplasty with acellular or seeded SIS.

Keywords: dog, ultrasound, tissue repair, cell therapy, cystoplasty

RESUMO

Entre os diferentes materiais utilizados para a reparação e o aumento da bexiga, a submucosa intestinal suína (SIS) é o mais amplamente investigado e se destaca por sua habilidade como um arcabouço celular. Nesse contexto, o exame ultrassonográfico possibilita a avaliação pós-operatória, bem como a detecção precoce de potenciais complicações. O objetivo deste artigo é descrever os principais achados ultrassonográficos em cães submetidos à cistoplastia utilizando a SIS acelular e semeada com células musculares lisas homólogas aos 30 (M30) e 60 (M60) dias de pós-operatório. As alterações ultrassonográficas pós-operatórias incluíram irregularidades e espessamento da parede da bexiga, especialmente aos M30. Além disso, foram visualizados sedimento urinário e urólitos em animais submetidos à cistoplastia utilizando a SIS acelular. A ecografia abdominal foi útil na avaliação pós-operatória dos animais submetidos à cistoplastia utilizando a SIS acelular e semeada com células musculares lisas homólogas.

Palavras chave: cão, ultrassom, reparo tecidual, terapia celular, cistoplastia

INTRODUCTION

The indications for reconstructive surgery of the bladder include severe trauma with extensive loss of tissue, neoplasms, recurrent interstitial cystitis, inflammatory pseudotumor, neurological disorders, and congenital genitourinary abnormalities (O'Sullivan e Barrett, 1993; Atala, 2000; Koh *et al.*, 2009). Various types of grafts have been used for bladder augmentation, as biodegradable or alloplastic grafts, seeded or not

with cells. Among the different materials used, porcine intestinal submucosa (SIS) is the most widely investigated and stands out for its ability as a cell scaffold to regenerate the bladder and other tissues of the urinary tract (Yiang and Guomin, 2008).

In this context, the ultrasound examination allows the detection of changes from surgical procedure, enabling the early verification of potential complications and evaluation of patient outcomes (Hertzberg *et al.*, 1987). The

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ultrasound examination of the bladder also allows the evaluation of the shape, location and internal anatomy of the bladder, and more often the detection of uroliths and wall thickening (Gallati e Iwasaki, 2004).

Due to the limited literature on the subject and in order to provide insight for postoperative follow-up of patients submitted to cystoplasty using acellular or seeded SIS, the aim of this paper is to describe the main sonographic findings in dogs submitted to cystoplasty using acellular SIS and seeded with homologous smooth muscle cells at 30 (M30) and 60 (M60) days postoperatively.

Ten female mongrel dogs were evaluated, aged between five and nine years old, spayed, weighing between 10 and 22 pounds, and in accordance with the Ethics Committee on Animal Use (Protocol 104/2009). The animals were randomly distributed into two groups: control group (CG), consisting of five animals submitted to cystoplasty using acellular SIS; and the treated group (TG), consisting of five animals submitted to cystoplasty using SIS seeded with homologous smooth muscle cells (HSMC).

The bladder smooth muscle cells were obtained from complete thickness fragments of the bladders of animals from the CG, collected at the time of the cystoplasty. The cells were cultivated in monolayer at the bottom of culture flasks filled with Ham F-12 culture medium, added with bovine fetal serum 20% and penicillin/streptomycin associated to amphotericin B 1%, kept at a temperature of 36.7° C and CO₂ pressure of 5%. To confirm the cell type and validate the cultivation, an immunocytochemistry technique by the LSAB (Labeled StreptAvidin Biotin) method was performed. After an average of three passages and about 70% of cell confluence, the smooth muscle cells were seeded above the SIS under the same conditions described before.

The resection of complete depth, 3cm length X 4cm width, at the ventral face of the bladder was performed to the cytoplasty of animals from both groups. We used the acellular SIS (SurgiSis, Cook Biotech, West Lafayette, USA) in the animals from the CG, and SIS seeded with HSMC in the animals from the TG, sutured to the edges of the remaining bladder wall with polyglactin 910 3-0 suture. The approximation or

fixation of the omentum to the bladder was not performed.

