

Research Article

Ovarian Reservation in Women with Ovarian Endometriosis Cyst after Laparoscopic Cystectomy and Leuprorelin Acetate Administration

Cadangan Ovarium pada Perempuan dengan Kista Endometriosis Ovarium setelah Laparotomi Kistektomi dan Leuprorelin Asetat

Reza F. Akhmad¹, Yuli Trisetiyono¹, I Edward K. Setiawan², Besari A. Pramono¹, Soerjo Hadijono¹, Inu Mulyantoro¹, Ediwibowo Ambari¹

¹Department of Obstetrics and Gynecology

²Department of Clinical Pathology

Faculty of Medicine Universitas Diponegoro

Dr Kariadi General Hospital Medical Center

Semarang

Abstract

Objective: To determine differences in ovarian reserve in women with ovarian endometrial cysts after laparoscopic cystectomy and leuprorelin acetate administration

Methods: Single cohort prospective pre and post-test design study with 25 research subjects. The independent variables were interventional laparoscopic cystectomy, and leuprorelin acetate administration. The dependent variable is ovarian reserve as measured by AMH levels.

Results: The research subjects had a mean age of 31 years and a BMI of 23.55 kg/m². There were 8 subjects with unilateral cysts and 17 subjects with bilateral cysts. Preoperative AMH levels had a median value of 1.32 ng/mL (0.88-5.13), postoperative AMH levels had a median value of 1.07 ng/mL (0.60-4.53), and postoperative AMH levels + Leuprorelin Acetate injection had a median value of 1.06 ng/mL (0.50-4.65). There was a significant difference between preoperative AMH and postoperative AMH ($p < 0.001$). There was no significant difference between postoperative AMH and postoperative AMH + Leuprorelin Acetate injection ($p = 0.149$). BMI has a relationship ($p = 0.048$) with pre-operative AMH levels with a weak and opposite relationship ($r = -0.399$).

Conclusion: There was a statistically significant 18.9% decrease between pre-operative post-operative AMH levels. Body Mass Index (BMI) and pre-operative AMH levels are associated with one another.

Keywords: anti-müllerian hormone, leuprorelin acetate, ovarian cystectomy, ovarian reserve.

Abstrak

Tujuan: Mengetahui perbedaan cadangan ovarium pada perempuan dengan kista endometrium ovarium setelah laparotomi kistektomi dan injeksi leuprorelin asetat.

Metode: Desain penelitian pre dan post-test prospektif kohort tunggal dengan 25 subjek penelitian. Variabel bebas adalah intervensi laparotomi kistektomi, dan injeksi leuprorelin asetat. Variabel terikat adalah cadangan ovarium yang diukur dengan kadar AMH.

Hasil: Subjek penelitian memiliki rerata usia 31 tahun dan IMT 23,55 kg/m². Terdapat 8 subjek dengan kista unilateral, 17 subjek dengan kista bilateral. Kadar AMH praoperasi memiliki nilai median 1,32 ng/mL (0,88-5,13), kadar AMH pascaoperasi memiliki nilai median 1,07 ng/mL (0,60-4,53), dan kadar AMH pascaoperasi + injeksi Leuprorelin Asetat memiliki nilai median 1,06 ng/mL (0,50-4,65). Ada perbedaan yang signifikan antara AMH pra operasi dan AMH pascaoperasi ($p < 0,001$). Tidak ada perbedaan bermakna antara AMH pascaoperasi dengan injeksi AMH + Leuprorelin Asetat pascaoperasi ($p = 0,149$). IMT memiliki hubungan ($p = 0,048$) dengan kadar AMH pra operasi dengan hubungan yang lemah dan berlawanan ($r = -0,399$).

Kesimpulan: Terdapat penurunan 18,9% antara tingkat AMH pra-operasi dan tingkat AMH pasca-operasi yang signifikan secara statistik. Ada hubungan antara BMI dan tingkat AMH pra-operasi

Kata kunci: cadangan ovarium, hormon anti-müllerian, kistektomi ovarium, leuprorelin asetat.

