

# The relationship of ovarian endometrioma and its size to the preoperative serum anti-Müllerian hormone level

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## ABSTRACT

**Objectives:** The aim of this study is to evaluate the impact of ovarian endometrioma according to its size on the serum anti-Müllerian hormone (AMH) levels compared to that of other benign ovarian cysts.

**Material and methods:** The current study retrospectively evaluated preoperative serum AMH level and its association to presenting ovarian cyst size which were measured in clinical setting. Women with surgically diagnosed endometrioma or other benign ovarian cysts were included. All patients underwent transvaginal or transrectal ultrasonography to determine the size of the ovarian cysts. Preoperative serum AMH level was checked and evaluated according to histologic type of the cyst, which were endometrioma or other benign ovarian cysts, respectively. Both groups were classified into  $\leq 4$  cm,  $> 4$  cm and  $\leq 8$  cm,  $> 8$  cm and  $\leq 12$  cm,  $> 12$  cm according to the diameter of cyst and analyzed the difference of mean AMH levels in both groups.

**Results:** There was no significant difference in preoperative serum AMH level between the two groups ( $3.36 \pm 2.3$  versus  $3.76 \pm 2.64$ ,  $p = 0.331$ ). The difference of preoperative AMH levels according to categorized cyst size also was not statistically significant in both groups.

**Conclusions:** Preoperative serum AMH levels were not statistically different between endometrioma and other benign ovarian cyst groups and were not related to the size of endometrioma.

**Key words:** AMH; endometriosis; endometrioma; ovarian cyst

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## INTRODUCTION

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein that belongs to the transforming growth factor-beta family [1]. It is involved in the regression of the Müllerian ducts during male fetal development. In the female, AMH is solely produced by the granulosa cells of primary, preantral and small antral follicles in the ovaries [2].

Clinical aspects of AMH have some exclusive characteristics; the serum AMH level is closely related with age, with insignificant intra- and inter-cycle variation throughout menstrual cycles [3, 4]. Because of such characteristics, AMH has been significantly used in several clinical practices such as assisted reproductive technologies [5, 6], prediction of menopause and diagnosis of PCOS [7–10]. Yet, while serum AMH level may be the best marker of ovarian reserve, the utility as a predictive marker for live births or timing of menopause is not reached to the definite conclusions [7].

In gynecological perspective with interests in clinical use of AMH, the impact of a benign ovarian cyst on physiological serum AMH level is also one of the clinical issues with indefinite conclusion [11–13]. Such debate could be considered in two points; influence of surgery for an ovarian cyst and that of an ovarian cyst itself on the serum AMH level.

Regarding the influence of surgery on ovarian cysts and serum AMH level, it is widely accepted that ovarian reserve measured with serum AMH level would be reduced after ovarian cystectomy [14–16]. Henes et al. reported that serum AMH level was significantly decreased after surgery on a follicular cyst and endometriosis, but not on dermoid and other cysts [15].

When considering the influence of endometrioma on the serum AMH level, several subordinate concepts are needed to be pondered: the stage, laterality and size of endometrioma. Still, conflicting results are being observed; some reports have suggested that the serum AMH level is relatively low in

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the endometrioma group [17, 18], but the opposite findings have been also reported [19, 20]. Karadag et al. [17] reported lower AMH levels in patients with bilateral endometrioma than patients with unilateral endometrioma and lower AMH levels in women with deep infiltrating endometriosis. Similarly, Uncu et al. [18] found that endometrioma patients have lower AMH levels and antral follicle count when compared to the control and suggested that the presence of endometrioma itself is associated with a reduction in ovarian reserve. On the other hand, Kim et al. [19] suggested that the serum AMH level were not different between the endometrioma and teratoma groups, but in age-body mass index matched stage IV endometriosis, the serum AMH level was lower in the endometrioma group. Despite the ongoing debate, general consensus is that higher stage and bilaterality of endometrioma are more likely to result low AMH [21–23].

