


Ovarian reserve after three-step laparoscopic surgery for endometriomas utilizing dienogest: A pilot study

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Funding information

Japan Society for the Promotion of Sciences, Grant/Award Number: 16K20197 and 18K09294

Abstract

Purpose: Surgery for endometriomas may cause detrimental effects on ovarian reserve. We evaluated the safety of three-step laparoscopic surgery for endometriomas utilizing dienogest in terms of post-surgical ovarian reserve.

Methods: Twelve women received first look laparoscopy (FLL) with fenestration and drainage. Immediately after the surgery, they took oral dienogest 2 mg for three months; then, they received second look laparoscopy (SLL) with cystectomy. We compared serum AMH levels between women had three-step management with dienogest, and another twelve women had conventional one-step surgery without medications. In women had three-step procedures, the changes in concentration of proinflammatory cytokines and chemokines in peritoneal fluids were evaluated.

Results: Serum AMH levels were significantly decreased after three months of dienogest following FLL. AMH levels were also significantly decreased 3-6 months both after SLL and after one-step surgery; however, recovery of serum AMH levels at 9-12 months after surgery was evident in women had three-step surgery comparing to those of one-step surgery. Proinflammatory cytokines and chemokines in peritoneal fluids were downregulated at the time of SLL comparing to those of FLL.

Conclusions: Three-step surgery with dienogest may be a beneficial approach to protect ovarian reserve. Dienogest may exert its effects in part by lowering proinflammatory cytokines and chemokines.

KEYWORDS

AMH, dienogest, endometrioma, laparoscopic three-step management, ovarian reserve

1 | INTRODUCTION

Endometriosis is a chronic pelvic inflammatory disease manifested by pain symptom and infertility. Ovarian endometriomas is one of the frequent disease phenotypes of endometriosis.¹ Surgery, such as cystectomy of endometriomas, is the main stay of the treatment but they

may cause serious damage on ovarian functions.² Serum AMH levels are widely used in the evaluation of ovarian reserve and the diagnosis of several endocrinopathy in women.³ They may also reflect the invasiveness of ovarian surgery though its usefulness yet to be determined.

Appropriate surgical approach to endometriomas has been a matter of debate. Three-step surgery utilizing GnRH agonist had been reported,⁴ and this procedure may be superior to one-step

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cystectomy in terms of post-surgical serum AMH levels.⁵ The advantage of this procedure may be associated with inter-surgical medical therapy and ablative surgical method in contrast to the one-step cystectomy, though the mechanism of the protective effect on normal ovarian tissue had not been clearly elucidated.

Dienogest is a progestin recently introduced to the treatment of endometriosis. As a fourth-generation progestin, dienogest possess high affinity to progesterone receptor and no androgenic effect, which make this medication potent long-term therapeutic means for women with endometriosis.⁶ Dienogest exerts anti-proliferative effects on endometriotic lesion via direct effects of progestational decidual changes of endometriotic tissue or indirectly via lowering circulating estradiol levels inhibiting central gonadotrophin secretions that result in altered follicular growth and ovulation.^{7,8} Dienogest may also alleviate pelvic inflammation that caused by endometriosis.⁹ Therefore, novel progestin may be a beneficial alternative in the three-step surgical management of endometriomas.

The aim of our study is to compare the effects on ovarian reserve evaluated by serum AMH levels between women had three-step surgery with dienogest and those had one-step surgery without peri-surgical medications for endometriomas. Serum AMH levels were followed until twelve months after surgery. In addition, we compared the changes of proinflammatory cytokines and chemokines in peritoneal fluid before and after the application of dienogest. The efficacy of three-step management with novel progestin was evaluated.

2 | MATERIALS AND METHODS

2.1 | Patient selection

From June 2012 to September 2015, infertile women and women who wish to preserve ovarian function under the age of 40 with suspected endometriomas, which diagnosed by vaginal ultrasonography and MRI had been allocated. The patients were counseled to choose three-step surgical management or one-step surgery, and prospectively followed up at least one year after surgery. Women who selected three-step management should be infertile and should not receive any hormonal treatment before allocation. Among the patients who selected one-step surgery, women received hormonal therapy within three months before the surgery were excluded. This study was accomplished at Nagasaki University Hospital. Institutional review board had been approved this study. Written informed consent was obtained from all patients.

