

# Is serum anti-Müllerian hormone (AMH) assay a satisfactory measure for ovarian reserve estimation? A comparison of serum and peritoneal fluid AMH levels

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## Conflict of interest

None declared

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## Abstract

**Background.** Clinical cases have been reported with women who got pregnant with confirmed low serum anti-Müllerian hormone (AMH) concentrations, thus demonstrating that low serum AMH concentration cut-points could be fairly specific for poor ovarian response (POR) to gonadotrophin stimulation, but not for pregnancy. That observation prompted the question whether serum AMH concentration accurately corresponded to the whole amount of AMH secreted by granulosa cells.

**Objectives.** To measure AMH levels in peritoneal fluid and their correlations with serum AMH concentrations.

**Material and methods.** The reported study involved 48 female patients, aged 18–40 years, diagnosed with benign ovarian cysts and qualified for a laparoscopic cystectomy. Prior to surgery, the ovarian reserve was assessed using serum AMH concentration assay. The peritoneal fluid was also collected during the laparoscopy and AMH concentrations in peritoneal fluid were measured.

**Results.** The AMH present in the peritoneal fluid strongly correlated with AMH levels in blood serum ( $r = 0.54$ ;  $p < 0.001$ ) and higher serum AMH concentrations corresponded to higher AMH concentrations in the peritoneal fluid. There was also a significant correlation between AMH levels in serum and in peritoneal fluid, collected from patients with endometrioma and other benign cysts ( $r = 0.61$ ;  $p = 0.001$  vs  $r = 0.43$ ;  $p = 0.03$ ).

**Conclusions.** The AMH is present in the peritoneal fluid and its concentrations significantly correlate with AMH levels in serum. The assessment of AMH concentration in the peritoneal fluid may be a valuable complement to the evaluation of ovarian reserve and the diagnosis of infertility after adnexal surgery.

**Key words:** anti-Müllerian hormone, peritoneal fluid, ovarian reserve, endometrioma, fertility

## Introduction

The anti-Müllerian Hormone (AMH) is produced in granulosa cells and its highest concentration is present in the granulosa cells of the secondary, preantral and small antral follicles.<sup>1</sup> It exerts an inhibitory effect on primordial follicle recruitment and may also inhibit, through its paracrine action, the follicle-stimulating, hormone-dependent selection of follicles for dominance.<sup>2</sup> It is secreted into the blood, which makes it possible to measure it in blood serum. The AMH concentration in serum reflects the pool of ovarian follicles, while a reduced amount of antral follicles may result in a decreased serum AMH level. That is why AMH concentration in serum seems to be a reliable marker in the evaluation of ovarian reserve.<sup>1–3</sup>

According to the opinions of both the American Society for Reproductive Medicine and the American College of Obstetricians and Gynecologists, no test of ovarian reserve is likely to be precise enough for an accurate evaluation of the chances for spontaneous conception. On the other hand, there is a prevailing opinion that AMH and the antral follicle count (AFC) are promising predictive markers, especially in the cases of poor ovarian reserve (POR), which corresponds to women in the reproductive age and with regular menses whose response to ovarian stimulation or fecundity is reduced compared to other women in a comparable age.<sup>3–6</sup> The studies revealed that AMH concentrations between 0.2 ng/mL and 0.7 ng/mL had been highly sensitive (40–97%) and specific (78–92%) to discriminate a diminished ovarian reserve (<3 follicles or 2–4 retrieved oocytes) or a poor response to gonadotrophin stimulation in a population of women qualified to in vitro fertilization (IVF); however, those cut-points were neither sensitive nor specific enough to precisely predict the chances for pregnancy.<sup>3,7,8</sup> The average AMH levels, which corresponded to normal ovarian reserve, ranged between 2.5 ng/mL and 2.7 ng/mL. Regarding those values, the AMH test demonstrated sensitivity and specificity to clinical pregnancy with 83% and 82% accuracy, respectively, with the positive predictive value of 67–77%, and the negative predictive value of 61–87%, respectively.<sup>9</sup>

As we can see, measuring serum AMH concentrations to estimate chances for clinical pregnancy at very low or even hardly detectable values does not always validate the actual status. There have been a few clinical cases of women who responded to gonadotrophin stimulation or got pregnant, regardless of very low AMH concentrations – below 1.1 ng/mL.<sup>3</sup>

Those findings prompted the question of whether serum AMH concentrations accurately reflect the amount of AMH secreted by granulosa cells, thereby corresponding to the woman's reproductive potential. In order to answer this question and assuming that ovarian follicles release their contents (including AMH) into the peritoneal cavity, a comparison juxtaposing AMH concentration levels in serum with those in the peritoneal fluid seemed necessary.

The aim of the study was to determine the presence of AMH in the peritoneal fluid and compare its concentration with that of blood serum.

