Anti-Müllerian hormone levels as a predictor of clinical pregnancy in \textit{in vitro} fertilization/intracytoplasmic sperm injection-embryo transfer cycles in patients over 40 years of age

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Objective: The aim of the current study was to determine the predictive value of anti-Müllerian hormone (AMH) levels for pregnancy outcomes in patients over 40 years of age who underwent \textit{in vitro} fertilization or intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) cycles.

Methods: We retrospectively analyzed the medical records of 188 women aged 40 to 44 years who underwent IVF/ICSI-fresh ET cycles due to unexplained infertility in the fertility center of CHA Gangnam Medical Center. Patients were divided into group A, with AMH levels $< 1.0$ ng/mL ($n = 97$), and group B, with AMH levels $\geq 1.0$ ng/mL ($n = 91$). We compared the clinical pregnancy rate (CPR) in the two groups and performed logistic regression analysis to identify factors that had a significant effect on the CPR.

Results: The CPR was significantly lower in group A than group B (7.2% vs. 24.2%, $p < 0.001$). In multivariate logistic regression analysis, AMH levels were the only factor that had a significant impact on the CPR (odds ratio, 1.510; 95% confidence interval, 1.172–1.947). The area under the receiver operating characteristic curve for AMH levels as a predictor of the CPR was 0.721. When the cut-off level of AMH was set at 1.90 ng/mL, the CPR was 6.731-fold higher in the group with AMH levels $\geq 1.90$ ng/mL than in the group with AMH levels $< 1.90$ ng/mL ($p < 0.001$).

Conclusion: Our study showed that AMH levels were predictive of clinical pregnancy in infertility patients over 40 years of age. Further prospective studies should be conducted to validate the predictive capability of AMH levels for the outcome of clinical pregnancy.

Keywords: Aged infertility patients; Anti-Müllerian hormone; Fertilization \textit{in vitro}; Pregnancy rate

Introduction

Anti-Müllerian hormone (AMH), a dimeric glycoprotein belonging to the transforming growth factor-beta family, is secreted by granulosa cells with preantral and early antral follicles [1]. AMH is a better marker than age, follicle-stimulating hormone (FSH) levels on day 3, estradiol (E2) levels or inhibin levels in predicting ovarian response to controlled ovarian stimulation (COS) prior to \textit{in vitro} fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The predictive value of AMH is known to be similar to that of the antral follicle count [2-4].

van Rooij et al. [5] reported that AMH levels correlate with the age-related decline in reproductive capacity. AMH levels are the especially valuable prognostic factor for pregnancy in women over 40 years of age who show a good response to COS [6]. Hence, AMH levels in women over 40 years of age can be hypothesized to be a predictive factor for pregnancy after IVF/ICSI cycles. However, according to a recently published meta-analysis, AMH levels were positively correlated with live birth after assisted reproduction techniques, but showed
poor prognostic accuracy [7].

The goal of this retrospective study was to determine the predictive value of AMH levels for pregnancy outcomes by investigating the clinical pregnancy rate (CPR) according to AMH levels in patients over 40 years of age who underwent IVF/ICSI cycles.

Methods

1. Patients

The medical records of women aged 40 to 44 years who underwent IVF/ICSI treatment from January 1, 2013 to September 1, 2014 in the fertility center of CHA Gangnam Medical Center were analyzed. The inclusion criteria were (1) a diagnosis of unexplained infertility without any other infertility-related diagnoses and (2) the performance of actual embryo transfer (ET). Cases in which the retrieval of oocytes failed and cases where no embryos were adequate for ET were excluded. The other exclusion criteria were male factor infertility as indicated by factors such as oligozoospermia and azoospermia in semen analysis; an abnormal uterine cavity on hysterosalpingography; and transvaginal sonographic findings with visible hydrosalpinx or endometrioma. A gonadotrophin-releasing hormone (GnRH) antagonist protocol with recombinant FSH (GONAL-f, Merck Serono, Darmstadt, Germany), Cetrotide (Merck Serono), and Ovidrel (Merck Serono) was used. No additional treatments or supplements such as dehydroepiandrosterone or growth hormone were used. Only cases with IVF/ICSI-fresh ET cycles were included in our analysis, and thawed ET cycles were excluded. This study was approved by the Institutional Review Board of our institution. We retrospectively analyzed the medical records of the patients who were enrolled in our study.