Expanding the issue to the relationship of size of endometrioma and serum AMH level, the controversies have been either insignificant or negative relationship of the two. However, the recent study of Marcellin et al. [20] has found strikingly positive association between the size of endometrioma and serum AMH level, introducing novel understanding of physiological characteristics of endometrioma and interpreting the associated AMH level. Currently, valuable scholarly investigations on AMH concentrations according to the size of endometrioma have been more complicated to be answered.

The aim of this study is to evaluate the impact of ovarian endometrioma according to its size on the serum anti-Mullerian hormone (AMH) levels compared to that of other benign ovarian cysts.

## MATERIAL AND METHODS

### Patients

This retrospective study was conducted at Department of Obstetrics and Gynecology, Pusan National University Hospital. Women with surgically diagnosed endometrioma between October 01, 2012 and October 01, 2019 were included as the study group. The exclusion criteria were prior ovarian surgery, irregular menstrual periods, the presence of polycystic ovary syndrome, hyperprolactinemia or abnormal thyroid function test (TFT), and medication history with dysmenorrhea management such as GnRH analogues, oral contraceptives or progestins during the past 3 months to the recruitment. The control group was comprised of women with benign ovarian cysts other than endometrioma. The same exclusion criteria were applied to the controls. BMI was calculated as the patient's weight in kilograms divided by her height in meters squared. All patients underwent laparoscopic or laparotomic ovarian cystectomy. All specimens obtained intraoperatively were submitted for pathologic examination, and pathologic confirmation was done in all cases. All patients agreed and provided informed consent

forms indicating that their medical records were to be used for the study. The study was approved by the ethics committee of Pusan National University Hospital.

### Measurement of ovarian cyst size

All patients underwent transvaginal or transrectal ultrasonography to determine the size of the endometriomas or other benign ovarian cysts using a 5–9 MHz transvaginal transducer or transrectally for virgin patients on the day before surgery (Voluson E6 General Electric, Milwaukee, Wauwatosa, WI, USA). The cyst size was recorded as the average of the largest and shortest diameters in centimeters. Each cyst diameters were measured vertically from outer-membrane to the opposite outer-membrane. Bilateral cysts were recorded as the sum of the two cyst sizes.

### Assay of AMH and inflammatory markers

All of women underwent blood sampling for preoperative AMH measurements and inflammatory markers within a month prior to surgery. Blood samples were obtained from patients after 8hrs of overnight fast, including following measurements, using Roche Modular DP (Tokyo, Japan): complete blood cell count including number of white blood cells (WBC), percentages of segmented\_neutrophils and lymphocytes and segmented neutrophil/lymphocyte (N/L) ratio. Serum AMH assay was performed using an anti-Mullerian hormone/Mullerian inhibiting substance Enzyme Immuno Assay (AMH/MIS EIA) kit (Immunotech version, Beckman Coulter, Marseille, France). The coefficients of variation of intra-assay and inter-assay were 12.3% and 14.2%, respectively. The distribution of AMH values for two or three days of the physiological cycle according to the patient's age is determined by using 5%, 10%, 25%, 50%, 75%, 90%, 95%, and mean values according to age was calculated [24].

### Statistics

All statistical data was organized into a computerized database. Variables were evaluated for clinical significance using chi-square test and Fisher's exact test for categorical variables or the independent t-test for continuous variables, where appropriate. One-way ANOVA was used to compare preoperative serum AMH levels according to categorized cyst sizes in the endometrioma group and control group. Pearson correlation coefficient was used for correlation between preoperative AMH level, age, ovarian cyst size and inflammatory markers. Using multiple linear regression analysis, factors affecting preoperative serum AMH level were studied. The statistical analysis was performed SPSS version 22.

