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Experimental Model of Intestinal Endometriosis: Pilot Study

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Authors' contributions

This work was carried out in collaboration between authors. Authors LGBR, RSH and MA designed the study, wrote the protocol, performed the procedures and wrote the first draft of the manuscript, authors RRZ and TDK performed the statistical analysis, and managed literature searches. Author AS performed the pathologic analysis. All authors above read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this study is to create an experimental model of intestinal endometriosis in pigs, which might allow better understanding of deep infiltrating endometriosis and development of new treatment techniques. As secondary objective, we intend to create endometrial implants accessible by transrectal ultrasonography (TRUS).
Study Design: Surgical experimental study in swine.
Place and Duration of Study: This study was performed at the Instituto de Ensino e Pesquisa do Hospital Sírio-Libanês, São Paulo, Brazil, between January 2012 and December 2012.
Methodology: Two sexually mature female *minipigBR* pigs underwent two laparotomies (each animal). The first laparotomy was performed to implant two fragments of autologous endometrium in the rectal wall. The second one was performed thirty days later to visualize, measure and obtain

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tissue of the site of the implants for histopathology study. A TRUS study was performed prior to the second surgery. The Institution's Animal Utilization Study Committee approved the study.
Results: In the first laparotomy a 5-cm segment of right uterine horn was resected. The endometrium was separated from the myometrium through sub-endometrial saline injection. Two endometrial fragments (1.0 x 2.0 cm) were dissected and sutured in the intra peritoneal anterior rectal wall of the animals. Thirty days later, all implants were identified during preoperative TRUS. "En-bloc" resection of the intestinal segment with the implants was performed during the second surgery. The autologous implants of endometrium invaded the muscular layer in one of the two animals.
Conclusion: We demonstrated that the creation of an animal model of deep infiltrating endometriosis with intestinal involvement is feasible through a simple surgical technique. We believe that this model can be applied in experimental and clinical studies but further studies are necessary to refine the technique.

Keywords: Endometriosis; deep infiltrating endometriosis; experimental animal model; swine.

1. INTRODUCTION

Endometriosis is a gynecological benign disease characterized by the presence of glandular or stromal endometrial tissue outside the uterine cavity with prevalence ranging from 7 to 10% in the general population and affecting women during reproductive age [1-3].

Deep infiltrating endometriosis occurs when the endometrial lesion infiltrates 5 mm or more into the peritoneal surface [4]. The intestinal endometriosis has an insidious evolution with unspecific signs and symptoms, such as abdominal pain and bowel habit alteration. The sigmoid, rectum and rectovaginal septum are usually affected [5].

Endometriosis is diagnosed through the histological analysis of the samples obtained by invasive methods such as laparoscopy or laparotomy [6]. Clinical treatment of deep infiltrating endometriosis is controversial; with partial and temporary relief of the symptoms during treatment. Surgical treatment with resection of the endometrial nodules or the affected segment of the bowel is the most effective treatment for symptomatic patients. Ultrasonography has been used to evaluate deep infiltrating endometriosis in the bowel and trans rectal endoscopic ultrasonography with fine needle aspiration allows cytological evaluation [7].

Experimental models of endometriosis use rabbits, mice and swine. Autologous tissue transplantation is used, with the animal's topic endometrium resected and usually implanted in the peritoneum of immune-competent animals [8-10]. To our knowledge, there is no animal experimental model of intestinal deep infiltrating endometriosis.

The objective of this study is to create an experimental model of intestinal endometriosis in pigs, which might allow better understanding of deep infiltrating endometriosis and development of new treatment techniques. As secondary objective, we intend to create endometrial implants accessible by transrectal ultrasonography.

2. MATERIALS AND METHODS

2.1 Animals

We used two female *minipig*BR1 animals from *Fazenda Tres Marias, Campina do Monte Alegre – Itapetininga, São Paulo, Brazil,* weighing approximately 30 kilograms (27-32 kilograms) and aged at least 11-months. These swine reach sexual maturity when they are 5 to 7 months old [11]. The animals were kept in rooms under light control, with 12 hours/day of light exposure, ambient temperature controlled between 20 and 25ºC, and variable relative humidity between 40 and 60%. The animals were fed with 400 g of daily ration of food divided in two meals and water *ad libitum*.

2.2 Anesthesia, Analgesia and Euthanasia Protocol

Midazolam (0.5 mg/kg) and Ketamine IM (5 mg/kg) were administrated as pre-anesthetic medication. Deep anesthesia was induced with 5 mg/kg propofol and 0.5 mg/kg morphine IV and maintained with inhalatory 2% isofluorane after orotracheal intubation. Post-surgery analgesia was performed with intravenous meloxicam 0.1 mg/kg daily, scopolamine 25 mg/kg twice a day

and tramadol 2 mg/kg every 12 hours for 3 days. Antibiotics prophylaxis was performed with azithromycin at 15 mg/kg through IV injection. Euthanasia was performed according to the institution´s protocol with 5% inhaled isofluorane and 19.1% potassium chloride IV at 6 mg/kg dose.

