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Different anti-Műllerian hormone (AMH) levels respond to distinct ovarian stimulation methods in assisted reproductive technology (ART): Clues to better ART outcomes

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Abstract

Purpose: We asked whether the relationship between anti-Műllerian hormone (AMH) value and the response to ovarian stimulation (OS) might be AMH value-related and differ for each regimen, aiming at getting clues as to how to choose OS protocols according to AMH levels. We further addressed how AMH value connects with ART outcome.

Methods: A total of 1112 women undergoing egg retrieval in ART were included. We adopted four OS protocols, that is, clomiphene, clomiphene + low-dose gonadotropins (Gns), GnRH (Gn-releasing hormone) + Gns (short), and GnRH antagonist.

Results: Anti-Műllerian hormone showed a stronger correlation with egg number compared with age over a wide age range. When patients were stratified into four groups by AMH value (<1, 1-2, 2-3, and 3≦ ng/mL), the relationship between AMH and egg number differed among differential OS regimes. The number of eggs rose as AMH and total doses of Gn increased. When analyzed for each AMH group, egg number, but not AMH, was associated with pregnancy rate.

Conclusion: Different AMH levels exhibit characteristic responses to distinct OS regimens. To improve ART outcomes, personalized OS should be selected so as to maximize egg number, which seems to be a more precise variable than AMH for predicting pregnancy.

KEYWORDS

age, anti-Műllerian hormone, assisted reproductive technology, egg number, ovarian stimulation

1 | INTRODUCTION

The outcome of assisted reproductive technology (ART) is largely dependent on the ovarian response to controlled ovarian stimulation. In other words, the number and quality of eggs obtained determine the success or failure of ART. The ovarian response is often paraphrased as ovarian reserve, which means how many eggs capable of fertilization and subsequent embryogenesis are remaining in the ovary. However, the latter is a rather broader concept including the former and, therefore, is extremely difficult to evaluate directly in clinical practice. At present, a range of ovarian reserve tests, which indirectly assess ovarian reserve, has been proposed for the prediction of a poor response. These include follicle-stimulating hormone (FSH), estradiol, anti-Műllerian hormone (AMH), inhibin B, antral follicular count (AFC), ovarian volume, and ovarian vascular flow.

There is accumulating evidence to suggest that AMH could be the most sensitive marker of ovarian reserve.[1-3] Hence, AMH is currently widely used to predict the response to ovarian stimulation before starting in vitro fertilization. However, so far, no established guideline has been put forth regarding which ovarian stimulation method to use according to AMH level. Thus, in the present study, we addressed the question of whether the relationship between AMH level and the response to ovarian stimulation might differ for each regimen in the hopes of getting some clues as to how to manage poor ovarian responders, presumably as identified by lower AMH levels. We further questioned whether AMH value could be directly related to ART outcomes.

2 | METHODS

All patients underwent the ART procedures in Women's Clinic Oizumigakuen, Tokyo, Japan, between October 2011 and December 2017, during which time the ART protocols were basically similar. We enrolled women who had natural menstruation and underwent the egg retrieval procedure. Patients diagnosed with polycystic ovary syndrome (PCOS) were excluded. The diagnosis of PCOS was essentially based on the diagnostic criteria approved by Japan Society of Obstetrics and Gynecology.[4] There was no limit on age as long as above conditions were met because we intended that the results obtained from this study could be relevant to actual clinical practice. Resultantly, 1112 patients were retrospectively studied. Multiple patients underwent a specific ovarian stimulation protocol up to three times at maximum. The major reasons for infertility were unexplained infertility, including diminished ovarian reserve mainly due to ovarian aging, male infertility, and female factor infertility, such as endometriosis and tubo-peritoneal factor.

In this study, we adopted four ovarian stimulation protocols including clomiphene (clomiphene protocol), clomiphene + low-dose gonadotropins (the modified mild protocol), gonadotropin-releasing hormone (GnRH) agonist flare-up (the short protocol), and GnRH antagonist (the antagonist protocol). Regarding the clomiphene protocol, we administered clomiphene 50 or 100 mg daily from days 2 to 3 of menstruation cycle until the day of ovulation induction by GnRH agonist nasal spray (buserelin 750 μg). The modified mild protocol utilized clomiphene in conjunction with gonadotropins (human menopausal gonadotropin: HMG/follicle-stimulating hormone: FSH) which were given from cycle days 6-7 every day or every other day. In both the short and the antagonist protocols, the injection of gonadotropins was initiated from days 2 to 3 and continued until the day of ovulation induction by the injection of human chorionic gonadotropin (hCG). In the short protocol, gonadotropins combined with GnRH agonist (buserelin 900 μg/d as nasal spray) were administered every day from day 2 onward. In the antagonist protocol, gonadotropins were started every day from day 2 with GnRH antagonist (cetrorelix acetate 0.25 mg/d) being administered together with gonadotropins after the leading follicle of 14-16 mm in diameter was present. Gonadotropins and the GnRH antagonist were given until the day of hCG injection. We injected hCG when the leading follicle reached 17 to 18 mm in diameter. Eggs were retrieved using transvaginal ultrasound-guided needle aspiration 34 hours after the injection of hCG.

Currently, there is no consensus as to how to select ovarian stimulation protocols according to the value of AMH. The protocol using antagonist is thought to be associated with a relatively low risk of ovarian hyperstimulation syndrome (OHSS) on the one hand, while it is prone to poor responses on the other hand.[5] When it comes to diminished ovarian reserve, which is supposedly reflected as a drop in AMH level, there seems to be no valid protocol at all.[6,7] So far, several papers attempting mild ovarian stimulation protocols in women with low ovarian reserve have been published from some clinical facilities.[8-10] Taking these currently widely practiced methods into consideration, we basically left the choice of an ovarian stimulation protocol to the request of the patients after fully explaining the pros and cons of each protocol.

