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Surgical adhesives and tubal sterilization: An experimental study

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Abstract

Aim: Our aim was to assess the application of three currently used surgical adhesives in the tubal lumen of rabbits, to promote sterilization, using a transvaginal approach.

Methods: Fifty-seven female albino New Zealand rabbits (114 uterine tubes), which became pregnant and delivered before the experiment, were divided into four groups: GS (sham-24 tubes), GEFIBRI (0.25 mL of fibrin adhesive in 30 tubes), GE-GRF (0.25 mL of resorcin adhesive in 30 tubes) and GEBUTYL (0.25 mL of *n*-butyl-2-cyanoacrylate adhesive in 30 tubes). The animals were mated with proven fertile males after the experiment and observed over 30, 90 and 180 days. Pregnancy and patency were macroscopically evaluated. The tubal diameter, tubal mucosa, myosalpinx, total optical density and inflammatory process were microscopically evaluated. The statistical analysis was performed by McNemar and Wilcoxon tests for the subgroups, and Fisher's exact test and Kruskal–Wallis test for the groups, the differences identified by Dunn's multiple comparisons test ($P = 5\%$).

Results: GS showed patency and pregnancies in all subgroups. GEFIBRI showed patency and pregnancies in all subgroups. GE-GRF did not show patency or pregnancies, but was associated with severe inflammatory process and tubal morphology alterations. GEBUTYL did not show patency, pregnancies or morphological tubal mucosa alterations.

Conclusions: The *n*-butyl-2-cyanoacrylate adhesive effectively promoted tubal obstruction, did not cause tubal morphological alterations, nor did it impair the rabbit pregnancy. The fibrin adhesive failed to cause the occlusion. The GRF adhesive, in spite of producing tubal occlusion, caused severe uterine tubes damage.

Key words: rabbits and experimental surgery, reproductive sterilization, surgical adhesive, uterine tube.

Introduction

Surgical sterilization is a method of birth control used by many women. More than 100 million women are believed to use this method to control their fertility, which is the world's most widely used method of birth prevention.[1]

The advances in new materials and surgical techniques, particularly the hysteroscopy procedure, allowed the introduction of substances or devices in the tubal lumen to prevent fecundation. Methods that use the transvaginal approach to promote sterilization are being continuously developed.[1]

For more than 100 years researchers have been trying to develop the ideal method of birth control, but none of them can be considered to be perfect.[2] Laparoscopic methods employing several techniques have been used, such as unipolar coagulation with or

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without uterine tube division; bipolar coagulation; cauterization; silastic band application; Spring, Hulka or Filshie clips placement; Yoon ring and Pomeroy surgical procedure. These methods account for nearly 50 000 laparoscopic sterilizations in the United Kingdom and approximately 1 million in the USA every year.[2]

On the other hand, hysteroscopy sterilization can be performed by electrocoagulation, cryocoagulation, Nd:YAG laser, chemical agents (quinacrine, tetracycline, methyl-cyanoacrylate) and also mechanical methods using ceramic, nylon, polyethylene, silicone, poly tetrafluorethylene devices. Recently, two new devices, Essure and Adiana, were reported.[1,2]

The aim of this study was to assess the application of three currently used types of surgical adhesives in the tubal lumen of rabbits, to promote sterilization, using a transvaginal approach. In order to achieve this objective, we used fibrin adhesive, gelatin-resorcin-formaldehyde (GRF) adhesive and *n*-butyl-2-cyanoacrylate adhesive.

Methods

The experimental protocol was approved by the Research Ethics Committee of the São Paulo Hospital of the Federal University of São Paulo, São Paulo's Medical School (CEP 835/01). All the procedures followed strictly the existing regulations about animal experimentation.

Fifty-seven female albino New Zealand rabbits (114 uterine tubes), aged 8 months, average weight 4 kg, which became pregnant and delivered before the experiment, were randomly divided into four groups as follows: (i) GS (sham group, with 24 uterine tubes); (ii) GEFIBRI (fibrin group, with 30 uterine tubes); (iii) GE-GRF (GRF group, with 30 uterine tubes); and (iv) GEBUTYL (butyl group, with 30 uterine tubes). Each group was divided into subgroups for a 30-day study (eight tubes in the GS group and 10 tubes in each of the other groups), a 90-day study (eight tubes in the GS group and 10 tubes in each of the other groups) and a 180-day study (eight tubes in the GS group and 10 tubes in each of the other groups).