2.2 | Surgical procedures

2.2.1 | Three-step surgical management

First look laparoscopy (FLL) was performed under general anesthesia with standard four ports placement. Peritoneal fluids were collected at the beginning of surgery. Portion of peritoneal fluids was sent to

cytological assessment and rest of them were centrifuged and aliquots were stored at -20°C . Endometriomas were fenestrated, then chocolate-like fluids were aspirated, and inner side of the cyst was irrigated. Small fragment of cyst wall was biopsied for pathological examination. Adhesiolysis was performed as much as possible. Staging of endometriosis by ASRM scoring system was recorded.¹⁰

After the first look laparoscopy, oral progestin, dienogest 2mg daily (b.i.d.) were prescribed and continued for three months until second look surgery.

Second look laparoscopy (SLL) was performed in similar way to first look surgery. Peritoneal fluids were collected at the beginning of surgery. Portion of peritoneal fluids was sent to cytological assessment and rest of them were centrifuged and aliquots were stored at -20°C . After the adhesiolysis, cystectomy with the care to conserve normal ovarian tissue was performed according to the procedures reported previously.¹¹⁻¹³ In the case with bilateral lesions, hemi-lateral cyst wall ablation with bipolar coagulator with reduced power setting was performed.

2.2.2 | One-step surgical management

Women who did not receive three-step procedure, one-step cystectomy without medications was performed as similar to second look surgery. Accordingly, in the case with bilateral lesions, cyst wall ablation with bipolar coagulator was performed instead of cystectomy after the complete adhesiolysis.

2.3 | AMH measurement and post-operative follow-up

Patient's blood was collected before and after the surgery, and serum AMH levels were determined by ELISA (AMH genII, Beckman-Coulter, Tokyo, Japan) at out-sourcing laboratory (SRL, Tokyo, Japan) irrespective of menstrual cycles. The patients were followed up 1-3 month intervals, and blood was collected at every their visits. In women had three-step management, serum AMH levels were determined at one month after FLL, three months after dienogest treatment just before SLL, and one month after SLL. For group comparison, serum AMH levels after surgery were categorized into three time points, that is, before surgery (time point 0), 3-6 months after surgery (time point 1), and 9-12 months after surgery (time point 2). If women had multiple assay during these periods, lower value was selected for analysis. In some women conceived during the follow-up periods, serum AMH levels were determined until they got conceived.

2.4 | Measurement of cytokines and chemokines in peritoneal fluids

Proinflammatory cytokines and chemokines, which may relate to the pathogenesis of endometriosis,^{14,15} such as interleukin (IL)-1 β , IL-6,

TABLE 1 Clinical backgrounds and severity of endometriosis

	Three-step surgery (n = 11)	One-step surgery (n = 12)
Age (years)	31.4 ± 4.7 (24-37)	31.6 ± 4.7 (24-39)
Nulliparous (n)	11 (100%)	10 (83%)
Infertility at the time of surgery (n)	9 (82%)*	3 (25%)*
Preoperative AMH (ng/mL)	3.44 ± 1.59 (1.09-5.28)	2.80 ± 1.74 (0.21-4.89)
Unilateral/bilateral (n)	5/6	8/4
Size of endometrioma at FLL (cm)	4.6 ± 1.4 (2-7) (n = 17)	5.5 ± 2.8 (1-10) (n = 16)
rASRM lesion score at FLL	35.2 ± 9.7 (23-50)	32.5 ± 9.4 (21-48)
rASRM adhesion score at FLL	34.5 ± 36.1 (4-96)	33.3 ± 33.6 (4-96)
rASRM total score at FLL	69.6 ± 38.7 (27-130)	65.8 ± 39.6 (25-138)
rASRM stage III/IV at FLL (n)	3/8	5/7
Operation time of FLL or one step (min)	116.6 ± 30.0 (82-188)**	179.9 ± 34.8 (111-232)**,*
Blood loss at FLL or one step (mL)	19.6 ± 14.4 (1-50)	56.8 ± 109.3 (10-400)
Size of endometrioma at SLL (cm)	2.5 ± 1.1 (1-5) (n = 16)****	NA
rASRM lesion score at SLL	28.8 ± 9.1 (18-44)****	NA
rASRM adhesion score at SLL	36.1 ± 29.7 (5-89)	NA
rASRM total score at SLL	64.9 ± 30.8 (27-113)	NA
rASRM stage III/IV at SLL (n)	3/8	NA
Operation time of SLL (min)	161.7 ± 62.4 (100-317)***	NA
Blood loss at SLL (min)	35.3 ± 30.3 (8-100)	NA
Conceived after surgery ***** (n)	2	1