## RESULTS

The mean age of the patients was higher in the endometrioma group compared to the control group, which

were  $30.45 \pm 5.99$  years and  $28.09 \pm 7.55$  years, respectively ( $p = 0.034$ ). Parity was lower in the endometrioma group than in the control group ( $0.09 \pm 0.35$  versus  $0.30 \pm 0.71$ ,  $p = 0.033$ ) and showed a regular menstrual cycle compared to the control group ( $79.66\%$  vs  $20.34\%$ ,  $p < 0.001$ ). In addition, dysmenorrhea in the endometrioma group were higher than the control group ( $82.2\%$  vs  $59.38\%$ ,  $p = 0.001$ ). There were no significant differences in preoperative serum

AMH level between the two groups ( $3.36 \pm 2.3$  vs  $3.76 \pm 2.64$ ,  $p = 0.331$ ). Serum WBC count and N/L ratio were significantly higher in endometriosis group than in control group (Tab. 1).

Table 2 shows the difference of preoperative AMH levels according to cyst size in endometrioma and control group. The groups were classified into  $\leq 4$  cm,  $> 4$  cm and  $\leq 8$  cm,  $> 8$  cm and  $\leq 12$  cm,  $> 12$  cm according to the cyst size. The difference of preoperative AMH levels accord-

**Table 1. Basic characteristics of the patients in control and endometrioma group**

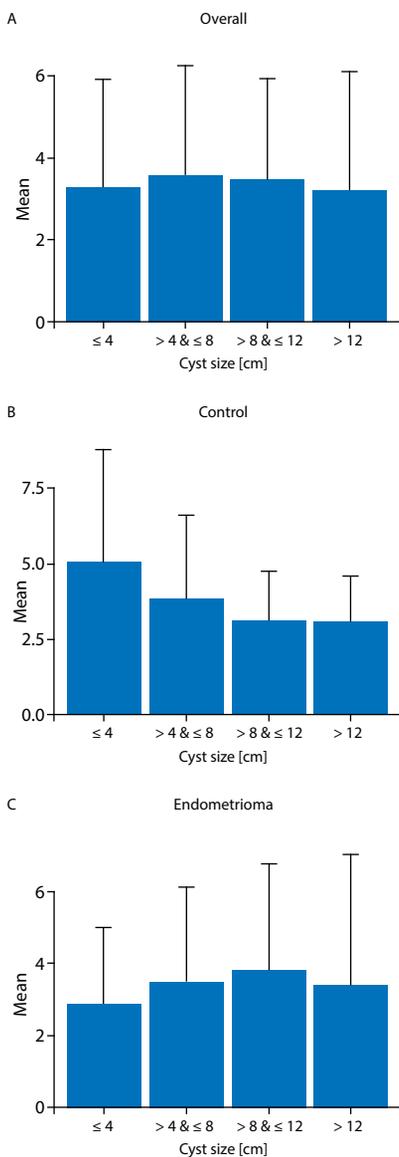
	Overall (n = 182)	Control (n = 64)	Endometrioma (n = 118)	p value
Age	29.62 (6.66)	28.09 (7.55)	30.45 (5.99)	0.034
BMI	21.90 (4.30)	22.51 (4.58)	21.56 (4.12)	0.154
Smoking history*				1.000
Never	175 (96.15)	62 (96.88)	113 (95.76)	
Current	7 (3.85)	2 (3.12)	5 (4.24)	
Previous	0 (0.00)	0 (0.00)	0 (0.00)	
Bilaterality*				0.310
No	128 (70.33)	48 (75)	80 (67.8)	
Yes	54 (29.67)	16 (25)	38 (32.2)	
Past history*				0.015
None	168 (92.31)	59 (92.19)	109 (92.37)	
HTN	3 (1.65)	2 (3.12)	1 (0.85)	
DM	1 (0.55)	0 (0.00)	1 (0.85)	
Hepatitis	2 (1.1)	2 (3.12)	0 (0.00)	
Thyroid disease	7 (3.85)	0 (0.00)	7 (5.93)	
Cancer	1 (0.55)	1 (1.56)	0 (0.00)	
Gravidity	0.37 (0.86)	0.52 (1.04)	0.30 (0.74)	0.139
Parity	0.16 (0.51)	0.30 (0.71)	0.09 (0.35)	0.033
OCs*				1.000
Never	175 (96.15)	62 (96.88)	113 (95.76)	
Current	4 (2.2)	1 (1.56)	3 (2.54)	
Previous	3 (1.65)	1 (1.56)	2 (1.69)	
Menstruation*				< 0.001
Regular	129 (70.88)	35 (54.69)	94 (79.66)	
Irregular	53 (29.12)	29 (45.31)	24 (20.34)	
Dysmenorrhea*				0.001
No	47 (25.82)	26 (40.62)	21 (17.8)	
Yes	135 (74.18)	38 (59.38)	97 (82.2)	
Menarche	13.32 (1.48)	13.19 (1.61)	13.40 (1.41)	0.381
AMH (ng/mL)	3.50 (2.63)	3.76 (2.64)	3.36 (2.63)	0.331
WBC ( $\times 103/uL$ )	6.66 (2.03)	6.23 (1.56)	6.90 (2.22)	0.019
Segmented_neutrophil (%)	59.35 (10.65)	58.07 (9.93)	60.05 (11.01)	0.218
Lymphocyte (%)	31.67 (9.41)	32.83 (8.78)	31.03 (9.71)	0.206
N/L	2.46 (3.46)	1.99 (0.99)	2.71 (4.22)	0.080
Cyst size (cm)	6.90 (4.03)	7.59 (4.10)	6.52 (3.96)	0.091