All animals underwent abdominal ultrasound examination prior to cystoplasty and at M30 and M60. The dogs were manually restrained and positioned in dorsal recumbence. The abdominal region was clipped with subsequent topical application of acoustic gel. An ultrasound GE ® device (General Electric Company, Logic Model 3, Winsconsin, EUA) was used with multifrequency linear transducer at a frequency of 6-10MHz, documented by printing on video printer (Sony, model UP - 890 MD) and CD-rom.

The abdominal images were obtained in transverse and longitudinal planes with special emphasis on the shape and location of the bladder, and evaluating the presence or absence of masses; urinary sediment; cystoliths; thickening of the bladder wall and at the site of graft implantation; areas of irregularities at the site of graft implantation; and compartmentalization of the bladder. The other internal organs and lymph nodes were evaluated for the presence of abnormalities. All the images were evaluated by qualified ultrasonographers.

All the tests were performed using the IBM SPSS 20 software, considering $P \leq 0.05$.

At M30 thickening of all bladder walls in three animals from GC and in four animals from GT was visualized, more pronounced on the ventral side of the bladder, surrounding the graft area. Besides this, two other animals from CG presented wall thickening only in this region. The average wall thickness of the bladder in the region adjacent to the graft was 0.95 in animals from CG, and 1cm in animals from TG ($P=1,00$). In four animals from CG urinary sediment was visualized, characterized by floating echogenic spots within the bladder (Figure 1a). The same, however, was not observed in any animal from GT ($P= 0.05$).

Additionally, irregularities were observed in the implanted graft area in all animals from GC and in four animals from GT ($P= 1.00$). Hyperechoic structures suggestive of uroliths, and abnormalities in bladder format, lymph nodes and other abdominal organs were not visualized in the both groups ($P= 1.00$).

At M60 thickening of all bladder walls in three animals from CG was visualized, especially around the implanted graft, but less intense when compared to the M30 (Figure 1b). Another animal from CG also presented only thickening in the implanted graft area. In GT, a dog presented thickening of all bladder walls, more pronounced around the implanted graft area, and other four animals had only thickening in the implanted graft area. The average thickness in the region adjacent to the graft was 0.52cm in animals from CG and 0.57cm in those from TG (P= 1.00).

Besides this, irregularities in the implanted graft area were observed in four animals from the GC and in two animals from GT. Associated to these

abnormalities hyperechogenic structures of variable sizes were observed, suggestive of uroliths, in three animals from GC (Figure 1c). These structures were not observed in the animals from GT (P= 0.17). In two animals from CG the presence of urinary sediment in mild and severe amount was visualized, characterized by floating echogenic spots within the bladder. This abnormality was not observed in the animals from GT (P= 0.44). Additionally, a dog from GC showed compartmentalization of the bladder due to dorsoventral flattening (Figure 1d). No urinary sediment or compartmentalization were verified in animals from GT, as well as changes in lymph nodes and other abdominal organs (P= 1.00).

