

# Ovarian reserve after three-step laparoscopic surgery for endometriomas utilizing dienogest: a non-randomized prospective pilot study.

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## Research

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# Abstract

**Background** Surgery for endometriomas may cause detrimental effects on ovarian reserve. In this study, we evaluated the safety of three-step laparoscopic surgery for endometriomas utilizing novel progestin, 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 2mg for three months, then they received second look laparoscopy (SLL) with cystectomy. We compared the effects on ovarian reserve by 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 surgery with dienogest, the changes in concentration of proinflammatory cytokines and chemokines (interleukin (IL)-1 $\beta$ , IL-6, IL-8, tumor necrosis factor (TNF)- $\alpha$ , monocyte chemotactic protein (MCP)-1) 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 month both after SLL and after one-step surgery, however, recovery of serum AMH levels at 9-12 months after surgery were evident in women had three-step surgery comparing to those of women had one-step surgery. Proinflammatory cytokines and chemokines in peritoneal fluids were down regulated 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.

## Background

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 have been a matter of debate. Three-step surgery utilizing GnRH agonist had been reported[4] and this procedure may be superior to one-step 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 safety of three step management with novel progestin were evaluated.

## Methods

### *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. After the provision of current information on risk and benefit of laparoscopic surgery for endometriomas, the subjects were counselled to choose three-step surgical management or one-step surgery, and prospectively followed up at least one year after surgery. Considering the surgical burden and to assess the safety of three-step management with dienogest on post-surgical fecundity, we planned that women who select three step management should be infertile or wishing the pregnancy in near future and should not receive any hormonal treatment before allocation. Infertile women also can choose one-step management after the provision of the information with shared decision. In one-step group, women without current desire to bear children were also included. Among the subjects 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 were obtained from all subjects.

### *Surgical procedures*

All surgeries were performed by single main surgeon (M.K.) with some assistant surgeons (K.M., A.H., and N.M.).

### **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, cystectomy was performed in larger cyst ( $\geq 3\text{cm}$ ) and cyst wall ablation with bipolar coagulator with reduced power setting was performed in contra-lateral cyst with smaller size ( $< 3\text{cm}$ ) .

### **One-step surgical management**

Women who did not receive three-step procedure, cystectomy was performed as similar to second look surgery. Accordingly, in the case with bilateral lesions, cystectomy was performed in larger cyst ( $\geq 3\text{cm}$ ) and cyst wall ablation with bipolar coagulator with reduced power setting was performed in contra-lateral cyst with smaller size ( $< 3\text{cm}$ ) after the complete adhesiolysis.

### *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 subjects were followed up 1-3month 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, i.e. before surgery (timepoint 0), 3-6month after SLL or one-step surgery (time point 1), and 9-12month after SLL or one-step surgery (time point 2). If women had multiple assay during these periods, lower value were selected for analysis. As most women in this study were infertile or wishing the pregnancy in near future, we do not restrict them from attempting pregnancy or infertility treatment during follow-up periods. Accordingly, some women conceived during the follow-up periods, serum AMH levels were determined until they got conceived.

### *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 $\beta$ , IL-6, IL-8, tumor necrosis factor (TNF)- $\alpha$ , monocyte chemotactic protein (MCP)-1 in peritoneal fluids were measured by electrochemiluminescence immunoassays (MESO QuickPlex SQ 120, MSD, Tokyo, Japan) at out-sourcing laboratory (KPSL, Fukuoka, Japan). One mL

aliquot of centrifugated peritoneal fluids were used for the assay. We calculated the percent changes of measured cytokines and chemokines levels by the formula described below.

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

### *Statistical analysis*

Continuous variables are compared with paired or unpaired student-t test and Wilcoxon signed-rank test. Categorical variables are compared with kai 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 0.05 were considered as statistical significance.

## **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 subjects were summarized in Table 1. There were significantly more women with infertility in three-step group. In three step management group, the size of endometriomas were 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 were significantly decreased comparing to those of FLL though we did not find significant difference in total ASRM score. Surgical time were longer in SLL and one-step surgery comparing to those of FLL, but we did not find significant difference between SLL and one-step surgery. We did not find significant difference in estimated blood loss.