2.3 Surgical Procedures

We performed two surgeries in each animal. The first one was the endometriosis induction surgery. The second laparotomy was performed thirty days later, to visualize and measure the endometrial implant and obtain tissue sample for histopathology study. A transrectal ultrasonography study was performed with a 7.5 MHz rigid endocavitary transducer, prior to the second surgery, to evaluate the endometrial implant.

2.3.1 First laparotomy – endometrial implants induction

After anesthesia, a 5-cm median incision was performed just above the pubic symphysis, to gain access to the abdominal cavity. After identifying the bladder, it was punctured with a 19G needle and drained. The uterus was identified and a 5-cm segment of the right uterine horn was resected. A longitudinal incision was performed in the uterine horn. The endometrium was separated from the myometrium through subendometrial injection of 5 ml of 0.9% saline solution with a 25G needle. The endometrium was dissected and cut with a pair of scissors and two fragments with 1.0 x 2.0 cm were delimited. The remaining portion of uterine fragment was sent for histopathology analysis. We used two fragments of endometrium per animal. To define the best position to suture the fragments, we introduced an ultrasound probe into the rectum and identified the rectouterine reflection through direct visualization of the compression of the ultrasound probe. We cut and opened up the serous layer of the rectal wall and suture the two endometrial fragments, one close to the rectouterine reflection and the other 1 cm apart to create subserous implants, as we wanted the implant to infiltrate into the muscular layer (Fig. 1). The abdominal wall was closed in layers. Animals showed no relevant post-operative complications.

2.3.2 Ultrasound evaluation and second laparotomy

After 30 days, the animals were anesthetized and a transrectal ultrasonography was performed (Hitachi EUB 405 – EUP V33 transducer). The second laparotomy was performed with the same anesthesia as the first one and complete rectal amputation was performed. The material obtained in the surgery was sent for pathology study. Euthanasia was performed after the second surgery.

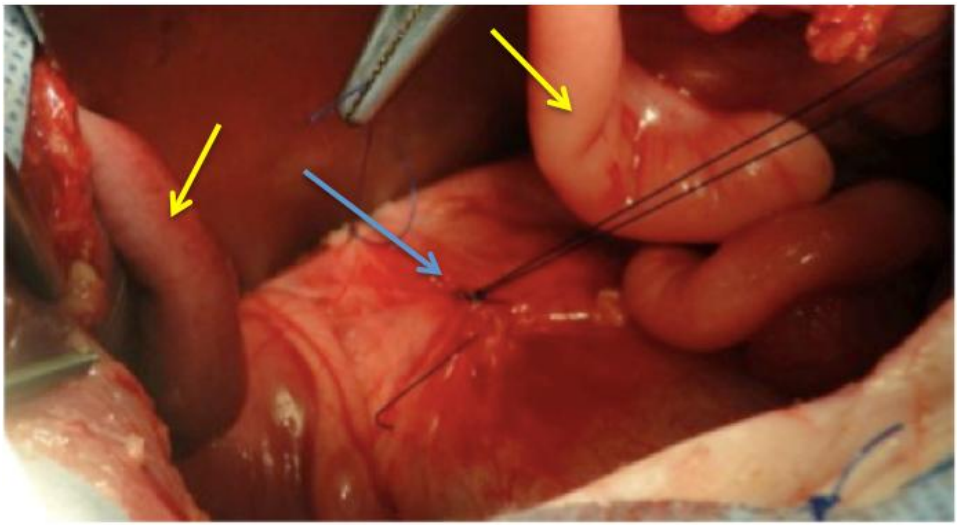

Fig. 1. Suture of the endometrial fragment in the rectal wall during 1st surgery

Fig. labels: yellow arrows: uterine horns; blue arrow: anterior rectal wall - point of endometrial implant suture

3. RESULTS AND DISCUSSION

3.1 Results

During transrectal ultrasonography, we tried to characterize the endometrial implant as hypoechoic lesions or thickening of the muscularispropria layer of the rectum. We gave liquid diet the day prior to the procedure and two rectal injections of 120-ml soluble sodium phosphate, one hour prior to the procedure. Endometrial lesions were easily identified by ultrasonography (Fig. 2) in both animals. Since the animals had implants close to the rectouterine reflection, we chose to perform rectal amputation. The implants were examined macroscopically and measured. The resected material was sent for histopathology study (data not shown).

