Different ovarian response by age in an anti-Müllerian hormone-matched group undergoing in vitro fertilization

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Abstract

Background Recently, serum anti-Müllerian hormone (AMH) has been used as a good marker of ovarian response during in vitro fertilization (IVF). However, in the clinical setting, we felt that ovarian response was clearly different by age with the same AMH level. Then in this study we evaluated the relationship between serum AMH, age and parameters related to ovarian response and compared these parameters in regard to age within serum AMH-matched group.

Methods and results The relationship of these parameters were evaluated retrospectively in patients undergoing their first IVF cycle under a GnRH agonist flare up protocol ($n=456$) between October 2008 and October 2010 in our clinic. To understand the relations between variables described above, principal component analysis (PCA) was performed. PCA revealed patients' age was at the different dimension from serum AMH and other variables. Therefore at first we segregated all patients into Low, Normal and High responder groups by their serum AMH using cut-off value of receiver operator characteristics curve analysis. Secondary, we divided each responder group into four subgroups

according to patients' age. The high aged subgroups required a significantly higher dose of gonadotropin and a longer duration of stimulation; however, they had significantly lower peak E2 and a smaller number of total oocytes as well as M2 oocytes compared to the low aged subgroups. **Conclusions** The influence of aging on the ovarian response was clearly seen in all groups; the ovarian response tended to decrease as patients' age increased with the same AMH level. Therefore serum AMH in combination with age is a better indicator than AMH alone.

Keyword AMH · Anti-Müllerian hormone · Age · IVF · GnRH agonist flare up protocol · Ovarian response

Introduction

A clear relationship exists between age and fertility [1]. In recent years, ovarian aging and reduced ovarian reserve can become critical factors for in vitro fertilization (IVF) treatment [2, 3]. One of the most important parameters to get better results from IVF is the forecasting factors for the ovarian response before these treatments. A number of parameters known as ovarian reserve markers (e.g., cycle day 3 serum FSH, antral follicle count, serum inhibin B, and patient age) have been used as predictive markers of ovarian responses to gonadotropin during IVF treatment [4–8].

Recently, serum anti-Müllerian hormone (AMH) has been used as a marker of ovarian reserve and ovarian response to gonadotropin during IVF treatment [9–12]. AMH is a dimeric glycoprotein that belongs to the transforming growth factor-beta superfamily. It induces regression of the Müllerian ducts during male fetal development [13]. In the female, AMH is exclusively produced by granulosa cells within preantral and small antral follicles;

Capsule Ovarian response was significantly different by age within AMH-matched groups; serum AMH in combination with age was more predictable ovarian response than AMH alone.

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however, it is not produced in either primordial follicles or atretic follicles. AMH inhibits initial primordial follicle recruitment and decreases the sensitivity of preantral and antral follicles to FSH [14, 15]. Therefore, AMH can serve as a marker of the primordial follicle pool, and may indicate ovarian reserve. In most studies, AMH levels are thought to be stable throughout the menstrual cycle [16, 17]; thus, AMH can serve as a simple and useful marker. Because it is able to predict how many oocytes collected, cycle cancellation or ovarian hyperstimulation syndrome (OHSS) by checking serum AMH level, AMH may be an ideal candidate for individualization of stimulation in IVF treatment [18, 19]

As described above, a number of studies had reported that AMH was a very good predictive marker of ovarian response and ovarian reserve. Since October 2008, we have been using serum AMH as an ovarian response marker for IVF treatment; the initial dose of gonadotropin was determined by serum AMH level. However in the clinical setting, we felt that the ovarian response was clearly different by patients' age with the same serum AMH level. Therefore we evaluated the relationship between serum AMH, age and parameters related ovarian response and compared those in regard to age within serum AMH-matched group. In this study we focused on the gonadotropin releasing hormone (GnRH) agonist flare-up protocol of their first IVF treatment to eliminate the variability of ovarian response with multiple protocols.