Note: The values are expressed as mean ± standard deviation, parenthesis indicates range.

Abbreviations: FLL, first look laparoscopy; SLL, second look laparoscopy; NA, not applicable.

*Significantly different between three-step surgery and one-step surgery ($P < .05$ by Fisher's exact test).

**Significantly different between FLL and one-step surgery ($P < .05$ by unpaired Student *t* test).

***Significantly increased comparing to those of FLL ($P < .05$ by paired Student *t* test).

****Significantly decreased comparing to those of FLL ($P < .05$ by paired Student *t* test)

*****Conceived within 12 months after the surgery (SLL or one-step laparoscopy).

IL-8, tumor necrosis factor (TNF)- α , and monocyte chemoattractant protein 1 (MCP)-1 in peritoneal fluids were measured by electrochemiluminescence immunoassays (MESO QuickPlex SQ 120, MSD, Tokyo, Japan) at out-sourcing laboratory (KPSL, Fukuoka, Japan). We applied this assay to paired samples of peritoneal fluids obtained in women had three-step management. One mL aliquot of centrifuged peritoneal fluids was used for the assay. We calculated the percent changes of measured cytokines and chemokines levels from FLL to SLL by the formula described below.

$$(\text{value of SLL} - \text{value of FLL}) / \text{value of FLL} * 100(\%)$$

2.5 | Statistical analysis

Continuous variables are compared with paired or unpaired Student *t* test and Wilcoxon signed-rank test. Categorical variables are compared with chi-square test and Fisher's exact test. All statistical analysis was performed with computer software (JMP Pro 14.0.0,

SAS institute Japan, Tokyo). *P* value under .05 was considered as statistical significance.

3 | RESULTS

Twelve women were allocated to each three-step management and one-step group. One woman in three-step management was excluded because post-surgical pathology was mixed with endometriomas and mucinous epithelial tumor of borderline malignancy. Clinical backgrounds of the patients were summarized in Table 1. In three-step management group, the size of endometriomas was significantly decreased due to the fenestrations and irrigations at FLL though newly pooled chocolate-like fluids and reformation of adhesion surrounding endometriomas were evident at SLL. Accordingly, rASRM lesion score at SLL was significantly decreased comparing to those of FLL though we did not find significant difference in total ASRM score.

