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Laparoscopic stripping of endometriomas: a randomized trial on different surgical techniques. Part II: pathological results

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BACKGROUND: The stripping technique for endometriomas excision has been reported to be associated with follicular loss. The objective of this trial was to evaluate the presence and nature of ovarian tissue adjacent to the endometrioma cyst wall obtained by stripping with different techniques. METHODS: Forty-eight patients with ovarian endometrioma were enrolled in two consecutive independent randomized trials. Two different techniques were analysed at the initial adhesion site (circular excision and subsequent stripping versus immediate stripping). Two different techniques were analysed at the ovarian hilus (stripping versus coagulation and cutting). Histology analysis was performed in three portions of the cyst wall (initial adhesion site, intermediate part of the specimen, ovarian hilus). RESULTS: Recognizable ovarian tissue was inadvertently excised together with the endometrioma cyst wall in most cases. At initial adhesion sites more ovarian tissue was removed with the circular excision technique (<0.001). No significant difference in quality of ovarian tissue (number and type of follicles) was found between specimens obtained with different surgical techniques at the initial or at the final part of the procedure. At the initial adhesion site and at the intermediate part of the cyst wall, the ovarian tissue removed along with the endometrioma wall was mainly constituted by tissue with no follicles or only primordial follicles (60% and 48% of the specimens from the initial part with both techniques, and from the intermediate part, respectively, had no follicles or only primordial follicles). Close to the ovarian hilus the ovarian tissue removed along with the endometrioma wall mostly consisted of tissue which contained primary and secondary follicles (69% of the cases, combining the two groups). CONCLUSIONS: Ovarian tissue is inadvertently excised together with the endometrioma wall in most cases. The excised tissue is at normal functional development stages only near the ovarian hilus. The different techniques used do not influence significantly the quality of the resected tissue.

Key words: endometrioma/endometriosis/laparoscopy/ovarian reserve

Introduction

Recent randomized trials have demonstrated that laparoscopy should be considered the best approach for the surgical treatment of benign ovarian cysts (Mais *et al.*, 1995; Yuen *et al.*, 1997). At laparoscopy, in the case of ovarian endometriotic cysts, the cyst wall can be excised with the stripping technique (Reich and McGlynn, 1986; Martin, 1991; Canis *et al.*, 1992, Beretta *et al.*, 1998) or ablated with bipolar coagulation or laser after cyst fenestration (Brosens *et al.*, 1996; Donnez *et al.*, 1996; Sutton *et al.*, 1997; Beretta *et al.*, 1998).

Recently, the stripping procedure has been reported to be preferable to cyst wall ablation since recurrence rates may be lower (Beretta *et al.*, 1998; Vercellini *et al.*, 2003). Nevertheless, a major concern that remains is the possible loss of follicles associated with the procedure (Brosens *et al.*, 1996; Donnez *et al.*, 1996). In addition, poorer performance in IVF protocols (Garcia-Velasco *et al.*, 2004) and reduced ovarian volumes (Exacoustos *et al.*, 2004) have been reported in patients who had undergone the stripping procedure for endometrioma excision.

In a recent report, we have reported that the stripping procedure may be a tissue-sparing technique (Muzii *et al.*, 2002). The pathological analysis of endometriotic cyst wall revealed that ovarian tissue was inadvertently excised together with the cyst wall in more than half of the cases, but in no case did this tissue show a normal follicular pattern as the one present in healthy ovaries (Muzii *et al.*, 2002). In the above study, however, only a 2 × 2 cm sample taken from

the intermediate part of the cyst wall, midway between the site of ovarian adhesion to the ovarian fossa and the hilus, was evaluated.

The objective of the present trial was to evaluate, in a randomized trial, the quantity and nature of ovarian tissue inadvertently resected along with the endometriotic tissue in the different parts of the cyst wall (i.e. at the cyst adhesion site with the ovarian fossa, in the intermediate part of the cyst wall, and where the cyst is closer to the ovarian hilus), using two different techniques at the initial adhesion site and two different techniques at the final part of the stripping procedure near the hilus.

Materials and methods

The primary objective of this trial was to evaluate the difference in ovarian tissue inadvertently removed together with the endometriotic cysts using different surgical techniques.