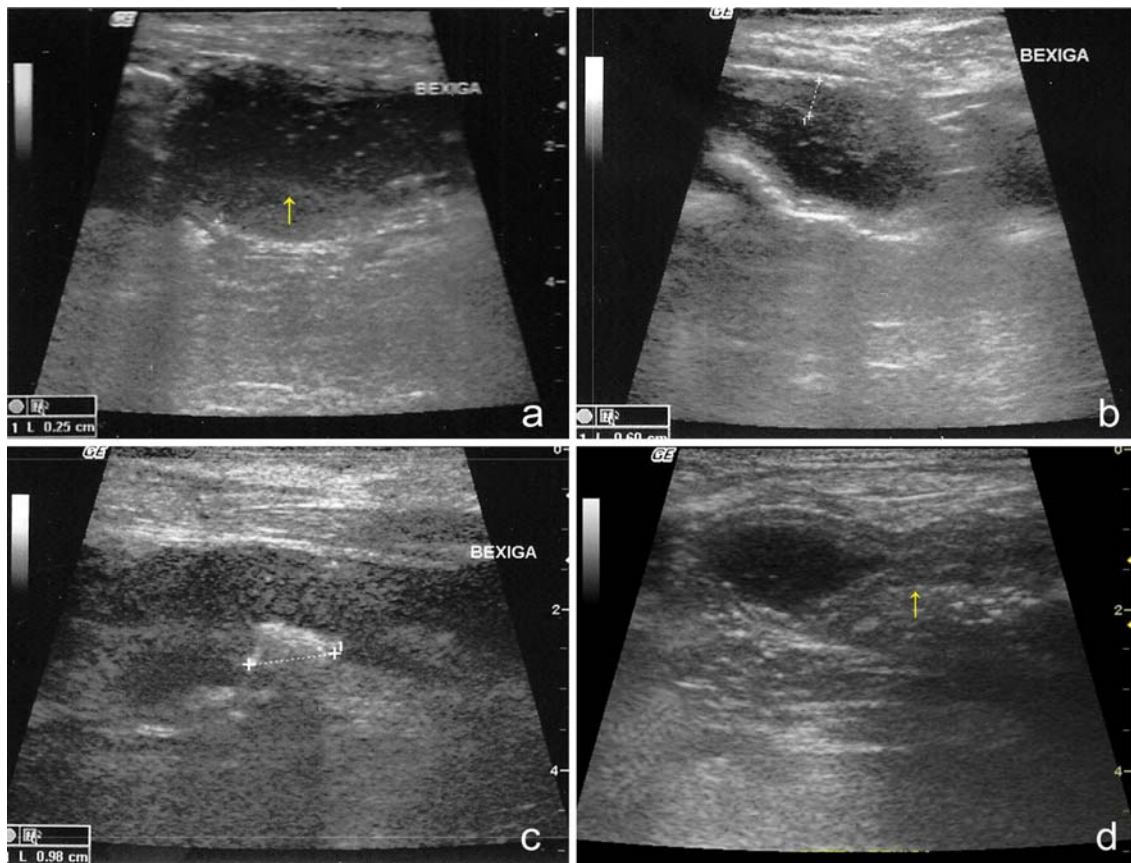


Figure 1. Dog. Ultrasonography examination of augmented bladders with acellular SIS. a) Sagittal ultrasonography plane of bladder at M30 in one animal from GC. Notice the floating echogenic spots within the bladder and the thickening of the bladder wall. b) Ultrasonography sagittal plane image of the bladder 60 days postoperatively of an animal from CG. Notice the thickened wall at the membrane implanted area. c) Sagittal ultrasonography plane of bladder at M60 in one animal from CG. Note at center the hyperechoic structures forming anacoustic shadow. d) Sagittal ultrasonography plane image of the bladder at M30 of an animal from the CG. Notice the dorsal-ventral flatness and compartmentalization of the medial third of the bladder (arrow).

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Abdominal ultrasonography performed at M30 and M60 can detect some changes arising from the surgical procedure and bladder tissue repair (Hertzberg *et al.*, 1987). These changes include irregularities and thickening of the bladder wall, and were visualized in animals from CG and TG, especially at M30 and around the implanted graft area. It is believed that these are caused by the inflammatory response inherent to the tissue repair process as well as the presence of cystitis in animals from the GC group. Dogs with cystitis or other inflammatory conditions of the bladder may present irregularities and focal or diffuse thickening of the bladder wall, whose appearance would return to normal as soon as the inflammation was interrupted (Finn-Bodner, 1995).