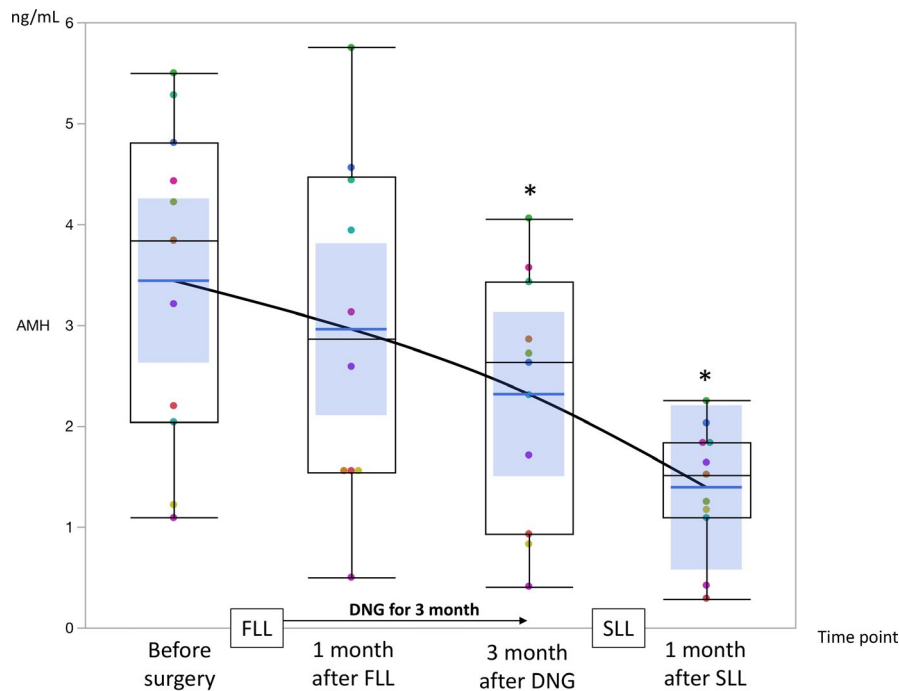


FIGURE 1 The changes of serum AMH levels in women had three-step management for endometriomas around two laparoscopies. This graph depicts the time course change in serum AMH levels from pre-surgical value to one month after second look laparoscopy (SLL). Serum AMH levels significantly decreased after three months of dienogest (DNG) treatment following first look laparoscopy (FLL), and further decreased one month after the SLL (* $P < .05$ at both time points by paired Student t test comparing to pre-surgical levels). Boxes represent the distance (interquartile range) between the first (25%) and third (75%) quartiles, and horizontal lines in the boxes represent median values. Blue horizontal line represents mean value, and blue colored square box represents 95% confidence interval. Each dot represents exact value of individual case. The line indicates cubic spline regression curve

In women had three-step management, serum AMH levels were significantly decreased after dienogest following FLL and further decreased one month after SLL ($P < .05$ by paired Student t test, Figure 1). Preoperative serum AMH levels were not significantly different between two groups. Comparing to preoperative AMH values, both three-step and one-step surgery showed significant decline at 3-6 months after surgery ($P = .02$ and $P = .008$ for one-step and three-step group, respectively; Figure 2). Two women and one woman were conceived in three-step management group and single step group, respectively, during the follow-up period. Two women and one woman were conceived in three-step management group (ART and spontaneous) and one-step group (spontaneous), respectively, during the follow-up period. One woman in three-step management group could not accomplish 12 months of follow-up due to a move. Accordingly, we could finally measure the serum AMH levels at 9-12 months after surgery in eight and eleven women in three-step management group and single step group, respectively. At 9-12 months after the surgery, the significant decline of serum AMH levels was remained in women had one-step surgery; however, we did not find significant difference between preoperative and 9-12 month value in women had three-step surgery. ($P = .01$ and $P = .16$ for one-step and three-step group, respectively; Figure 2).

Although we could obtain peritoneal fluid samples in all surgeries, one woman could not provide paired samples of peritoneal fluid for technical reasons, and after excluding woman with borderline

malignancy as mentioned previously, proinflammatory cytokines and chemokines that involved in pelvic inflammation in peritoneal fluids were measured in 10 women had three-step management. Four women showed decline in all five measured cytokines and chemokines at SLL (after 3 months of dienogest) but the degree of decline was not homogenous among the patients (Table 2). On the other hand, six women showed increase in one of the measured cytokines and chemokines at SLL. The degree of increase was either homogenous among the patients. One woman showed increase in all five measured cytokines. We did not find significant correlation between the depth and distributions of decline in cytokines and chemokines in peritoneal fluids and the decline in serum AMH levels after SLL.