Correspondence author. Reza F. Akhmad. Department of Obstetrics and Gynecology.

Faculty of Medicine Universitas Diponegoro. Dr. Kariadi General Hospital Medical Center. Semarang.

Email: reza.poji@gmail.com

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INTRODUCTION

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus. Approximately 30-50% of women with endometriosis are infertile and ovarian endometrial cysts are commonly found during infertility screening examinations.¹⁻³ Ovarian endometriosis cysts can cause chronic pelvic pain and infertility.¹

Treatment of endometriosis consists mainly of hormonal therapy or surgical therapy. Milder forms of endometriosis can be treated with oral contraceptive pills, whereas severe forms of endometriosis, such as ovarian endometriosis cysts, require surgical management with ovarian cystectomy.¹ It is suspected that ovarian cystectomy surgery can cause damage to the ovarian follicles resulting in decreased ovarian reserve. Hormonal therapy using GnRH agonist is thought to also affect ovarian reserve.⁴

One of the reliable markers of ovarian reserve is the measurement of Anti-Müllerian Hormone (AMH) levels. AMH is thought to play a role in the transition phase from inactive primordial follicles to follicles which then develop due to gonadotropin stimulation.⁵

This study aims to assess the ovarian reserve by measuring AMH levels in patients with ovarian endometriosis cysts before surgery, after laparoscopic cystectomy surgery, and three months after administration of leuporelin acetate injection.

METHODS

This research was a prospective single cohort study of pre and post-test design conducted at the Gynecology Polyclinic of the Merpati Installation RSUP Dr. Kariadi Semarang. The research subjects consisted of 25 patients with ovarian endometrial cysts who underwent laparoscopic cystectomy followed by injection of leuporelin acetate. All subjects met inclusion criteria and had no exclusion criteria. The inclusion criteria were women aged 18-45 years, diagnosed with endometrial cysts through history taking, physical examination, and supporting examinations according to the protocol, desiring a pregnancy, and willing to participate in the study by signing an informed consent. Exclusion criteria were pregnant or breastfeeding women, a woman using hormonal contraception, with a history of taking Vitamin D in the past year, a history of

previous ovarian surgery, and currently having a malignancy. The drop-out criteria for the study were the mismatched in anatomical pathology results with ovarian endometriosis cysts, and lost follow-up during the study.

The research sample was taken using consecutive sampling technique. All patients with clinical endometriosis cysts who would undergo surgery were included in the prospective research subjects. Prospective research subjects who met the inclusion criteria and did not have the exclusion criteria, would be subjected to blood collection and pre-operative AMH serum examination. Following the surgery, an anatomical pathology examination was done. If the results of anatomical pathology did not show endometriosis cysts, the prospective research subjects would be excluded. Prospective research subjects with anatomical pathology results in accordance with endometriosis cysts would be included as research subjects. The subjects received leuporelin acetate injection every month. Blood samples from the subjects were taken for AMH serum examination upon receiving leuporelin acetate injection and two months after. Data collected was analyzed and reported.

The data with a numerical scale was tested for the distribution of Shapiro Wilk's data. If the data distribution was normal, the data would be presented using the mean \pm SD, while if it was not normal it would be presented in the median (minimum-maximum). Nominal scale data was presented as a percentage in each category.

Differences in serum AMH levels before and after laparoscopic cystectomy, before laparoscopic cystectomy and after leuporelin acetate injection, after laparoscopic cystectomy and after leuporelin acetate injection were tested by the Wilcoxon difference test. The relationship between age and BMI on AMH levels was tested by the Spearman test.

The research was carried out after obtaining ethical clearance from Dr. Kariadi Semarang with ethical clearance no. 938/EC/KEPK-RSDK/2021.