The animals were preanesthetized[3] with diazepam 0.5 mg/kg i.m. (Cristália, São Paulo, Brazil), and 15 min later anesthetized with 25 mg/kg tiletamine/zolazepan i.m. (Zoletil; Virbac do, São Paulo Brazil). Fentanyl citrate (Fentanil; Cristália) 0.05 mg/kg i.m. was used as analgesic. Antibiotic prophylaxis was provided by a single dose of chloramphenicol hemisuccinate 50 mg/kg i.m. (Vixmicina; União Quimíca, São Paulo, Brazil).

The animals were placed on a single operating table. Videovaginoscopy and tubal catheterization were performed according to the method previously described by Thurmond and Ross.[4,5]

In the GS animals, both tubal isthmi were catheterized, no substance was administered and the catheter was removed. In the GEFIBRI group, the uterine tubes were catheterized and 0.25 mL of fibrin biological adhesive (Beriplast; Aventis-Boheringer, São Paulo, Brazil) was injected in each tubal isthmus. In the GE-GRF group, the uterine tubes were catheterized and 0.25 mL of gelatin-resorcin-formaldehyde biological adhesive was injected (Colagel; Cirumédica, São Paulo, Brazil) in each tubal isthmus. In the GEBUTYL, 0.25 mL of *n*-butyl-2-cyanoacrylate synthetic adhesive (Histoacryl; B. Braun, Melsungen, Germany) was injected in each tubal isthmus, after catheterization.

Female rabbits were mated to males of proven fertility (breeders from the Bioterism Center). All the females that had previously mated with these same male rabbits became pregnant and delivered before the experiment.

According to the protocol, in the 30th, 90th or 180th day of observation, the animals were anesthetized and submitted to laparotomy and resection of uterus, uterine tubes and ovaries in monobloc. The anesthetized animals were subjected to euthanasia through the administration of 20 mL of 100% magnesium sulfate. In case of pregnancy, the laparotomy was performed 1 week after the delivery.

The *in vitro* patency test was done with a 5-Fr polyethylene probe that was introduced and tied in the uterine ostium by the inflated ballonet. The two-way probe (Fig. 1) was connected into a Y device, with a syringe (20 mL) used to inject the air and a manometer (Welch Allyn Tycos, Asheville, NC, USA) used to measure intrauterine pressure. The uterus and uterine tubes were immersed in saline solution and the patency of uterine tubes was tested by air insufflation until it reached at least the pressure of 40 mmHg. The burst pressure test was considered to be positive when air bubbles came out of the fimbriae.

The uterine tubes were removed and fixed in 10% formalin solution, embedded in paraffin wax and 5 μm sections were cut and stained with hematoxylin and eosin. Three histological cuts were made for all the groups (one cut in the middle of the adhesive or manipulated segment, another one upstream, and the last one downstream). Cuts were 5 μm thick, and for

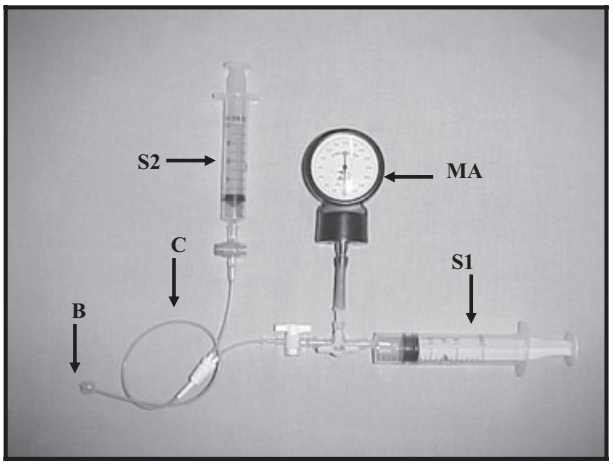

Figure 1 The burst pressure test system. B, ballonet; C, catheter 2-way; MA, manometer; S1, syringe 20 mL; S2, syringe 10 mL.

the GEBUTYL group, a diamond microtome blade was used to cut the polymer inside the tubes. Histological evaluation was undertaken by an independent pathologist who had no knowledge of the experimental groups from which the specimens were derived. The microscopic assessment was performed using a grading scoring: 1 (no changes), 2 (mild), 3 (moderate) and 4 (severe) to parameters of damages in epithelium caused by the adhesive, presence or adherence of the adhesive in tube lumen and the degree of inflammatory process. The images were captured through a high resolution camera from a Carl Zeiss Axilab optic microscope (Zeiss, Oberkhoen, Germany). The software Image Proplus version 4.5 program (Media Cybernetics, Silver Spring, MD, USA) was used for analysis of morphometry data values of tube diameter, the mucosa and the myosalpinx thickness.

