Effect of fibrin glue and comparison with suture on experimental induction of endometriosis in a rat endometrial autograft model

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Summary

Objective: The effects of fibrin glue (FG) and suture were investigated and compared with experimental induction in an endometriosis model. *Material and Methods:* A randomized, controlled, and double-blind study was performed with 25 adult female Wistar Albino rats. Two autologous endometrial grafts were obtained from each of the rats. The endometrial grafts were transplanted by gluing with FG on the right abdominal wall and suturing with only 5/0 prolene on the left in ten rats. Gluing+suturing and after suturing over the covering with FG of the endometrial graft were performed, respectively, on the right and left in another ten rats. Covering with FG glue of the endometrial graft was performed in another five rats. The endometriosis-like lesions and intraperitoneal adhesions were evaluated macroscopically and histopathologically. *Results:* The mean volume (31.4 ± 17.3), adhesion (0.8 ± 0.7) and inflammatory reaction (1.2 ± 0.7) score of the implants in the group using only FG were significantly lower than in the group using suture [respectively, (49.2 ± 20.6), (2.4 ± 0.8), (2.2 ± 0.8)] ($p < 0.05$). *Conclusions:* Our results demonstrate the general feasibility of reproducible and reliable endometrial graft fixation with FG onto the inner abdominal surface in rats. Furthermore, several advantageous characteristics could be demonstrated such as less inflammation and fewer adhesions.

Key words: Fibrin glue; Suturing; Animal model; Endometriosis induction.

Introduction

Fibrin sealants can be used for hemostasis, wound closure, and tissue sealing and they are not associated with inflammation, foreign body reactions, tissue necrosis, or extensive fibrosis [1]. Fibrin sealants contain the active components thrombin and fibrinogen that, when mixed together, form a fibrin clot. The wound healing properties of fibrin sealant may be attributed to the ultrastructure of the fibrin sealant matrix which, like a normal fibrin clot, allows for diffusion of nutrients and cytokines and subsequent vascularization [2, 3]. Fibrin sealants which have been established include cardiovascular surgery, thoracic surgery, neurosurgery, plastic surgery, and dental surgery. There are, as yet, few clinical reports of the application of fibrin sealants to endometriosis surgery [4, 5].

The conventional method of an animal endometriosis model after endometrial excision is mostly the fixation of endometrial grafts using suture [6]. Sutures provide point fixation of the graft but not continuous adherence to and between endometrial and peritoneal surfaces. This situation draws attention to the use of fibrin glue (FG) for skin grafts [7]. On the other hand the use of FG for induction of endometriosis is interesting for us. This study was designed to observe the possible effect of FG on induction of endometriosis in an animal model.

Materials and Methods

The Cumhuriyet University Committee on the Use and Care of Animals approved the experiments, and all investigations complied. Twenty-five mature, female, non-pregnant Wistar Albino rats weighing between 220 and 280 g were used. Animals were provided by Cumhuriyet University Animal Reproduction Centre and housed in the Animal Laboratory of Cumhuriyet University. They were caged in a controlled environment at 22°C with 12 h light/dark cycles. Standard rat feed and water were provided ad libitum. All rats were allowed to acclimatize to this environment for one week before the experiment.

Rats were anesthetized by intraperitoneal administration of 60 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey). Before surgery, the abdominal skin was shaved and antisepsis was obtained with 10% povidone iodine solution. Using a sterile technique, a 3-4 cm ventral vertical incision was made to expose the reproductive organs. A 12 mm segment of the right uterine horn was excised after devascularization and ligation using 4-0 prolene and placed in sterile phosphate-buffered saline (PBS) at 37°C. Ectopic endometrium was induced surgically in rats by transplanting an autologous frag-