The concentrations of AMH, FSH, luteinizing hormone (LH), thyroid-stimulating hormone (TSH), prolactin, and estradiol were determined in serum samples taken on days 2-4 of the menstrual cycle before treatment. The concentrations of AMH were evaluated using method of Kumar et al (AMH GenII assay) with minor modifications.[11] Blastocysts were graded according to Gardner's classification.[12] The main outcomes analyzed were the number of eggs retrieved and clinical pregnancy rate.

The data were analyzed by using EZR software (a modified version of R commander). Correlations were calculated using Spearman's rank correlation coefficient. We used Steel-Dwass nonparametric test for nonparametric multiple comparisons. Multiple regression analysis was used to identify variables associated with the number of eggs retrieved and pregnancy rate. $P < 0.05$ was considered to be statistically significant. The study was approved by the institutional ethics committee managed in Lenia Medical Corporation. Patient data were analyzed in an anonymous manner. All the patients gave informed consent to participate in this study.

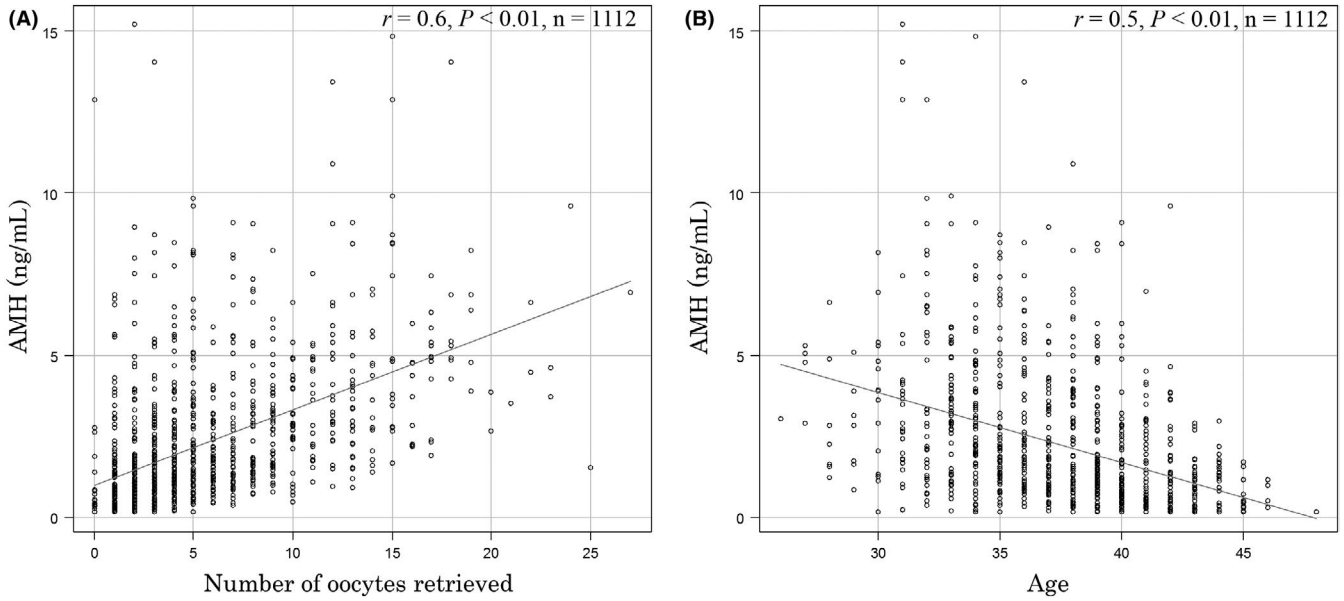

FIGURE 1 Relationship between AMH level and number of oocytes retrieved (A) and between AMH level and age (B). Spearman's correlation coefficient (*r*) is shown. AMH, anti-Műllerian hormone

3 | RESULTS

First, we examined how AMH is related to the number of eggs retrieved. The results given in Figure 1A show that a moderate positive correlation was observed between AMH level and the number of eggs (correlation coefficient $r = 0.6$, $P < 0.01$). It must be pointed out here that these data include the results of all ovarian stimulation protocols. Accordingly, the egg number considerably scattered even with the same AMH level.

Figure 1B compares AMH level with age. An inverse moderate correlation was found between the two (correlation coefficient $r = -0.5$, $P < 0.01$). One may, therefore, argue that age might be one way to explain why the number of eggs was smaller in women with lower AMH level. However, looking closely at the data, we noticed that AMH levels varied among women of the same age. Viewed this way, the question arises as to whether AMH and age might be involved in the egg yield through different mechanisms. To address this, we stratified the patients by age to see whether or not AMH is related to the number of eggs retrieved the same way as age. We classified the patients into three groups, that is, 34 years old or younger, 35-39 years old, and 40 years old or above, and looked into the number of eggs in each ovarian stimulation protocol for each age subgroup (ie, ≦34, ≦35-39, and 40≦). Figure 2 illustrates the number of eggs for each ovarian stimulation protocol in the subgroups stratified by age. It is to be noted that the number of eggs after the clomiphene and the modified mild protocols was smaller compared with the short and the antagonist protocols across all subgroups with different age. In addition, women aged 40 years or older, relative to women under 40 years, had smaller number of eggs collected as a whole. Analysis of the relationship between age and egg number for women under 40 years old showed a very weak negative correlation (correlation coefficient $r = -0.18$, $P < 0.01$). Thus, it appears that, apart from whether or not age exceeds 40, age is of low significance in predicting egg number.