Other histological sections were stained for densitometry by the Feulgen reaction (Merck, Rio de Janeiro, Brazil). The mucosa cells densitometry (total optical density) expressed the amount of DNA ploidy, measured in 400 cells per section.[6,7]

The statistical methodology represented the qualitative variables by absolute (n) and relative (%) frequency, and the quantitative variables by means, standard deviation and median. The right and left sides were compared in relation to qualitative variables, by means of the McNemar test and in relation to quantitative variables by the Wilcoxon test for related samples. Groups and subgroups were compared with respect to changes in qualitative variables through the generalization of Fisher's exact test, and with respect to changes in quantitative variables by the Kruskal–Wallis test for independent samples. After the application of the Kruskal–Wallis test, the difference was identified by Dunn's multiple comparison test. The 0.05 significance level ($\alpha = 5\%$) was adopted and descriptive levels (P) lower than this value were considered significant and represented by an asterisk (*).

Results

No animal death occurred in any groups due to anesthetic or experimental procedures.

The animals in all groups had a weight increase, except in the GE-GRF group in which a significant loss of weight occurred, mainly at the 30th observation day (Table 1). This fact was associated with the systemic toxicity of the GRF adhesive. Those animals showed fever, insufficient food intake, diarrhea and difficulty in making spontaneous movements in their cages. They needed parenteral fluid and saline support in the first 2 weeks.

According to the study of pregnancies, all GS rabbits, as expected, became pregnant in each one of the uterus. The catheterization of the uterine tubes was not harmful and, consequently, it did not impair the pregnancy.

For the animals of GEFIBRI group, no pregnancy occurred in five uteri (50%) in the 30 days subgroup, but pregnancy was present in all uteri of the 90 and 180 days subgroups. The pregnancies showed that the fibrin adhesive can neither obstruct the uterine tubes nor cause any harm to tubal morphology and physiology. In the GE-GRF and GEBUTYL groups there were no pregnancies (Table 2).

The burst pressure test indicated that the uterine tubes of animals in the GS (Fig. 2) and GEFIBRI groups were patent, whereas the tubes of animals in the GE-GRF and GEBUTYL groups were obstructed (Table 2). Therefore, it was found that both the GRF and the *n*-butyl-2-cyanoacrylate adhesives obstructed the tubes and impaired the passage of air.

The macroscopic evaluation showed that *n*-butyl-2-cyanoacrylate adhesive was not absorbable and was not expelled from any uterine tube in any of the observation periods (Fig. 3). Although the GRF adhesive also impaired the pregnancy in all tubes and in all observation periods, an evident inflammatory process with dark areas of vascular suffering, scar retraction and tubal deformity occurred (Fig. 4).

Table 1 Mean and standard deviation of initial weight minor the final weight of the animals of four groups and the three periods of observation

Observation time	Initial weight *minor* final weight (±SD)			
	GS	GEFIBRI	GE-GRF	GEBUTYL
30 days	295.0 (±140.6)	120.0† (±216.8)	–740.0‡ (±270.2)	100.0† (±122.5)
90 days	400.0 (±267.7)	300.0 (±308.2)	20.0§ (±614.0)‡	440.0 (±260.8)
180 days	665.0 (±134.2)	440.0 (±384.7)	–780.0¶ (±311.4)	446.0 (±151.6)

Kruskal–Wallis test with Dunn's multiple comparison test. †GEFIBRI = GEBUTYL < GS ($P = 0.005$). ‡GE-GRF < GS ($P = 0.005$). §GE-GRF < GS ($P = 0001$). ¶GE-GRF < GS ($P = 0.009$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

Table 2 Number and percentage of pregnancy (uteri) and patency (uterine tubes) in all of four groups (sham, GRF, fibrin and *n*-butyl-2-cyanoacrylate) and in all observation periods (sum of 30, 90 and 180 observation days)

	GS	GEFIBRI	GE-GRF	GEBUTYL
Pregnancy +	24 (100%)†	25 (83.3%)‡§	0 (0%)	0 (0%)
Patency +	24 (100%)†	30 (100%)‡§	0 (0%)	0 (0%)
Total	24 (100%)	30 (100%)	30 (100%)	30 (100%)