3.1.1 Macroscopic and histopathological results

The endometrium fragments obtained in the first surgery had different macroscopic characteristics. The endometrium had bluish-purple color in animal 1 and whitish color in animal 2 (Fig. 3). The histopathology analysis of the uterine segments resected in the first surgery demonstrated proliferative endometrium (tubular glands with columnar cells) in both animals, even though the macroscopic appearance were different.

In the second surgery, all implants were identified visually. The endometriosis implants invaded the muscularis propria layer of the rectum in one of the two animals (Fig. 4). The details are shown in Table 1.

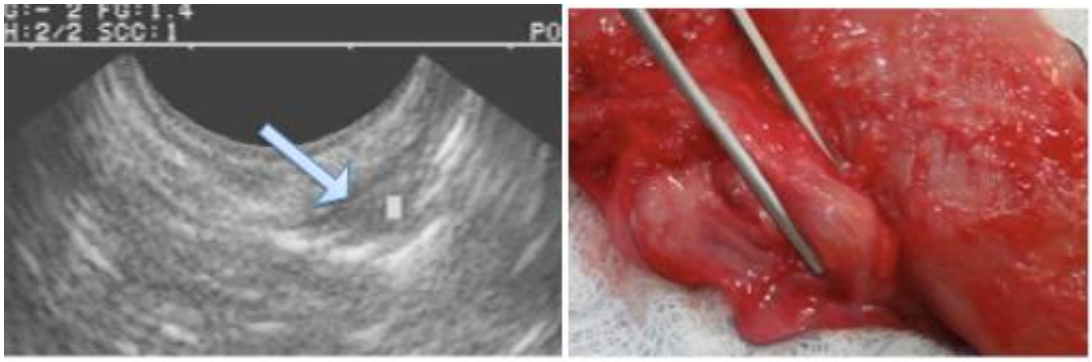

Fig. 2. Preoperative TRUS identification of the endometriosis implant in the rectal wall (left arrow) and macroscopic identification during 2nd surgery (right) - animal 1

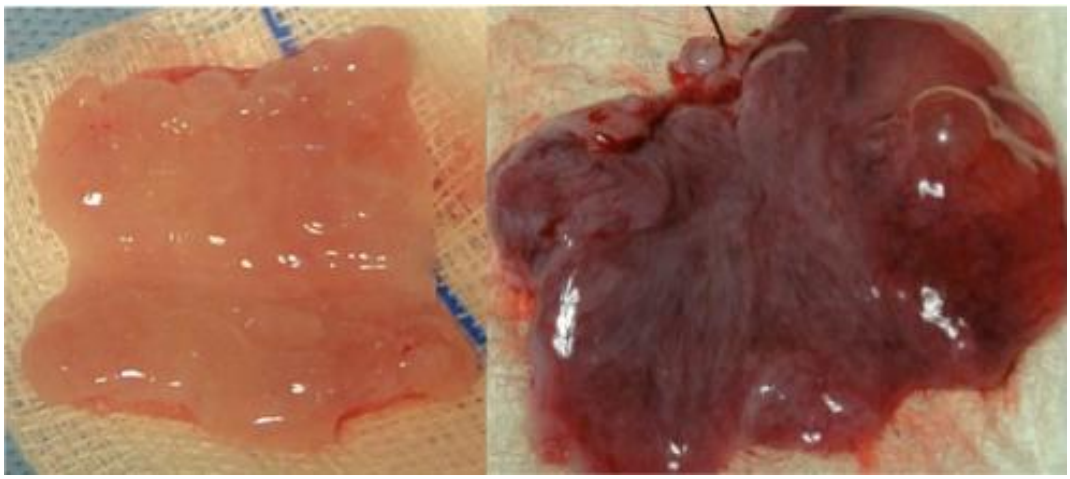

Fig. 3. Macroscopic appearance of the endometrium: left: animal 2, whitish; right: animal 1, bluish-purple

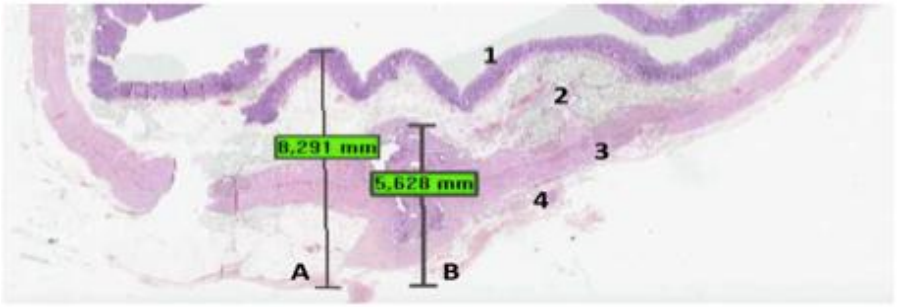

Fig. 4. Histology slide of the endometrial implant – 2nd surgery
A: intestinal wall, B: endometrial implant invading the submucosal layer.
1: mucosa, 2: submucosa, 3: muscularispropria, 4: serosa/subserousspace.