In order to compare the contribution of AMH and age to the number of eggs retrieved in each age subgroup, a multiple regression analysis was carried out with AMH, age, and respective ovarian stimulation protocols as covariates and the number of eggs as a target variable (Table 1). When analyzing for each age subgroup, AMH but not age was significantly correlated with the number of eggs retrieved. Regarding the relation between ovarian stimulation protocols and egg number, no significant differences were found between the clomiphene protocol and the modified mild protocol across the subgroups. What was common to all groups is that the number of eggs was larger in the short and the antagonist protocols compared with the clomiphene and the modified mild protocols, with egg number being larger for the antagonist protocol relative to the short protocol only in the subgroup under 35 years.

In an attempt to see characteristic hormonal profiles and differences in responsiveness to each ovarian stimulation protocol according to different AMH levels, we classified all patients into four subgroups depending on AMH levels, that is, <1, ≦1-2, ≦2-3, and 3≦ ng/mL. Table 2 shows age and basal levels of fertility-related hormones in respective subgroups with different AMH levels. Age of the patients decreased as AMH levels increased. The median FSH concentration was higher in the order of <1, ≦1-2, and ≦2-3 ng/mL with no difference between ≦2-3 and 3≦ ng/mL. Regarding LH, the variation of the median LH level in each subgroup was relatively slight with the median LH level in women with AMH <1 ng/mL being significantly lower than that of AMH 3≦ ng/mL. The median estradiol level was lowest in the subgroup with AMH 3≦ ng/mL with significant differences being observed compared with those in the

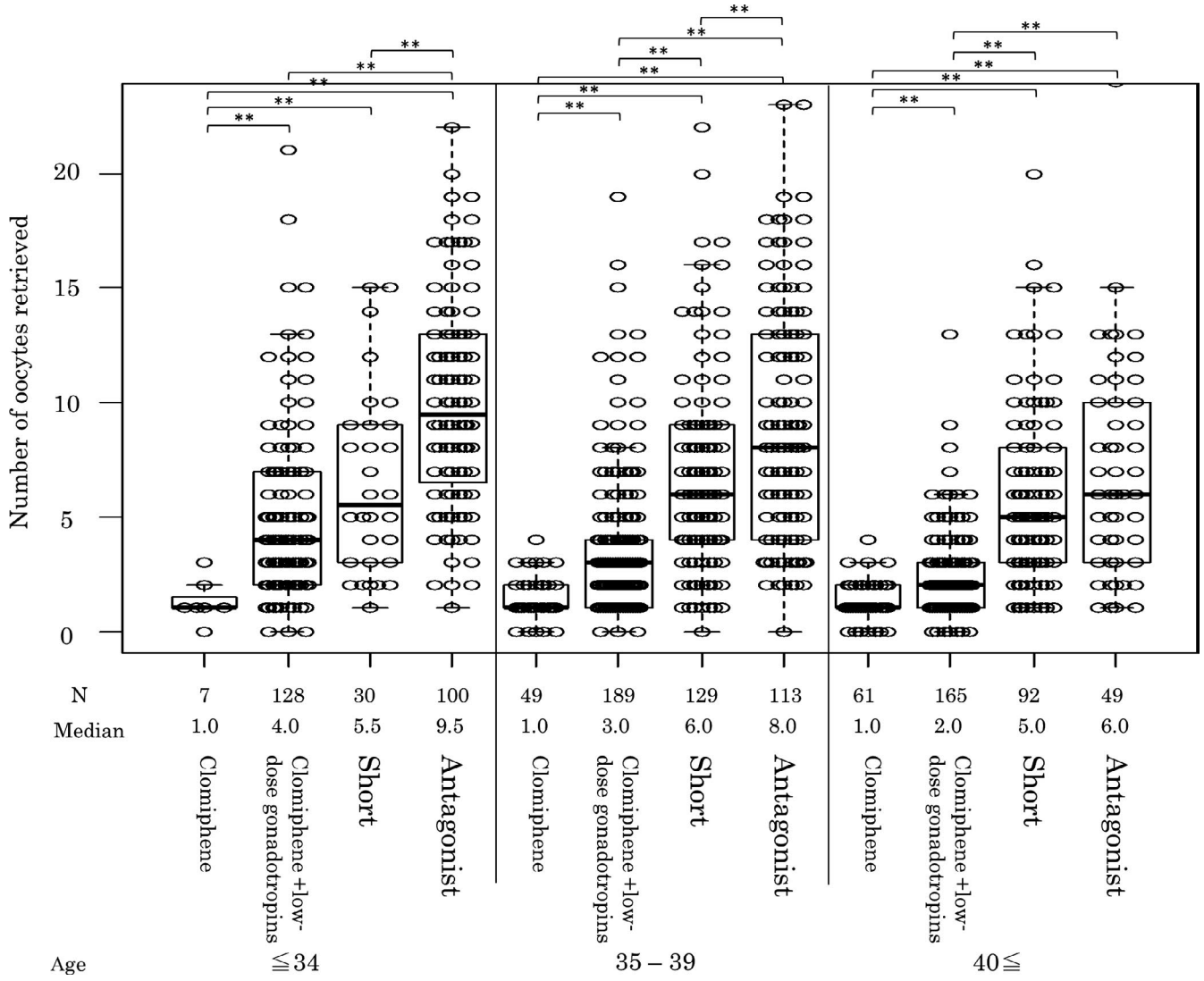

FIGURE 2 Number of oocytes retrieved for each ovarian stimulation protocol in each subgroup stratified by age. Steel-Dwass test was used to evaluate differences between groups. $^{**}P < 0.01$

TABLE 1 Multiple regression modeling explaining factors associated with egg number in each subgroup stratified by age