## Material and methods

The study was carried out at the Department of Operative Gynecology and Gynecological Oncology at the Polish Mother's Memorial Hospital Research Institute, Łódź, Poland, during the years 2014–2016. Peritoneal fluid samples were collected from female patients at the age of 18–40 years to assess AMH concentration levels. All those patients were qualified for a laparoscopic cystectomy for benign unilateral ovarian cysts. The exclusion criteria eliminated women at the age below 18 or above 40.

During laparoscopy, when a camera and 2 trocars were inserted, a volume of 5 mL of peritoneal fluid was aspirated from the Douglas cavity. The fluid samples were collected before the onset of cystectomy procedure and the cyst sac opening, to avoid contamination with either blood or cyst secretions. Prior to the operation, each patient provided 5 mL of blood for serum AMH concentration assay. The collected peritoneal fluid was centrifuged and serum was separated from the whole blood, transferred to sterile polypropylene tubes and stored at –20°C until assay. Serum AMH concentrations were measured using a Beckman Coulter Gen II enzyme immunoassay kit (Beckman Coulter Poland, Warszawa, Poland; www.beckman.com), according to its manufacturer's instructions. A total of 48 patients were recruited, out of whom 24 were diagnosed with endometrioma and 24 with ovarian cysts other than endometrioma.

The study was approved by the Ethics Committee of the Medical University of Lodz, Poland, and informed consent forms were obtained from all the included patients.

## Statistical analysis

All the data was analyzed using STATISTICA v. 12 software package (StatSoft, Inc., Tulsa, USA). The Mann–Whitney U test was applied instead of Student's t test, when variables did not pass the normality test. Spearman's correlation coefficient and the simple regression test were used to reveal relations among variables. P-value <0.05 was considered statistically significant.

## Results

Table 1 presents clinical characteristics of the recruited patients, including their obstetric and gynecological history, histopathological evaluation of operated cysts and the clinical stage of endometriosis observed during laparoscopy. The mean age of the recruited patients was 30.3 ±5.2 years.

Table 1. Characteristic of the study group

Age [years] mean SD	30.3 ±5.2	
Previous pregnancies	n	%
Intrauterine *childbirth	18	37.5
*miscariages	3	6.2
ectopic pregnancy	0	0
Primary infertility	5	10.4
Secondary infertility	4	8.3
Previous abdominal surgery	5	10.4
Adnexitis in medical history	0	0
Histopathological diagnosis of the operated cysts: *endometriomas	24	50.0
*Other than endometrioma: simple cysts	7	14.6
mature teratoma	12	25.0
other	5	10.4

The study confirmed the presence of AMH in the peritoneal fluid samples with a concentration range of  $4.69 \pm 3.14$  ng/mL. The concentration of AMH in the peritoneal fluid did not differ significantly from that in blood serum, assayed before the laparoscopic cystectomy ( $4.69 \pm 3.14$  ng/mL vs  $4.58 \pm 3.54$  ng/mL,  $p = 0.58$ ). A strong and statistically significant correlation was thus established between serum AMH concentrations before surgery and AMH concentrations in the peritoneal fluid ( $r = 0.54$ ,  $p < 0.001$ ) (Fig. 1). The higher was serum AMH concentration, the higher was the concentration of AMH in the peritoneal fluid.

When the study group was divided into patients with endometrial cysts and with cysts other than endometrioma, no significant difference was found between the mean AMH concentrations in serum and in the peritoneal fluid ( $4.12 \pm 2.7$  ng/mL vs  $5.56 \pm 3.4$  ng/mL,  $p = 0.3$ ; and  $4.05 \pm 2.71$  ng/mL vs  $5.55 \pm 3.26$  ng/mL,  $p = 0.12$ ). A significant correlation was found in both groups between serum and peritoneal fluid AMH concentrations. However, the correlation strength was definitely higher in the endometrial cyst group, in comparison to the group of patients with conditions other than endometrioma ( $r = 0.61$ ,  $p = 0.001$  vs  $0.43$ ,  $p = 0.03$ ).

Regarding the Bologna criteria, in which POR is defined as AMH  $< 1.1$  ng/mL, there were only 3 such patients in our

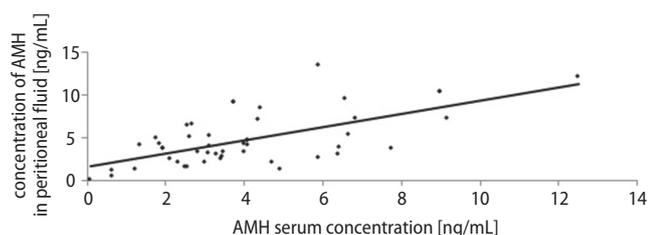


Fig. 1. Correlation of AMH concentration in peritoneal fluid with serum concentration before surgery

study group.<sup>10</sup> In only 1 patient was the concentration of AMH in the peritoneal fluid significantly higher than that in serum (1.22 ng/mL vs 0.6 ng/mL, respectively).