Materials and methods

Patients and treatment

Patients undergoing their first assisted reproduction cycles of ($n=1026$) between October 2008 and October 2010 were retrospectively evaluated. Inclusion criteria for this study were as follows: (1) the patient was in her first cycle of IVF treatment; (2) her age was ≤ 45 years; (3) there was no evidence of an endocrinological disorder (normal prolactin and thyroid stimulating hormone); (4) basal serum FSH levels were ≤ 13.0 mIU/ml; and (5) body mass index (BMI) was ≤ 30.0 kg/m². In addition to the patient's age, the IVF protocol selection was based on her basal serum FSH level, as follows (FSH level, protocol): serum FSH=8.0–13.0 mIU/ml, GnRH agonist flare up protocol; serum FSH ≤ 8.0 mIU/ml, GnRH agonist long protocol; serum FSH ≤ 8.0 mIU/ml with suspected of polycystic ovary syndrome (PCOS) and OHSS, GnRH antagonist flexible protocol. Patients with an irregular menstrual cycle and suspected of premature ovarian failure were not selected. There were no cycle cancellations because of impending OHSS or lack of response in these patients. The patients received oral sequential estradiole (E2) and progesterone treatment in pretreatment

cycle [20]. A GnRH agonist flare-up protocol, which began with daily nasal buserelin 600 μ g/day (Buserecure[®], Fuji Pharmaceutical, Tokyo, Japan) on day 3 of the stimulation cycle. The GnRH agonist was continued until the day of HCG administration. The initial daily gonadotropin was either Menopur[®] (Ferring Pharmaceuticals, Tokyo, Japan) or GONAL-f[®] (Merck Serono, Tokyo, Japan).

The starting dose of gonadotropin was according to the serum AMH level as follows; patients of serum AMH 0.0 to 10.0 pM was initially administered 300 to 450 IU of gonadotropin; patients of serum AMH 10.1 to 20.0 pM was initially administered 225 to 300 IU of gonadotropin; and patients of serum AMH over 20.0 pM was initially administered 150 to 225 IU of gonadotropin. Gonadotropin was administered from day 3 of the stimulation cycle for 4 to 5 days, after which the dose was adjusted according to the ovarian response. The ovarian response was monitored by transvaginal ultrasound and serum E2 levels. When three or more follicles reached a maximum diameter of 18 mm, urinary HCG (5000 or 10,000 IU) was administered. To avoid OHSS the dose of HCG was determined by peak E2 and number of follicles at the day of HCG administered. Transvaginal oocyte retrieval was performed 34 to 35 h after HCG injection as previously described [21]. Patients' age and BMI was determined at the pretreatment cycle of day 2–5. This study was approved by the Institutional Review Board and conducted in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from all patients for AMH measurement and a description of their treatment outcome.

Hormonal assays

The peripheral blood serum was collected from these patients on day 2–5 of the pretreatment cycle and serum AMH, basal FSH and basal LH were determined. Serum sample of peak E2 was collected at the day of HCG administered. Serum samples were stored at -20°C and these archived samples were retrieved for hormonal assays. The AMH concentration was determined in duplicate with Enzyme-Linked Immunosorbent Assay (ELISA) using a commercial kit (EIA AMH/MIS A16507; Immunotech, Beckman-Coulter, Marseille, France) with a sensitivity of 0.7 pM and the intra- and inter-assay coefficients of variation were 12.3% and 14.2%, respectively. Serum FSH, LH, E2, and progesterone were measured using the automated Electrochemiluminescence Immunoassay via the Cobas e 411 Analyzer (Roche Diagnostics K.K., Tokyo, Japan).

Statistics

The distribution of variable data was assessed and normal or abnormal distributions were treated appropriately. To

understand the relations between variables related to COS, principal component analysis (PCA) was performed. PCA is an unsupervised decomposition method to reduce multi-dimensional data. Each dimension represents a principal component with a certain percentage of variance. PCA recognize patterns and clusters in data sets in multiple dimensions and is a variable visualization and summarizing tool [22]. The correlation of ovarian response in relation to COS and parameters of patients' characteristics were analyzed by Spearman's rank correlation coefficient. Analysis across groups and subgroups were by Kruskal-Wallis one-way analysis of variance (Kruskal-Wallis analysis). The ROC curve was computed to assess the predictive accuracy of measured variables for Low responder and High responder. The cut-off value was decided by Youden's Index [23]. These data analyses were performed using the statistical package StatView Ver. 5.0 (SAS Institute Inc., NC, USA) and Excel Statistics (SSRI, Tokyo, Japan). Significance was determined with $P < 0.05$.