	≦34			≦35-39			40≦		
Age	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
AMH	0.6	0.44-0.82	<0.01	0.9	0.76-1.12	<0.01	1.2	1.00-1.40	<0.01
Age	−0.2	−0.43-0.10	0.21	0.1	−0.11-0.37	0.28	0.0	−0.19-0.11	0.62
Clomiphene vs clomiphene + low-dose gonadotropins	2.0	−1.02-5.00	0.19	0.8	−0.33-2.00	0.16	0.4	−0.3-1.14	0.26
Short	5.0	1.79-8.21	<0.01	4.7	3.49-5.87	<0.01	3.4	2.62-4.24	<0.01
Antagonist	7.4	4.4-10.44	<0.01	5.2	3.92-6.52	<0.01	3.5	2.52-4.48	<0.01
Clomiphene + low-dose gonadotropins vs short	3.0	1.41-4.62	<0.01	3.8	3.02-4.67	<0.01	3.0	2.38-3.64	<0.01
Antagonist	5.4	4.41-6.45	<0.01	4.4	3.52-5.25	<0.01	3.1	2.26-3.91	<0.01
Short vs antagonist	2.4	0.78-4.05	<0.01	0.5	−0.44-1.52	0.28	0.1	−0.78-0.93	0.87

AMH, anti-Műllerian hormone; CI, confidence interval.

TABLE 2 Hormonal profiles in each subgroup stratified by AMH level

AMH (ng/mL)	<1 (n = 384)	≦1-2 (n = 306)	≦2-3 (n = 148)	3≦ (n = 274)
Age	40 (29-48)[a]	39 (28-46)[b,c]	36 (27-44)[b,d,e]	35 (26-42)[b,d,f]
FSH (mIU/mL)	8.3 (0.4-24.4)[a]	7.5 (3.4-21.7)[b,c]	6.7 (3.7-19.6)[b,d]	6.7 (0.3-13.5)[b,d]
LH (mIU/mL)	4.4 (0.1-13.3)[a]	4.6 (0.7-19.5)	4.9 (0.6-14.0)	5.2 (0.1-12.7)[b]
E2 (pg/mL)	29.4 (20-156)[a]	30.4 (20-254)[c]	27.7 (20-223)	25.3 (20-110)[b,d]
TSH (μU/mL)	1.8 (0.1-6.6)	1.7 (0.04-13.6)	1.6 (0.4-7.7)	1.7 (0.08-10.9)
PRL (ng/mL)	13.3 (1.6-34.3)	13.1 (1.6-39.5)	13.3 (3.7-36.1)	12.8 (0.6-39.8)

Data represent the median (minimum − maximum).
Data were analyzed using chi-square test with Bonferroni correction.
FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; TSH, thyroid-stimulating hormone; PRL, prolactin; TSH, thyroid-stimulating hormone.
[a,b,c,d]$P<0.01$.
[e,f]$P<0.05$.

subgroups with AMH below 2 ng/mL. The median values of TSH and prolactin were essentially equivalent among the subgroups.

We then examined the relationship between AMH levels and the number of eggs retrieved for each stimulation protocol (Figure 3). In addition, we analyzed the relationship between the median egg number and different AMH levels for each stimulation protocol. First, we show the data on the clomiphene protocol (Figure 3A). When the data on the clomiphene protocol being analyzed collectively, AMH levels exhibited a very weak positive correlation with the number of eggs without a statistical significance. The median value of egg number in women with AMH levels <1, ≦1-2, and ≦2-3 ng/mL was one, while that in women with AMH levels 3≦ ng/mL was 3. We looked at the association of the number of eggs collected with AMH levels using Steel-Dwass test. We could not detect a significant difference in the median egg number when compared in any combination of the four subgroups with different AMH levels.

Next, we studied the relationship between AMH levels and the number of eggs with the modified mild protocol (Figure 3B). A moderate positive correlation was found between the number

