Decreased serum anti-Müllerian hormone level is associated with vitamin D deficiency in healthy Japanese women

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Title	Full Title	Decreased serum anti-Müllerian hormone level is associated with vitamin D deficiency in Japanese healthy women
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1. Study design

1) In this study, results and its interpretations are so complicated because they have not compared each item under matched ages or the similar age group (Table 3,4). They should analyze them in age < 30 years and >30 years, respectively. Obese women and women complicated with PCOS should be excluded in this study.

We have completely removed obese women (BMI ≥25 kg/m^2 and/or %Fat ≥35%) from the study population, and have re-analyzed the data for age < 30 years and ≥30 years.

Although PCOS patients were only screened by medical interview and obese women were excluded to reduce the risk of including PCOS patients, we cannot completely rule out the possibility that some cases were included, since medical checks using ultrasonography were not performed.

We have mentioned this issue in the Discussion section as a limitation.

2) Looking at relations and association between AMH levels and BMI or body fat ratio, the relation between AMH levels and physical and nutritional indices in < 30 years and >30 years are different (Table 2). This interpretation are very complicated and difficult. It is not simply due to age and body weight loss because obese women and PCOS women who were included in this study, also might be effect on AMH levels. In this paper, discussion is poor and too simple. Obese women and women complicated with

PCOS should be excluded in this study.

As suggested, we have excluded obese women from the present study. After this step, no association between AMH level and BMI or body fat ratio was seen. The conclusions have been simplified and made clearer.

2. Recommendation

1) Study design should be more simplified. For example, data for body fat (Table 2) and data for 5 obese women might be excluded. After data for 5 obese women were excluded, the relation between vitamin D levels and AMH levels should be re-analyzed.

We have re-analyzed the relationship between serum 25OH-D and AMH levels after excluding the data for obese women.

2) In Table 4, we could not understand which categories or groups authors have analyzed statistically. So authors should mark the significant figures; as below.

5.1+2.7a 4.3+2.5 5.8+2.0a <0.01a

6(31.6)b 12(63.2)c 1(5.3)d

<0.01b,c,d

26(26.0)b 41(41.0)c 33(33.0)d

We have made the original Table 4 the new Table 3, and have re-performed statistical analyses using the χ^2 test for each condition.

Original Article

Decreased serum anti-Müllerian hormone level is associated with vitamin D deficiency in healthy Japanese women

Running Title: Serum AMH level is associated with 25OH-D

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***Abbreviations**:*

25OH-D, 25-hydroxyvitamin D

AMH, anti-Müllerian hormone

BIA, bioelectrical impedance analysis

BMI, body mass index

DEXA, dual-energy X-ray absorptiometry

BDHQ, brief-type self-administered diet history questionnaire

PCOS, polycystic ovary syndrome

Abstract

Objective: The aim of this study was to investigate the associations of anti-Müllerian hormone (AMH) levels with physique and 25-hydroxyvitamin D (25OH-D) levels in healthy women of reproductive age based on measurements of nutritional status and physical constitution.

Materials and Methods: Subjects comprised 108 non-obese women (age range, 21–39 years) who underwent examination of their physique, blood biochemistry and nutritional state. For data analysis, subjects were first divided by age. AMH levels were grouped by serum 25OH-D concentration using Holick's classification: deficiency, <30 ng/mL; and sufficiency, ≥30 ng/mL.

Results: Mean levels were 25.2±8.4 ng/mL for serum 25OH-D and 4.9±2.4 ng/mL for AMH. Overall, 76 women (70.4%) were diagnosed with 25OH-D deficiency. Serum AMH levels were significantly lower in subjects with 25OH-D deficiency (4.5±2.5 ng/mL) than in those with 25OH-D sufficiency (5.7±1.9 ng/mL; $P<0.01$). Significant differences were seen in the frequency of subjects with 25OH-D deficiency and sufficiency between low AMH (<2.2 ng/mL) status and normal AMH (≥2.2 ng/mL) (16/17 [94.1%] vs. 1/17 [5.9%] for low AMH status; 60/91 [65.9%] vs. 31/91 [34.1%] for normal AMH status, respectively; $P<0.05$). Independent predictors of serum AMH levels ≥2.2 ng/mL were serum 25OH-D level ($P<0/05$) and age ($P<0.05$) according to binary logistic regression analysis.