Revised manuscript accepted for publication October 20, 2011

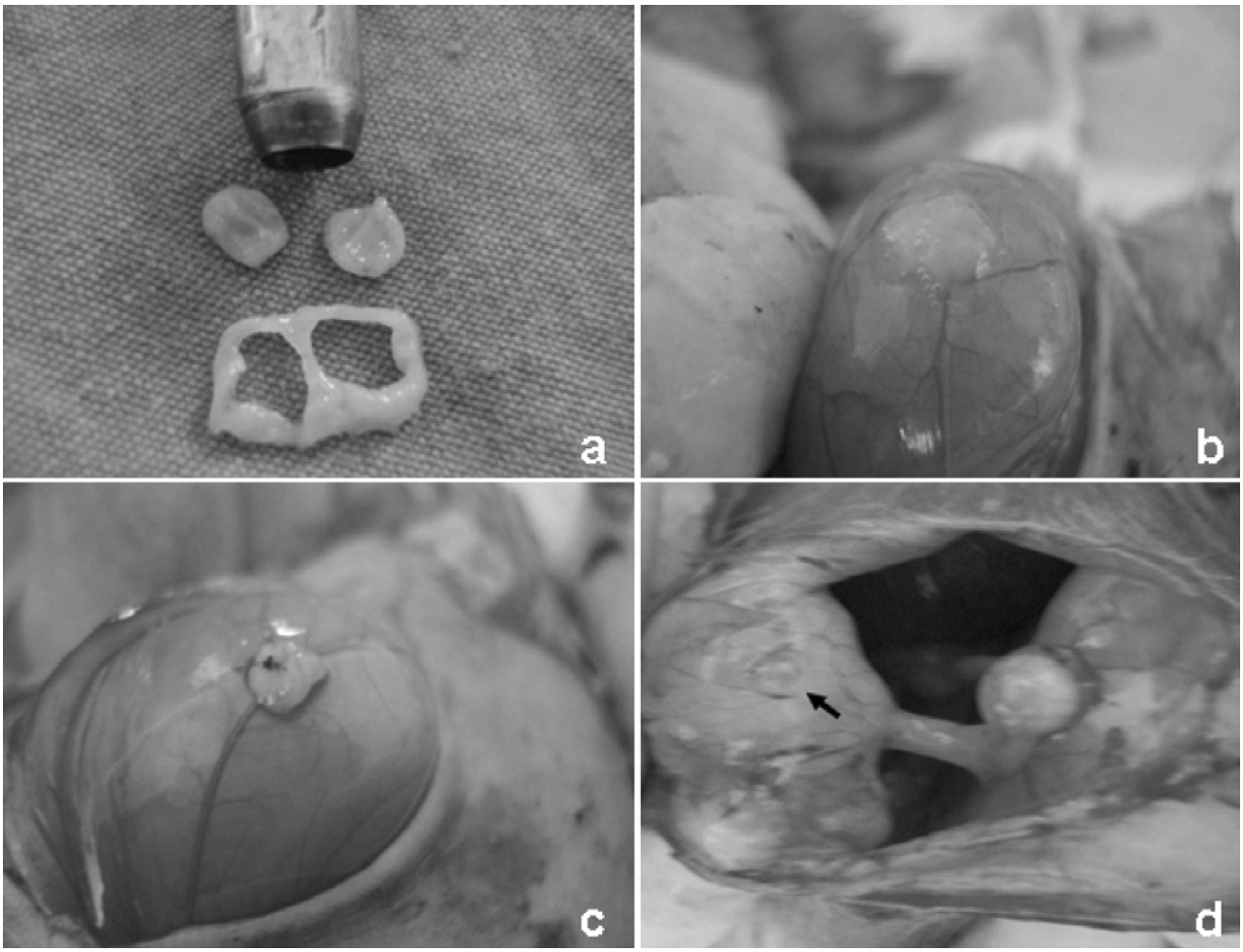

Figure 1.
a) Graft preparation from the rat endo-myometrium. b) Gluing of the graft to the lateral inner abdominal wall with fibrin glue. c) Fixation of the graft to the lateral inner abdominal wall with suturing. d) Endometriosis-like lesions in group PFG (marked with arrow) and in group OS in the same rat.

ment of endometrial tissue onto the inner surface of the abdominal wall. Five mm in diameter circular endometrial pieces (surface area = 19,625 mm^2) were made by a cutting device without removing the myometrium (Figure 1a). The cutting device was designed by one of the authors and made by a gun repair workshop in Sivas. In ten rats, two pieces of uterine tissue were transplanted respectively, by gluing with FG (PFG group) and only suturing (OS group) with single non-absorbable 5-0 prolene sutures onto a vascular area of the inner surface of the right and left abdominal wall with the endometrial surfaces (Figure 1b-c). In another ten rats, endometrial grafts were transplanted with FG+suturing (PFG-S group) on the right and transplanted then by suturing over the covering with glue (S-OCFG group) on the left. In five rats, endometrial grafts were transplanted over the covering with FG (OCFG group) on the right and left abdominal inner wall. The vertical abdominal incision was closed with the use of two-layer polyglactin sutures. After the operation, all rats were observed for 14 days without medication.