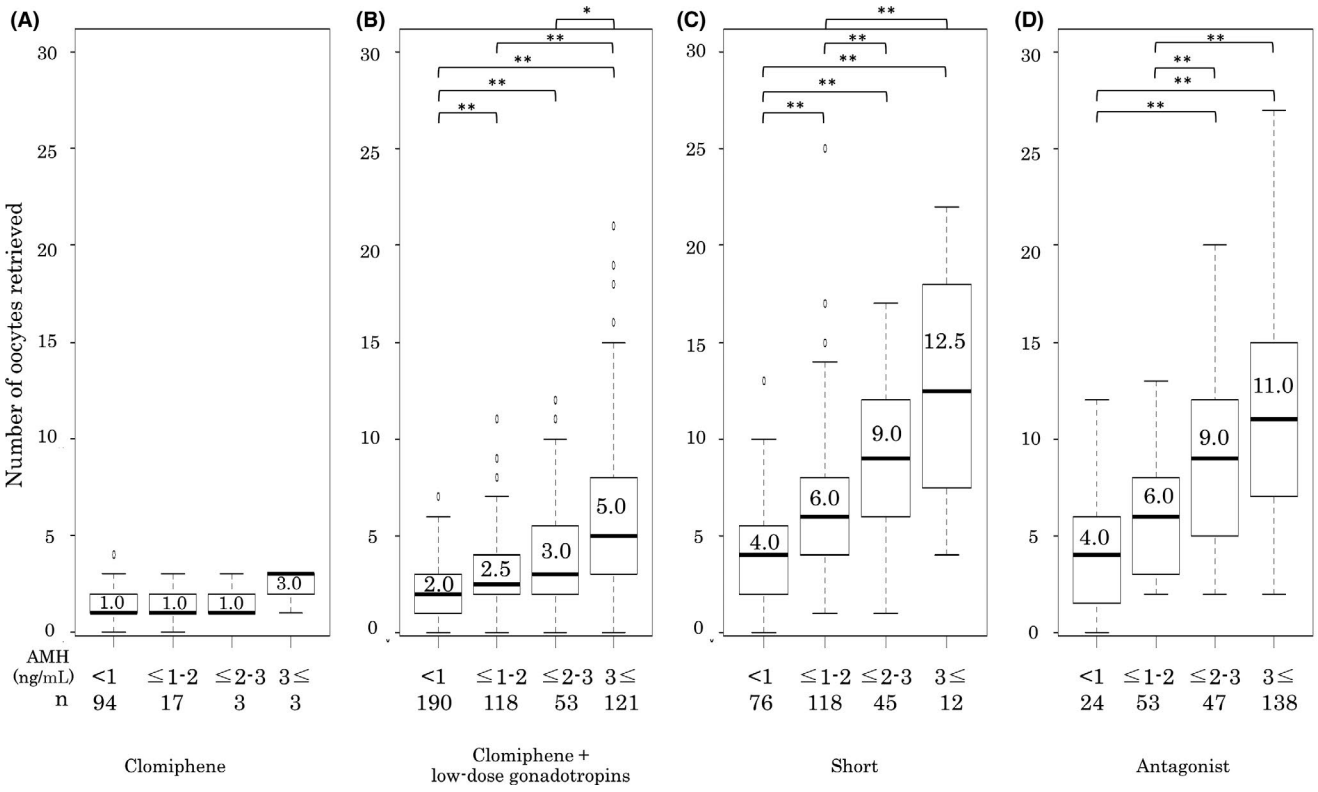

FIGURE 3 The median of egg number for each ovarian stimulation protocol in each subgroup stratified by AMH level. A, B, C, and D depict the data on the clomiphene, clomiphene+low-dose gonadotropins, short, and antagonist protcols respectively. Standard box plot, in which the horizontal thick line represents the median, thin horizontal line represents the interquartile range, and whiskers above and below represent the maximum and the minimum, respectively. Steel-Dwass test was used to evaluate differences between the subgroups. AMH, anti-Műllerian hormone. $^{**}P < 0.01$, $^{*}P < 0.05$

TABLE 3 The percentage of relatively good quality blastocysts with 3BC, 3CB, or better to the eggs cultured to grow into a blastocyst in each subgroup with different AMH levels

AMH (ng/mL)	<1	≦1-2	≦2-3	3≦
Clomiphene (%)	37.3	56.3	100	50.0
Clomiphene + low-dose gonadotropins (%)	39.7	50.5	53.3	47.3
Short (%)	39.9	40.1	42.5	35.1
Antagonist (%)	28.3	54.2	46.6	44.0
Total (%)	38.1[a]	46.1[b]	46.4[b]	44.4

Data were analyzed using chi-square test with Bonferroni correction.
[a,b]$P<0.05$.

of eggs collected and AMH levels (correlation coefficient $r = 0.5$, $P < 0.01$). The median number of eggs collected was 2 for AMH <1 ng/mL, 2.5 for AMH ≦1-2 ng/mL, 3 for AMH ≦2-3 ng/mL, 5 for AMH ≦3 ng/mL. In the three subgroups with AMH 1≦ ng/mL, the number of eggs collected was significantly larger compared with AMH <1 ng/mL ($P < 0.01$). Furthermore, the number of the eggs in the subgroup with AMH 3≦ ng/mL was larger compared with subgroups with AMH ≦1-3 ng/mL ($P < 0.05$).

We then focus on the short protocol (Figure 3C). The number of eggs collected exhibited a moderate positive correlation with AMH levels (correlation coefficient $r = 0.5$, $P < 0.01$). The median egg number was 4 for AMH <1 ng/mL, 6 for AMH ≦1-2 ng/mL, 9 for AMH ≦2-3 ng/mL, and 12.5 for AMH 3≦ ng/mL. In the three subgroups with AMH 1≦ ng/mL, the number of eggs collected was significantly larger compared with AMH <1 ng/mL ($P < 0.01$). Significant differences were further observed between AMH ≦1-2 ng/mL and AMH ≦2-3 ng/mL, and between AMH ≦1-2 ng/mL and AMH 3≦ ng/mL ($P < 0.01$). Although, in both the modified mild protocol and the short protocol, egg number increased with increasing AMH values, the short protocol, in which relatively higher doses of gonadotropins were administered, yielded an even more pronounced increase in egg number with increasing AMH values.

Finally, we describe the data on the antagonist protocol (Figure 3D). A moderate positive correlation was found between the number of eggs collected and AMH level (correlation coefficient $r = 0.5$, $P < 0.01$). The median of egg number collected was 4 for AMH <1 ng/mL, 6 for AMH ≦1-2 ng/mL, 9 for AMH ≦2-3 ng/mL, and 11 for AMH 3≦ ng/mL. The number of eggs collected was significantly larger in the two subgroups with AMH ≦2 ng/mL, when compared with AMH <1 ng/mL ($P < 0.01$). In addition, significant differences were observed in the number of eggs between AMH ≦1-2 ng/mL vs AMH ≦2-3 ng/mL and between AMH ≦1-2 ng/mL vs AMH 3≦ ng/mL ($P < 0.01$). Thus far, we mentioned the relationship between AMH value and the response to each ovarian stimulation protocol. If focusing on the data of women with AMH <1 ng/mL, egg number increased in the order of the clomiphene, the modified mild, and the short, with the antagonist protocol being comparable with the short protocol.