Table 1: Clinical backgrounds and severity of endometriosis

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

FLL, first look laparoscopy; SLL, second look laparoscopy; NA, not applicable

\* Significantly different between three-step surgery and one-step surgery (p<0.05 by Fisher's exact test)

† Significantly different between FLL and one-step surgery (p<0.05 by unpaired student-t test)

‡ Significantly increased comparing to those of FLL (p<0.05 by paired student-t test)

\*\* Significantly decreased comparing to those of FLL (p<0.05 by paired student-t test)

§ Conceived within 12months after the surgery [SLL or one-step laparoscopy]

In women had three-step management, serum AMH levels were significantly decreased after dienogest following FLL, and further decreased one month after SLL (P<0.05 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 month after surgery (P=0.02 and P=0.008 for one-step and three-step group, respectively; Figure 2). 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

	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) <sup>†</sup>	179.9±34.8 (111-232) <sup>††</sup>
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) <sup>‡</sup>	NA
Blood loss at SLL (min)	35.3±30.3 (8-100)	NA
Conceived after surgery <sup>§</sup> (n)	2	1

group could not accomplish 12 months of follow-up due to a move. Accordingly, we could finally measure the serum AMH levels at 9-12month after surgery in eight and eleven women in three step management group and

single step group, respectively. At 9-12 month after the surgery, the significant decline of serum AMH levels were remained in women had one-step surgery, however, we did not find significant difference between pre-operative and 9-12month value in women had three-step surgery. (P=0.01 and P=0.16 for one-step and three-step group, respectively; Figure 2).

Because one woman could not provide paired samples of peritoneal fluid, 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 subjects (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 subjects. 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.

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.

\*IL-1β: interleukin-1 beta, IL-6: interleukin-6, IL-8 : interleukin-8, TNFα: tumor necrosis factor alpha, MCP-1: monocyte chemotactic protein 1,

%change was calculated by the formula: (value in first look laparoscopy - value in second look laparoscopy)/value in first look laparoscopy\*100

Values within parenthesis indicate negative number (declined, Cell with gray shadow)□

## Discussion

Case No.	%change of intraperitoneal cytokines and chemokines*				
	IL-1 $\beta$	IL-6	IL-8	TNF $\alpha$	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	15566.7	113126.2	18.1

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 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 with cystectomy[5]. Women at the risk of diminished ovarian reserve, such as bilateral and large endometriomas, 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 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 (i.e. IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , 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 subjects. 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.

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 safety of novel progestin in three step management in surgical intervention in endometriomas, small numbers of subjects 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.

## 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 down regulating the proinflammatory cytokines and chemokines, reducing the activity of endometriotic lesion to maintain smaller cyst size, then protective surgical procedures can be achieved.

## **Declarations**

### **Ethics approval and consent to participate**

This study was approved by Nagasaki University Hospital Clinical Research Ethics Committee. Written informed consent were obtained from all subjects.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Author's contributions**

MK was a chief surgeon, study organizer, and main contributor in writing the manuscript. KM was an assistant surgeon and analysed the data. NM managed out-patient follow up and analysed the data. AH was an assistant surgeon and managed in-patient care. YK managed out-patient follow up and analysed the data. HM was a contributor in correcting the manuscript. KM was a major contributor in writing and correcting the manuscript. All authors read and approved the final manuscript.

