

# Serum concentration of bone morphogenetic proteins (BMPs) is not linked to serum anti-mullerian hormone (AMH) level

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## Abstract

Serum concentration of anti-mullerian hormone (AMH) is used as a biomarker in practical clinics. As bone morphogenetic protein (BMP) cytokines induce AMH expression in human granulosa cells (GC) *in vitro*, serum concentrations of BMP cytokines and AMH were evaluated whether there would be a relationship between BMP cytokines and AMH. Serum concentrations of BMP-2, -6 -7, AMH and FSH were measured in 25 infertility patients using EIA or ELISA kits. Among 25 infertile patients, serum BMP-2, -6, and -7 were detected only in 10, 3, 7 patients respectively, while AMH was detected in 24 out of 25 patients. There was no relation between BMP cytokines and AMH concentration in serum. The detection rate of these BMPs in serum was much lower than that of AMH. Serum concentration of BMP-2, -6, -7 could not estimate serum AMH level.

## Introduction

In infertility treatments, the precise assessment of ovarian reserve is necessary to plan a treatment strategy for each patient. Among all available markers of ovarian reserve, much interest has been given to anti-mullerian hormone (AMH) as a reliable, accurate and reproducible predictor [1,2]. AMH, which belongs to the transforming growth factor (TGF)- $\beta$  superfamily, is a product of the granulosa cells (GC) in pre-antral and small antral follicles [3]. As serum concentration of AMH which is derived from GC, is considered an excellent biomarker in practical clinics [1,2], it is important to elucidate the mechanism by which AMH is regulated in GC. Previously, we found that bone morphogenetic protein (BMP)-2, 6, 7 and 15 increased the expression of AMH in human GC [4,5]. BMP cytokines which are member of TGF- $\beta$  superfamily, are known to regulate ovarian physiology; including gonadogenesis [6,7], folliculogenesis, ovulation and luteinization in various species [8]. In the human ovary, it has been reported that GC express BMP-2 and -6, theca cells express BMP-7 and oocytes express BMP-6, -15 respectively [4,9-13]. Given that BMP cytokines regulate ovarian function, including AMH expression, serum concentrations of BMP cytokines could be a useful marker for the ovarian function. In the present study, we validated the hypothesis whether the measurement of the BMP cytokines in serum could be a predictor for ovarian reserve.

## Materials and methods

### Reagents and materials

The concentrations of serum BMPs were measured using ELISA kits for BMP-6 and -7 from RayBiotech (Norcross, GA), and for BMP-2 from R&D Systems. Serum AMH concentrations were measured using the EIA kit from Beckman Coulter (Tokyo, Japan).

Written informed consent was obtained from all of the study

participants. Ethical approval was given by Tokyo University Ethics Committee.

### Serum sample

A total of 25 infertile women were recruited for the study. The mean age of subjects was 35.0 years (20-43 years). All of them had regular menstrual cycles of around 28 days duration. On day 3-5 of a spontaneous menstrual cycle, blood samples were obtained by venipuncture. The FSH concentration were measured using the immulite semiautomated assay system. Plasma for assay of AMH, BMP-2, 6, and 7 was separated immediately from blood and frozen in aliquots at -80°C until thawed and assayed. A concentration of sensitivity, intra and inter-assay coefficients of variation respectively were as follow; BMP-2: 10 pg/ml, <5%, <10%, BMP-6: 80 pg/ml, <10%, <12%, BMP-7: 10 pg/ml, <10%, <12%, AMH: 70 pg/ml, <13%, <15%.

### Statistical analysis

Data were analyzed by Pearson's correlation coefficient using Statview software (SAS Institute Inc., Cary, NC). A p-value of less than 0.05 was considered statistically significant.

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## Result

Knowing that BMPs significantly induced AMH mRNA in GC *in vitro* [4,5], we examined concentrations of BMP-2, -6, -7 and AMH in serum of infertile patients. Among 25 infertile patients, serum BMP-2, -6, and -7 were detected only in 10, 3, 7 patients respectively (Table 1), while AMH was detected in 24 out of 25 patients. Median serum levels of BMP-2, BMP-6, BMP-7, AMH and FSH were 0 (range 0-118) pg/ml, 0 (range 0-673) pg/ml, 2.1 (range 0-37) pg/ml, 3.0 (0-16.2) ng/ml and 9.2 (1.0-38) mIU/ml, respectively. The concentrations of serum BMP-2 and -7 were shown in Figure 1.

There was no correlation between the concentration of serum BMP-2, -6, -7 and concentration of AMH (data not shown). The concentration of serum FSH exhibited negative correlation with AMH concentration (Figure 2  $r=-0.41$ ,  $p=0.02$ ), but no correlation with BMP-2, -6, or -7 (data not shown).