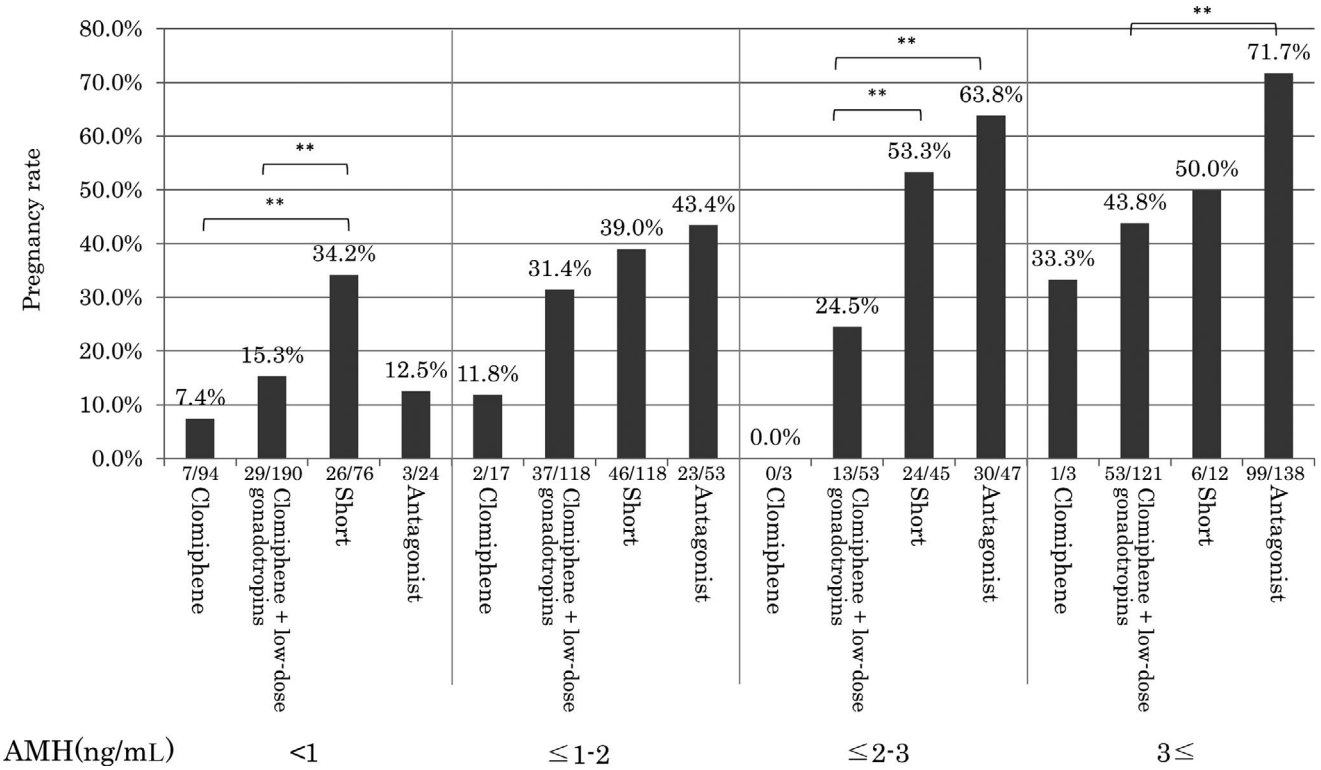

FIGURE 4 Clinical pregnancy rate for each ovarian stimulation protocol in each subgroup stratified by AMH level. Data were analyzed using chi-square test with Bonferroni correction. AMH, anti-Müllerian hormone. $^{**}P < 0.01$

So far, we have seen the relationship between AMH value and the number of eggs collected. Next major issue concerns how the egg quality relates to AMH level. To address this, we evaluated the developmental potency of the egg as the surrogate of egg quality. More specifically, we compared the percentage of relatively good quality blastocysts with 3BC, 3CB, or better to the eggs cultured to grow into a blastocyst in the subgroups with different AMH levels (Table 3). In the subgroup with AMH <1 ng/mL, 38.1% of the eggs cultured grew into the blastocyst stage, which was significantly lower than the subgroups with AMH ≦1-2 ng/mL (46.1%) and ≦2-3 ng/mL (46.4%), while no differences in the percentage of eggs reaching the blastocyst stage were found among the three subgroups with AMH ≦1 ng/mL. Then, one may wonder that egg quality might depend on ovarian stimulation protocols. We, therefore, compared the blastocyst rate by each stimulation protocol. The data, all in all, suggested that the quality of eggs seemed to be unaffected by the stimulation protocols.

Lastly, we questioned whether or not the value of AMH could be related to pregnancy rate per egg collection procedure. As depicted in Figure 4, the clinical pregnancy rate in the subgroup with AMH <1 ng/mL was generally low. The pregnancy rate tended to increase with the rise in AMH value. This relationship seems to be similar to that between AMH value and egg number. Thus, assuming that egg number might be the link between AMH value and pregnancy rate, we wished to know how AMH level and egg number are associated with pregnancy rate in each subgroup with different AMH levels. To address this, we utilized the logistic regression analysis with AMH, egg number, and age as covariates, and clinical pregnancy as a target variable. The data in Table 4 indicated that the number of eggs, but not AMH, was significantly associated with pregnancy rate in all subgroups with different AMH values. It is further noteworthy that, when limiting to the subgroups with AMH below 2 ng/mL, age was negatively related to pregnancy rate with a statistical significance. To rephrase, both age and the egg number, but not AMH, were related to pregnancy rates in the subgroups with AMH < 2 ng/mL. In contrast, when AMH levels exceeded 2 ng/mL, age was no longer associated with pregnancy rates. The reason for age showing a negative correlation with pregnancy rates in the subgroups with AMH < 2 ng/mL might be that these subgroups had a higher percentage of elderly women. What is further noteworthy here is that a certain ovarian stimulation protocol did not produce higher pregnancy rates than other protocols.

