# Influence of Anti-Mullerian Hormone on ART Outcomes in Infertility Patients of Different Ages

Research Article

JO Sohn[1,2], MJ Kim[1], JH Jeon[1], YM Jo[1], SH Kim[1], JM Lim[2,3*], HJ Song[1*]

[1] Fertility Medical Center, Seoul Women's Hospital, Bucheon, Korea.
[2] Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea.
[3] Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Korea.

## Abstract

Aging-related intervention by anti-mullerian hormone (AMH) on ART profile were retrospectively examined, and ART outcome of different AMH categories (<1.0, 1.0-3.5, >3.5) was compared. Overall, increased AMH accompanying with endogenous FSH decrement improved oocyte retrieval and maturation. Of those 112 cycles recruited, 77% had ≥1 ng/ml AMH, which accompanied with a significant (p<0.05) reduction in endogenous FSH and age. Improved oocyte retrieval and maturation resulted in better pregnancies than did cycles with <1 ng/ml AMH. A significant decrease in AMH was detected in patients ≥35 years old and great increase in the number of ET embryos in patients maintaining high AMH levels resulted marked increase of the pregnancy rate (11% vs. 69%). However, most AMH effects were disappeared in the 30-34 years group. These results demonstrated that endogenous AMH influenced ART outcomes, and the effect was prominent in patients aged beyond 35 years old, which can predict ART outcomes in middle-aged, infertile patients.

**Keywords:** AMH; Infertility; Oocyte Maturation; Fecundity; Age.

**Abbreviations:** ART: Applied Reproductive Technology; AMH: Anti-Mullerian Hormone; FSH: Follicle Stimulating Hormone; ICSI/ET: Intracytoplasmic Sperm Injection/Embryo Transfer; PCOS: Polycystic Ovary Syndrome.

## Introduction

Changes in life patterns in most developed countries have resulted in marriage at a later age. The mean age of couples has increased gradually, which may expose them to an age-related fertility disorder. Such social and clinical cases are considered a serious problem that leads to population decline. Aging accompanies diminished ovarian reserves and further understanding of reproductive physiology at middle age is critical for mobilizing developmentally competent oocytes during infertility treatment in aging women. Thus, establishing new parameters to precisely predict fecundity of a patient contributes to developing an efficient applied reproductive technology (ART) program for couples that marry late.

Aging induces a gradual decrease in the level of endogenous anti-Mullerian hormone (AMH), which directly influences the number of primordial follicles [1-3] and the sensitivity of the ovarian follicles to the follicle stimulating hormone (FSH)-dependent follicular wave [4] and folliculogenesis [5, 6]. Due to its relationship with endogenous FSH levels, AMH has been used as a parameter to evaluate the status of the controlled ovarian hyperstimulation cycle [7-13]. Based on previous reports, we evaluated whether measuring blood AMH levels would be useful to predict ART outcomes in middle-aged, infertile patients. Two major factors, the AMH level and patient age, were used to categorize the patients, and various patient profiles and ART outcomes including

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oocyte maturity and intracytoplasmic sperm injection/embryo transfer (ICSI/ET) results were employed for the evaluation. A retrospective cohort study was conducted for the analysis.

## Materials and Methods

### Patients

Collection of data was made from the total 113 cases undergoing ICSI-ET undergoing at Fertility Medical Center of Seoul Women's Hospital between September 2012 and July 2014. The study was approved by the Institutional Review Board of the Seoul Women's Hospital. Serum AMH level of each patient was measured without relating of cyclicity. According to AMH level and ages, the cycles examined were classified into three or four groups. The selection criteria were (i) within the range of 25-48 years old, (ii) only ICSI attempt, (iii) no evidence of endocrinological disorders, (iv) no evidence of polycystic ovary syndrome (PCOS) and (v) regular menstrual cycles. Informed consent was retrieved from all the patients participating in this study.

### IVF Regimen

All women underwent controlled ovarian hyperstimulation with recombinant FSH or highly purified hMG with preventing of premature ovulation by GnRH agonist down-regulation or antagonist suppression. hCG (Ovidrel, 250ng/0.5ml; Merck Serono, UK) was administered when there were at least three follicles measuring 18 mm or more in diameter and transvaginal ultrasound-guided oocyte retrieval performed 36 hours later. ICSI was performed by using described techniques and instruments [14]. Embryo transfer was performed 2 days later using embryo transfer catheter under transvaginal ultrasound. The luteal phase was supported by progesterone (90 mg of Crinone Gel; Merck Serono, UK) beginning on the day of embryo transfer. Pregnancy was determined by the increase of plasma β-hCG more than 50 mIU/mL 14 days after embryo transfer. An ultrasound scan 3 weeks after a positive pregnancy test confirmed a clinical pregnancy.