Data are presented as the means (SD); \* values are presented as the number of patient (%); p value by independent t-test for continuous variable; \* Chi-square test or Fisher's exact test for categorical variable; BMI — body mass index; HTM — hypertension; DM — diabetes mellitus; OCs — oral contraceptives; AMH — anti-Mullerian hormone; WBC — white blood cell; N/L — segmented neutrophil/lymphocyte ratio

**Table 2. Comparisons of serum AMH level according to cyst size in control and endometrioma group**

Cyst size (cm)	≤ 4	> 4 & ≤ 8	> 8 & ≤ 12	> 12	p value
Overall (n = 182)	41, 3.39 (2.68)	89, 3.62 (2.68)	35, 3.50 (2.47)	15, 3.27 (2.89)	0.948
Control (n = 64)	9, 5.04 (3.75)	34, 3.82 (2.78)	15, 3.11 (1.62)	6, 3.08 (1.50)	0.336
Endometrioma (n = 16)	32, 2.92 (2.16)	55, 3.49 (2.64)	20, 3.79 (2.97)	9, 3.39 (3.63)	0.675

Data are presented as the number of patients, means (SD)



**Figure 1.** The value of the serum AMH according to average ovarian cyst size; A. Overall group; B. Control group including benign ovarian cyst except endometrioma; C. Endometrioma group

ing to categorized cyst size was not statistically significant in both groups ( $p = 0.336$ ,  $p = 0.675$ , respectively), regardless of bilaterality (Fig. 1).

Table 3 shows the results of the correlation between inflammatory markers and preoperative AMH levels. Pear-

**Table 3. Correlations between preoperative AMH and other parameters including all groups**

(n = 182)	Rho value	p value
Age [years]	-0.357	< 0.0001
Average of cyst size [cm]	-0.051	0.492
N/L ratio	-0.022	0.766
Number of WBC	-0.009	0.907

AMH — anti-Mullerian hormone; N/L — segmented neutrophil/lymphocyte; WBC — white blood cell

son correlation coefficient showed only age factors have a significantly negative correlation (Rho value = -0.357 with  $p < 0.0001$ ). Serum WBC count and N/L ratio had Rho values of -0.009 ( $p = 0.907$ ) and -0.022 ( $p = 0.766$ ), respectively.