4 | DISCUSSION

The present study provides strong support for the view that AMH is a reliable index to estimate the egg number collected during ART in comparison with age. This study further emphasizes that the number of eggs obtained by ovarian stimulation is AMH value-dependent and differs for each regimen. In addition, egg number could be a crucial variable associated with the establishment of pregnancy. As such, the date presented here could yield clues on how to improve ART outcomes.

TABLE 4 Logistic regression analysis explaining factors associated with clinical pregnancy in each subgroup stratified by AMH level

	<1			≦1-2			≦2-3			3≦		
AMH (ng/mL)	AOR	95% CI	P	AOR	95% CI	P	AOR	95% CI	P	AOR	95% CI	P
Egg number	1.19	1.04-1.38	<0.03	1.29	1.17-1.44	<0.01	1.26	1.13-1.42	<0.01	1.19	1.11-1.28	<0.01
AMH	1.47	0.44-4.94	0.53	1.26	0.5-3.17	0.62	0.80	0.21-3.01	0.74	0.98	0.86-1.12	0.75
Age	0.86	0.79-0.94	<0.01	0.87	0.82-0.94	<0.01	0.91	0.81-1.02	0.10	0.87	0.8-0.95	<0.01
Clomiphene vs clomiphene + low-dose gonadotropins	1.93	0.79-4.71	0.15	1.77	0.37-8.5	0.48	2.E + 06	0-Inf	0.99	0.79	0.07-9.46	0.85
Short	3.48	1.26-9.63	<0.03	1.12	0.22-5.75	0.89	4.E + 06	0-Inf	0.99	0.49	0.03-8.36	0.63
Antagonist	0.67	0.13-3.41	0.63	1.31	0.24-7.07	0.76	5.E + 06	0-Inf	0.99	1.42	0.12-17.5	0.78
Clomiphene + low-dose gonadotropins vs short	1.81	0.9-3.64	0.10	0.63	0.32-1.24	0.18	1.53	0.54-4.34	0.42	0.62	0.15-2.62	0.52
Antagonist	0.35	0.08-1.45	0.15	0.74	0.33-1.63	0.45	2.00	0.73-5.47	0.18	1.80	0.93-3.49	0.08
Short vs antagonist	0.19	0.05-0.77	<0.03	1.17	0.56-2.45	0.68	1.30	0.49-3.45	0.60	2.88	0.76-11	0.12

AMH, anti-Müllerian hormone; AOR, adjusted odds ratios; CI, confidence interval.

It is well known that AMH value correlated positively with the number of eggs obtained by ovarian stimulation in ART.[13] In the present study, like AMH, age was also related to the number of eggs. Furthermore, AMH and age were mutually interrelated. Based on these observations, what is the superiority of AMH as an indicator to predict the number of eggs in ART procedures in comparison with age? As for age, the number of eggs changed drastically around the age of 40 years. But, in women under 40 years, the accuracy of age as an indicator to estimate the number of eggs was less precise relative to AMH. Unlike age, AMH showed a relatively high correlation with the number of eggs over a wide concentration range of AMH. Besides, AMH value was predictive of the number of eggs in women over a wide reproductive age range. When it comes to FSH, the data not shown here revealed that FSH values below 10 mIU/mL could not discern how much ovarian reserve is. On the other hand, when FSH value exceeded 10 mIU/mL, there was a marked decline in the number of eggs whatever the value of FSH. Collectively, AMH can be regarded as a useful index for the vast majority of women with different backgrounds in order to estimate the egg number compared with age and FSH.

Notably, lower AMH levels were associated with the rise in FSH and estradiol levels. Although a cardinal regulator of FSH synthesis and secretion is GnRH, other peptide hormones, including activin, inhibins, and follistatin, modulate the secretion of FSH. Activin can directly stimulate FSH secretion, whereas inhibins and follistatin negatively regulate FSH secretion.[14] Inhibins are composed of two biologically active forms, that is, inhibin A and inhibin B. Inhibin B has been shown to possess greater biological potency compared with inhibin A. The rise in FSH in women approaching menopause is associated with a decrease in inhibin B and a rise in activin A, while follistatin is unchanged.[15] Given the age of women in the subgroup with lowest AMH value being highest, the observed rise in FSH could be explained by a decrease in inhibin B. It is intriguing to assume another mechanism for the association of the low AMH level with the rise in FSH. Both AMH and inhibins are produced by granulosa cells. Interestingly, the gene expressions of these hormones in granulosa cells are up-regulated by a common factor, bone morphogenic protein.[16] From this perspective, the decline in both AMH and inhibins might be caused by perturbed actions of bone morphogenic protein in the ovary which is not necessarily related to aging, resulting in the rise in FSH. Another noteworthy result is that the women with lowest AMH exhibited higher estradiol levels. Elevated FSH levels might provide an explanation for this. Alternatively, AMH suppresses the sensitivity of granulose cells to release estradiol in response to FSH.[17] This may offer another explanation for the rise in estradiol associated with lowest AMH.