4 | DISCUSSION

In infertile women with ovarian endometriomas, the surgery may be the choice of treatment though the decline in ovarian reserve should be taken into consideration.² The different type of surgical methods, such as fenestration and irrigation, ablation of cyst wall with various surgical equipment, cystectomy, and combined methods had been reported,¹⁶ and these procedures may affect residual ovarian reserve. In terms of disease recurrence and histological assurance, and even regarding post-surgical natural fecundity, cystectomy may be advocated.² On the other hand, the three-step management had

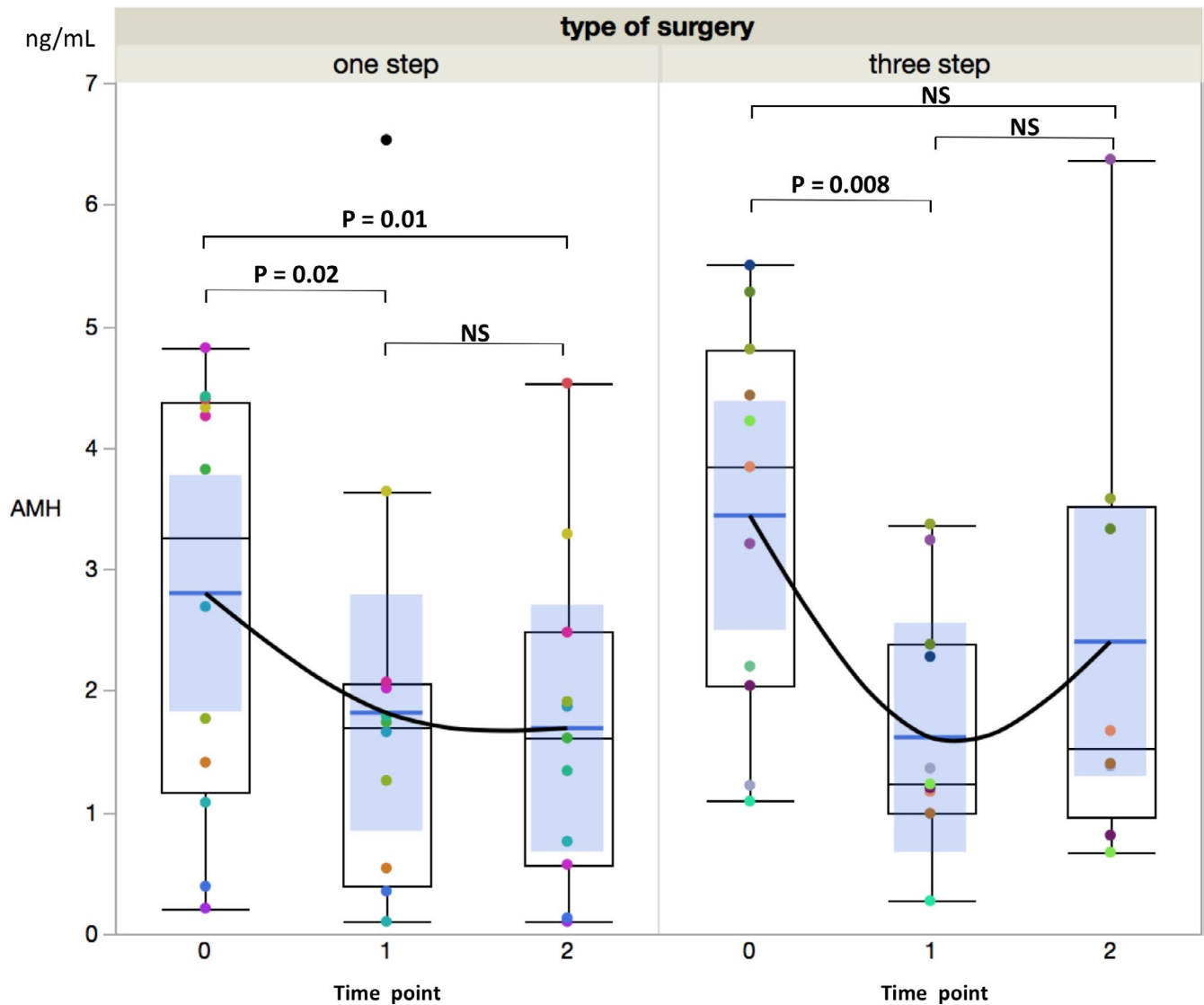


FIGURE 2 The changes of serum AMH levels after the surgery according to the surgical methods. This graph depicts the change in serum AMH levels from pre-surgical value (time point 0) to 3-6 months after the surgery (time point 1) and 9-12 months after surgery (time point 2) in women had one-step laparoscopy and women had three-step management. Both groups showed significant decline in serum AMH levels at 3-6 months after the surgery (the significance of difference determined by Wilcoxon signed-rank test was $P = .02$ and $P = .008$ for one-step and three-step group, respectively). In women had one-step surgery, significant decline was persistent until time point 2 (9-12 months after surgery). Serum AMH levels showed recovery, and there was no statistically significant difference between time points 0 and 2 in women had three-step management. Boxes represent the distance (interquartile range) between the first (25%) and third (75%) quartiles, and horizontal lines in the boxes represent median values. Blue horizontal line represents mean value, and blue colored square box represents 95% confidence interval. Each dot represents exact value of individual case. The line indicates cubic spline regression curve. NS: non-significant

been reported as less invasive surgical procedures for ovarian endometriomas.^{4,5} Originally, this is the method to treat large ovarian endometriomas with CO₂ laser ablation of cyst wall. GnRH agonist were used for three months to lower the estrogen levels and to maintain the effect of drainage of the cyst. One randomized study revealed that post-surgical AMH levels were higher in women had three-step management comparing to those of women had one-step surgery.⁵ Women at the risk of diminished ovarian reserve, such as bilateral and large endometriomas, and three-step surgery may be beneficial.¹⁷