Rats were euthanized by ketamine anesthesia and a second laparotomy was performed on day 15. Then all the observations about adhesions were scored according to Blauer's scoring system [8]: 0 = no adhesion; 1 = thin, easily separable adhesions; 2 = thick adhesions limited to one area; 3 = thick and widespread adhesions; 4 = thick and widespread adhesions plus adhesions of viscera to the anterior/or posterior of the abdominal wall. During laparotomy, implant volumes were calculated in vivo by measuring their dimensions (length, width, height) with a micrometer and using the ellipsoid volume formula (π/6x length x width x height). The implants were then excised and fixed in 10% formalin for histopathological examination. All operations, scoring of adhesions and measurements were performed by physicians blinded to the study treatment.

Samples from the endometriosis areas were fixed in 10% formaldehyde for a minimum of 12 h. After routine procedures, specimens were embedded in paraffin and cut into 5 µm sections. Sections were stained with hematoxylin and eosin according to standard laboratory procedures. The amounts of glandular tissue (GT) and stromal tissue (ST), inflammatory reaction (IR) and angiogenesis in the implants were histopathologically examined. The GT and ST was scored from 0 to 3. The GT score was zero if there was no gland per 10 hpf, 1 in one gland, 2 in two to three glands, and 3 in four glands. The ST score was zero if there was no ST per 10 hpf, 1 in < 25%, 2 in 25-50%,

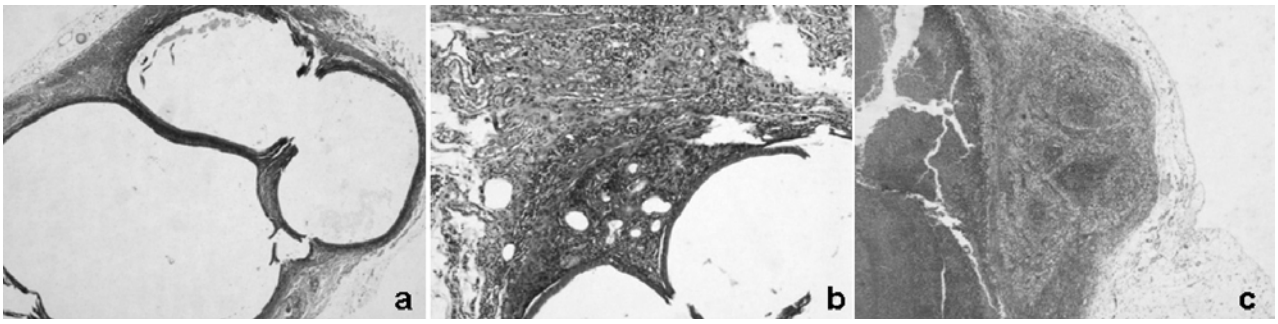

Figure 2.
a) Endometriosis-like lesion with light inflammation in group PFG (H-E; x 40). b) Endometriosis-like lesion with moderate inflammation in group OS (H-E; x 100). c) Massive inflammation that destructs the glandular tissue of endometriosis-like lesion in group OS (H-E; x 40).

Table 1. — *Comparison of macroscopic and histopathologic results*.

Groups	PFG (N: 10)	OS (N: 10)	PFG-S (N: 10)	S-OCFG (N: 10)	OCFG (N: 10)
Volume mm^3	34.4 ± 16.6	54.7 ± 20.9	48.4 ± 19.4	44.5 ± 22.2	28.3 ± 18.4
Adhesion[b] scores	1 ± 0.8	2.6 ± 0.9	2.2 ± 0.7	2.5 ± 0.9	0.7 ± 0.6
IR[c] scores	1.5 ± 0.7	2.6 ± 0.6	2.2 ± 1.0	1.9 ± 0.8	1 ± 0.6
GT scores	1.8 ± 0.7	1.1 ± 0.8	1.6 ± 0.	1.7 ± 0.9	1.5 ± 0.7
ST scores	0.9 ± 0.5	1.4 ± 0.8	1.2 ± 0.9	1.6 ± 0.8	1.2 ± 0.4
Microvessel density	12.9 ± 3.3	11.0 ± 4.1	13.0 ± 4.3	12.2 ± 5	11.7 ± 4.7

N: number of endometriotic lesions; GT: glandular tissue; ST: stromal tissue; IR: inflammatory reaction; [a, b, c] *p* value for Kruskal-Wallis; [a]p = 0.03; [b]$p < 0.001$; [c]p = 0.002.