At M60, the bladder wall of the animals in both groups remained thickened, but its value was reduced and closer to normal. This may be due to the maintenance of the inflammatory response of lower intensity at this moment of the evaluation period. It is believed, therefore, that the bladder wall would tend to normality if the time of postoperative evaluation could be extended. In a study evaluating seven dogs submitted to cystoplasty with homologous bladder, the thickness of the bladder wall returned to normal after seven months postoperatively (Probst *et al.*, 2000). According to the authors, after this period, the bladder wall showed a similar aspect to that seen in the preoperative sonographic examination.

Associated to irregular and thickened areas, at M30 a hypoechoic cystic structure was visualized in one animal from GC, attached to the graft and compatible with a mass. This structure could be a clot secondary to cystitis or surgical manipulation, since it was not visible again at M60. The sonographic appearance of clots depends on its size and chronicity, but those adhered to the bladder wall generally have tracery internal aspect, similar to cloth (Finn-Bodner, 1995). Wall polyps, abscesses or tumors are included in the differential diagnoses, but the evolution is usually progressive (Hertzberg *et al.*, 1987; Finn-Bodner, 1995). Moreover, these structures typically exhibit vascularization, capable of identification by Doppler, which was not observed in the present study.

Additionally, four animals from the CG showed urinary sediment at M30, characterized by floating echogenic spots within the bladder, usually visualized in patients with acute bacterial cystitis due to the presence of blood, pus, and/or cellular debris in the urine (Green, 1996). Small echogenic spots are also reported in patients without infection of the urinary tract, and due to exacerbated presence of mucus (Hertzberg *et al.*, 1987).

In the present case, the abdominal ultrasonography associated with clinical and laboratory findings allowed the diagnosis of bacterial cystitis in animals from GC at M30. However, the presence of urinary sediment was also observed at 60 days postoperatively in two animals of CG without clinical and laboratory findings compatible with hematuria or cystitis. It is believed that another condition, such as urolithiasis was related to the maintenance of the inflammatory process and therefore the visualization of the urinary sediment. In fact, in three animals from CG were visualized with uroliths. Another study evaluating bladder repairing with SIS also reported concomitant presence of urolithiasis and infection of the urinary tract, and attributed the urolithiasis to the incomplete resorption of the graft, urine stasis due to loss of contractile function of the bladder or intermittent bladder catheterization (Zhang *et al.*, 2006). According to the authors, the remnants of the SIS act as nests for the aggregation of crystals and urolith formation (Zhang *et al.*, 2006). The uroliths in turn lead to irritation to the bladder wall and perpetuation of inflammation (DiBartola and Westropp, 2014), resulting in the floating echogenic spots on the ultrasonographic examination. Thus, it is suggested that no urinary sediment and urolithiasis were visualized on ultrasound assessment of the animals from TG due to a possible protective effect of seeded cells over the SIS. The cells form a protective barrier preventing the crystals dissolved in the urine to precipitate between the collagen fibers of the graft (Atala, 2000; Zhang *et al.*, 2006).

Additionally, at the histopathological evaluation, the CG showed fewer muscle fibers than the TG, arranged in an irregular manner. This could result in lower contractile bladder capacity and therefore higher residual, thus contributing to the

occurrence of cystitis and urolithiasis (Rossetto et al., 2013).

As for intermittent bladder catheterization, this does not apply to this study, since the urine collection was made through cystocentesis guided by ultrasound at specific moments.

In an animal of the GC group at M60 a compartmentalization of the bladder was visualized. It is believed that this is due to incomplete and deficient resorption of the membrane, which was protruded into the bladder lumen. In a study, compartmentalization is reported due to adhesion formation in a patient undergoing multiple surgeries and recurrent urinary tract infection (Hertzberg et al., 1987). The animal in question, however, underwent one procedure, despite having had bacterial cystitis, the infection is cleared without complications after appropriate medical therapy instituted.

Abdominal ultrasonography was useful in the postoperative evaluation of animals undergoing cystoplasty with acellular SIS and seeded with HSMC bladder, and allows early identification of complications such as cystitis, urolithiasis and compartmentalization of the bladder.

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