In a separate paper the study design and eligibility and exclusion criteria have been described, and a more thorough description of the surgical procedure is also provided (Muzii *et al.*, 2005). Briefly, two surgical techniques (direct stripping versus circular excision) were analysed at the initial adhesion site, and two different techniques (stripping versus coagulation followed by cutting) were analysed for the management of the cyst near the ovarian hilus (Muzii *et al.*, 2005).

Postoperatively, the surgeon removed three separate specimens from the cyst wall for thorough pathological analysis: specimen 1, removed around the site of the original parietal adhesion, obtained with either surgical procedure (stripping or circular excision) (Figure 1A); specimen 2, removed from the intermediate part of the cyst (midway between specimen 1 and specimen 3) (Figure 1B); and specimen 3 removed from the cyst wall pedicle (close to the ovarian hilus), with either surgical procedure (stripping or coagulation and cutting) (Figure 1C).

The remaining specimen was sent for routine pathological analysis. The pathologist was not informed on which technique was adopted to remove the endometrioma. The same blinded pathologist evaluated each specimen separately. The specimen thickness and the presence of ovarian tissue were recorded. When ovarian tissue was present, the tissue thickness and the morphologic characteristics were recorded. Morphologic characteristics of this tissue were graded on a semiquantitative scale of 0 to 4 (0, complete absence of follicles; 1, primordial follicles only; 2, primordial and primary follicles; 3, some secondary follicles; 4, pattern of primary and secondary follicles as seen in the normal ovary) (Maneschi *et al.*, 1993).

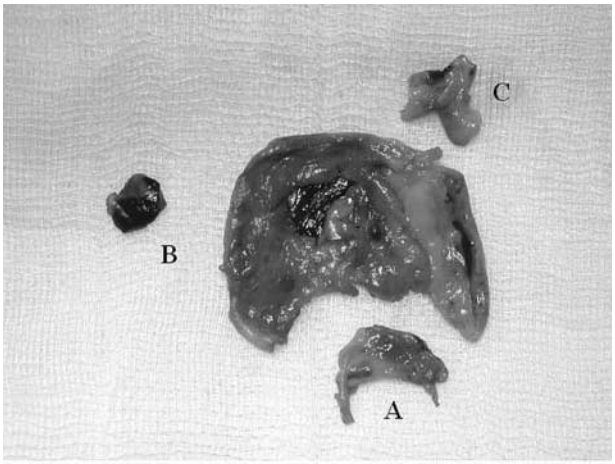

Figure 1. Excised endometrioma with the three specimens removed. (**A**) Specimen 1: removed around the site of the original parietal adhesion. (**B**) Specimen 2: removed from the intermediate part of the cyst. (**C**) Specimen 3: removed from the cyst wall pedicle (close to the ovarian hilus). Scale 1:2.

Statistical analysis

The sample size utilized (24 versus 24 observations) was selected in order to detect, with 80% power at the 0.05 alpha level, a difference of 34% in the rate of presence of ovarian tissue inadvertently excised, given a reference rate for inadvertently removed ovarian tissue of 54%, reported in our previous study (Muzii *et al.*, 2002).

Parametric tests were used after having evaluated the normal distribution of the data to be analysed. The Student's *t*-test was used to evaluate the difference between continuous variables. The χ^2-test was used to evaluate the difference between the presence or absence of ovarian tissue between groups, at different cyst sites. The Mann–Whitney test was used to evaluate the difference in quality of ovarian tissue removed with the different surgical techniques. Analysis of variance (ANOVA) was used to compare specimens obtained at the three different cyst sites. When significant, Fisher's post-hoc test was used to identify significant comparisons. The Kruskal–Wallis test was used to evaluate the difference in quality of ovarian tissue removed at the three different cyst sites. Statistical significance was set at $P < 0.05$.

Results

Between 1 January 2000 and 31 December 2001, 48 patients were enrolled in the trial and evaluated. Patients characteristics and the study flow chart have been previously reported (Muzii *et al.*, 2005).