### Measures

Serum was separated from blood samples within 2 hours and was frozen in aliquots at -20°C until used. AMH was measured with Immunotech Enzyme Immunoassay kit (Bechman-Caulter, France). All samples were concomitantly assessed for minimizing intra assay variation. The analytical sensitivity for AMH was 0.09 ng/mL, and the intra- and inter assay coefficients of variation with serum controls were approximately 4.5%–5.6% and 3.6%–5.4%, respectively.

### Statistics Analysis

Statistical analyses were performed with Statistical Analysis Software (SAS Institute, Cary, NC) using general linear models with analysis of variance. When a significant model effect was detected, each treatment effect was compared using the least-squares method. The level of significance was determined when P value was less than 0.05.

## Results and Discussions

### Overall Profiles of Patients

Total 112 cycles comprised of 111 patients were employed for the analysis, which was undertaken during the period of September 2012 to July 2014. Mean age of patients were 36.7 years old and average level of AMH was 3.1 ng/ml. A mean of 6.6 oocytes were retrieved from a patient and ET of an average of 2.6 embryos per patient resulted the pregnancy rate of 37%.

Two categories of ART cycles according to AMH levels (less or more than 1 ng/ml AMH) were initially designed without considering of age factors. 77% of the cycles had the patients of more than 1 ng/ml AMH (49 cases consisting of 1-3.4 and 37 cases of >3.5 ng/ml). In the cycles of >1 ng/ml AMH, a significant difference was detected in the number of cycle (26 cases vs. 86 cases; p<0.0001), age (39.2 years old vs. 35.9 years old; p=0.0034), FSH level (10.9 ng/ml vs. 6 ng/ml; p=0.0006), oocyte retrieval (2.7 oocytes vs. 7.8 oocytes per patient; p<0.0001), maturation (oocytes provided for ICSI: 2.3 oocytes vs. 6.2 oocytes per patient; p<0.0001), mean number of ET embryos (1.8 embryos vs. 2.8 embryos; p=0.0006), endometrial thickness (8.8 mm vs. 10 mm; p=0.04) and pregnancy rate (18% vs. 41%; p=0.0226) compared with the cycles of <1 ng/ml AMH. The patients with high AMH of >1 ng/ml yielded better pregnancy rate without increasing of offspring number (1.3 to 1.4 babies per delivery) than the patients with low AMH.

### The Comparison between Ages

As shown in Table 1, total cases were divided into two groups according to age (30-34 vs. over 35) to search the effective AMH levels on IVF-ET outcomes. BMI and levels of serum FSH were not difference in two groups, however, significant difference was detected in endometrial thickness and levels of serum AMH. No significant difference among infertility factors was detected in two groups (Table 2). Considering IVF-ET outcomes in two groups (Table 3), no significant difference was detected without clinical pregnancy rate (51.6% vs. 28.8%; p=0.0291).

As shown in Figure 1, the cycles consisting of the patients of 30-34 years old had higher AMH level (4.17 ng/ml vs. 2.32; p=0.0006) than the cycles consisting of the patients of >35 years old. The percentile value of the number of the cycles of >1 ng/ml AMH significantly increased in the cycles of the ≥ 35 years old (15 to 29%) compared with the cycles of the 30-34 years old. As shown in Table 4, the comparison among three AMH levels (<1, 1-3.4 and ≥ 3.5) was undertaken separately within two groups. In the cycle of ≥ 35 years old, a significant difference was detected in the parameters of cycle number (21 cases vs. 15-35 cases; p=0.0011), FSH levels (12.1 ng/ml vs. 5.6-6.4 ng/ml; p=0.0098), oocyte retrieval (2.7 oocytes vs. 6-9.6 oocytes; p<0.0001), maturation (oocytes provided for ICSI: 2.2 oocytes vs. 5-6.9 oocytes; p=0.0002), mean number of ET embryos (1.8 embryos vs. 3-3.3 embryos; p=0.0005) and pregnancy rate (11% vs. 23-69%; p=0.019). In the cycles consisting of the patients aged ≥ 35 years old, the cycles consisting of the patients maintaining AMH level of 1 to 3.4 was predominant (p < 0.05). Significant decrease in FSH level (12.1 ng/ml to 5.6 ng/ml; p=0.0098) and increase in oocyte retrieval

**Table 1. Comparison of the characteristics of the patients between different ages.**

|  | 30-34 | ≥35 | P value |
|---|---|---|---|
| No. of cycles | 34 | 71 |  |
| Age (years) | 32.0 ± 0.2 | 39.8 ± 0.4 | <.0001 |
| Body mass index (kg/m²) | 22.3 ± 0.6 | 21.8 ± 0.3 | 0.3748 |
| Serum AMH (ng/ml) | 4.2 ± 0.6 | 2.3 ± 0.2 | 0.0006 |
| Serum FSH (mIU/ml) | 5.9 ± 0.5 | 6.5 ± 0.3 | 0.1300 |
| Endometrial thickness(mm) | 10.9 ± 0.5 | 9.0 ± 0.3 | 0.0004 |