Table 2. — *Groups created based on the use of suture. Comparison of the macroscopic and histopathologic results*.

Group	FG (N: 20)	S (N: 30)
Volume mm^3	31.4 ± 17.3	49.2 ± 20.6
Adhesion[b] scores	0.8 ± 0.7	2.4 ± 0.8
IR[c] scores	1.2 ± 0.7	2.2 ± 0.8
GT scores	1.6 ± 0.7	1.4 ± 0.8
ST scores	1 ± 0.5	1.4 ± 0.8
Microvessel density	12.3 ± 4	12 ± 4.4

N: number of endometriotic lesions; GT: glandular tissue; ST: stromal tissue; IR: inflammatory reaction; [a, b, c] *p* value for Kruskal-Wallis; [a]p = 0.03; [b]$p < 0.001$; [c]p = 0.002.

and 3 in > 50%. At magnifications of x10 and x100 the IR was scored as mild (score 1), moderate (score 2) or severe (score 3). To evaluate angiogenesis, vessels were immunohistochemically highlighted using CD31 antibody. Immunostaining was performed on 5 µm-thick, formalin-fixed, paraffin-embedded tissue sections of endometrosis. Sections were deparaffinized in xylene and dehydrated through graded concentrations of ethanol. After blocking of endogenous peroxidase activity with 3% hydrogen peroxide for 15 min, the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 min. The slides were allowed to cool to room temperature, and non-specific binding was blocked with normal horse serum for 20 min at room temperature. The sections were further incubated with the primary antibody against CD31 (rabbit polyclonal, Cat. #RB-10333-R7 Thermo Scientific USA) for 30 min. The sections were then stained using the avidin-biotin complex (ABC) immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA), for demonstration of binding sites where AEC chromogen was applied. Phosphate buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer's hematoxylin. Microvessel density was determined by counting the number of CD31 positive microvessels in an entire 1-mm core. A microvessel was defined as any endothelial cell or endothelial cell cluster staining positive for CD31.

All tissues were evaluated by the same pathologist, who was blinded to the origin of the samples.

Statistical analysis: for statistical analysis the Kruskal–Wallis test and one-way analysis of variance (ANOVA) Tukey's method were used.

When the groups were created based on the use of suture, two groups were formed. One was the FG group (FG group = group PFG+ group OCFG) and the other was group S (group S = group OS+ group PFG-S + group S-OCFG). The data of these groups were analyzed by the Mann-Whitney test.

Spearman's test was used for correlations. Values are expressed as mean ± SD; significance was defined as $p < 0.05$.

Results

The standardized surgical procedures were well tolerated by all animals and none of them died. There was an infected area on one laparotomy site in the form of an abscess. In the group OCFG, using fibrin adhesive without sutures, one endometrial graft did not remain attached to the abdominal wall. It was found near the cecum whereas the rest of the grafts remained stable in groups PFG and OCFG.

There were significant differences between groups for volume, adhesions and inflamation reaction (IR). There were no differences between groups for GT, ST and microvessel density (Table 1). When the five groups were compared in pairs the adhesion score of the implants was lower in group PFG and OCFG than in all other groups ($p < 0.05$). There were no other differences between group PFG and OCFG and between each other group. The mean volume of the implants in group OCFG was significantly lower than in group OS (respectively 28.3 mm^3 and 54.7 mm^3) ($p < 0.05$). The IR score was significantly lower in Group OCFG than in group OS and group PFG-S ($p < 0.05$). There was no correlation for data in these five groups

When the groups were created based on the use of sutures, the mean adhesion score, volume and IR score of the implants was lower in group FG than in group S ($p <$