When the IVF-ET cycle using a GnRH antagonist regimen started, serum FSH and AMH levels were measured on the second and third days of menstrual cycle. The Beckman-Coulter second-generation AMH assay was used to estimate AMH levels [8].

2. GnRH antagonist protocol

Ovarian stimulation was initiated on the third day of the menstrual cycle with 150 to 300 IU of recombinant FSH. Ovarian response monitoring was performed using serial vaginal ultrasonography. When dominant follicles reached 14 mm in mean diameter, 0.25 mg/day of a GnRH antagonist (Cetrotide, Merck Serono) was initiated and was continued until the day of recombinant human chorionic gonadotropin (r-hCG) injection. When at least two follicles with a mean diameter of 17 mm were observed, 250 μg of r-hCG (Ovidrel, Merck Serono) was injected subcutaneously. Serum E2 levels were measured on the day of r-hCG injections. Oocyte retrieval was performed 34 to 36 hours after r-hCG injection using a 17-gauge needle under transvaginal ultrasonography guidance. Conventional IVF or ICSI was performed according to previously published protocols. A maximum of three embryos were transferred. The luteal phase was supported by daily vaginal administration of 90 mg of progesterone (Crinone gel, Merck Serono) after oocyte retrieval.

High-quality embryos were defined as grade 1 or 2 embryos with at least eight cells for 3-day, embryos that had passed the morula phase or were at the morula phase for 4-day ET, and embryos at the blastocyst phase for 5-day ET [9,10].

3. Data collection and analysis

Several patient characteristics were analyzed in this study. The continuous variables included age, body mass index (BMI), infertility duration, the number of IVF treatments, serum FSH and AMH levels on days two and three of the menstrual cycle, serum E2 levels on the r-hCG trigger day, the number of retrieved oocytes, and the number of oocytes in metaphase II (MII). Categorical data included history of previous ovarian surgery, CPR, and high-quality ET. Patients were divided into group A (AMH levels < 1.0 ng/mL) and group B (AMH levels ≥ 1.0 ng/mL), based on the criteria established by Nardo et al. [11], and the data from both groups were compared and analyzed.

4. Statistical analysis

The data were analyzed using PASW ver. 18 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation and were evaluated for statistical significance using the Student’s t-test or the Mann-Whitney U test. Categorical data were expressed as number (percent) and were compared using the chi-square test. Logistic regression analysis was performed to determine the effect of individual variables on the CPR. The p-values < 0.05 were considered to indicate statistical significance.

Results

A total of 188 patients who underwent IVF/ICSI-fresh ET cycles were analyzed. Group A included 97 patients with AMH levels < 1.0 ng/mL, while group B included 91 patients with AMH levels ≥ 1.0 ng/mL. The patients in group B were on average 0.51 years younger than those in group A (p = 0.009). No significant differences were found between the two groups in BMI, infertility duration, the number of IVF treatments, and history of previous ovarian surgery (Table 1). However, a significant difference was found between both groups with regard to FSH levels on days 2 and 3 of the menstrual cycle and E2 levels on the r-hCG trigger day (p < 0.001) (Table 1).

A significant difference was found between group A and group B in the number of retrieved oocytes (3.61 ± 2.21 vs. 8.81 ± 5.20, p < 0.001) and the number of oocytes at MII (2.07 ± 1.73 vs. 5.16 ± 3.76, p < 0.001). A marginally significant difference between groups A and B was
found with regard to the frequency of at least one instance of high-quality ET (72/97 [74.2%] vs. 77/91 [84.6%], p = 0.079). The CPR was significantly lower in group A than in group B (7/97 [7.2%] vs. 22/91 [24.2%], p < 0.001). The frequency of clinical pregnancy in patients with high-quality ET was significantly lower in group A than in group B (6/72 [8.3%] vs. 21/77 [27.3%], p = 0.003).