Results

From October 2008 through October 2010, 1026 of their first IVF treatment cycles including 456 of GnRH agonist flare-up protocol, 69 of GnRH agonist Long protocol and 501 of GnRH Antagonist protocol were performed. Within these cycles, 456 cycles of GnRH agonist flare-up protocol were studied retrospectively.

The results of PCA

In this study to examine the characteristics of serum AMH, patients' age and parameters related to ovarian response (duration of stimulation, total gonadotropin dose, peak E2 level, number of total oocytes and number of metaphase 2 (M2) oocytes) we analyzed feature of these variables by the principal component analysis (PCA) (Table 1). We could consider principal component (PC) 1 included high eigenvector of AMH, total gonadotropin dose, peak E2, number of oocytes and M2 oocytes. In other words PC1 represented typical ovarian response in IVF treatment. Furthermore PC1 had high proportion of variance and AMH had higher eigenvector than age in PC1. This showed AMH had stronger correlation than age in ovarian response. PC2 had high eigenvector of duration of stimulation and total gonadotropin dose. Therefore PC2 almost represented stimulation days. PC3 and PC4 had high eigenvector of age and AMH and represented age and AMH, respectively. The directions of the vector of serum AMH and patients' age were different from each other. Notably it could be said that age was a variable in a different dimension (PC) from serum AMH and other parameters in this study.

Cut-off value of low responder and high responder

We divided all of the patients by serum AMH using ROC analysis for the discrimination of poor (≤ 4 oocytes retrieved) and high responder (≥ 20 oocytes retrieved) [24]. The cut off value of Low responder was 10.0 pM (ROC AUC=0.758, sensitivity: 0.722, specificity: 0.757, Odds ratio: 5.81 (95% CI 2.67–12.65)). That of High responder was 17.6 pM (ROC AUC=0.764, sensitivity: 0.692, specificity: 0.752, Odds ratio 8.00 (95% CI 3.73–18.15)). Then we decided as follows; Low responder: 0.0–10.0 pM, Normal responder: 10.1–17.5 pM, High responder: over 17.6 pM.

Baseline patient information and information of Low Normal and High responders

Table 2 presents the patients' clinical characteristics and parameters in relation to ovarian response during the first treatment cycle. Data are shown as median (inter-quartile) of each group. The median age and BMI of the total patients were 37.0 (34.0–40.0) years and 20.5 (19.2–22.2) kg/m². The median serum AMH, FSH and peak E2 concentrations were 12.8 (7.7–18.4) pM, 8.7 (7.1–10.4) mIU/l and 4,636 (2,912–6,862) pg/ml, respectively. The median duration of stimulation was 12.0 (11.0–13.0) days and the median total gonadotropin dose was 3,787 (3,300–5,100) IU. The median number of total oocytes and the number of M2 oocytes was 9.0 (6.0–14.0) and 7.0 (4.0–11.5), respectively. Table 2 also presents the clinical characteristics and parameters of Low, Normal and High responder groups.

We examined the differences of each parameter between these groups by the Kruskal-Wallis analysis. The serum AMH ($P < 0.0001$), serum FSH ($P = 0.0176$), peak E2 ($P < 0.0001$), duration of stimulation ($P < 0.0001$), total dose of gonadotropins ($P < 0.0001$), the number of total oocytes ($P < 0.0001$) and M2 oocytes ($P < 0.0001$) were significantly different between three groups. There was a clear relationship that as the serum AMH level rose, the peak E2 level rose and the total gonadotropin dose decreased; furthermore the duration of stimulation was shorter and the number of oocytes and M2 oocytes increased. Age and BMI of the patients were not significantly different between these groups.