0.05) (Table 2). There was a positive correlation between adhesion score and IR score in group FG (R = 0.53, $p < 0.05$). Also when the data were not grouped there was a positive correlation between adhesion score and IR score (R = 0.51, $p < 0.001$)

We observed more glandular tissue in endometriosis-like lesions in the FG group than in the S group (respectively, 1.6 ± 0.7, 1.4 ± 0.8), and vice versa for stromal tissue (respectively, 1 ± 0.5, 1.4 ± 0.8) (Figures 5/6). Also we observed that nearly all glandular tissue could be destroyed in highly inflammed lesions (Figure 7). Thus we noted endometriosis-like lesions to be histopathologically cleaner in the FG group than in group S. However this finding was observed histopathologically.

Discussion

In this study, it was observed that foci of endometriosis obtained by using only FG were smaller, clearer, less inflammed and less adherent to other intraabdominal tissue than by suturing (Figure 4). Autologous fragments of endometrial tissue were transplanted onto inner surfaces of the abdominal wall by FG and/or suturing. Thus, two endometriosis foci were obtained by two different methods in one rat. Transplanted endometrial fragments were achieved in a circular form and, as well, as exactly equal by instrument design. With these methods, the numbers of subjects used in the study were reduced, while at the same time in every way equal to the creation of a model of endometriosis. These methods made it easy to compare and evaluate the results of the experiment.

Most homologous murine models of endometriosis are based on surgical implantation of endometrial tissue at different sites within the peritoneal cavity of recipient animals [6]. Implantation of endometrial tissue on peritoneal surfaces is done mostly with the help of a suture. Endometriosis foci can be obtained via intraperitoneal injection or by subcutaneously placing endometrial fragments without suturing [9, 10]. However, the purpose of suture is to determine in advance the location of the focus of endometriosis. On the other hand suturing provides only point fixation, if there is a large endometrial fragment, and it may fully prevent "touching" by between the endometrium and peritoneum.

Gibran *et al*. [7] used fibrin sealant for sheet skin grafts in patients with burns thus drawing attention to this situation, and continuous adherence to the wound bed which is optimal for adhesions and vascularization. In this study endometrial fragments were designed as circular and their surface area was 19,625 mm^2. In some studies, endometrial fragments were designed as square and sized 25 mm^2 [11]. Use of endometrial fragments in that form and prolene suture may create "touching" problems in corners.

Hiratu *et al*. [12] drew attention to the fact that in homologous and heterologous types of animal models, endometriotic-like lesions which are identified histologically are sometimes too unclear to distinguish from surrounding normal tissue. We concluded that inflammation contributes to this situation. On the other hand, inflammation may cause difficulty for histopathological evaluation. In this study histopathological examinations showed more intense adhesions and inflammation in all groups in which suturing was used and these findings suggest that suturing induces inflammation and adhesions. We concluded that the reason that larger implants were obtained when suturing was used induced more inflammation. In groups, in which only FG was used, there was no adhesion in seven of 20 subjects. Grade 1 adhesion was observed in nine and grade 3 and 4 adhesions were not seen in those subjects. The findings of the study supports the argument that FG may be a barrier to adhesions, as proposed in other experimental studies [13, 14]. Another conclusion extracted from the results of the study is that FG may have an adhesion preventing effect, at least it does not induce adhesions.

Although Brown *et al*. [2] reported that FG does not impair the supply and vascularization in wound healing, it was uncertain whether attachment of endometrial and peritoneal surfaces via FG impaired the supply and vascularization in endometrial grafts. Findings of the study showed that endometriosis-like lesions may successfully be obtained by using FG. Microvessel density was similar in all groups, which means that FG does not have a deleterious effect on angiogenesis and vascularization.

Two clinical studies were reported that had reduction of adhesions with FG after laparoscopic excision of large ovarian endometriomas [4, 5]. However two reviews about clinical use of FG did not mention the use of FG in gynecology [15, 16]. Endometriosis surgery is a difficult operation and it may cause excessive bleeding, whereas postoperative adhesions create major problems in infertility. When considering the hemostatic features of FG, it can be concluded that it will have more use in endometriosis surgery.