Fisher's exact test generalization. †GS > GE-FIBRI ($P = 0001$). ‡GEFIBRI > GE-GRF ($P = 0001$). §GEFIBRI > GE-GRF ($P = 0001$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

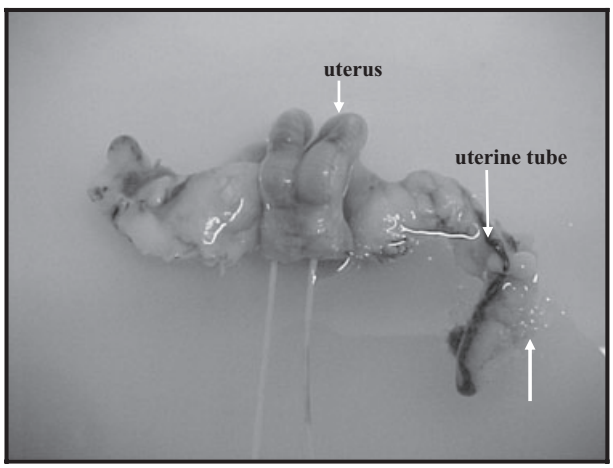

Figure 2 Photograph of the burst pressure test of fibrin glue in an animal on the 180th observation day. The arrows indicate the bubbles flowing through the fimbriae.

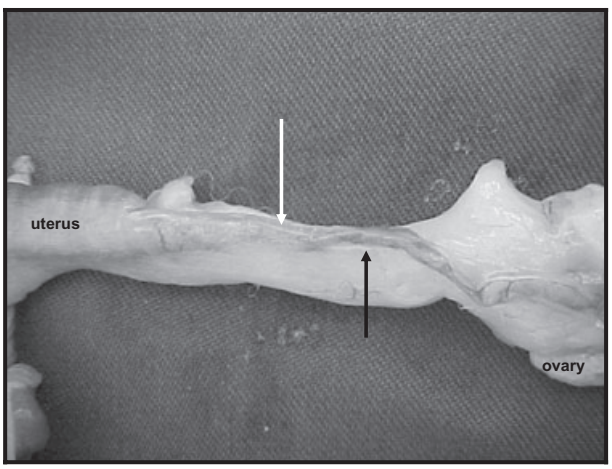

Figure 3 Photograph of the *n*-butyl-2-cyanoacrylate plug adhesive in uterine tube an animal on the 180th observation day, showing the distal diameter increase (black arrow) and the plug adhesive (white arrow).

There was a significant difference in the proximal tubal diameter (Table 3) compared to the distal tubal diameter in the GE-GRF and GEBUTYL at 30th, 90th and 180th observation day. Probably the insertion of GRF or *n*-butyl-2-cyanoacrylate adhesive increased the diameter of the uterine tube by a mechanical distension caused by the volume of the adhesive applied to the tubal lumen.

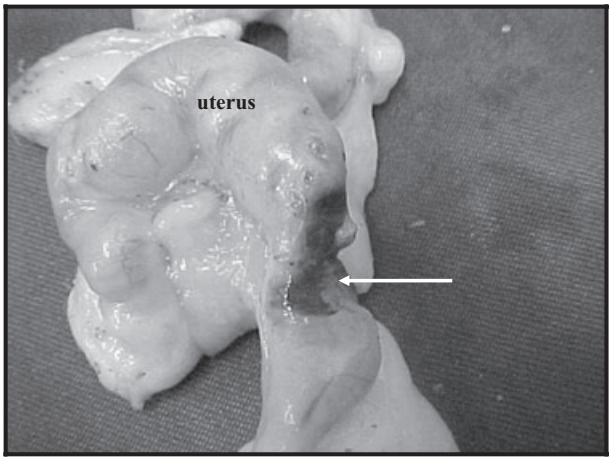

Figure 4 Photograph of the inflammatory process with necrosis (arrow) in the uterotubal junction promoted by the gelatin-resorcin-formaldehyde adhesive in an animal on the 180th observation day.

The mucosa in the GE-GRF was partially destroyed by the adhesive toxicity and showed a significant difference in thickness compared to that in the GEBUTYL group (Table 4). On the other hand, the mucosa thickness in the GEBUTYL was significantly enlarged in comparison with that in the SG and GEFIBRI groups.