Analysis of specimen 1: at original adhesion site with the ovarian fossa (direct stripping versus circular excision)

Forty-eight patients were evaluated, 24 in each arm. Overall the mean tissue thickness of specimen 1 was 1.2 ± 0.4 mm. The thickness of specimen 1 obtained in patients subjected to circular excision was significantly greater than the one obtained from patients subjected to direct stripping (1.4 ± 0.4 mm versus 1.0 ± 0.4 mm, $P < 0.05$). Ovarian tissue was present in 64% (31/48) of the patients. As expected, there was a tendency of finding ovarian tissue removed with the endometriosis more frequently after circular excision than after direct stripping (79% versus 50%, $P = 0.07$). When ovarian tissue was present, the mean thickness was 0.3 ± 0.2 mm. The circular excision technique removed significantly more ovarian tissue, when present, if compared with the direct stripping technique (0.5 ± 0.1 mm versus 0.1 ± 0.03 mm, respectively, $P < 0.001$). Figure 2 shows a specimen removed with the circular excision technique.

Ovarian tissue inadvertently excised with the endometrioma wall was primarily constituted by tissue with no (grade 0) or only primordial (grade 1) follicles (29/31, 94%). In one case (2%) grade 3 ovarian tissue was present, whereas in another case (2%) grade 4 was present. No difference was

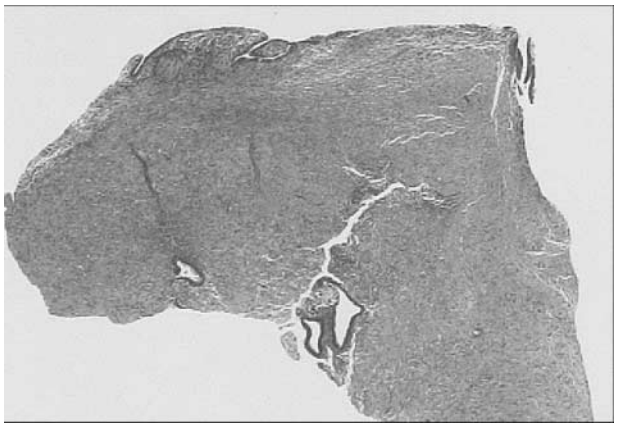

Figure 2. Specimen 1. At the top of the specimen, the thin mesothelium is visible, whereas at the bottom, endometrial glands can be seen. Hematoxylin and eosin; magnification ×40.

present in morphological characteristics of the ovarian tissue between the specimens obtained through the two different surgical techniques (Table I).

Analysis of specimen 2: intermediate part of the cyst wall (midway between specimen 1 and specimen 2)

Forty-eight specimens were analyzed. The surgical technique for the intermediate part was presumably the same stripping technique in all 48 patients. In fact, this part of the procedure was not part of any randomized trial, but was carried out in the usual manner (Muzii *et al.*, 2002). Overall, the mean tissue thickness of specimen 2 was 1.4 ± 0.5 mm. Ovarian tissue was present in 54% (26/48) of the patients. When ovarian tissue was present, the mean thickness was 0.3 ± 0.1 mm. Ovarian tissue inadvertently excised with the endometrioma wall primarily consisted of tissue with no or only primordial follicles (23/26, 88%). In three cases (12%), tissue with secondary (grade 2) follicles was found. No difference in quality or quantity of ovarian tissue was identified on the basis of the technique used in trial 1. This specimen represents the tissue that constitutes the greatest part of the cyst wall.

Analysis of specimen 3: close to the ovarian hilus (completion with the stripping technique versus bipolar coagulation followed by cutting with scissors)

Forty-eight patients were evaluated, 24 in each arm. The mean tissue thickness of specimen 3 was 1.6 ± 0.6 mm. No difference in thickness was present between specimens obtained in the two groups (1.6 ± 0.5 mm versus 1.5 ± 0.6 mm for stripping versus bipolar coagulation and cutting, respectively). Ovarian tissue was present in 71% (34/48) of the patients. In particular, ovarian tissue was present in 18/24 (75%) versus 16/24 (67%) of patients who underwent direct stripping versus bipolar coagulation and cutting, respectively. Ovarian tissues mean thickness was 0.8 ± 0.4 mm, with no difference between the two groups. When ovarian tissue was present, this primarily consisted of tissue with primary and secondary follicles, i.e. grade 3 and 4 (29/34, 85%). No significant difference was present between the specimens achieved through the two different surgical techniques (Table II). Figure 3 shows a specimen removed with the direct stripping technique.