The correlation of serum AMH and parameters of patients' characteristics were analyzed by Spearman's rank correlation coefficient. Serum AMH significantly correlated with ovarian response; duration of stimulation ($r = -0.267$; $P < 0.0001$), total dose of gonadotropins ($r = -0.373$, $P < 0.0001$), peak E2 on the day of hCG ($r = 0.531$; $P < 0.0001$), number of total oocytes ($r = 0.509$; $P < 0.0001$), and number of M2 oocytes ($r = 0.497$; $P < 0.0001$). In this study, correlation coefficient between serum AMH and age was low ($r = -0.108$; $P = 0.0209$).

Table 1 Results of principal component analysis

Variables	PC1	PC2	PC3	PC4
Age (years)	-0.321	-0.316	-0.888	-0.095
AMH (pM)	0.652	-0.295	0.086	0.683
Duration of stimulation (days)	-0.650	-0.655	0.275	0.018
Total gonadotropin dose (IU)	-0.770	-0.529	0.181	-0.032
Peak E2 (pg/ml)	0.828	-0.252	0.007	-0.036
Number of oocytes	0.881	-0.314	0.041	-0.254
Number of M2 oocytes	0.884	-0.291	0.053	-0.266
Standard deviation	3.786	1.143	0.908	0.614
Proportion of Variance	54.087	16.322	12.969	8.771
Cumulative Proportion	54.087	70.409	83.378	92.149

To understand the relations

between variables of serum AMH, age and parameters related to COS (duration of stimulation, total gonadotropin dose, peak serum estradiol (E2), number of total oocytes and number of metaphase 2 (M2) oocytes), principal component analysis (PCA) was performed. PC: principal component; Peak E2: Serum estradiol level on the day of HCG administration

Group information categorized by age

In accordance with the age stratification used by the Society for Assisted Reproductive Technology (SART) we divided all of the patients into four age groups; <35 years, 35–37 years, 38–40 years, and >40 years [25]. Table 3 presents the clinical characteristics and parameters of the groups according to age. We also examined the differences of each parameter between groups by the Kruskal-Wallis analysis. Serum AMH, BMI and FSH of the patients were not significantly different between the four groups. Age ($P<0.0001$), peak E2 ($P=0.0002$), the duration of stimulation ($P=0.0034$), total dose of gonadotropins ($P<0.0001$), the number of total oocytes ($P<0.0001$) and M2 oocytes ($P<$

0.0001) were significantly different between four groups. Moreover the correlation of age and parameters of patients' characteristics were analyzed by Spearman's rank correlation coefficient. Age significantly but weakly correlated with total gonadotropin dose ($r=0.252$; $P<0.0001$), number of total oocytes ($r=-0.207$; $P<0.0001$) and number of M2 oocytes ($r=-0.217$; $P<0.001$).

Ovarian response between subgroups categorized by age in each responder group (AMH-matched group)

By the results of PCA that age was in a different dimension from serum AMH, we divided each responder group into four subgroups by age (a, 34 y<; b, 35–37 y; c, 38–40 y; d, >40 y).

Table 2 Patients' basic characteristics and group characteristics according to serum AMH level