In the GEBUTYL group, the myosalpinx thickness was significantly enlarged at the 90th and 180th observation days in comparison with all other groups (Table 5). Probably the presence of adhesive polymer in the tubal lumen, acting as a stimulus, increased the peristaltic movements in an attempt to expel the adhesive and was responsible by the hypertrophy of muscular layer (Fig. 5).

The total optical density was significantly higher in the GE-GRF group at the 30th, 90th and 180th observation days (Table 6). Our hypothesis was that the toxic and harmful action of the GRF adhesive caused a significant cell hyperplasia at the mucosa, not present in

Table 3 Mean and standard deviation of uterine tube diameter (cm) in all animals of four groups (sham, GRF, fibrin and *n*-butyl-2-cyanoacrylate) and in all observation periods (30, 90 and 180 observation days)

Observation time	Tubal diameter (cm ± SD)			
	GS	GEFIBRI	GE-GRF	GEBUTYL
30 days	0.757† (±0.331)	1.158 (±0.316)	0.935 (±0.628)	0.947 (±0.234)
90 days	0.856 (±0.428)	1.068 (±0.444)	1.083 (±0.507)	1.159‡ (±0.382)
180 days	0.766 (±0.409)	0.946 (±0.295)	1.042 (±0.385)	1.118§ (±0.117)

Kruskal–Wallis test with Dunn's multiple comparison test. †GS < GEFIBRI = GE-GRF = GEBUTYL ($P = 0023$). ‡GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0045$). §GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0047$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

Table 4 Mean and standard deviation of mucosa uterine tube thickness (mm) in all animals of four groups (sham, GRF, fibrin and *n*-butyl-2-cyanoacrylate) and in all observation periods (30, 90 and 180 observation days)

Observation time	Mucosa thickness (mm ± SD)			
	GS	GEFIBRI	GE-GRF	GEBUTYL
30 days	0.194† (±0.083)	0.241 (±0.120)	0.160 (±0.043)	0.300 (±0.210)
90 days	0.249 (±0.121)	0.184 (±0.057)	0.193 (±0.054)	0.430‡ (±0.259)
180 days	0.211 (±0.070)	0.203 (±0.059)	0.280 (±0.047)	0.447§ (±0.247)

Kruskal–Wallis test with Dunn's multiple comparison test. †GS = GEFIBRI = GE-GRF = GEBUTYL ($P = 0334$). ‡GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0004$). §GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0009$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

Table 5 Mean and standard deviation of myosalpinx thickness (mm) in all animals of four groups (sham, GRF, fibrin and *n*-butyl-2-cyanoacrylate) and in all observation periods (30, 90 and 180 observation days)

Observation time	Myosalpinx thickness (mm ± SD)			
	GS	GEFIBRI	GE-GRF	GEBUTYL
30 days	0.274† (±0.108)	0.356 (±0.149)	0.297 (±0.238)	0.442 (±0.143)
90 days	0.475 (±0.281)	0.316 (±0.115)	0.213 (±0.107)	0.721‡ (±0.196)
180 days	0.335 (±0.168)	0.397 (±0.074)	0.275 (±0.144)	0.853§ (±0.097)

Kruskal–Wallis test with Dunn's multiple comparison test. †GS = GEFIBRI = GE-GRF = GEBUTYL ($P = 0158$). ‡GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0001$). §GEBUTYL > GS = GEFIBRI = GE-GRF ($P = 0001$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

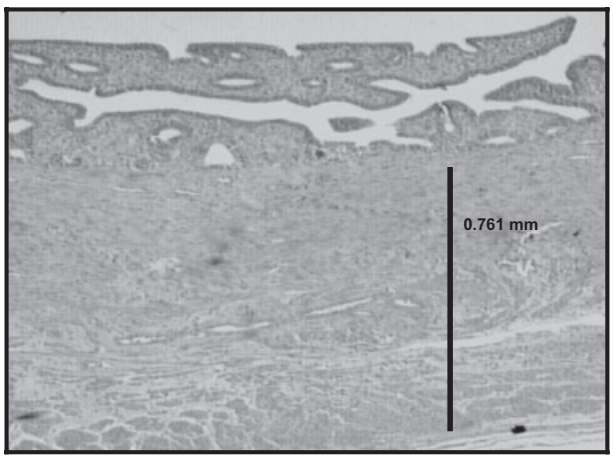

Figure 5 Photomicrograph of a cross-section of the uterine tube, upstream of the application of *n*-butyl-2-cyanoacrylate, at the 90th observation day. See the myosalpinx layer enlargement (black marked). (HE ×50). Bar, 0.761 mm.

the other groups. The toxicity of the GRF adhesive was probably caused by the formaldehyde present in the adhesive formulation, which originated an inflammatory reaction and consequent cellular hyperplasia that persisted even in the longest observation period of 180 days.