Conclusions: Decreased serum AMH level is associated with vitamin D deficiency, but is unrelated to physique state in this population.

Key words: Anti-Müllerian hormone, 25-hydroxyvitamin D, Body fat (%), Body mass index

Objective

Anti-Müllerian hormone (AMH) is one of the most reliable markers of ovarian reserve [1)]. This hormone is produced in prenatal follicles with little influence from the estradiol cycle, and is affected by the number of growing eggs. AMH is commonly used in useful marker to predict ovarian reserve and to adjust controlled ovarian stimulation [2)]. Previous reports have indicated that nutritional status may influence circulating AMH levels. Lower serum AMH levels have been found in obese women compared with normal-weight non-PCOS women, and a strong association between AMH levels and body mass index (BMI) has been shown [3,4)]. It has been also reported that AMH level increases with increasing serum 25-hydroxyvitamin D (25OH-D) level in women ≥40 years of age [5, 6)] In addition to serum vitamin D levels, premenopausal women show seasonal variations in serum AMH levels, showing an 18% decrease in serum AMH levels in winter compared to summer [5)]. Vitamin D has also been shown to affect AMH gene expression in vitro [7)]. However, few reports have examined nutritional status and serum levels of AMH and vitamin D in the younger population of women before pregnancy.

The aim of the present study was to investigate the prevalence of vitamin D deficiency and the relationship between serum AMH levels and 25-hydroxyvitamin D (25OH-D) in healthy, Japanese, non-obese women between 21 and 39 years old based on measurements of nutritional status and physical constitution.

Materials and Methods

1. Subjects

A total of 136 healthy Japanese female volunteers between 20 and 39 years old were recruited through the internet from October to November 2012 in Tokyo, Japan (located at 35.4°N, 139.4°E). AMH is usually elevated in women with polycystic ovary syndrome (PCOS), and correlates with the severity of this syndrome [3, 8]. It is also known that the prevalence of obesity in patients with PCOS is nearly 30% [9]. Of these, 28 subjects were excluded based on the following criteria that influences ovarian AMH synthesis: history of POCS (n=3); endometriosis or gynecological disease (n=2); history or ovarian insufficiency or failure (n=2); during pregnancy (n=4); diabetes (n=1); intake of vitamin D supplements (n=3); and obesity (BMI ≥25 kg/m^2 or %Fat ≥35%) (n=13). Study approval was obtained from the institutional review board at Juntendo University (No. 26-368). Each participant was provided with detailed information about the study protocol, and written informed consent was obtained from all participants prior to enrollment.

To determine serum AMH and 25OH-D levels, blood samples were collected from a cubital vein after 6 h of fasting. Body weight was noted, and body composition was measured via bioelectrical impedance analysis (BIA) [10,11]. Nutritional information on energy intake, vitamin D and gynopathy was obtained from a questionnaire that was distributed to each subject [12].

2. Serum analysis

Within 6 h of collection, blood samples were centrifuged for 10 min at 3000 rpm to separate the serum, then stored at -80°C until measurement. Recipient vitamin D status was measured by assessing circulating levels of 25OH-D in serum samples that had been stored frozen, having never been previously thawed, using radioimmunoassay (25-hydroxyvitamin D 125I RIA Kit; DiaSorin, Saluggia, Italy) [13,14]. Subjects were also divided into two groups according to clinically accepted ranges for vitamin D deficiency (<20 ng/mL) and insufficiency (20-29.9 ng/mL) into a Deficient group (<30 ng/mL), and a Sufficient group (≥30 ng/mL) [15].

AMH was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (AMH Gen II ELISA; Beckman Coulter and R&D Systems, Fullerton, CA) [16]. The resulting measurements of 25OH-D and AMH were expressed in nanograms per milliliter. Following the age-related normative model [17], we used the value for 40-year-olds (2.2 ng/mL) as the criterion to categorize AMH results into 2 groups: low AMH (<2.2 ng/mL) and normal AMH (≥2.2 ng/mL).