Our study found significant differences in AMH levels between pregnant patients (n = 29) and non-pregnant patients (n = 159) (2.51 ± 2.02 ng/mL vs. 1.26 ± 1.26 ng/mL, p < 0.001) and in FSH levels on days two and three of the menstrual cycle (7.99 ± 3.15 IU/L vs. 10.20 ± 5.29 IU/L, p = 0.038). In addition, a significant difference was found in the prevalence of high-quality ET between pregnant patients and non-pregnant patients (28/29 [96.6%] vs. 122/159 [76.7%], p = 0.015), which suggests that AMH levels may be associated with

Table 1. Patient characteristics and COS-IVF/ICSI outcomes

| Variable                        | Group A (AMH < 1.0 ng/mL) | Group B (AMH ≥ 1.0 ng/mL) | p-value |
|---------------------------------|---------------------------|---------------------------|---------|
| Number                          | 97                        | 91                        |         |
| Age (yr)                        | 41.76 ± 1.33              | 41.25 ± 1.32              | 0.009a  |
| BMI (kg/m²)                     | 21.54 ± 2.77              | 21.14 ± 2.55              | 0.305   |
| Infertility period (yr)         | 5.82 ± 4.30               | 5.16 ± 3.56               | 0.252   |
| History of previous ovarian surgery | 10/97 (10.3)            | 4/91 (4.4)                 | 0.123   |
| No. of IVF/ICSI cycles          | 3.95 ± 2.97               | 3.40 ± 2.20               | 0.147   |
| AMH (ng/mL)                     | 0.50 ± 0.27               | 2.46 ± 1.55               | <0.001a |
| FSH level on day 2–3 (IU/L)     | 11.48 ± 6.07              | 8.13 ± 2.89               | <0.001a |
| E2 level on the trigger day (pg/mL) | 804 ± 657              | 1,991 ± 1,573             | <0.001a |
| Retrieved oocytes (n)           | 3.61 ± 2.21               | 8.81 ± 5.20               | <0.001a |
| MII oocytes (n)                 | 2.07 ± 1.73               | 5.16 ± 3.76               | <0.001a |
| High-quality ETb                | 72/97 (74.2)              | 77/91 (84.6)              | 0.079   |
| Clinical pregnancy rate among patients with high-quality ET | 6/72 (8.3) | 21/77 (27.3) | 0.003a |
| Clinical pregnancy rate         | 7/97 (7.2)                | 22/91 (24.2)              | <0.001a |

Values are presented as mean ± standard deviation. Continuous variables (mean ± standard deviation) were evaluated for significance using the Student’s t-test, while categorical data (n, %) were compared using the chi-square test. COS, controlled ovarian stimulation; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; E2, estradiol; MII, metaphase II; ET, embryo transfer.

Table 2. Baseline and cycle characteristics of pregnant and non-pregnant patients

| Variable                        | Pregnant group | Non-pregnant group | p-value |
|---------------------------------|----------------|--------------------|---------|
| Number                          | 29             | 159                |         |
| Age (yr)                        | 41.38 ± 1.37   | 41.54 ± 1.34       | 0.507   |
| BMI (kg/m²)                     | 21.09 ± 2.58   | 21.39 ± 2.68       | 0.315   |
| Infertility period (yr)         | 5.28 ± 4.02    | 5.55 ± 3.96        | 0.615   |
| History of previous ovarian surgery | 0/29 (0.0)    | 14/159 (8.8)       | 0.132a  |
| No. of IVF/ICSI cycles          | 2.97 ± 2.28    | 3.81 ± 2.68        | 0.106   |
| AMH (ng/mL)                     | 2.51 ± 2.02    | 1.26 ± 1.26        | <0.001a |
| FSH level on day 2–3 (IU/L)     | 7.99 ± 3.15    | 10.20 ± 5.29       | <0.001a |
| E2 level on the trigger day (pg/mL) | 2,334 ± 2,110 | 1,204 ± 1,050      | <0.001a |
| Retrieved oocytes (n)           | 9.76 ± 5.10    | 5.47 ± 4.35        | <0.001a |
| MII oocytes (n)                 | 6.10 ± 4.24    | 3.11 ± 2.85        | <0.001a |
| High-quality ETc                | 28/29 (96.6)   | 122/159 (76.7)     | 0.015a  |

Values are presented as mean ± standard deviation or number (%). Continuous variables (mean ± standard deviation) were evaluated for significance using the Mann-Whitney U test because non-parametric testing methods were required due to the sample size of the two groups. Categorical data (n, %) were compared using the chi-square test.

BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; E2, estradiol; MII, metaphase II; ET, embryo transfer.

aStatistically significant; bHigh-quality embryos were defined as grade 1 or 2 embryos with eight or more cells on day 3, the morula phase on day 4, and the blastocyst phase on day 5.
Embryo quality in infertility patients over 40 years of age (Table 2). Logistic regression tests found that age did not have a significant effect on the CPR (odds ratio [OR], 0.912; 95% confidence interval [CI], 0.675–1.234; p = 0.552) by univariate analysis. In univariate analysis, AMH (OR, 1.581; 95% CI, 1.230–2.032; p < 0.001), and FSH levels (OR, 0.890; 95% CI, 0.800–0.990; p = 0.032) had a significant impact on the CPR; whereas, in multivariate analysis, AMH levels were the only factor found to have a significant impact on the CPR (OR, 1.510; 95% CI, 1.172–1.947; p = 0.001) (Table 3).