Group	Total	Low	Normal	High	<i>p</i>	Correlation coefficient
Number of patients	456	173	156	128	—	—
Age (years)	37.0 (33.5–39.0)	37.0 (34.0–40.0)	36.0 (33.0–39.0)	37.0 (34.0–39.0)	N.S.	-0.108
AMH (pM)	12.8 (7.7–18.4)	6.9 (4.8–8.4)	13.9 (12.2–15.7)	21.7 (19.2–28.3)	<0.0001	—
BMI (kg/m ²)	20.5 (19.2–22.2)	20.8 (19.3–22.6)	20.3 (19.0–21.9)	20.6 (19.3–22.3)	N.S.	-0.032
Basal serum FSH (mIU/ml)	8.7 (7.1–10.4)	8.9 (7.4–11.3)	8.6 (6.8–10.2)	8.6 (7.1–9.8)	0.0176	0.096
Duration of stimulation (days)	12.0 (11.0–13.0)	12.0 (11.0–14.0)	12.0 (11.0–13.0)	11.0 (11.0–12.0)	<0.0001	-0.266
Total gonadotropin dose (IU)	3787 (3300–5100)	4800 (2076–4442)	3600 (3300–4800)	3300 (3000–3881)	<0.0001	-0.373
Peak E2 (pg/ml)	4636 (2912–6862)	3024 (2076–4442)	5151 (3524–6873)	6652 (4854–8836)	<0.0001	0.533
Number of oocytes	9.0 (6.0–14.0)	7.0 (4.0–15.0)	10.0 (7.0–14.0)	13.0 (10.0–19.0)	<0.0001	0.51
Number of MII oocytes	7.0 (4.0–11.5)	4 (3.0–7.0)	8.0 (5.0–12.0)	11.0 (7.0–15.0)	<0.0001	0.498

Patients were grouped by serum AMH level. Low responder group: serum AMH 0–10.0 pM; Normal responder group: serum AMH 10.1–17.5 pM; High responder group: AMH over 17.6 pM. Correlation coefficient: the correlations of serum AMH in relation to COS were analyzed by Spearman's rank correlation coefficient. Data were shown as median (inter-quartile) of each group and significance between groups was analyzed by Kruskal-Wallis analysis. BMI: body mass index; Peak E2: Serum estradiol level on the day of HCG administration. N.S.: not statistically significant. $P<0.05$: statistically significant

Table 3 Group characteristics according to patients' age

Group	Under 34 y	35–37 y	38–40 y	Over 41 y	<i>P</i>	Correlation coefficient
Number of patients	144	124	117	71	–	–
Age (years)	32.0 (31.0–33.0)	37.0 (36.0–38.0)	39.0 (38.0–40.0)	41.0 (40.0–42.0)	<0.0001	–
AMH (pM)	13.6 (8.3–18.4)	12.7 (8.1–18.5)	11.2 (7.3–18.1)	10.7 (6.9–17.4)	N.S	–0.108
BMI (kg/m ²)	20.2 (19.1–21.5)	20.5 (19.3–22.1)	20.5 (19.3–22.1)	21.2 (19.4–23.2)	N.S	0.101
Basal serum FSH (mIU/ml)	9.0 (7.7–10.5)	8.4 (6.8–10.1)	8.4 (6.8–10.1)	7.9 (6.6–10.2)	N.S	–0.73
Duration of stimulation (days)	12.0 (11.0–13.0)	12.0 (11.0–13.0)	12.0 (11.0–13.0)	13.0 (11.0–14.0)	0.0034	0.187
Total gonadotropin dose (IU)	3600 (3225–4800)	3750 (3300–4800)	3600 (3300–5250)	4800 (3600–5400)	<0.0001	0.250
Peak E2 (pg/ml)	5720 (3519–7760)	4512 (3052–6637)	4566 (3201–6534)	3824 (2506–5729)	0.0002	–0.187
Number of oocytes	12.0 (7.0–16.0)	9.0 (6.0–14.0)	9.0 (6.0–13.0)	8.0 (5.0–11.0)	<0.0001	–0.207
Number of MII oocytes	9.0 (5.5–13.0)	7.0 (4.0–11.0)	7.0 (4.0–11.0)	6.0 (3.0–9.0)	<0.0001	–0.214

Patients were grouped by their age in accordance with the Society for Assisted Reproductive Technology. Correlation coefficient: the correlations of patients' age in relation to COS were analyzed by Spearman's rank correlation coefficient. Data were shown as median (inter-quartile) of each group and significance between groups was analyzed by Kruskal-Wallis analysis. BMI: body mass index; Peak E2: Serum estradiol level on the day of HCG administration. N.S.: not statistically significant. $P < 0.05$: statistically significant

Then we examined the differences between four subgroups by Kruskal-Wallis analysis in each responder group. The characteristics of all subgroups were summarized in Table 4.