The result of multiple linear regression analysis including age, cyst size, serum WBC count and N/L ratio showed non-significant statistical correlation except age (coefficient  $\beta = -0.15$ ,  $p < 0.0001$ ).

### DISCUSSION

The surgery-related decrease of ovarian reserve in endometriosis is relatively well-established finding [2, 15, 16, 18]. However, the impact of ovarian endometrioma itself on ovarian reserve — one of the most important preoperative characteristics to decide further treatment method — showed inconsistent results. Uncu et al. [18] reported that women with endometrioma had lower AMH levels and antral follicle count than controls, and Pacchiarotti et al. [25] also observed the negative effect of endometriosis on the ovarian reserve, especially in cases of severe endometriosis. The possible underlying mechanism suggested by the authors was that increased peritoneal macrophages in endometriosis could have caused substantial damage to ovarian tissue and oxidative stress, inducing oocyte degeneration and apoptosis by disturbing the meiotic spindle [25, 26]. Yet, in these studies, the control groups comprised women of reproductive age who did not have any ovarian cysts [18]. Thus, it would be unclear to conclude whether such reduced serum AMH level in the endometrioma group was caused specifically by endometrioma or generally by the presence of cystic lesion on the ovary. When the control group had benign ovarian cysts, some studies have reported the insig-

nificant difference in AMH level between the control and the patient group with endometrioma [12, 19]. More detailed evaluation and analysis according to the characteristics of endometrioma itself and suitable control group are required to interpret AMH level in endometrioma patients.

Recently, Marcellin et al. [20] evaluated serum AMH levels in endometrioma patients according to the size of the cyst; interestingly, they reported that serum AMH levels in women with no prior history of surgery for endometrioma increased with the cyst size of endometrioma. In the control group, non-endometrioma benign ovarian cyst group, serum AMH levels was not related with the size of cyst. The authors concluded that serum AMH level had positive correlation with endometrioma size and was not affected by laterality of endometrioma or deep infiltrating endometriosis, which was strikingly different from previous literature.

In this study, to investigate such inconclusive effect of endometrioma itself on serum AMH level according to its size, we compared the preoperative AMH level between endometrioma and other benign ovarian cyst group, conducting subgroup analysis according to corresponding ovarian cyst size.

First, we observed that the effect of benign ovarian cyst on serum AMH level was not different between the endometrioma and the other benign ovarian cyst group. Such result of ours was in agreement with the study of Streuli et al. [13], in which they reported that the serum AMH levels was not different between women with endometriosis and women with benign gynecological condition (endometrioma group,  $3.6 \pm 3.1$  ng/mL and control group,  $4.1 \pm 3.4$  ng/mL,  $p = 0.06$ ). Therefore, preoperative serum AMH level seems to be similar between endometrioma and other benign ovarian cysts, as previously known [13, 20].

Next, we compared the relationship of ovarian cyst size and serum AMH level. In our study, there was no statistical difference between endometrioma size and serum AMH level, which was inconsistent with the results of Marcellin et al. However, except for the endometrioma with size of more than 12 cm, the serum AMH level tended to be increased with the increasing cyst size. Additionally, we have run the same statistical analysis on unilateral endometrioma and bilateral endometrioma separately, and, interesting enough, the similar pattern was observed (data not shown). Despite statistical insignificance, in unilateral endometrioma, serum AMH level tended to be increased with increasing cyst size up to 6 cm and dropped thereafter; in bilateral endometrioma, the same tendency was observed up to the cyst size of 12 cm. At this point, we supposed that serum AMH level increased with increasing endometrioma size up to a certain limit — in this study, the upper limit was 12 cm in diameter when the unilateral and bilateral endometrioma were combined — and became insignificant thereafter.