The presence of a moderate and accentuated inflammatory process (Fig. 6) at the 180th observation day in the GE-GRF group pointed to its deleterious action and the occurrence of chronic inflammation. The GRF adhesive showed persistent inflammatory process at all observation periods. The inflammatory process detected seemed to be a chronic one, which was not observed in the other groups. The inflammatory process in the other groups was mild or absent. Fibrin glue caused an inflammatory reaction until day 30, but it was not identified any further during the observation period, which indicates its absorption occurred at an earlier stage (Table 7).

Discussion

Tubal sterilization has become one of the most used methods for definite contraception or family planning throughout the world.[2] There is an ongoing consensus that the hysteroscopy approach is the ideal procedure because it can be fitted under local anesthesia in an ambulatory setting, may lessen postoperative pain and may allow faster recovery than incisional surgery with general anesthesia.[8] Nevertheless, until now, no method has been widely adopted in the procedure of fallopian tube occlusion by the transcervical route owing to safety or effectiveness limitations.[8] We proposed a procedure that, if suitable for use in human beings, may be performed in an outpatient setting and should propitiate a significant reduction of public health costs.

Our results support the idea that the hysteroscopic approach and tubal catheterization are safe and feasible procedures that do not cause a morphological or a functional uterine injury, as was demonstrated by the results of sham group. The effectiveness for sterilization purposes depended on the type of adhesive used. Further technical details on tubal catheterization shall be provided and adapted to other animal species, and particularly to human beings.

The animal model proposed using the female rabbits was the one currently referred to by the biomedical literature as the gold standard in sterilization studies.[4]

Table 6 Mean and standard total optical density (DNA ploidy amount) in all animals of four groups (sham, GRF, fibrin and *n*-butyl-2-cyanoacrylate) and in all observation periods (30, 90 and 180 observation days)

Observation time	Total optical density (DNA ploidy amount ± SD)			
	GS	GEFIBRI	GE-GRF	GEBUTYL
30 days	0.309† (±0.035)	0.304 (±0.016)	0.300 (±0.013)	0.274 (±0.042)
90 days	0.309‡ (±0.024)	0.309 (±0.021)	0.297 (±0.024)	0.284 (±0.032)
180 days	0.295 (±0.046)	0.296 (±0.022)	0.377§ (±0.012)	0.306 (±0.014)

Kruskal–Wallis test with Dunn's multiple comparison test. †GS = GEFIBRI = GE-GRF = GEBUTYL ($P = 0.391$). ‡GS = GEFIBRI = GE-GRF = GEBUTYL ($P = 0.634$). §GE-GRF > GS = GEFIBRI = GEBUTYL ($P = 0.037$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

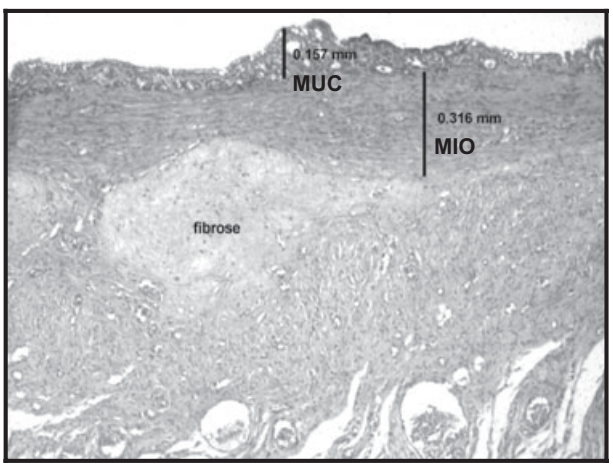

Figure 6 Photomicrograph of a cross-section of the uterine tube, upstream of the application of gelatin-resorcin-formaldehyde adhesive at the 90th observation day. See in the right up of the figure the details of the mucous (MUC; bar, 0.157 mm) and the myosalpinx (MIO; bar, 0.316 mm) morphometry. See at the center of the figure the severe area of fibrosis (fibrose). (HE ×50).