Note: Data are mean ± SE, NS = not significant

**Table 2. Infertility factors in different ages.**

| Etiology | 30-34 (n=34) | ≥35(n=71) | P value |
|---|---|---|---|
| Unexplained | 5 | 11 | 0.9174 |
| Male | 7 | 14 | 0.9179 |
| Tubal | 9 | 16 | 0.6614 |
| Ovulation | 6 | 11 | 0.7817 |
| Multiple | 8 | 19 | 0.7261 |

**Table 3. IVF/ET outcome of the patients with different ages.**

| Parameters | 30-34 (n=34) | ≥ 35(n=71) | P value |
|---|---|---|---|
| No. oocytes | 7.5±1.0 | 5.8±0.5 | 0.1112 |
| ICSI | 6.1±0.9 | 4.6±0.4 | 0.0827 |
| Fertilization rate(%) | 86.9±4.4 | 85.4±3.1 | 0.5939 |
| Patients with embryo transfer (n) | 31 | 66 | 0.7204 |
| No. embryos transferred | 2.3±0.2 | 2.7±0.2 | 0.1073 |
| Clinical-pregnancy rate | 51.6%(16/31) | 28.8%(19/66) | 0.0291 |
| Live birth rate | 62.5% (10/16) | 47.4%(9/19) | 0.3856 |
| Offspring/delivery | 1.5±0.3 | 1.3±0.1 | 0.4903 |

**Figure 1. Mean AMH level and number of the patients with different ages. (A) Mean AMH level (30-34 years old vs. ≥ 35 years old) (B) Percentile values of the patients of the same age category (patient number).**
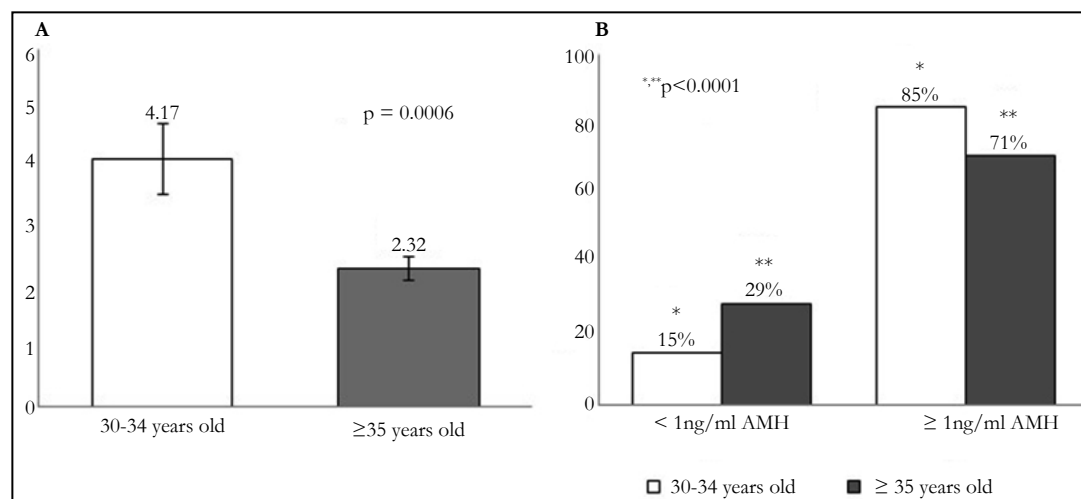


(2.7 to 9.6 oocytes per patient; p<0.0001), maturation (2.2 to 6.9 oocytes per patient: p=0.0002), ET embryos (1.8 to 3.3 embryos per cycle; p=0.005) and pregnancy rate (11 to 69%; p=0.0019) was detected in the cycles of the patients with increased AMH level. However, no significant difference among AMH level was detected in mean number of offsprings per delivery (1 to 2 offsprings). In the 30-34 years old group, significant model effect was detected only in the parameter of cycle number (5 cases vs. 14-15 cases; p=0.0173) and the >1 ng/ml AMH group was predominant. Although the tendency was similar to the cycles consisting of the patients of ≥35 years olds, no significant difference was detected in all comparisons among AMH groups except for oocyte retrieval (2.6 to 9.9 oocytes per patient). In vitro-development of embryos prior to ET was subsequently assessed (Table 4) and regardless of age and AMH categories, no significant difference was detected among comparisons.