The duration of stimulation and total gonadotropin dose were significantly different between the subgroups of both Low and Normal responder group. Peak E2 level, number of total oocytes and number of M2 oocytes were significantly different between the subgroups of all three groups (Fig. 1). Serum AMH was not significantly different between subgroups of each responder group. The influence of aging on the ovarian response was clearly seen in all groups; the ovarian response tended to decrease as patient age increased within the same AMH level.

Discussion

This retrospective study involved relatively a large population undergoing a single IVF protocol of their first IVF treatment cycle. Our study firstly revealed that age was at a different dimension from serum AMH within ovarian response and was an important factor to predict ovarian response with the same AMH levels. Therefore serum AMH level in combination with patients' age might more accurately predict ovarian response in the first treatment cycle. Furthermore, our study reconfirmed many other studies, which found that the serum AMH was a reliable and accurate predictive marker of ovarian response in IVF treatment [9–12].

We examined the differences of parameters related to COS between groups classified by serum AMH level. It was clear that despite the fact that low serum AMH population (Low responder) required a larger amount of

gonadotropin and a longer duration of stimulation, they had lower serum E2 levels, less total oocytes, and less M2 oocytes compared to the high serum AMH population (High responder). We also examined the differences of these parameters between groups classified by patients' age. It was evident that ovarian response among high aged patients was significantly poorer than that among low aged patients as previously reported. Moreover, the correlation coefficient between serum AMH, patients' age and these parameters also indicated that the serum AMH was superior marker for predicting ovarian response than patients' age.

Patients' age was a very significant predictive marker of ovarian response with IVF for about 20 years [5, 26]. Recently serum AMH was thought to be a reliable and accurate predictive marker of ovarian response in IVF treatment [9–12]. However, in the clinical setting, we sometime consider that ovarian response might be different by age with the same serum AMH population. PCA revealed that patients' age was at a different dimension from serum AMH in the ovarian response. Therefore, to compare ovarian response by age within AMH-matched group we firstly categorized all of the patients to three groups by serum AMH level by ROC analysis. Secondly, each group was divided into four subgroups according to patients' age. In the patients' background characteristics, age was significantly different and serum AMH were not significantly different between subgroups of each group. Notably, ovarian response was clearly different between subgroups of each group. The ovarian response tended to decrease with increased patients' age. Despite increasing the total gonadotropin dose and the duration of stimulation, peak E2, the number of total oocytes and M2 oocytes decreased in the high aged patients. In Low and Normal