RESULTS

Table 1. Characteristics of Research Subjects

Variable	n (%)	Mean ± SD	Median (min-max)
Age (years)	-	31.04±6.20	-
Body Mass Index (kg/m ²)	-	23.55±2.14	-
Cysts Number	-	-	-
Unilateral	8 (32)		
Bilateral	17 (68)		
AMH before Surgery (ng/mL)	-	-	1.32 (0.88-5.13)
AMH after Surgery (ng/mL)	-	-	1.07 (0.60-4.53)
AMH after Surgery + Leuprorelin Acetate (ng/mL)	-	-	1.06 (0.50-4.65)

Table 1 shows the characteristics of research subjects. Research subjects had an age with a mean ± standard deviation: 31.04 ± 6.20 years. Body mass index had a mean ± standard deviation: 24.19 ± 2.19 kg/m². Based on the number of cysts, 8 subjects (32%) had unilateral cysts and 17 subjects (68%) had bilateral cysts. AMH before surgery had a median value of 1.32 ng/mL, the smallest value of 0.88 ng/mL and the largest value of 5.13 ng/mL. AMH after surgery had a median value of 1.07 ng/mL, the smallest value of 0.60 ng/mL and the largest value of 4.53 ng/mL. AMH after surgery + leuprorelin acetate had a median value of 1.06 ng/mL, the smallest value of 0.50 ng/mL and the largest value of 4.65 ng/mL.

Table 2. Differences in AMH levels before surgery, after surgery, and after surgery + leuprorelin acetate injection

Variable	P-value
AMH before Surgery	<0.001
AMH after Surgery	0.149
AMH after Surgery + Leuprorelin Acetate	

Table 2 shows there was a significant difference ($p < 0.001$) between preoperative AMH levels and postoperative AMH levels. There was a decrease in AMH value of 17% between AMH before surgery and AMH after surgery. There was no significant difference ($p = 0.149$) between postoperative AMH levels and postoperative AMH + leuprorelin acetate levels.

Table 3. Age and BMI on AMH Levels before Surgery

	AMH before Surgery	
	r	P-value*
Age	0.074	0.725
BMI	-0.399	0.048

Table 3 shows Body mass index had a relationship ($p = 0.048$) with AMH levels before surgery with a weak and opposite relationship ($r = -0.399$). This means that an increase in body mass index value is associated with a decrease in AMH levels before surgery. There was no relationship between age and AMH levels before surgery ($p = 0.725$).

DISCUSSION

The average age of the subjects in this study was 31 years. Most of the subjects had bilateral cysts (68%). The mean of subjects with BMI more than 24 kg/. Thirty- fifty percent of women with endometriosis will experience infertility. The incidence of endometriosis mainly occurs between the phases of menarche and menopause. The peak incidence occurs between the ages of 25 and 45 years. Recent data show that there is generally no association between BMI and the incidence of endometriosis, but there has been a significant increase in the incidence of endometriosis in obese women compared with normal weight women.⁶

When compared with healthy ovaries, ovaries with endometrial cysts had a reduced response to exogenous gonadotropin stimulation, lower antral follicle counts, lower follicle density in the cortex, and increased follicular atresia. Further assessment showed that the density of the primordial follicles was reduced, and the general morphology and vascular network were also distorted. Follicular loss may occur even in the early stages of cyst development.⁷