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**Table 4. IVF/ET outcome of the patients with different ages and anti mullerian hormone (AMH) levels.**

| Parameters | 30-34 years old (n=34) | | | P value | ≥ 35 years old (n=71) | | | P value |
|---|---|---|---|---|---|---|---|---|
| | <1.0 | 1.0-3.4 | ≥ 3.5 | | <1.0 | 1.0-3.4 | ≥ 3.5 | |
| No. cycles | 5 | 14 | 15 | 0.0173 | 21 | 35 | 15 | 0.0011 |
| FSH (ng/ml) | 6.4 ± 1.9 | 5.7 ± 0.2 | 4.9 ± 0.4 | 0.3138 | 12.1 ± 2.5ᵃ | 6.4 ± 0.6ᵇ | 5.6 ± 0.8ᵇ | 0.0098 |
| No. oocytes | 2.6 ± 0.9ᵃ | 6.6 ± 1.1ᵃᵇ | 9.9 ± 1.9ᵇ | 0.0456 | 2.7 ± 0.5ᵃ | 6.0 ± 0.7ᵇ | 9.6 ± 1.4ᶜ | <.0001 |
| ICSI | 2.4 ± 0.9 | 5.6 ± 0.7 | 7.9 ± 1.7 | 0.0937 | 2.2 ± 0.6ᵃ | 5.0 ± 0.5ᵇ | 6.9 ± 1.2ᵇ | 0.0002 |
| Fertilization rate(%) | 91.7 ± 8.2 | 88.5 ± 4.4 | 96.3 ± 1.8 | 0.4468 | 95.2 ± 2.3 | 89.6 ± 2.6 | 92.3 ± 2.3 | 0.3843 |
| Embryo score (day3) | | | | | | | | |
| All embryos | | | | | | | | |
| ≤ 10% fragmentation | 1.5 ± 0.5ᵃ | 1.9 ± 0.5ᵃ | 3.9 ± 0.6ᵇ | 0.0236 | 1.2 ± 0.5ᵃ | 1.5 ± 0.6ᵃ | 3.5 ± 0.6ᵇ | 0.0071 |
| Patients with embryo transfer (n) | 4 | 14 | 13 | 0.3046 | 18 | 35 | 13 | 0.0731 |
| No. embryos transferred | 1.8 ± 0.6 | 2.6 ± 0.2 | 2.4 ± 0.3 | 0.3284 | 1.8 ± 0.3ᵃ | 3.0 ± 0.2ᵇ | 3.3 ± 0.4ᵇ | 0.0005 |
| Clinical-pregnancy rate | 50%(2/4) | 42.9%(6/14) | 61.5%(8/13) | 0.8186 | 11.1%(2/18) | 22.9%(8/35) | 69.2%(9/13) | 0.0019 |
| Live birth rate | 100%(2/2) | 33.3%(2/6) | 75.0%(6/8) | 0.1617 | 50%(1/2) | 25%(2/8) | 66.7%(6/9) | 0.2586 |
| Offspring/delivery | 1.5 ± 0.5 | 1.5 ± 0.5 | 1.4 ± 0.2 | 1.000 | 1.0 ± 0.0 | 2.0 ± 0.0 | 1.5 ± 0.2 | 0.0723 |

ᵃᵇᶜ Different superscripts within the same parameter of each age range indicate significant difference, p<0.05.

In addition, embryo score at the day 3 showed better scores in ≥ 3.5 category (p=0.0236, p=0.0071) than other categories of two groups.

## Discussion

Our results strongly suggest that measuring serum AMH levels is a powerful parameter for predicting ART outcomes in middle-aged, infertile patients. Feasibility of the AMH measurement is based on its positive relationship with the retrieval of developmentally competent oocytes, which directly improve clinical pregnancy rates. Maintenance of endogenous FSH levels within a presumptively optimal range is one of the principal roles of AMH, which is particularly critical for patients aged > 35 years.

AMH is a dimeric glycoprotein expressed at the beginning of the perinatal period [15]. AMH expression continues throughout sexual maturity, but endogenous AMH levels begin to decline near the end of reproductive life [16] and completely disappear after menopause [17]. The major function of AMH is to inhibit the initiation of primordial follicle recruitment and to maintain the FSH-dependent follicular wave [4, 18-20]. AMH antagonizes FSH function in pre-antral and early antral follicles [21]. Our results confirmed the relationship between FSH and AMH and further suggest that this relationship is critical for maintaining ART-based fecundity in middle-aged women with declining AMH levels. Accordingly, concomitantly measuring AMH and FSH is an efficient strategy for predicting the ovarian response and ART outcome in aging patients, although further studies are necessary to elucidate the complications of AMH-related physiological events.

## Conclusion

Our study demonstrated endogenous AMH levels influenced ART outcomes, and the effect was prominent in patients >35 years of age. Measuring endogenous AMH may be a sensitive parameter for predicting ART outcomes in middle-aged, infertile patients.

## Acknowledgement & Declarations

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