Pathological characteristics of the three specimens obtained by the 48 evaluated patients are reported in Table III. Specimens obtained from the hilus (specimen 3) were significantly thicker than the ones obtained from the initial adhesion site (specimen 1). Specimen 2 tended to be thicker than specimen 1 and thinner than specimen 3, but not in a significant way. No difference was present between specimens in the proportion of specimens with identifiable ovarian tissue. A significant difference was present in the thickness of ovarian tissue inadvertently removed between specimens 1–2 and specimen 3 ($P < 0.05$) (Figure 3), with specimen 3 having more ovarian tissue than the other two

Table I. Pathological characteristics of specimen

Pathological data		Direct stripping (24 patients)		Circular excision (24 patients)		*P*
Tissue thickness Specimen 1 (mean ± SD) [mm]		1.0 ± 0.4		1.4 ± 0.4		<0.05
Presence of ovarian tissue Specimen 1		12 (50%)		19 (79%)		N.S.
Ovarian tissue thickness Specimen 1 (mean ± SD) [mm]		0.1 ± 0.03		0.5 ± 0.11		<0.001
Ovarian tissue quality Specimen 1(G)	0	5	21%	9	37%	N.S.
	1	6	25%	9	37%	
	2	0	0	0	0	
	3	0	0	1	4%	
	4	1	4%	0	0	

G = Grade 0, complete absence of follicles; 1, primordial follicles only; 2, primordial and primary follicles; 3, some secondary follicles; 4, pattern of primary and secondary follicles as seen in the normal ovary (Maneschi *et al.*, 1993).

Table II. Pathological characteristics of specimen

Pathological data		Completion with stripping ($n = 24$)		Bipolar coagulation and subsequent cutting ($n = 24$)		*P*
Tissue thickness Specimen 3 (mean ± SD) [mm]		1.6 ± 0.5		1.5 ± 0.6		NS
Presence of ovarian tissue		18 (75%)		16 (67%)		NS
Ovarian tissue thickness (mean ± SD) [mm]		0.72 ± 0.29		0.82 ± 0.42		NS
Ovarian tissue quality Specimen 3 (G)	0	0	0	0	0	NS
	1	0	0	1	4%	
	2	2	8%	2	8%	
	3	6	25%	5	21%	
	4	10	40%	8	33%	

G = Grade 0, complete absence of follicles; 1, primordial follicles only; 2, primordial and primary follicles; 3, some secondary follicles; 4, pattern of primary and secondary follicles as seen in the normal ovary (Maneschi *et al.*, 1993).

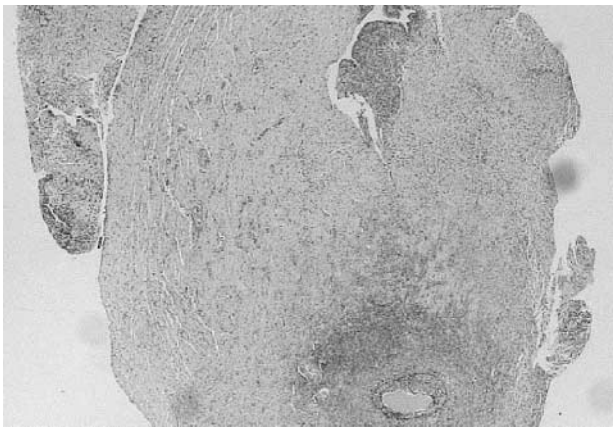

Figure 3. Specimen 3. At the top of the slide, endometrial tissue can be seen, whereas at the bottom, a secondary follicle is present. Hematoxylin and eosin; magnification ×40.

specimens. No difference in quality of ovarian tissue was present between specimen 1 and specimen 2. Specimen 3 contained significantly more primary and secondary follicles compared to the other two specimens ($P < 0.05$).