3. Measurement of body weight and body composition

Body weight and body composition were measured by the 8-electrode BIA method using a multifrequency body composition analyzer (MC-190EM; Tanita, Tokyo, Japan). BIA is a significantly more cost-effective method of measuring body composition than

dual-energy X-ray absorptiometry (DEXA). BIA is also portable, and easier to use than other technologies [10, 11]. The BIA method offers a high correlation with mean regional lean soft tissue and whole-body skeletal muscle mass estimates using the reference method of DEXA for non-pregnant women [11]. According to the World Health Organization guideline for the measurement of BMI, all subjects were divided into two groups: underweight (BMI <18.5 kg/m^2), and normal weight (18.5 ≤ BMI < 25 kg/m^2).

4. Diet history questionnaire

Dietary/nutritional habits from the previous month were reported on the brief-type self-administered diet history questionnaire (BDHQ) [12]. The BDHQ was developed as a scaled-down version of the self-administered diet history questionnaire [18]. In recording food eaten for the questionnaire, examinees tend to underestimate the volume of food eaten [19]. To increase the accuracy of the BDHQ, the present study indicated the vitamin D dietary intake for every 1,000 kcal, in accordance with previous reports.

5. Statistical analysis

Results are reported as means ± standard deviation. Categorical variables are reported as positive percentages. Differences in continuous variables between subjects <30 and ≥30 years old were assessed using the unpaired Student's t-test. Distributions among serum 25OH-D levels, serum AMH levels, age, and BMI were compared using

the χ^2 test in each condition. Binary logistic regression analysis was used to determine factors associated with decreased concentrations of serum AMH levels (≥2.2 ng/mL) and to calculate odds ratios (OR) with 95% confidence intervals (95%CIs). All statistical analyses were performed using IBM SPSS Statistics version 21 (Statistical Package for Social Science Japan, Tokyo, Japan). The tests were two-sided, and values of $P<0.05$ were considered statistically significant.

Results

1. Serum AMH levels and 25-hydroxyvitamin D

Table 1 compares participant characteristics by age. Of the 108 participants, 57 were under 30 years old and 51 were ≥30 years old (mean age, 29.2±4.2 years). Mean BMI was 19.9±1.9 kg/m^2, and mean body fat percentage was 25.2±4.2%. Mean serum 25OH-D concentration was 25.6±8.4 ng/mL, and mean AMH level was 4.9±2.4 ng/mL. In the study population, 70.4% of women were categorized to the Deficient group and 29.6% to the Sufficient group. Regarding AMH, 15.7% of the entire group showed low levels (<2.2 ng/mL) and 84.3% had normal levels (≥2.2 ng/mL). In age comparisons, subjects ≥30 years old showed significantly lower AMH levels ($P<0.01$) than subjects <30 years old, with a higher frequency of low AMH levels among subjects ≥30 years old ($P<0.01$). No differences in other categories were observed between age levels.

2. Weight, BMI, body fat percentage, and nutrition of all participants separated by 25OH-D status

Table 2 indicates the comparisons of weight, BMI, body fat percentage and dietary intake of vitamin D by serum 25OH-D levels. Although weight and BMI showed no correlations with serum vitamin D, intake of vitamin D per 1,000 kcal was lower in the Deficient group (6.3±4.8 μg/1000 kcal) than in the Sufficient group (8.2±4.0 μg/1000 kcal; $P<0.05$).

3. AMH status between 25OH-D Deficient and Sufficient groups

Mean serum AMH level was lower in the Deficient group (4.5±2.5 ng/mL) than in the Sufficient group (5.7±1.9 ng/mL; $P<0.01$) (Table 3). When distinguishing between AMH status (<2.2 ng/mL and ≥2.2 ng/mL), significant differences in the frequency of 25OH-D deficient and sufficient subjects were seen between two groups (16/17 [94.1%] vs. 1/17 [5.9%] for low AMH status; 60/91 [65.9%] vs. 31/91 [34.1%] for normal AMH status, respectively; $P<0.05$).

4. Binary logistic regression for AMH

We used binary logistic regression modeling to evaluate independent variables showing strong correlations with normal serum AMH levels (≥ 2.2 ng/ml) in subjects. The following covariates were included in the model: serum 25OH-D level ≥30 ng/mL,

age ≥30 years, BMI, and body fat (%). Binary logistic regression analysis revealed serum 25OH-D levels (≥30 ng/mL) (odds ratio [95%CI] = 8.270 [1.033–66.199], $P<0.05$) and age (≥30 years) (odds ratio [95%CI] = -1.164 [0.099–0.985], $P<0.05$) as independent predictors of AMH levels (≥ 2.2 ng/ml) (Table 4).