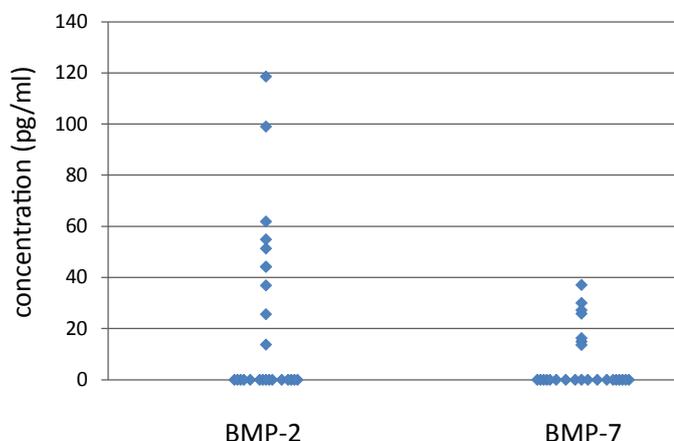
## Discussion

Recently, AMH has been demonstrated to play an important role in ovarian function with its inhibitory effect on follicle recruitment, the process by which primordial follicles enter the growing pool of primary follicles [3]. Moreover, serum concentration of AMH is considered an excellent biomarker to estimate ovarian reserve of individuals in practical clinics [1,2]. In addition to AMH, BMP cytokines are known to be important intra-ovarian factors in the regulation of ovarian function [8]. We have reported that BMP-2, -6, -7 contribute to folliculogenesis by inducing FSH receptor and suppressing LH receptor expression in human GC [4,10,14]. Also, in the previous study, we have found that treatment with BMP-2, -6, -7 and -15 (100 ng/ml) significantly increased AMH mRNA expression in human GC [4,5].

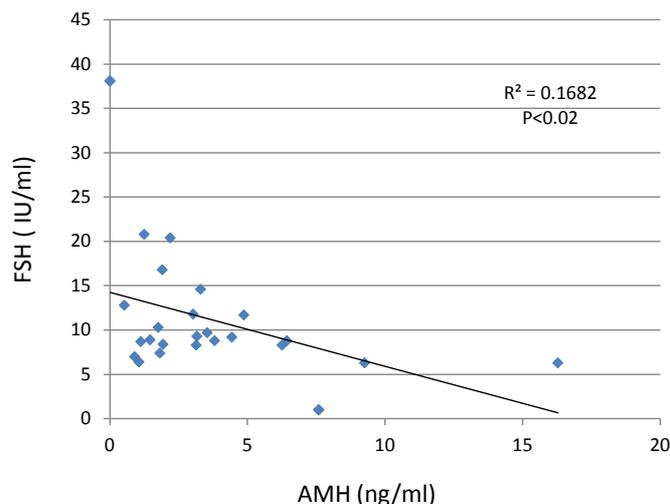
In the present study, we investigated whether these BMPs, which have a potential to induce AMH expression, could be a marker

**Table 1.** The serum concentrations of bone morphogenetic protein (BMP)-2, -6, -7 and anti-mullerian hormone (AMH) of 25 infertility patients were measured. The cut off level and the patients' number of positive or negative for each cytokines were shown.

|       | cut off  | total | negative | positive |
|-------|----------|-------|----------|----------|
| BMP-2 | 10 pg/ml | 25    | 15       | 10       |
| BMP-6 | 80 pg/ml | 25    | 22       | 3        |
| BMP-7 | 10 pg/ml | 25    | 18       | 7        |
| AMH   | 70 pg/ml | 25    | 1        | 24       |



**Figure 1.** The serum concentrations of bone morphogenetic protein (BMP) -2 and -7 of 25 infertility patients were measured. The distribution of data was shown.



**Figure 2.** The serum concentrations of anti-mullerian hormone (AMH) and FSH of 25 infertility patients were shown. The concentration of serum FSH exhibited negative correlation with AMH concentration ( $r=-0.41$ ,  $p=0.02$ ).

for ovarian reserve. Among BMPs, we evaluated BMP-2, 6, and -7 concentrations in the serum of infertile patients. Among 25 infertility patients, serum BMP-2, -6, -7 were detected in 10, 3, 7 patients respectively, while AMH was detected in 24 patients.

As AMH is known to be produced exclusively by GC, serum concentration of AMH might be a good marker [1,2]. We confirmed that the serum concentration of AMH showed negative correlation with that of FSH, which is consistent with previous studies [1,2]. Although *in vitro* study showed that AMH expression could be induced with BMP stimuli in GC [4,5], the detection rate of BMP-2, -6 and -7 in serum was low, suggesting that comparing to AMH level, intra-ovarian BMP cytokines are not high enough to be reflected to serum level. Also, there was no relationship between serum BMP cytokines and AMH concentration. Since BMP-2, -6 and 7 are also produced in various organs including bone, adrenal gland, kidney, pituitary and pulmonary artery [14], the origin of these BMP cytokines in serum might not be specific to the ovary.

It is known that serum AMH is a good marker to predict a patient's ovarian reserve, but this has not been used successfully to predict the chance of achieving pregnancy [15,16]. Gode *et al.* reported that higher levels of mature growth differentiation factor (GDF)-9 protein, a member of BMP cytokine, in the follicular fluid were significantly correlated with oocyte nuclear maturation and embryo quality [17]. There is no study regarding the relevance between clinical outcome and levels of BMP-2, -6, and 7 in follicular fluid. Further study is needed to determine whether BMPs could be good markers to predict clinical outcome.

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