The tubal diameter is about one-third to one-half the size of human tubes.[4] This represented a limitation to our study when we tried to apply our findings to human beings.[4] To solve that, we ran another study using the sheep as animal model, in which the tubal diameter is similar to the human uterine tube.[9]

Several substances or devices were referred to promote the tubal obstruction by transvaginal or laparotomy.

An experimental study in female rabbits using transvaginal uterine tube catheterization and the application of ethanol, hydrogel and occlusive emulsion showed that the ethanol reached the peritoneal cavity in all animals. After 2 days and 30 days of proven occlusion in one out of six tubes with the use of ethanol, hydrogel obstructed three out of six tubes and the occlusive emulsion obstructed five out of six tubes.[10] In another study, hydrogel with sclerosing substance was used and a plug was placed in the lumen of the eight tubes of eight female rabbits, for 25–28 observation days. The plug was totally eliminated in one animal. In four animals hydrogel was inside the tube and ovary causing adherences between them and the intestine, and in one animal, the parietal peritoneum was also involved. Pregnancy did not occur in six uteruses, but an inflammatory reaction occurred in all tubes with hydrogel.[11] In a report of tube sterilization in female rabbits by transvaginal application of methyl-cyanoacrylate, the presence of cellular necrosis, inflammatory cells, fibrin and fibrosis were found after 2 weeks. After 2 and 6 months, besides the findings described, the total absorption of the adhesive occurred and all tubes showed a segment of wall fibrosis and a chronic inflammatory process.[12] Another two studies referred to the insert of fibrin adhesive inside 10 tubes of female rabbits and in other 14 tubes bipolar coagulation and fibrin adhesive performed by laparotomy. The occlusion rate was 71.4% in the tubes where coagulation and adhesive were used, whereas in the tubes where only fibrin adhesive was used there was no occlusion at all.[13] In a report of application of 0.15 mL of methyl-cyanoacrylate in the tubal lumen of female rabbits, for 6 weeks, by means of incision in the uterine cornus 2 cm from the uterotubal junction, all tubes were obstructed, and histological study showed the destruction of tubal epithelium, with extensive

Table 7 The number and percentage of the different grades (absent, mild, moderate and severe) of inflammatory response of uterine layers to the three different adhesives (fibrin glue, resorcin and *n*-butyl-2-cyanoacrylate) in the three observation days (30, 90 and 180 days)

Periods	Inflammatory grade	Groups			
		GS	GEFIBRI	GE-GRF	GEBUTIL
30 days	0 (absent)	4 (50%)	3 (30%)	0 (0%)	6 (60%)
	1 (mild)	4 (50%)	2 (20%)	0 (0%)	2 (20%)
	2 (moderate)	0 (0%)	4 (40%)	5 (50%)†	2 (20%)
	3 (severe)	0 (0%)	1 (20%)	5 (50%)‡	0 (0%)
90 days	0 (absent)	7 (87.5%)	6 (60%)	0 (0%)	8 (80%)
	1 (mild)	1 (12.5%)	4 (40%)	3 (30%)	2 (20%)
	2 (moderate)	0 (0%)	0 (0%)	6 (60%)§	0 (0%)
	3 (severe)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
180 days	0 (absent)	7 (87.5%)	8 (80%)	0 (0%)	9 (90%)
	1 (mild)	1 (12.5%)	2 (20%)	4 (40%)	1 (10%)
	2 (moderate)	0 (0%)	0 (0%)	5 (50%)¶	0 (0%)
	3 (severe)	0 (0%)	0 (0%)	1 (10%)	0 (0%)

Kruskal–Wallis test with Dunn's multiple comparison test. †GE-GRF > GS = GEFIBRI ($P = 0.001$). ‡GE-GRF > GS = GEBUTYL > GEFIBRI ($P = 0.001$). §GE-GRF > GS = GEBUTYL = GEFIBRI ($P = 0.001$). ¶GE-GRF > GS = GEBUTYL = GEFIBRI ($P = 0.001$). GS, sham group; GEFIBRI, fibrin adhesive group; GE-GRF, gelatin-resorcin-formaldehyde adhesive group; GEBUTYL, *n*-butyl-2-cyanoacrylate adhesive group.

fibrosis and absence of the adhesive. Polymer particles were found on the macrophages.[14]