Though further statistical analysis on subdivided sizes of endometrioma is warranted, based on the current results, the size of endometrioma might have affected the serum AMH level, not necessarily reflecting the ovarian reserve, which concurs with the results of Marcellin et al. [20].

Marcellin et al. suggested three hypotheses to explain their results [20]. First is selection bias. In case of women with low AMH levels, surgical management tended to be avoided. Second, they suggested that there may be increased secretion of AMH into the circulation by the ovaries as the size of the endometrioma increases; inflammation and neoangiogenesis in endometriosis could boost the local blood clearance from the ovaries. Third, the toxicity of endometrioma on the ovarian reserve may contribute improved primordial follicular stimulation and consequently an increase in serum AMH levels.

Of the three hypotheses of Marcellin et al. [20], regarding the selection bias, we also excluded infertile patients, suggesting the potential of selection bias may exist. Streuli et al. [13] excluded infertile patients who were more likely to have lower basal AMH in their study as well, and they reported similar results to ours. Considering the effect of infertile patient, Kim et al. [19] reported that the preoperative serum AMH was not different between endometrioma and mature cystic teratoma, including infertile patients in their subject group (endometrioma group 9.8% and control group 7.8%,  $p =$  not significant, respectively); additionally, in case of stage IV endometriosis, the serum AMH level was lower in the endometrioma group. Thus, regardless of including infertile patient to the subject group, the basal serum AMH level was not significantly different between patients with endometrioma or other benign ovarian cysts. Still, more detailed and larger studies are needed considering infertility history and endometrioma severity.

For evaluating the effect of inflammation, we analyzed the relationship of inflammation markers; WBC count and N/L ratio. The results showed no statistical relevance. However, it is hard to define that the number of serum WBCs and N/L ratio reflected the inflammatory environment of peritoneal cavity related with endometrioma. It is necessary to analyze the inflammatory marker of peritoneal fluid.

Last, as reported elsewhere, an increase in endometrioma size might cause increasing toxicity on the ovarian reserve, and short-term exposure of human ovarian follicles to cyclophosphamide metabolites seems to promote follicular activation *in vitro* [27, 28]. However, such studies were conducted *in vitro*, and the toxicity of cyclophosphamide metabolite and endometrioma might differ in clinical setting.

The current study has several limitations. First, the number of patients included in this study was relatively small. However, data were collected in a single institute, and all patients were firmly diagnosed by laparoscopic surgery.

We clearly excluded minimal or mild endometriosis patients in control group by laparoscopy, leading the consistency of data to be quite valuable. Second, the mean age of control group was significantly lower than the endometrioma group. Despite the slightly higher mean age, the mean AMH of the endometrioma group was comparable to the control group. Thus, we could logically conclude that the preoperative AMH were not different between the endometrioma and control groups. Lastly, since the current study is retrospective, all statistical analysis was inevitably performed using previously measured data; yet, Marcellin et al. [20] adopted the sum of the largest diameters for bilateral ovarian cysts in their study of increasing serum AMH level with endometrioma size, when, in previous literature, women with bilateral ovarian cysts had been reported to have lower serum AMH levels, irrespective of the nature of the cyst [12]. In the current study, regardless of bilaterality of ovarian cysts, increasing tendency of serum AMH level with ovarian cyst size was observed, giving the important perspective of relationship between serum AMH level and the size of cyst in endometrioma.

## CONCLUSIONS

The preoperative serum AMH levels were not statistically different between the endometrioma and other benign ovarian cyst groups and were not related to the size of endometrioma. Nevertheless, except for the cysts larger than 12 cm in diameter, the serum AMH level tended to have positive relationship with increasing endometrioma size. With further analyses with larger number of patients and more suitable inflammatory markers, future findings on the association between serum AMH level and endometrioma size could innovatively suggest proper interpretation of preoperative serum AMH level and clear management direction for treatment in endometrioma patients.

### Conflict of interest

None of the authors have direct or indirect conflict interest associated with publishing the article.

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