Conclusion

Finally, endometriosis-like lesions can be obtained by FG in animal models. The use of FG does not impair the supply and vascularization of endometrial grafts. The endometriosis-like lesions obtained via this method were smaller, cleaner, and included fewer inflammatory reactions and adhesions to other organs. Therefore wider clinical use of FG for endometriosis surgery may be possible.

References

[1] Jackson M.R.: "Fibrin sealants in surgical practice: An overview". *Am. J. Surg.,* 2001, *182* (2 suppl.), 1S.

[2] Brown D.M., Barton B.R., Young V.L., Pruitt B.A.: "Decreased wound contraction with fibrin glue–treated skin grafts". *Arch. Surg*., 1992, *127,* 404.

[3] Grabosch A., Bogusch K., Plogmeier K., Ollinger R.: "In vivo studies of fibrin sealant ultrastructure". In: Schlag G., Redl H. (eds.). Fibrin Sealing in Surgical and Nonsurgical Fields. Vol. 1: Wound Healing. Berlin: Springer-Verlag, 1994, 67.

[4] Takeuchi H., Awaji M., Hashimoto M., Nakano Y., Mitsuhashi N., Kuwabara Y.: "Reduction of adhesions with fibrin glue after laparoscopic excision of large ovarian endometriomas". *J. Am. Assoc. Gynecol. Laparosc.,* 1996, *3,* 575.

[5] Donnez J., Nisolle M.: "Laparoscopic management of large ovarian endometrial cyst: use of fibrin sealant". *J. Gynecol. Surg.,* 1991, *7*, 163.
[6] Tirado-González I., Barrientos G., Tariverdian N., Arck P.C., García M.G., Klapp B.F., Blois S.M.: "Endometriosis research: animal models for the study of a complex disease". *J. Reprod. Immunol*., 2010, *86,* 141.
[7] Gibran N., Luterman A., Herndon D., Lozano D., Greenhalgh D.G., Grubbs L. *et al*.: "FS 4IU Clinical Study Group. Comparison of fibrin sealant and staples for attaching split-thickness autologous sheet grafts in patients with deep partial- or full-thickness burn wounds: a phase 1/2 clinical study". *J. Burn Care Res*., 2007, *28,* 401.
[8] Kocak I., Unlu C., Akcan Y., Yakin K.: "Reduction of adhesion formation with cross linked hyaloronic acid after peritoneal surgery in rats". *Fertil. Steril.,* 1999, *72*, 873.
[9] Somigliana E., Viganò P., Rossi G., Carinelli S., Vignali M., Panina-Bordignon P.: "Endometrial ability to implant in ectopic sites can be prevented by interleukin-12 in a murine model of endometriosis". *Hum. Reprod.,* 1999, *14,* 2944.
[10] Xu H., Lui W.T., Chu C.Y., Ng P.S., Wang C.C., Rogers M.S.: "Anti-angiogenic effects of green tea catechin on an experimental endometriosis mouse model". *Hum. Reprod.,* 2009, *24*, 608.
[11] Akkaya P., Onalan G., Haberal N., Bayraktar N., Mülayim B., Zeyneloglu H.B.: "Doxycycline causes regression of endometriotic implants: a rat model". *Hum. Reprod.,* 2009, *24,* 1900.
[12] Hirata T., Osuga Y., Yoshino O., Hirota Y., Harada M., Takemura Y. *et al*.: "Development of an experimental model of endometriosis using mice that ubiquitously express green fluorescent protein". *Hum. Reprod.,* 2005, *20,* 2092.
[13] Toosie K., Gallego K., Stabile B.E., Schaber B., French S., de Virgilio C.: "Fibrin glue reduces intra-abdominal adhesions to synthetic mesh in a rat ventral hernia model". *Am. Surg*., 2000, *66,* 41.
[14] Lima A.G., Taha M.O., Rivoire H.C., Fagundes A.T., Fagundes D.J.: "Fibrin adhesive and the vaginal vault synthesis on female rabbits abdominal hysterectomies". *Acta Cir. Bras.,* 2009, *24*, 30.
[15] Jackson M.R.: "Fibrin sealants in surgical practice: An overview". *Am. J. Surg.,* 2001, *182* (2 suppl.), 1S.
[16] Spotnitz W.D.: "Fibrin sealant: past, present, and future: a brief review". *World J. Surg.,* 2010, *34,* 632.

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