Table 4 Subgroup characteristics of each group

Group	Low		Normal		High	
Number of patients	<i>a</i>	49	<i>a</i>	56	<i>a</i>	40
	<i>b</i>	41	<i>b</i>	45	<i>b</i>	33
	<i>c</i>	50	<i>c</i>	34	<i>c</i>	38
	<i>d</i>	33	<i>d</i>	21	<i>d</i>	17
Age (years)	<i>a</i>	32.0 (31.0–33.0)	<i>a</i>	32.0 (30.0–33.0)	<i>a</i>	32.0 (30.0–33.0)
	<i>b</i>	36.0 (35.8–37.0)	<i>b</i>	36.0 (35.0–37.0)	<i>b</i>	36.0 (35.0–37.0)
	<i>c</i>	38.0 (39.0–40.0)	<i>c</i>	38.0 (39.0–40.0)	<i>c</i>	38.0 (39.0–40.0)
	<i>d</i>	42.0 (42.0–43.0)	<i>d</i>	42.0 (41.0–42.3)	<i>d</i>	42.0 (41.0–42.0)
	$p<0.0001$		$p<0.0001$		$p<0.0001$	
AMH (pM)	<i>a</i>	6.6 (5.3–8.4)	<i>a</i>	14.4 (12.5–15.9)	<i>a</i>	23.1 (19.2–35.1)
	<i>b</i>	6.9 (4.8–8.4)	<i>b</i>	13.9 (12.1–15.8)	<i>b</i>	20.4 (18.9–25.4)
	<i>c</i>	7.0 (4.7–8.5)	<i>c</i>	13.0 (11.8–15.2)	<i>c</i>	21.4 (18.8–27.7)
	<i>d</i>	6.2 (4.3–7.7)	<i>d</i>	13.9 (11.4–15.6)	<i>d</i>	23.6 (19.9–29.9)
	N.S.		N.S.		N.S.	
Duration of stimulation (days)	<i>a</i>	12.0 (11.8–13.0)	<i>a</i>	11.0 (11.0–12.0)	<i>a</i>	11.0 (11.0–12.0)
	<i>b</i>	12.0 (11.0–13.0)	<i>b</i>	12.0 (11.0–13.0)	<i>b</i>	11.0 (11.0–12.0)
	<i>c</i>	12.0 (11.0–13.0)	<i>c</i>	12.0 (11.0–13.0)	<i>c</i>	11.0 (10.0–12.0)
	<i>d</i>	14.0 (12.0–15.0)	<i>d</i>	12.0 (11.0–13.0)	<i>d</i>	11.0 (11.0–13.0)
	$p=0.0252$		$p=0.0053$		N.S.	
Total gonadotropin dose (IU)	<i>a</i>	4500 (3600–5325)	<i>a</i>	3300 (3000–3900)	<i>a</i>	3300 (2812–3712)
	<i>b</i>	4800 (4125–5287)	<i>b</i>	3900 (3300–4650)	<i>b</i>	3300 (3075–3900)
	<i>c</i>	3900 (3300–5550)	<i>c</i>	3712 (3525–5100)	<i>c</i>	3300 (3010–4125)
	<i>d</i>	5550 (4912–6300)	<i>d</i>	4800 (3525–5137)	<i>d</i>	3600 (3300–4987)
	$p<0.0001$		$p=0.0003$		N.S.	
Peak E2 (pg/ml)	<i>a</i>	2974 (1727–4978)	<i>a</i>	5814 (4089–7312)	<i>a</i>	8172 (6421–9997)
	<i>b</i>	3489 (2405–4285)	<i>b</i>	5135 (3156–6974)	<i>b</i>	6001 (3865–8072)
	<i>c</i>	3589 (2629–5076)	<i>c</i>	4945 (3477–6476)	<i>c</i>	6501 (4430–9408)
	<i>d</i>	2438 (1583–3241)	<i>d</i>	4545 (2812–6589)	<i>d</i>	5385 (4788–7099)
	$p=0.0079$		$p=0.0469$		$p=0.0046$	
Number of oocytes	<i>a</i>	7.0 (4.0–12.3)	<i>a</i>	12.0 (12.5–15.9)	<i>a</i>	16.0 (12.0–20.0)
	<i>b</i>	7.0 (5.0–9.3)	<i>b</i>	10.0 (6.8–15.3)	<i>b</i>	11.5 (9.0–17.0)
	<i>c</i>	8.0 (5.0–10.0)	<i>c</i>	9.5 (6.0–12.0)	<i>c</i>	12.0 (8.8–20.3)
	<i>d</i>	5.0 (3.8–7.7)	<i>d</i>	8.0 (5.0–9.3)	<i>d</i>	11.0 (10.0–19.0)
	$p=0.0415$		$p=0.0055$		$p=0.0169$	
Number of M2 oocytes	<i>a</i>	4.0 (3.0–10.3)	<i>a</i>	9.0 (7.0–12.5)	<i>a</i>	12.5 (10.0–15.5)
	<i>b</i>	5.0 (3.0–7.0)	<i>b</i>	7.0 (4.0–13.0)	<i>b</i>	10.0 (7.0–14.0)
	<i>c</i>	5.0 (3.0–8.0)	<i>c</i>	7.0 (4.0–10.0)	<i>c</i>	11.0 (7.0–15.5)
	<i>d</i>	3.0 (1.8–4.3)	<i>d</i>	5.0 (3.8–8.3)	<i>d</i>	10.0 (6.0–14.3)
	$p=0.0032$		$p=0.0118$		$p=0.0362$	

Patients were grouped by serum AMH level as Table 2. Then each group was divided into four subgroups by age; a: under 34 years; b: 35–39 years; c: > 40 years. Data were shown as median (inter-quartile) each subgroup and significance between subgroups was analyzed by Kruskal-Wallis analysis. Peak E2: Serum estradiol level on the day of HCG administration. N.S.: not statistically significant. $P<0.05$: statistically significant

responder group all of the parameters were significantly different according to patients' age. In High responder group, not all parameters were significantly different; however the same tendency was seen in the less significant

parameters. We considered the fact that Low and Normal responder group had a greater number of included patients; therefore, High responder group might exhibit a significant difference with the inclusion of more patients.