DISCUSSION

The present study found that 70.4% of the study population was suffering from either vitamin D insufficiency or deficiency according to Holick's classification [15] and decreased serum AMH level was associated with vitamin D deficiency. Although previous studies have investigated serum 25OH-D concentrations in Japanese women by age group, few have studied this topic specifically in women of childbearing age. A study of Japanese women (mean age, 32.9±11.3 years) in Niigata Prefecture (latitude 37°48 to 59°N), found that women <30 years old had significantly lower mean serum 25OH-D levels compared with women ≥30 years old, and 42.1% of these younger women were vitamin D-deficient [20]. In the present study, although no significant differences in serum 25OH-D levels were evident between women under or over 30 years old, women of childbearing age in Tokyo (located at 35.4°N, 139.4°E) were commonly vitamin D-deficient.

Serum 25OH-D level and body fat percentage have previously been reported as inversely proportional to each other [21, 22]. According to a meta-analysis of 21 studies

conducted in North America and Europe, when BMI increases by 10%, blood levels of vitamin D decrease by a mean of 4%. For obese individuals, weight loss, exposure to sunlight, and vitamin D intake from diet are recommended [23]. In underweight individuals, when body fat percentage decreases due to body weight loss, the frequency of menstrual abnormalities rises [24]. Decreased body weight is linked to abnormalities in sex hormone secretions from the hypothalamus and pituitary, which can lead to diminished ovarian function [25]. In the present study, multiple regression analysis showed that both BMI and body fat percentage had no significant impact on AMH. The influence of physical constitution on AMH could have been lower among non-obese women in this population.

In the present study, levels of serum 25OH-D depended on the oral intake of vitamin D, which was 6.3±4.8 μg/1000 kcal in the Deficient group and 8.2±4.0 μg/1000 kcal in the Sufficient group, showing a significantly lower level in the Deficient group (P<0.01). While we investigated <40-year-old women in the present study, we also found more women with low AMH levels among those who were vitamin D-deficient. Vitamin D deficiency has recently been reported as common among women who have experienced recurrent pregnancy losses, and low serum 25OH-D levels suppress peripheral blood NK cells in recurrent pregnancy loss [6]. This indicates that vitamin D is an extremely important nutrient in preparing for pregnancy. Although the reference intake for vitamin D has been defined as that required to achieve a serum 25OH-D level

above 20 ng/mL by the Institute of Medicine in the United States [26] and 30 ng/mL by the International Society of Endocrinology [27], no reference intake has been established for vitamin D with respect to pregnancy potential. An investigation concerning serum vitamin D and a reference intake for vitamin D with consideration for pregnancy potential represent future research challenges.

Some limitations to this study must be considered when interpreting the present findings. Serum levels of AMH in women are influenced by conditions such as PCOS. Although PCOS patients were screened in the medical interview and obese women were excluded to reduce the risk of including patients with PCOS, the possibility of some patients with this pathology being included could not be completely ruled out, since medical checks using ultrasonography were not performed. Second, seasonal changes are seen in serum vitamin D levels [28] and the promotor of the *AMH* gene contains a vitamin D-response element that is active in cultured cells [29]. Consideration must be given in the next study to the fact that latitudinal and regional differences [30], as well as seasonal variations [28], are seen with respect to serum 25OH-D concentrations.

Conclusions

Vitamin D deficiency or insufficiency were commonly observed in healthy women of reproductive age in Tokyo area. The current study suggests that pre-pregnancy serum 25OH-D status affects AMH levels. In subjects with decreased serum AMH levels,

vitamin D deficiency may need to be considered. Nutritional support that considers the appropriate nutritional status at childbearing age may be warranted.

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Declaration of Conflicting Interests

All the authors of this study declare that they have nothing to disclose regarding conflict of interest with respect to this manuscript.

Finding

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References

1) Durlinger AL, Gruijters MJ, Kramer P, et al: Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology, 2002;143:1076-84.

2) de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril, 2002;77:357-2.

3) Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss JF, 3rd: Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Fertil Steril, 2007;87:101-6.