The area under the curve (AUC) value of the receiver operating characteristic curve for AMH levels as a predictor of clinical pregnancy was 0.721, which was indicative of a fair predictive model (Figure 1). When an AMH level of 1.90 ng/mL was set as the cut-off point, the CPR was 6.731-fold higher in the group with AMH ≥ 1.90 ng/mL than in the group with AMH < 1.90 ng/mL (19/54 [35.2%] vs. 10/134 [7.5%]; OR, 6.731; 95% CI, 2.867–15.791; p < 0.001).

**Table 3. Logistic regression analysis of factors related to the clinical pregnancy rate (%)**

| Variable                              | B    | OR   | 95% CI (OR) | p-value |
|---------------------------------------|------|------|-------------|---------|
| **By univariate analysis**            |      |      |             |         |
| Age (yr)                              | −0.092 | 0.912 | 0.675–1.234 | 0.552   |
| BMI (kg/m²)                           | −0.045 | 0.956 | 0.819–1.117 | 0.956   |
| Infertility period (yr)               | −0.018 | 0.982 | 0.885–1.090 | 0.734   |
| Previous ovarian surgery (yes/no)     | 19.593 | 3.231 × 10⁻¹⁰ | 0.000 *   | 0.999   |
| No. of IVF/ICSI cycles (n)            | −0.145 | 0.865 | 0.721–1.036 | 0.116   |
| AMH levels (ng/mL)                    | 0.458 | 1.581 | 1.230–2.032 | < 0.001*|
| Basal FSH levels (IU/L)               | −0.116 | 0.890 | 0.800–0.990 | 0.032   |
| **By multivariate analysis**          |      |      |             |         |
| AMH levels (ng/mL)                    | 0.412 | 1.51  | 1.172–1.947 | 0.001 * |
| Basal FSH levels (IU/L)               | −0.087 | 0.916 | 0.815–1.030 | 0.916   |

B, regression coefficient; OR, odds ratio; CI, confidence interval; BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone.

*Statistically significant.

**Discussion**

Serum AMH levels may be a potential predictor of ovarian response in COS, pregnancy outcomes, and live birth, although these associations remain controversial [12-14]. In particular, serum AMH levels are known to be the best indicator of ovarian reserves [2,11,15]. Since previous studies were not able to predict the outcomes of IVF/ICSI-ET cycles accurately due to the high level of variability in fertility between individuals according to age [16-21], it may be meaningful that setting an appropriate cut-off value of serum AMH levels in infertile women over 40 years of age could allow an individualized approach to treatment by stratifying the patients according to prognosis.

Age is the primary determinant of pregnancy outcomes in IVF/ICSI cycles [22,23]. However, the relationship between the chronological age of women and their reproductive capacity is very complex [24]. Roest et al. [6] reported that patients over 40 years of age who showed a good response to ovarian stimulation had better outcomes in pregnancy. In our study, significant differences in the number of retrieved oocytes at MII and the CPR were observed in patients aged over 40 years between those with AMH levels ≥ 1.0 ng/mL and those with AMH levels < 1.0 ng/mL. Moreover, the CPR was significantly higher in patients who underwent high-quality ET (Table 1). The CPR was 6.731-fold higher in patients with AMH levels ≥ 1.90 ng/mL (35.2%, 19/54) than in patients with AMH levels < 1.90 ng/mL (7.5%, 10/134), which was found to be a significant difference.
Positive correlations between serum AMH levels and embryo quality, fertilization rates, and pregnancy rates have been reported [12,13,25,26]. Some studies have found AMH levels to be a poor predictor of pregnancy and live birth [20,21]. However, the current study demonstrated that AMH levels in patients over 40 years of age are a valuable predictive tool for pregnancy outcomes (Figure 1). Twisk et al. [27] reported that even morphologically normal embryos can have abnormal chromosomes, resulting in a low pregnancy rate. We found a significant difference in the CPR of patients with high-quality ET based on AMH levels. Hence, high AMH levels can function as a predictor of the embryo quality in patients over 40 years of age.