In this study, we used novel progestin, dienogest instead of GnRH agonist, between FLL and SLL. Although the reformation of some fluid collection in drained endometriotic cysts could not be avoided in most of the cases, dienogest could maintain less lesion size at the time of SLL. This may be related to less traumatic surgical procedures for large endometriomas at the time of SLL. Dienogest can be used similar to GnRH agonist in three-step management of endometriomas, especially women want to avoid the side effects of GnRH agonist. Although there was some continuous spotting bleeding within expectation in women had dienogest, we did not

TABLE 2 The changes in cytokines and chemokines in peritoneal fluids between first look laparoscopy and second look laparoscopy after 3 months of oral dienogest

Case No.	%change of intraperitoneal cytokines and chemokines				
	IL-1 β	IL-6	IL-8	TNF α	MCP-1
1	(29.7)	(72.2)	(97.8)	(99.4)	(37.2)
2	(96.8)	(77.1)	(75.8)	(93.6)	(57.0)
3	(16.5)	(84.3)	(54.7)	(28.2)	(12.5)
5	(78.7)	(97.3)	(90.1)	(0.5)	(52.2)
4	944.2	(52.0)	(57.9)	(80.7)	(48.9)
6	89.7	156.0	(60.6)	(10.6)	(61.7)
7	(23.4)	43.9	(19.4)	21.3	(43.7)
8	(8.5)	56.3	15.5	(44.4)	227.7
9	284.6	(27.9)	1187.0	4.8	73.2
10	416.2	211.7	15 566.7	113 126.2	18.1

Note: Values within parenthesis indicate negative number (declined, Cell with gray shadow).

%change from FLL to SLL was calculated by the formula: (value in second look laparoscopy – value in first look laparoscopy)/value in first look laparoscopy*100.

Abbreviations: IL-1 β , interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8; TNF α , tumor necrosis factor alpha, and MCP-1, monocyte chemotactic protein 1.

found women discontinued the medication in this study periods. As dienogest can be used during peri-surgical periods in contrast to oral contraceptives, it can be alternative medications for three-step surgery for endometriomas.

We also could compare the changes of cytokines and chemokines in peritoneal fluids before and after the administration of oral dienogest in this study. These cytokines and chemokines are related to pelvic inflammatory environment caused by endometriosis (ie, IL-1 β , IL-6, IL-8, TNF- α , and MCP-1).^{14,15} Although some women showed decline in all these five molecules, the degree of decline and distributions of declined cytokines and chemokines were not homogenous among the patients. These results may relate to the individual difference in response against dienogest, which may be hypothesized as progesterone-resistance in the pathogenesis of endometriosis.¹⁸ On the other hand, we did not find correlation between the changes in these cytokines and chemokines in peritoneal fluids and the surgical findings at SLL or the changes in serum AMH levels. The relationship between alleviation of pelvic inflammation by dienogest and the effects on ovarian reserve remains to be clarified in future study.