Discussion

Laparoscopy should be considered the gold-standard approach for the treatment of ovarian endometriomas, whereas the choice of which laparoscopic technique to use is still a matter of debate (Jones and Sutton, 2000; Vercellini *et al.*, 2003). There are strong supporters and detractors for both the technique of cyst wall stripping and the technique of cyst fenestration and subsequent vaporization or coagulation of the inner wall. Recent reports have compared the two techniques, both in prospective (Beretta *et al.*, 1998) and retrospective (Hemmings *et al.*, 1998; Saleh and Tulandi, 1999) studies. In a randomized controlled study by Beretta *et al.* (1998), cystectomy with the stripping procedure proved to be significantly better than fenestration and bipolar coagulation of the cyst wall, both in terms of symptom recurrence and subsequent fertility rates. In uncontrolled series however, higher pregnancy rates were reported with the fenestration and coagulation/ablation technique (Jones and Sutton, 2001). Retrospective series yielded conflicting results. In a retrospective series by Hemmings *et al.* (1998), patients submitted to laparoscopic fenestration and bipolar coagulation of the cyst wall achieved pregnancy in a shorter period of time (1.4 years) than patients undergoing laparoscopic cystectomy (mean time to first pregnancy 2.2 years) or ovarian cystectomy by laparotomy and microsurgical techniques (mean time to pregnancy 2.4 years). Cumulative pregnancy rates at 36 months and recurrence rates at follow-up were not significantly different between the three groups. In a retrospective study by Saleh and Tulandi (1999), laparoscopic fenestration and ablation of the cyst wall yielded poorer results when compared with laparoscopic cyst excision (reoperation rates at 18 and 42 months were 22% and 58%, respectively, for fenestration, versus 6% and 24% for cyst excision).

Therefore, a consensus on which technique should be considered the gold standard as to postoperative reproductive performance and recurrences has not been reached.

Some authors (Brosens *et al.*, 1996; Donnez *et al.*, 1996) have recently questioned the laparoscopic technique of endometrioma stripping, since stripping of the cyst wall may result in removal of ovarian tissue in excess, with possible loss of follicles. Some indirect evidence from the literature

Table III. Pathological analysis

Pathological data		Site of the original parietal adhesion Specimen 1		Intermediate part of the cyst Specimen 2		Cyst wall pedicle at ovarian hilus Specimen 3		*P*
Tissue thickness [mm] (mean ± SD)		1.2 ± 0.4		1.4 ± 0.5		1.6 ± 0.6		<0.005[a] NS[b] <0.05[c] NS[d]
Presence of ovarian tissue		31		26		34		NS
Ovarian tissue thickness [mm] (mean ± SD)		0.30 ± 0.20		0.29 ± 0.13		0.80 ± 0.36		<0.001[a] NS[b] <0.05[c] <0.05[d]
Ovarian tissue quality (G)	0	14	29%	10	21%	0	0	<0.001[e]
	1	15	31%	13	27%	1	2%	NS[b]
	2	0	0	3	6%	4	8%	<0.05[c]
	3	1	2%	0	0%	11	23%	<0.05[d]
	4	1	2%	0	0%	18	37%	

[a]ANOVA.
[b]Specimen 1 versus specimen 2.
[c]Specimen 1 versus specimen 3.
[d]Specimen 2 versus specimen 3.
[e]Test of Kruskal Wallis.
G = Grade 0, complete absence of follicles; 1, primordial follicles only; 2, primordial and primary follicles; 3, some secondary follicles; 4, pattern of primary and secondary follicles as seen in the normal ovary (Maneschi *et al.*, 1993).

show that ovaries submitted to excision of ovarian cysts perform worse than nonoperated ovaries when the patients undergo ovarian stimulation for assisted reproduction techniques (Nargund *et al.*, 1996; Loh *et al.*, 1999) or in monitored natural cycles (Loh *et al.*, 1999). This hypothesis is supported mostly by studies that have primarily analysed the response of previously operated ovaries to clomiphene or to gonadotropin agonists (Nargund *et al.*, 1996; Loh *et al.*, 1999; Donnez *et al.*, 2001; Ho *et al.*, 2002; Somigliana *et al.*, 2003). On the contrary, in similar settings, other groups were unable to find clinically significant differences (Canis *et al.*, 2001; Marconi *et al.*, 2002).