4) Olszanecka-Glinianowicz M, Madej P, Owczarek A, Chudek J, Skalba P: Circulating anti-Mullerian hormone levels in relation to nutritional status and selected adipokines levels in polycystic ovary syndrome. Clin Endocrinol (Oxf), 2015;83:98-104.

5) Dennis NA, Houghton LA, Jones GT, van Rij AM, Morgan K, McLennan IS: The level of serum anti-Mullerian hormone correlates with vitamin D status in men and women but not in boys. J Clin Endocrinol Metab, 2012;97:2450-5.

6) Ota K, Dambaeva S, Han AR, Beaman K, Gilman-Sachs A, Kwak-Kim J: Vitamin D deficiency may be a risk factor for recurrent pregnancy losses by

increasing cellular immunity and autoimmunity. Hum Reprod, 2014;29:208-19.

7) Merhi Z, Doswell A, Krebs K, Cipolla M: Vitamin D alters genes involved in follicular development and steroidogenesis in human cumulus granulosa cells. J Clin Endocrinol Metab, 2014;99:E1137-45.

8) Sahmay S, Atakul N, Aydogan B, Aydin Y, Imamoglu M, Seyisoglu H: Elevated serum levels of anti-Mullerian hormone can be introduced as a new diagnostic marker for polycystic ovary syndrome. Acta Obstet Gynecol Scand, 2013;92:1369-74.

9) Alvarez-Blasco F, Botella-Carretero JI, San Millan JL, Escobar-Morreale HF: Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. Arch Intern Med, 2006;166:2081-6.

10) Heitmann BL: Impedance: a valid method in assessment of body composition? Eur J Clin Nutr, 1994;48:228-40.

11) Pietrobelli A, Rubiano F, St-Onge MP, Heymsfield SB: New bioimpedance analysis system: improved phenotyping with whole-body analysis. Eur J Clin Nutr, 2004;58:1479-4.

12) Kobayashi S, Murakami K, Sasaki S, et al: Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr, 2011;14:1200-11.

13) Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL: Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. Clin Chem, 1993;39:529-33.

14) Kobayashi T, Okano T, Shida S, et al. Variation of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 levels in human plasma obtained from 758 Japanese healthy subjects. J Nutr Sci Vitaminol (Tokyo), 1983;29:271-81.

15) Holick MF: Vitamin D deficiency. N Engl J Med, 2007;357:266-81.

16) Kumar A, Kalra B, Patel A, McDavid L, Roudebush WE: Development of a second generation anti-Mullerian hormone (AMH) ELISA. J Immunol Methods, 2010;362:51-9.

17) Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH: A validated model of serum anti-mullerian hormone from conception to menopause. PLoS One, 2011;6:e22024.

18) Kobayashi S, Honda S, Murakami K, et al: Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol, 2012;22:151-9.

19) Okubo H, Sasaki S, Rafamantanantsoa HH, Ishikawa-Takata K, Okazaki H, Tabata I: Validation of self-reported energy intake by a self-administered diet history questionnaire using the doubly labeled water method in 140 Japanese adults. Eur J Clin Nutr, 2008;62:1343-50.

20) Nakamura K, Nashimoto M, Matsuyama S, Yamamoto M: Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. Nutrition, 2001;17:921-5.

21) Lucas JA, Bolland MJ, Grey AB, et al: Determinants of vitamin D status in older women living in a subtropical climate. Osteoporos Int, 2005;16:1641-8.

22) Rosen CJ, Adams JS, Bikle DD, et al: The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. Endocr Rev, Jun 2012;33:456-92.

23) Vimaleswaran KS, Berry DJ, Lu C, et al: Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med, 2013;10:e1001383.

24) Carlberg KA, Buckman MT, Peake GT, Riedesel ML: Body composition of oligo/amenorrheic athletes. Med Sci Sports Exerc, 1983;15:215-7.

25) Dale E, Gerlach DH, Wilhite AL: Menstrual dysfunction in distance runners. Obstet Gynecol, 1979;54:47-53.

26) Ross AC, Manson JE, Abrams SA, et al: The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab, 2011;96:53-8.

27) Holick MF, Binkley NC, Bischoff-Ferrari HA, et al: Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab, 2011;96:1911-30.

28) Pasco JA, Henry MJ, Kotowicz MA, et al: Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong Osteoporosis Study. J Bone Miner Res, 2004;19:752-8.