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## **References**

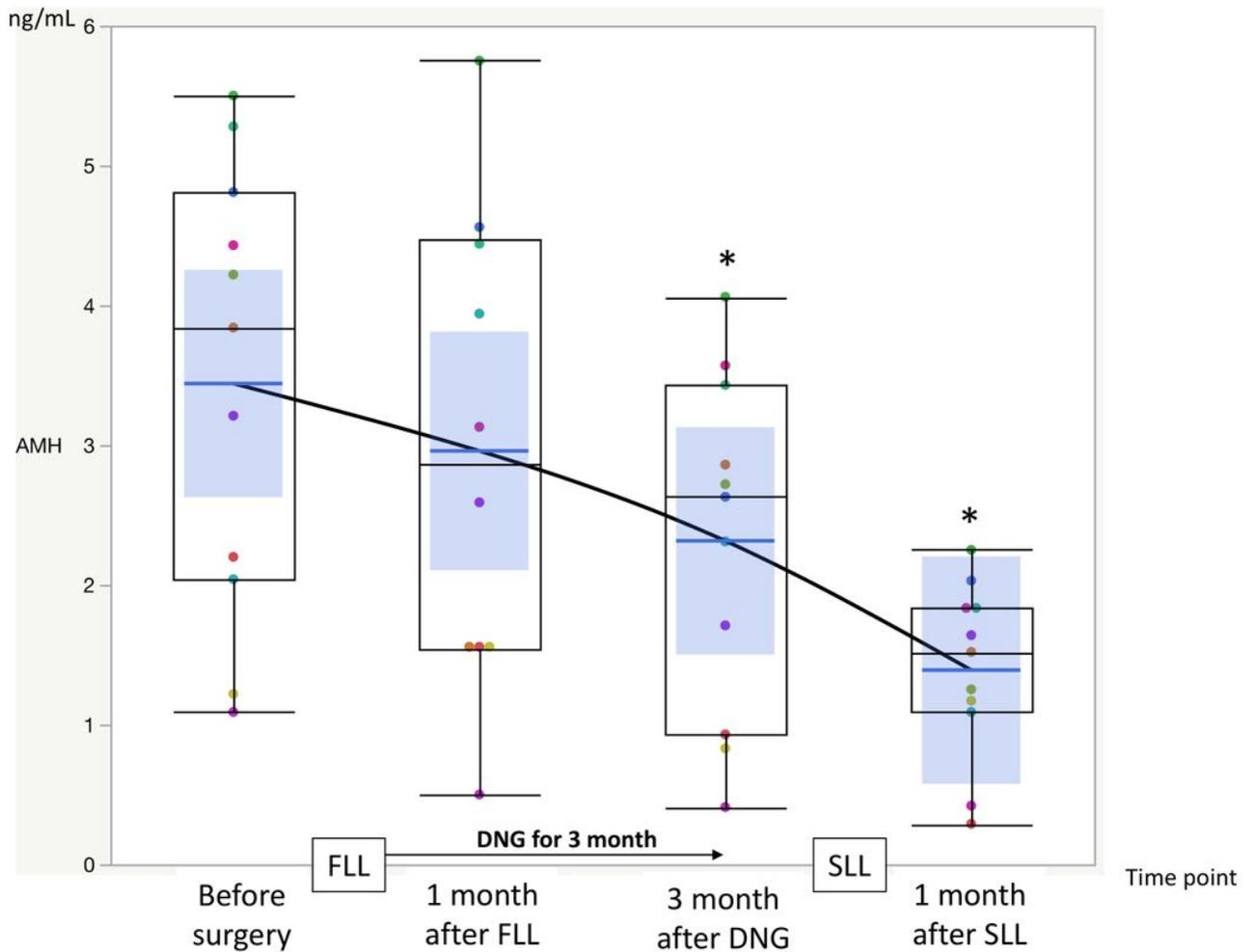
1. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril.* 1997;68:585-96.

[https://doi.org/10.1016/S0015-0282\(97\)00191-X](https://doi.org/10.1016/S0015-0282(97)00191-X).

2. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: management of women with endometriosis. *Hum Reprod*. 2014;29:400-12. [https://doi: 10.1093/humrep/det457](https://doi.org/10.1093/humrep/det457).
3. Seifer DB, Maclaughlin DT. Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril*. 2007;88:539-46. <https://doi.org/10.1016/j.fertnstert.2007.02.014>
4. Donnez J, Nisolle M, Gillet N, Smets M, Bassil S, Casanas-Roux F. Large ovarian endometriomas. *Hum Reprod*. 1996;11:641-6. <https://doi.org/10.1093/HUMREP/11.3.641>.
5. Tsolakidis D, Pados G, Vavilis D, Athanatos D, Tsalikis T, Giannakou A, et al. The impact on ovarian reserve after laparoscopic ovarian cystectomy versus three-stage management in patients with endometriomas: a prospective randomized study. *Fertil Steril*. 2010;94:71-7. <https://doi.org/10.1016/j.fertnstert.2009.01.138>.
6. Harada T, Taniguchi F. Dienogest: a new therapeutic agent for the treatment of endometriosis. *Womens Health (Lond)*. 2010;6:27-35. <https://doi.org/10.2217/WHE.09.72>.
7. Mabrouk M, Paradisi R, Arena A, Del Forno S, Matteucci C, Zannoni L, et al. Short-term histopathological effects of dienogest therapy on ovarian endometriomas: in vivo, nonrandomized, controlled trial. *Gynecol Endocrinol*. 2018;34:399-403. <https://doi.org/10.1080/09513590.2017.1405932>.
8. Miyashita M, Koga K, Takamura M, Izumi G, Nagai M, Harada M, et al. Dienogest reduces proliferation, aromatase expression and angiogenesis, and increases apoptosis in human endometriosis. *Gynecol Endocrinol*. 2014;30:644-8. <https://doi.org/10.3109/09513590.2014.911279>.
9. Ichioka M, Mita S, Shimizu Y, Imada K, Kiyono T, Bono Y, et al. Dienogest, a synthetic progestin, down-regulates expression of CYP19A1 and inflammatory and neuroangiogenesis factors through progesterone receptor isoforms A and B in endometriotic cells. *J Steroid Biochem Mol Biol*. 2015;147:103-10. <https://doi.org/10.1016/j.jsbmb.2014.12.008>.
10. American Society for Reproductive Medicine, Revised American society for reproductive medicine classification of endometriosis: 1996. *Fertil Steril*. 1997;67:817-21. [https://doi.org/10.1016/S0015-0282\(97\)81391-X](https://doi.org/10.1016/S0015-0282(97)81391-X).
11. Donnez J, Lousse JC, Jadoul P, Donnez O, Squifflet J. Laparoscopic management of endometriomas using a combined technique of excisional (cystectomy) and ablative surgery. *Fertil Steril*. 2010;94:28-32. <https://doi.org/10.1016/j.fertnstert.2009.02.065>.
12. Canis M, Kondo W, Botchorishvili R, Bourdel N. Surgical arrows should be identified on the cyst wall. *Fertil Steril*. 2013;99:e7. <https://doi.org/10.1016/j.fertnstert.2012.11.026>.
13. Lewis M, Baker V, Nezhat C. The impact on ovarian reserve after laparoscopic ovarian cystectomy versus three-stage management in patients with endometriomas: a prospective randomized study. *Fertil Steril*. 2010;94:e81-2; author reply e83. <https://doi.org/10.1016/j.fertnstert.2010.08.010>.
14. Banu SK, Lee J, Starzinski-Powitz A, Arosh JA. Gene expression profiles and functional characterization of human immortalized endometriotic epithelial and stromal cells. *Fertil Steril*.