## Discussion

In practice, the procedures related to assistive reproductive technology (ART) require a reliable marker to evaluate the quality of obtained oocytes. Consequently, a concept was developed to determine AMH concentration in the secretion of ovarian follicles. The secretions from smaller follicles provided a 3 times higher AMH titer, compared to larger follicles, which proved a reduced AMH secretion during ovarian follicle maturation. In addition, the AMH levels from follicular secretion correlated strongly with the number of AFC observed in the early first phase of the menstrual cycle. The high levels of AMH in the follicular secretion correlated strongly with the number of clinically observed pregnancies. However, the quality of obtained embryos did not significantly differ in the group with low and high AMH levels in follicular secretions. Such reports gave the authors the basis for a hypothesis about certain importance of AMH as a qualitative marker in the assessment of ovarian reserve.<sup>4,11–14</sup>

Evaluating only AMH in blood serum seems incomplete when trying to assess the ovarian reserve. For example, young girls may have a high ovarian reserve, but their serum AMH concentrations are low. Therefore, it may be suspected that the concentration of AMH in blood serum may not entirely reflect the produced AMH volume. Hence, an idea occurred to assess the presence and concentration of AMH in peritoneal fluid. In fact, we found only 1 report in the available medical literature, evaluating the concentration of AMH in peritoneal fluid, but it was published after we started the collecting of samples. Our findings are confirmed in that study, carried out by Hipp et al. The authors compared AMH concentrations in plasma and in peritoneal fluid from women, operated for endometriosis and from a control group. They did not observe any significant differences in plasma or peritoneal fluid AMH concentrations in women with endometriosis compared to the controls. Moreover, they also found a significant correlation between AMH concentrations in plasma and in peritoneal fluid.<sup>15</sup> In addition, the authors claim that AMH concentrations in plasma may be a marker for peritoneal AMH in studies evaluating the local effects of AMH, especially in endometriosis.<sup>15</sup> Our results confirm that statement with the observed strong correlation between AMH concentrations in serum and in peritoneal fluid in our group of patients with endometriomas.

Except for 1 study, all the other found reports described assessments of AMH levels in ovarian follicles. The first study in which the concentration of AMH was measured in follicular secretion, was the work of Fallat et al. from 1997.<sup>16</sup> In their study, only polycystic ovaries syndrome (PCOS) and endometrial cysts were evaluated and

a higher concentration of AMH in the follicular secretion in women with PCOS was found, compared to women with endometrial cysts. The authors did not, however, compare the concentrations of AMH in follicles and in blood serum.

Currently, a report can be found in which AMH was assayed in secretion from the ovarian follicles during qualification for ART.<sup>4,11–13</sup> As mentioned above, those studies showed a decrease in AMH secretion during ovarian follicular maturation and a strong correlation of AMH concentration with the number of antral follicles in the early first phase of the menstrual cycle.<sup>4,11–13</sup> However, the follicular secretion of AMH was not compared either with AMH concentration in serum or peritoneal fluid.

The results of our study did not show any significant difference between AMH concentrations in serum and in the peritoneal fluid. On the other hand, a strong and statistically significant correlation was found between serum AMH concentration before surgery and the concentration of AMH in peritoneal fluid. The higher was the concentration of AMH in the peritoneal fluid, the higher was the concentration of AMH in blood serum. These results prove that the amount of AMH secreted to peritoneal fluid corresponds to AMH concentration in serum, which means that serum AMH concentration correctly reflects the secretion of AMH by granulosa cells and, therefore, the ovarian reserve.

Our results also suggest that our patient, whose AMH concentration levels in peritoneal fluid significantly differed from those in serum AMH concentration before surgery, may have had an abnormal secretion of AMH into blood serum. We thus believe that peritoneal fluid collection during laparoscopic cystectomy for AMH assay may be a valuable complement to ovarian reserve evaluation and a fairly reliable prognosis of fertility after surgery, especially in patients with low serum AMH values (<1.1 ng/mL) prior to surgery and in patients with endometriomas, in whom the surgery may potentially diminish the ovarian reserve. However, this hypothesis requires further research.

The weakness of our study was the rather small number of patients with low AMH in whom the difference between serum and peritoneal fluid AMH levels was evident. The strength of the study was the measurement of AMH in peritoneal fluid and its comparative juxtaposition with serum AMH levels in a very homogeneous group of patients.

## Conclusions

The AMH is present in the peritoneal fluid and its concentrations significantly strongly correlate with AMH levels in blood serum. The assay of AMH levels in peritoneal fluid may then be a valuable complement to ovarian reserve evaluation and a valuable marker for the prognosis of infertility after adnexal surgery.

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