Although, AMH correlates with the number of eggs retrieved; however, until now, it has been poorly studied whether the relationship between AMH and the number of eggs is the same across multiple ovarian stimulation methods. The present study demonstrated that the relationship between the value of AMH and egg yield differed depending on the stimulation protocols. To be more specific, the egg number was small when using the clomiphene protocol with no significance difference in the number observed across the subgroups with different AMH levels. On the other hand, when the protocols include gonadotropins, the egg yield was AMH value-dependent until the value of AMH reached 3 ng/mL. Interestingly, even though in the group with AMH < 1 ng/mL, the egg yield increased in the order of the clomiphene < the modified mild < the short. The number of eggs was almost the same between the short protocol and the antagonist protocol with the total doses of gonadotropins being essentially the same between the two. From these observations, it appears that, as long as the value of AMH is <3 ng/mL, the number of eggs seems to be proportional to the amount of exogenous gonadotropins whatever AMH level is. Furthermore, as the amounts of gonadotropins administered were increasing, the difference in the number of eggs for each AMH value subgroup was more amplified, reflecting that AMH is indeed a genuine index for ovarian reserve. Looking from different perspectives, a reduction in ovarian reserve, which could be regarded as almost synonymous with the low value of AMH, is difficult to detect in physiological situations and could be unveiled only after ovarian stimulation by exogenous gonadotropins.

In this study, the decision making whether gonadotropins were used or not in women with low AMH value depended on their informed choice following the doctor's suggestions considering their clinical profiles, the data on past treatment, economical issues, and so on. Thus, several biases might be introduced in the treatment choice, which might temper the scientific implication of the results of this study. Viewed in this light, we do not intend to recommend ovarian stimulation methods using gonadotropins unconditionally to get a certain number of eggs in women with lower AMH values. However, it is worth mentioning that, even if AMH is low, there are women in which a considerable number of eggs could be obtained in response to ovarian stimulation regimens including gonadotropins. On the contrary, we sometimes encounter a woman who has a poor response despite the value of AMH being not low. Thus, although it is certain that AMH value relates to the ovarian response stochastically, nevertheless, we should not stick to the stereotypical notion in the real clinical setting that ovarian stimulation methods should be strictly selected according to the value of AMH exclusively.

Ovarian reserve is a term that implies the capacity of the ovary to produce eggs capable of fertilization resulting in a successful pregnancy, or the number and quality of the ovarian primordial follicular pool. However, in any case, the definition of ovarian reserve is too conceptual to develop a decisive tool for assessing it. Although opinion has been divided regarding the relation between AMH value and ART outcome, the low value of AMH is generally thought to link with the low pregnancy rate. However, it is still of dispute as to whether the mechanism for poor ART outcomes might be due to a decrease in the number of eggs collected or a decrease in both the number of eggs and the quality of eggs. The data presented here revealed that lower AMH levels were associated with smaller egg number and presumably poor egg quality as judged by poor developmental potency of cultured eggs,

suggesting that a reduction in the amount and quality of eggs might be responsible for the decline in pregnancy rate. But what we would like to emphasize here is that only the value of AMH <1 ng/mL was associated with a decline in developmental potency of eggs whereas the number of eggs collected was well correlated with a wider range of AMH values including AMH <1 ng/mL. Thus, it is tempting to speculate that the mechanisms by which AMH is related to the number and quality of eggs would not be the same. The mechanism for this could be, in part, explained by age. The median age was the highest in the AMH <1 ng/mL subgroup. A certain number of eggs, although not enough, can be collected by ovarian stimulation in women of relatively high age. However, perhaps it may fail to improve the deteriorated egg quality accompanied by aging.

As mentioned above, one thing for sure is that the low AMH value is associated with a low pregnancy rate. However, it remains still controversial whether AMH could really correlate with the success of ART over a wide concentration range. Some papers were in support of AMH as a predictor for ART outcomes,[18,19] while others could not confirm this.[20,21] In the present study, when studying all women with widely distributed AMH levels together, the value of AMH seems to be associated with pregnancy rate. However, when analyzing in each subgroup with different AMH values, there is no association between AMH and pregnancy rate. Accordingly, the association of AMH with pregnancy rate may differ depending on whether the women with a limited range of AMH values are surveyed, or women with a wide range of AMH levels are exhaustively examined. This may yield clues to understanding conflicting conclusions on the association of AMH value with pregnancy rate.

We have shown that the number of eggs was a crucial factor for a successful outcome of ART in keeping with previous reports.[22,23] In this regard, it is interesting to note that, when focusing on the group with AMH <1 ng/mL, AMH value was correlated with the number of eggs whereas, regarding pregnancy rate per egg collection, egg number, but not AMH, showed a statistically significant association, implying that egg number might play a key role for ART outcome. The implication of this fact could be as follows. It is true that AMH values less than 1 ng/mL are correlated with the number of eggs. However, if AMH values of this range predicted the number of eggs with high accuracy, egg number and AMH value alike should be associated with pregnancy rate. However, this is not the case. The explanation for this is that even though in women with AMH values <1 ng/mL, egg number varies depending on ovarian stimulation methods, or even the same stimulation method brings about differences in egg number from person to person. It seems difficult to overcome individual differences. Nevertheless, if a considerable number of eggs could be obtained by choosing an appropriate stimulation method for respective women with the low AMH levels, an increase in pregnancy rate could be expected.

This paper deals with a retrospective clinical study analyzing ART results and, therefore, entails some limitations such as inherent biases common to observational studies. The allocation of women to ovarian stimulation protocols was not randomized. Some women, therefore, had been led to a specific protocol based on the judgment of doctors. Moreover, the sample size was not enough to deduce a conclusive treatment guideline. In the future, a large-scale prospective, randomized controlled study will be desirable to establish treatment policy of ART for each AMH value with diverse clinical backgrounds taken into account.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institute and national) and with the Helsinki Declaration of 1964 and its later amendment. Our institutional review board approved the study protocol and its consent form, and we obtained informed consent for this study from all participants.

ANIMAL STUDIES

Not applicable.

DISCLOSURE

Conflict of interest: All the authors state explicitly that there are no conflicts of interest in connection with this article.

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