29) Malloy PJ, Peng L, Wang J, Feldman D: Interaction of the vitamin D receptor with a vitamin D response element in the Mullerian-inhibiting substance (MIS) promoter: regulation of MIS expression by calcitriol in prostate cancer cells. Endocrinology, 2009;150:1580-7.

30) Lips P, Duong T, Oleksik A, et al: A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab, 2001;86:1212-21.

Table 1. Characteristics of subjects categorized by age

Age group	Total	< 30 years (n=57)	≥ 30 years (n=51)	*P*
Demographics				
Age (years)	29.2±4.2	26.1±2.4	32.7±2.7	<0.01
Weight (kg)	50.5±5.5	50.9±5.1	50.1±5.9	NS
BMI (kg/m^2)	19.9±1.9	19.9±1.8	19.9±1.9	NS
Underweight (<18.5)	23 (21.3)	12 (21.1)	11 (21.6)	NS
Normal (18.5-24.9)	85 (78.7)	45 (78.9)	40 (78.4)	
Body fat (%)	25.2±4.2	25.3±3.7	25.0±4.8	NS
Serum biological parameters				
25OH-D (ng/mL)	25.6±8.4	25.7±8.5	25.6±8.4	NS
25OH-D status, n (%)				
Deficient group	76 (70.4)	39 (68.4)	37 (72.5)	NS
Sufficient group	32 (29.6)	18 (31.6)	14 (27.5)	
AMH (ng/mL)	4.9±2.4	5.4±2.4	4.3±2.3	<0.01
AMH status, n (%)				
Low (<2.2)	17 (15.7)	5 (8.8)	12 (23.5)	<0.05
Normal (≥2.2)	91 (84.3)	52 (91.2)	39 (47.2)	

Results are given as mean±SD; percentages are given in parentheses.

n, number; NS, not significant; BMI, body mass index, 25OH-D, 25-hydroxyvitamin D; AMH, anti-Müllerian hormone; SD, standard deviation

Deficient group, 25OH-D < 30 ng/mL; Sufficient group, 25OH-D ≥ 30 ng/mL.

Table 2. Weight, BMI, body fat %, and nutrition of all participants according to 25OH-D status

	Deficient group (n=76)	Sufficient group (n=32)	*P*
Age (years)	29.4±3.8	28.8±5.0	NS
Weight (kg)	50.4±5.3	50.8±5.9	NS
BMI (kg/m^2)	19.9±1.9	20.0±1.8	NS
Body fat (%)	25.6±4.1	24.0±4.4	NS
Vitamin D (μg/day)	10.0±7.2	10.9±5.4	<0.01
(μg/1000 kcal)	6.3±4.8	8.2±4.0	<0.05
25OH-D (ng/mL)	21.5±4.8	35.5±6.6	<0.01

Results are given as mean±SD; percentages are given in parentheses.

BMI, body mass index; 25OH-D, 25-hydroxyvitamin D; NS, not significant

Deficient group, < 30 mg 25OH-D (ng/mL); Sufficient group, ≥ 30 mg 25OH-D (ng/mL).

Table 3. AMH status in 25OH-D deficient and sufficient groups

25OH-D levels	Deficient group (n=76)	Sufficient group (n=32)	*P*
AMH (ng/mL)	4.5±2.5	5.7±1.9	<0.01
AMH status, n (%)			
<2.2 ng/mL (n=17)	16 (94.1)	1 (5.9)	<0.05
≥2.2 ng/mL (n=91)	60 (65.9)	31 (34.1)	

Results are given as mean±SD; percentages are given in parentheses.

AMH, anti-Müllerian hormone; 25OH-D, 25-hydroxyvitamin D; SD, standard deviation

Deficient group, <30 mg 25OH-D (ng/mL); Sufficient group, ≥ 30 mg 25OH-D (ng/mL).

Table 4. Results of binomial logistic regression analyses for categories of serum AMH level (≥ 2.2 ng/ml)

Independent variable	*Standardized partial regression coefficient (ß)*	Odds ratio (95% confidence interval)	*P*
25OH-D (≥30)	2.113	8.270 (1.033-66.199)	<0.05
Age (≥30)	-1.164	0.312 (0.099-0.985)	<0.05

The Hosmer-Lemeshow test was used to test the goodness-of-fit of the model (X^2 = 2.653, P= 0.265).