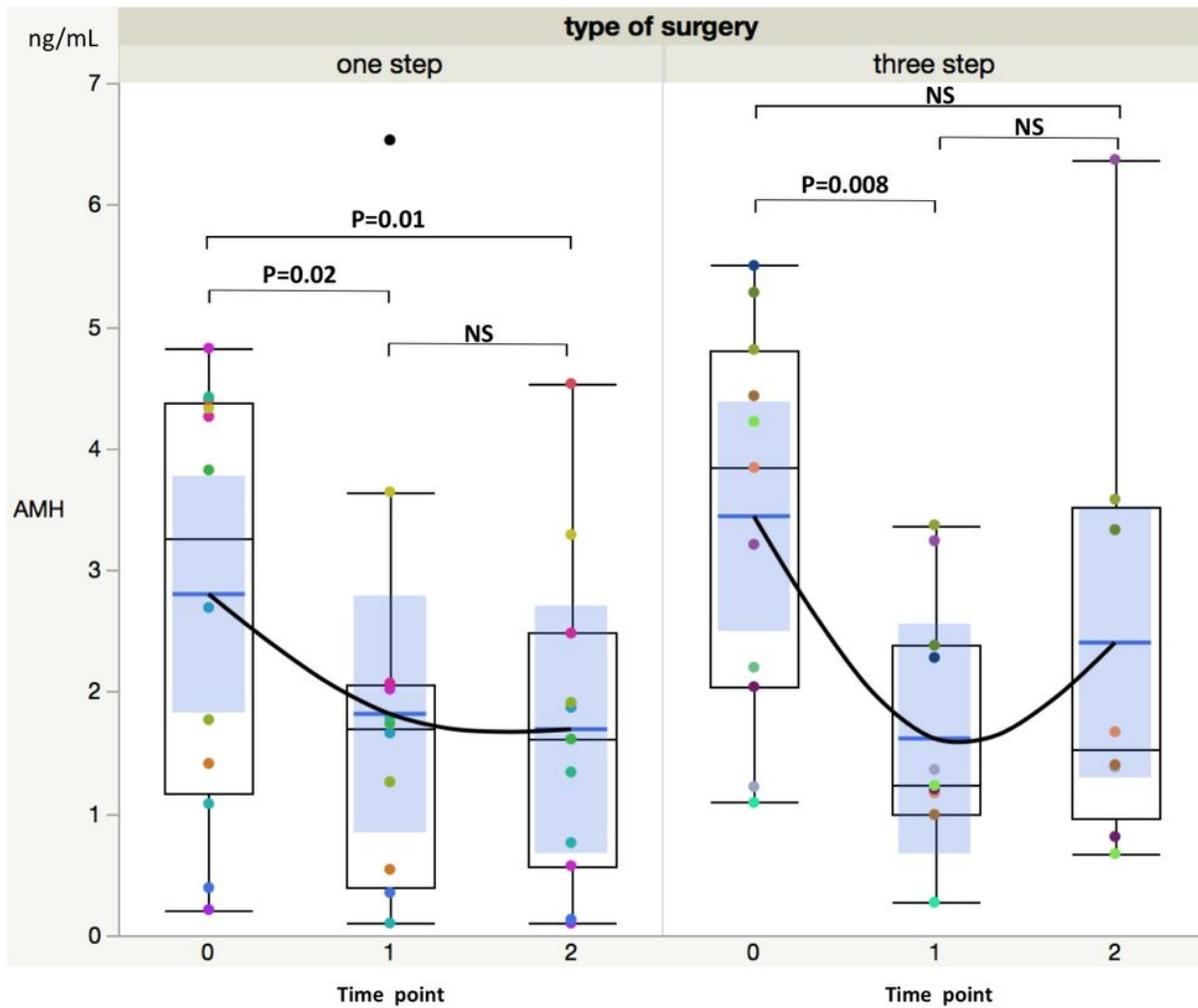
- 2008;90:972-87. <https://doi.org/10.1016/j.fertnstert.2007.07.1358>.
15. Borrelli GM, Abrão MS, Mechsner S. Can chemokines be used as biomarkers for endometriosis? A systematic review. *Hum Reprod*. 2014;29:253-66. <https://doi.org/10.1093/humrep/det401>.
  16. Jadoul P, Kitajima M, Donnez O, Squifflet J, Donnez J. Surgical treatment of ovarian endometriomas: state of the art? *Fertil Steril*. 2012;98:556-63. <https://doi.org/10.1016/j.fertnstert.2012.06.023>.
  17. Roman H. Endometriosis surgery and preservation of fertility, what surgeons should know. *J Visc Surg*. 2018;155 Suppl 1:S31-S36. <https://doi.org/10.1016/j.jviscsurg.2018.03.002>.
  18. Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: origins, consequences and interventions. *Acta Obstet Gynecol Scand*. 2017;96:623-632. <https://doi.org/10.1111/aogs.13156>.
  19. Sugita A, Iwase A, Goto M, Nakahara T, Nakamura T, Kondo M, et al. One-year follow-up of serum antimüllerian hormone levels in patients with cystectomy: are different sequential changes due to different mechanisms causing damage to the ovarian reserve? *Fertil Steril*. 2013;100:516-22.e3. <https://doi.org/10.1016/j.fertnstert.2013.03.032>.
  20. Del Forno S, Mabrouk M, Arena A, Mattioli G, Giaquinto I, Paradisi R, et al. Dienogest or Norethindrone acetate for the treatment of ovarian endometriomas: Can we avoid surgery? *Eur J Obstet Gynecol Reprod Biol*. 2019;238:120-4. <https://doi.org/10.1016/j.ejogrb.2019.04.010>.
  21. Tamura H, Yoshida H, Kikuchi H, Josaki M, Mihara Y, Shirafuta Y, et al. The clinical outcome of Dienogest treatment followed by in vitro fertilization and embryo transfer in infertile women with endometriosis. *J Ovarian Res*. 2019;12:123. <https://doi.org/10.1186/s13048-019-0597-y>.

## Figures



**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 (\* $P < 0.05$  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 represent 95% confidence interval. Each dot represents exact value of individual case. The line indicates cubic spline regression curve.



**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  $P=0.02$  and  $P=0.008$  for one-step and three-step group, respectively). In women had one-step surgery, significant decline was persistent until time point 2 (9-12 month after surgery). Serum AMH levels showed recovery and there was no statistically significant difference between time point 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 represent 95% confidence interval. Each dot represents exact value of individual case. The line indicates cubic spline regression curve. NS: non-significant.