Ovarian reserve reflects, both qualitatively and quantitatively, reproductive potential and oocyte function, in a patient. The most reliable and widely used quantitative marker of ovarian reserve is serum anti-Müllerian hormone (AMH) levels. Endometriotic cysts can affect ovarian reserve in two ways: impairing circulation in the ovarian

cortex by compression of the cyst and thereby causing follicle loss and/or through regulation of inflammation within the cyst wall leading to follicular destruction.¹ A systematic review and meta-analysis examining 17 articles with a total sample of 968 women with endometriotic ovarian cysts found that AMH levels were significantly lower in the endometriotic cyst group compared to women with non-endometriotic benign ovarian cysts or healthy ovaries (mean difference: -0.84 ng/ml; CI: -1.16 to -0.52; $P < 0.01$). In the secondary analysis, women with endometriotic cysts were separately compared with women with non-endometriotic ovarian cysts (mean difference: -0.61 ng/ml; CI: -1.37 to -0.32) and women with normal ovaries. (mean difference: -0.61 ng/ml; CI -0.99 to -0.24). Both comparisons showed a significantly lower AMH in patients with endometrial cysts. These findings suggest that the presence of endometrial cysts is associated with reduced AMH levels in women.⁸

In this study, there was a significant difference between AMH levels before and after surgery. There was no significant difference between postoperative AMH levels and postoperative AMH + leuprorelin acetate.

Regarding the assessment of ovarian reserve through measurement of follicular density based on ovarian biopsy and Anti-Müllerian Hormone (AMH) in endometriosis patients, it was found that AMH levels were shown to decrease with age in untreated endometriosis patients, but AMH levels were significantly lower in endometriosis cases than in endometriosis patients with the control group only occurred in patients over the age of 36 years. The decrease in AMH was more rapid in endometriosis than that in controls. The number of primordial follicles based on ovarian biopsy decreased as AMH levels decreased in the intervention and control groups. Therefore, it can be concluded that AMH is a reliable marker of ovarian reserve in endometriosis patients.⁹

The effect of endometrial cysts and laparoscopic cystectomy on AMH hormone levels by assessing 1,642 patients who were divided into 4 groups, namely group 1 (1,232 infertile patients without endometriosis cysts), group 2 (141 patients with cysts). endometriosis), group 3 (147 patients who underwent unilateral or bilateral laparoscopic cystectomy due to endometrial cysts for more than 6 months), and group 4 (31 patients who underwent cystectomy during the study phase). The mean level of AMH was significantly lower in patients with bilateral cystectomy compared

with patients with unilateral cystectomy. The mean serum AMH level was also significantly lower in patients with bilateral endometrial cysts compared with patients with unilateral endometrial cysts. In group 4, the mean AMH level decreased significantly from 3.95 ± 0.42 preoperatively to 2.01 ± 0.21 ng/ml at 3 months postoperatively.¹⁰

Laparoscopic ovarian cystectomy in cases of endometrial cysts has a negative effect on ovarian capacity.¹¹ Similar to previous studies that have been shown, ovarian cystectomy can affect ovarian reserve.^{10,12-14} This side effect occurs immediately after surgery and affects patients over the medium term, at least 6 months.¹⁵ The cause of decreased ovarian reserve not only due to loss of follicles during endometriotic cyst removal but also blood loss during surgery. The additional effect of total abdominal hysterectomy on serum AMH is the loss of ovarian reserve more than 30%. Surgery, hysterectomy, can reduce the ovarian blood supply and result in a temporary decrease in ovarian reserve.¹⁶

In a meta-analysis that discussed the effect of unilateral and bilateral laparoscopic surgery in endometriosis cases on AMH hormone levels after 3 months and 6 months found that unilateral and bilateral laparoscopic endometriosis surgery affected AMH levels. AMH levels were reduced in both comparisons, and the decrease in AMH levels was influenced more by the effect of laparoscopic surgery for bilateral endometriosis than laparoscopic surgery for unilateral endometriosis. In addition, the number in AMH will increase after 6 months.¹⁷