Fig. 1 Box plots of Peak E2, number of total oocytes and number of M2 oocytes in AMH-matched group. Ovarian response about Peak E2 (**a**), number of total oocytes and number of M2 oocytes (**b**) between subgroups categorized by age were analyzed by Kruskal-Wallis analysis. Bars indicate 5th–95th centiles; boxes indicate 25th–75th centiles and medians. Peak E2; serum E2 at the day of HCG administered. Groups categorized by serum AMH: Poor, Poor responder; Normal, Normal responder; High, High responder. Subgroups categorized by age: a, 34 y<; b, 35–37 y; c, 38–40 y; d, >40 y. Significance was determined with $P < 0.05$

The mechanisms underlying these results observed in this study remain to be elucidated. Nelson et al. reported that age influenced to the proportion of follicles from primordial to recruitable follicles; however AMH reflect the number of FSH-sensitive recruitable follicles [27]. Therefore they mentioned older women with low AMH would yield fewer follicles than younger women with the same AMH. It has been also said the pool of growing follicles produces AMH, which could impact the remaining primordial follicles and inhibiting their recruitment [14, 15]. In this study the high aged subgroups required a significantly higher dose of gonadotropin and a longer duration of stimulation in low-high AMH population; however, they

had significantly lower peak E2 and a smaller number of total oocytes compared to the low aged subgroups. Considering from above, we suspect that FSH-sensitive follicle recruitment by gonadotropin might be also decreased in older patients irrespective of serum AMH level; in other words there was a possibility that the effect of serum AMH inhibiting follicle recruitment was different dependent on patients' age. Of interest, a recent study by Reichman et al. found that extending exposure to HCG for oocytes maturation may be beneficial to patients aged over 40 in IVF treatment. They considered reproductive aging might be associated with altered gene expression in luteinized granulosa cells [28]. We also consider our results might be related

to reproductive aging. Further studies are needed to clarify the role of AMH in follicle recruitment and the basic physiology of AMH produced by granulosa cells; these studies might result in the improvement of the treatment outcome of low responder patients undergoing IVF treatment.

It has been reported that serum AMH has a significant correlation with age [29, 30]. However, in this study, correlation coefficient between serum AMH and age was lower compared to previous studies; the median of the serum AMH levels was relatively low (12.8 pM), while that of age was high (37.0 years). A tendency for the selection of the GnRH agonist flare-up protocol was found in both relatively normal and low responder patients [31]. Moreover, the GnRH antagonist protocol has clear advantages for the prevention of OHSS in high responders, such as patients with polycystic ovary syndrome (PCOS) and high serum AMH levels [32].

Therefore, in this study, a normal or low serum AMH population might be primarily selected as candidates for the GnRH agonist flare-up protocol; in these limited patients serum AMH might weakly correlate with age and might be at the different dimension from age by PCA. It is necessary to confirm these results with multiple IVF protocols.

Conclusions

We reconfirmed that serum AMH was a good predictive marker of ovarian response. Our study firstly revealed that patients' age was at the different dimension from serum AMH in ovarian response in IVF treatment. Furthermore ovarian response was significantly different according to age within AMH-matched groups; high aged patients responded poorly when compared to low aged ones irrespective of serum AMH level. Therefore serum AMH in combination with patients' age is a superior predictor of ovarian response than AMH alone. The cutoff values of the low responders or high responders in regard to serum AMH according to age in multiple IVF protocols needs to be determined. These values could be used more accurately to individualize in IVF treatment.

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