Although dienogest might have reduced the activity of endometriotic lesion after FLL, we found total rASRM score was not improved at the time of SLL. This could be due to reformation of endometriotic adhesion in the pelvis as adhesion score was not significantly changed between two surgeries. In addition, serum AMH levels continuously decreased after the dienogest treatment following FLL, and these declines persist shortly after SLL. Dienogest may

not have enough potency to prevent adhesion formation after surgery. Similarly, dienogest may not affect the fate of atresia of AMH secreting growing follicles, which might have provoked by surgical stress. To avoid the mechanical damage to normal ovarian tissue, adhesiolysis surrounding ovaries at FLL should be as minimum as possible.

Comparing to conventional one-step surgery, three-step surgery showed recovery of serum AMH levels at around one year after surgery. Although both surgical techniques showed acute decline shortly after the surgery, the diminished AMH levels were persisted in women with one-step surgery. Sugita, et al reported that women had surgery for endometriomas may be classified as women with or without recovery in serum AMH levels one year after surgery.¹⁹ If ovarian reserve was protected against surgical invasion, reconstruction of follicular cohort may result in recovery of serum AMH levels at around one year after surgery. If ovarian reserve was severely demised or very low before the surgery, serum AMH levels may not recover. Thus, the results of this pilot study indicate three-step management may be beneficial for protecting ovarian reserve.

Although three-step surgery with dienogest may have potential benefit on protecting ovarian reserve, the dependence of this effects on surgical techniques or medication (dienogest) is unclear. Since dienogest may maintain reduced size of the cyst and may decrease intrapelvic inflammation, it may be able to minimize the harm of invasive surgical technique. In addition, as long-term administration of dienogest may decrease the size of endometrioma without surgery,²⁰ the application of dienogest before conventional one-step surgery may also have potential benefit in terms of protecting ovarian reserve. On the other hand, suppressive hormonal medication in women with endometriosis may not have benefit in terms of pregnancy rate.² Moreover, the administration of dienogest before the follicle aspiration in IVF in women with endometriomas did not show significant benefit.²¹ In the view of infertility treatment, peri-operative medication should be minimized. However, infertile women who have pain symptom, short-term medication with dienogest may have clinical benefit avoiding the detrimental effect of surgery.

Although we could determine the post-operative changes in serum AMH levels according to the surgical methods and the efficacy of novel progestin in three-step management in surgical intervention in endometriomas, small numbers of patients in non-randomized setting may be the limitations of this study. The study of three-step management of endometriomas with lager sample size to confirm the benefit of this surgical methods is warranted.

In conclusions, three-step surgical management of endometriomas with dienogest may be beneficial for infertile women at the risk of diminished ovarian reserve after surgery. Although the acute decline of serum AMH levels shortly after the surgery may occur, the recovery of serum AMH levels at around one year after the surgery can be expected. Dienogest may exert its effects via downregulating the proinflammatory cytokines and chemokines, reducing the activity of endometriotic lesion to maintain smaller cyst size; then, protective surgical procedures can be achieved.

ACKNOWLEDGEMENTS

This research was supported in part by the Grants-in-Aid for Scientific Research (grant no. 18K09294 and 16K20197 to MK and NM) from Japan Society for the Promotion of Sciences.

DISCLOSURES

Conflict of interest: The authors declare that they have no conflict of interests. **Human rights statements and informed consent:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declarations of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study. **Approval by Ethics Committee:** This study was approved by Nagasaki University Hospital Clinical Research Ethics Committee. **Clinical Trial Registry:** This study was not a clinical trial.

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How to cite this article: Kitajima M, Matsumoto K, Murakami N, et al. Ovarian reserve after three-step laparoscopic surgery for endometriomas utilizing dienogest: A pilot study. *Reprod Med Biol.* 2020;19:425-431. <https://doi.org/10.1002/rmb2.12349>