There are several studies that discuss the effect of using leuproline acetate or GnRH agonists (GnRHa) on women's AMH levels. AMH decreased 7 days after GnRHa administration by a median of 24% (1.7 ng/mL) and then increased above the pre-intervention level 14 and 30 days after GnRHa by 13% (2.5 ng/mL) and 32% (3.1 ng/mL). Significant changes in AMH levels occurred in the first 4 weeks after GnRHa administration, suggesting that AMH may not be used as a good marker of ovarian reserve during this interval. Administration of GnRHa causes an initial increase in LH and FSH, which can stimulate follicular development and E2 secretion (flare), followed by gonadotropin desensitization and continued suppression of gonadotropins and ovarian steroids. The GnRH receptor is expressed by human granulosa cells and is up regulated, in part, by GnRH. The decrease in AMH after 7 days

of treatment was due to upregulation of the GnRH receptor combined with the antiproliferative and apoptotic effects of short-term GnRHa exposure on granulosa cells.¹⁸ Studies in rodents have shown that GnRH receptor expression in granulosa cells is decreased with prolonged administration of GnRHa. This explains the continued increase in AMH levels starting from day 14 of GnRHa administration. The decrease in AMH that occurred at day 7 allowed the expansion of the pool of small preantral and antral follicles that secrete AMH, resulting in an increase in AMH levels on days 14 and 30. The initial decrease in AMH resulted from an undetectable gonadotropin flare which'll then mature follicular development in antral follicles. gonadotropin-reponsive bulk, thereby reducing AMH¹⁹. Following a flare, suppression of FSH and LH inhibits FSH-dependent maturation of follicles, thereby increasing the total number of AMH-secreting gonadotropin-responsive follicles.

An increase in body mass index has a relationship with a decrease in AMH levels before surgery. The effect of obesity on AMH levels in women of reproductive age (18-48 years) stated that from 13 studies (n = 1214 women consisted of 811 non-obese women (body mass index; BMI <30 kg/m²) and 403 obese women. (BMI > 30 kg/m²), five of which reported a reduction in AMH levels with obesity, whereas eight showed comparable AMH levels between groups.²⁰ Environmental changes that occur in ovarian follicles have been confirmed in obese women and involve disturbances in several system, including steroidogenic, metabolic, and inflammatory, all of which can impact folliculogenesis and ovulation potential.²¹ A single meta-analysis demonstrated a negative association between AMH and BMI.²²

Obesity is generally associated with systemic insulin resistance and compensatory hyperinsulinemia. Excessive insulin levels have been shown to alter granulosa cell uptake, and subsequently, AMH production.²³ In addition, the increased leptin production associated with obesity may directly suppress AMH production. This condition stems from the inhibitory effect of leptin on the expression of the AMH receptor gene.²⁴ Obesity may lead to increased apoptotic effects at the ovarian follicle level, which is a mechanism that occurs in animal models. Although this mechanism could explain the decrease in ovarian follicle count and AMH levels, this seems less likely based on available data.²⁵ The limitations of this study include ovarian

reserve in this study was only measured by a single marker, namely AMH levels. The antral follicle count (AFC) is another useful marker for assessing ovarian reserve. European Society of Human Reproduction and Embryology (ESHRE) strongly recommends that to predict the response to ovarian stimulation, AFC or AMH assessment is recommended over other ovarian reserve tests, the size of the endometriosis cyst in this study was not specifically measured using a standard measuring instrument, the measurement is only subjectively carried out by the operating operator during the operation therefore the variable size of the cyst is invalid to be used.

CONCLUSIONS

Differences in ovarian reserve in women with ovarian endometrial cysts after laparoscopic cystectomy and injection of leuprorelin acetate had a median value of 1.06 ng/mL, the smallest value of 0.50 ng/mL and the largest value of 4.65 ng/mL. A decrease of 18.9% between AMH levels before surgery and AMH levels after surgery was statistically significant. There is a relationship between BMI and AMH levels before surgery.

This study suggests that a significant decrease in AMH occurs after cystectomy surgery, therefore it is necessary to perform cystectomy surgery to better maintain ovarian reserves and educate patients about the risk of decreased ovarian reserves after cystectomy surgery. Further study is required to measure the number of antral follicles (AFC) as an alternative in assessing ovarian reserve.

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