In a recent study (Muzii *et al.*, 2002) we demonstrated, by histology analysis of specimens excised at operative laparoscopy for ovarian cysts, that some ovarian tissue is inadvertently removed together with the endometrioma 'pseudo-capsule' in 54% of the cases. From the quoted study (Muzii *et al.*, 2002), and from other indirect evidence obtained at laparotomy (Maneschi *et al.*, 1993), it appears that the ovarian tissue adjacent to the endometrioma wall is morphologically different from the normal ovarian tissue, since it never shows the normal follicular pattern that can be observed in normal ovaries. In this perspective, removal of a thin layer of ovarian tissue, if any, at the time of laparoscopic stripping of the cyst wall may not represent an overtreatment, since it may be morphologically altered (and possibly non functional) tissue that is being removed. At partial variance with our recent experience (Muzii *et al.*, 2002), Hachisuga and Kawarabayashi (2002) reported that two groups of endometriomas could be identified at the stripping technique. In a group, the endometrioma capsule could be easily stripped from the ovarian parenchyma, and in this setting primordial follicles could be identified in 69% of the cases. In a second group, where the cyst capsule was densely attached to the ovarian parenchyma, primordial follicles were never found in the cyst wall specimens. The thickness of the cyst wall was higher in the first group (2.1 mm) than in the second group (1.8 mm). In our series (Muzii *et al.*, 2002), the mean thickness of the cyst wall was only 1.3 mm. These data probably reflect different surgical techniques, with possibly less tissue trauma in our series and in the second group in Hachisuga's series, rather than different natures of the endometriomas.

In the above-mentioned study by our group (Muzii *et al.*, 2002), only specimens from the intermediate part of the stripping procedure were evaluated. No information is present in the literature as to histology analysis of specimens from the various sites of the excised cyst wall where surgeons performing the stripping procedure may adopt different surgical techniques, in particular at the beginning of the cyst excision procedure at the initial adhesion site, and at the end of the procedure, near the ovarian hilus (Martin and Berry, 1990).

In the present study, we evaluated the different techniques that can be used when stripping the endometrioma wall from the ovary. As a first result of the study, we confirm that a rim of ovarian tissue is inadvertently removed together with the cyst wall in the majority of the cases. This strip of ovarian tissue excised along with the endometrioma wall is ~0.1–0.3 mm thick in the whole specimen, except near the hilus, where it becomes thicker, measuring on average 0.8 mm. In addition, in most of the specimens from the initial and the intermediate parts of the procedure, the ovarian tissue appears to be devoid of follicles, or only scanty primordial follicles can be recognized. On the other hand, when approaching the hilus, in nearly 70% of the specimens, higher functional stages of follicular development can be recognized, resembling the normal pattern of primordial, primary and secondary follicles that can be seen in healthy ovarian tissue.

During any part of the procedure, the surgeon may visually notice small follicles on the side of the cyst wall that is being excised. This finding invariably corresponds histologically to higher stages of follicular development. In a few cases, this has occurred at the initial part of the stripping procedure, possibly reflecting the presence of a cyst wall more adherent to the healthy ovarian parenchyma, or else the development of a wrong cleavage plane. When follicles are observed on the side of the excised cyst wall at the beginning or at the intermediate part of the procedure, the surgeon should consider the possibility of an incorrect surgical plane, and he/she should go back in the developed plane in order to find a cleavage plane deeper toward the cyst wall. When follicles are encountered near the hilus, this represents instead a common occurrence, and the two techniques used do not influence the surgical performance.

In conclusion, in the majority of the cases some ovarian tissue, ~0.1–0.8 mm in thickness, is excised along with the 'pseudo-capsule' of the endometrioma, whichever the technique used; this tissue, however, shows the morphologic characteristics seen in normal ovarian tissue only when approaching the hilus. Since the stripping of the greatest part of the pseudo-capsule is not associated with removal of healthy tissue, the stripping procedure, whichever the technique used, appears to be a tissue-preserving procedure.

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