Table 1. Characteristics and results of the animals used in the experiment

Animal	Endometrium macroscopic appearance (1st surgery)	Implant size after surgery (in cm)	Pathology after 2nd surgery	Muscularis propria invasion	Lymph node status
1	Bluish-purple coloration	0.8 x 0.6 x 0.6 1.5 x 1.4 x 0.6	Mixed and poorly differentiated histological pattern	Yes 2/2 implants	2/4 positive for endometrial cells
2	Whitish coloration	2.8 x 1.5 x 1.2 2.1 x 1.5 x 1.2	Mixed histological pattern	No	Non found

3.2 Discussion

The objective of this study was to create an animal model of the intestinal involvement of the deep infiltrating endometriosis.

The non-human primate is the only animal species that presents spontaneous endometriosis, similar in histology and location to the human endometriosis in 25% of cases. But the high cost to obtain and maintain primates, transforms its use for research impracticable [9].

There are animal models of peritoneal endometriosis in rabbits and rats but the small abdominal and pelvic cavities of these animals do not allow for the use of instruments designed for use in humans and would not allow us to adequately perform the surgical technique to create the endometrium implant in the intestinal wall [9,10].

We elected swine as the animal model as it is widely used as models for other surgical experiments and extensive experience in maintaining these animals in captivity. The costs of obtaining and maintaining swine are relatively low as compared to other animals, like non human primates. The wider pelvic cavity of swine, when compared with smaller animals, facilitates the manipulation and surgical implant of endometrial tissue in the intestinal surface. There is also the possibility of using devices designed for human use, like ultrasonography transducers that we used to evaluate the implants before the surgery in the second procedure.

In our sample, although we identified the implants in the second laparotomy in both animals, we could induce endometrial implants that invaded the muscular layer in only one of the two animals.

In animal 2, there was only adherence to the muscular layer without invasion. Reasons for these findings are unknown. Additional experiments with more animals are needed for conclusive interpretations.

The histopathology showed proliferative endometrium in the samples of the endometrium used to create the implants in both animals

although they had different macroscopic aspect during the first surgery. This proliferative endometrium lead us to believe that we reproduced active endometriotic tissue which is similar to histological patterns of endometriosis. We used sexually mature animals but did not evaluate or manipulate the animals' estrus phase. There is hormone and inflammatory interaction with a complex microenvironment in endometriosis and it must have to be taken into account that hormone status is fundamental for the development of endometriosis and only the suture of small fragments of endometrium into the intestinal wall may not be sufficient to simulate the disease seen in humans [4]. The hormonal induction may improve growth and provide better quality implants [9].

Animal 1 had implants that invaded muscular layer and presented poorly differentiated mix pattern endometriosis in the histology analysis. This pattern may explain the invasion of muscular layer and lymph node involvement. Further studies may explain why the animals had different degrees of subtypes of endometriosis.

The intention of creating two implants in each animal was to demonstrate that each implants might have unique behavior. In our study, both implants had the same behavior, but this finding could not be sustained in larger samples.

We demonstrated that the creation of an animal model of deep infiltrating endometriosis with intestinal involvement is feasible through a simple surgical technique. Further studies are necessary to refine the technique, which can prove useful in the future. One of the main purposes for the development of an experimental model is to test the feasibility and safety of local treatment. Local treatment for endometriosis may be very useful in cases of rectovaginal septum or distal rectal wall involvement, in which cases the standard surgical treatment, rectosigmoidectomy, involves serious risks of complications like sphyncter dysfunction. Since the endometriosis implants were identified by transrectal ultrasound, one of the methods for local treatment could be ultrasound-guided injection of drugs or hormone particles. Local hormone treatment could prevent the use of high dose systemic hormones and its side effects and induce reduction on endometriosis lesions' size as neoadjuvant therapy, providing a more conservative surgical approach.

4. CONCLUSION

We demonstrated that the creation of an animal model of deep infiltrating endometriosis with intestinal involvement is feasible through a simple surgical technique. We believe that this model can be applied to experimental and clinical studies but further studies are necessary to refine the technique.

ETHICAL APPROVAL

This study was approved by the Institution´s Animal Utilization Study Committee, under number CEUA 2012/22.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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