Experimental studies with laparotomy and a complementary procedure for tubal sterilization of female rabbits have been widely reported. Endoluminal coagulation gave 98% successful results,[15] laser photocoagulation, 94%,[16] copolymer of ethylene-vinyl-alcohol was shown to be ineffective,[17] and the efficiency of radiofrequency was not proved.[18] A large intestine device was recanalized in 180 days in 50%.[19] Polyethylene glycol showed 57% efficiency,[20] and polydocanol in rats and monkeys showed no significantly results for tubal obstruction.[21]

Our data showed that the GRF and cyanoacrylate adhesive was effective in promoting tubal obstruction, but GRF was associated with severe systemic toxicity and local necrosis, which impaired their current use. On the other hand, the fibrin glue was ineffective in promoting sterilization on the 90th or 180th observation days.

No reports of the GRF adhesive for experimental use or use in human beings for tubal sterilization were found in the medical literature. Our results demonstrated that the systemic toxicity and the severe inflammatory reaction do not recommend its use. Nonetheless, the proposed alteration in its chemical composition by changing the polymerization substance may suggest new possible uses for this adhesive.[22]

Due to its fast and complete absorption, the fibrin glue does not meet the basic requirements to provide the desired tubal occlusion, ensuring permanent sterilization. The only report in medical literature of its use in female rabbits showed its inefficiency in all cases.[13] Our results demonstrated that around the 30th day the fibrin glue is not present in the tubal lumen anymore and 50% of animals get pregnant. Its application for sterilization purposes should be reconsidered, according to the present research method, since all pertinent studies conducted so far did not recommend its use.

Concerning specifically the *n*-butyl-2-cyanoacrylate, our previous experimental research on surgical adhesives used for uterine tube occlusion and video hysteroscopy in female rabbits showed, after 30 days of observation, the absence of pregnancy as well as the absence of tubal patency in the pressure burst test or dye instillation test. The adhesive plug remained in the original application site. No histological alterations were found in the tubal morphology.[23]

There is only one medical literature report describing the use of *n*-butyl-2-cyanoacrylate in human beings. Two women were submitted to transvaginal application of the adhesive and after 4 years of follow up, including the performance of hysterosalpingography, their tubes remained occluded.[24] We were informed by the researchers who performed this procedure that the

continuation of the investigation did not occur and that control was made by image alone, so they did not know anything about the adhesive behavior in the uterine tube structure and function (J. Pelage, personal communication, 2005).

Conversely to methyl-cyanoacrylate, the *n*-butyl-cyanoacrylate was not absorbed by the uterine tube mucosa until the 180th observation day. The adhesive plug remained intact in the tubal lumen and no macrophage cells were identified in optical microscopy with casts of the polymers inside. The hypertrophy of myosalpinx may be associated with the effort of peristaltic movements in removing the adhesive plug. No animals, in the all observation periods, were pregnant, and all of the uterine tubes were negative for the burst pressure test. Those findings showed that *n*-butyl-2-cyanoacrylate was a reasonable option for a transvaginal sterilization procedure.

Experimental studies shall be conducted for longer periods of time, although a 6-month period might be considered to be long, in view of the female rabbit life cycle. Other larger, non-rodent mammals, such as the sheep, shall be used in further studies to test these adhesives for 6- or 12-month periods, since the question that remains unanswered concerns the long-term permanence of the cyanoacrylate adhesive plug.

Therefore, the conduction of new experimental studies to attest to the real efficiency of this method shall ensure its large scale clinical application, thus providing the possible use of an efficient, cost-effective, safe sterilization method with minimum morbidity, which does not require invasive surgical and anesthetic procedures and that can be performed at an outpatient setting.

The cost of a laparoscopic sterilization procedure in the USA is reported to be US$3449 and the implant of an endo-uterus-tubal implant costs US$1374,[2] whereas the procedure proposed by the present research was estimated to vary from US$200–300.

We concluded that the fibrin adhesive failed to cause the occlusion of the rabbit uterine tubes and the GRF adhesive, in spite of having produced tubal occlusion, caused important cellular damage and chronic inflammation of the uterine tubes. The *n*-butyl-2-cyanoacrylate adhesive was effective in promoting the uterine tube obstruction, did not cause morphological alterations in the uterine tubes, nor impaired the rabbit pregnancy. The transvaginal approach was not harmful and effective in providing the adhesive insert in the tubal isthmus.

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