The limitations of this study were as follows. First, this was a retrospective study, meaning that it was not possible to control for all confounding factors that could have impacted the clinical outcomes. However, the study was strengthened by the consistent application of a unified IVF/ICSI protocol with GnRH antagonist cycles. Second, the basal characteristics indicated that the patients with AMH levels < 1.0 ng/mL were 0.51 years older on average than the patients with AMH levels ≥ 1.0 ng/mL, which was a statistically significant difference (Table 1). However, since age was not found to have a significant effect on the CPR by logistic regression analysis, it should not be considered to have had a major influence on the study results, despite the slight difference in age between the two groups.

In conclusion, our study found AMH levels to be predictive of clinical pregnancy in infertility patients over 40 years of age. Furthermore, we determined the AUC value and the optimal cut-off value of AMH levels for predicting pregnancy. However, our study had the limitation of being a retrospective study. Thus, a well-designed prospective study should be conducted to validate the predictive capability of AMH levels.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea (grant number: HI12C-0055-020014).

References

1. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004;10:77-83.
2. Fanchin R, Mendez Lozano DH, Louafi N, Achour-Frydman N, Frydman R, Taieb J. Dynamics of serum anti-Mullerian hormone levels during the luteal phase of controlled ovarian hyperstimulation. Hum Reprod 2005;20:747-51.
3. Patrelli TS, Gizzo S, Sianesi N, Levati L, Pezzuto A, Ferrari B, et al. Anti-Mullerian hormone serum values and ovarian reserve: can it predict a decrease in fertility after ovarian stimulation by ART cycles? PLoS One 2012;7:e44571.
4. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113-30.
5. van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002;17:3065-71.
6. Roest J, van Heusden AM, Mous H, Zeilmaker GH, Verhoef A. The ovarian response as a predictor for successful in vitro fertilization treatment after the age of 40 years. Fertil Steril 1996;66:969-73.
7. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 2014;20:560-70.
8. 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.
9. Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, et al. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999;14:2345-9.
10. Sakkas D, Gardner DK. Noninvasive methods to assess embryo quality. Curr Opin Obstet Gynecol 2005;17:283-8.
11. Nardo LG, Gelbaya TA, Wilkinson H, Roberts SA, Yates A, Pember ton P, et al. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. Fertil Steril 2009;92:1586-93.
12. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. Serum antimullerian hormone/mullerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril 2004;82:1323-9.
13. Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, et al. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod 2005;20:3178-83.
14. Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles: implications for individualization of therapy. Hum Reprod 2007;22:2414-21.

15. Penarrubia J, Fabregues F, Manau D, Creus M, Casals G, Casamitjana R, et al. Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist–gonadotropin treatment. Hum Reprod 2005;20:915-22.

16. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002;8:141-54.

17. Guerif F, Lemseffer M, Couet ML, Gervereau O, Ract V, Royere D. Serum antimullerian hormone is not predictive of oocyte quality in vitro fertilization. Ann Endocrinol (Paris) 2009;70:230-4.

18. Riggs R, Kimble T, Oehninger S, Bocca S, Zhao Y, Leader B, et al. Anti-Mullerian hormone serum levels predict response to controlled ovarian hyperstimulation but not embryo quality or pregnancy outcome in oocyte donation. Fertil Steril 2011;95:410-2.

19. Mutlu MF, Erdem M, Erdem A, Yildiz S, Mutlu I, Arisoy O, et al. Antral follicle count determines poor ovarian response better than anti-Mullerian hormone but age is the only predictor for live birth in in vitro fertilization cycles. J Assist Reprod Genet 2013;30:657-65.

20. Yao L, Zhang W, Li H, Lin W. The role of serum AMH and FF AMH in predicting pregnancy outcome in the fresh cycle of IVF/ICSI: a meta-analysis. Int J Clin Exp Med 2015;8:1755-67.

21. Smeenk JM, Sweep FC, Zielhuis GA, Kremer JA, Thomas CM, Braat DD. Antimullerian hormone predicts ovarian responsiveness, but not embryo quality or pregnancy, after in vitro fertilization or intracytoplasmic sperm injection. Fertil Steril 2007;87:223-6.

22. Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. Lancet 1996;348:1402-6.

23. Hunault CC, Eijkemans MJ, Pieters MH, te Velde ER, Habbema JD, Fauser BC, et al. A prediction model for selecting patients undergoing in vitro fertilization for elective single embryo transfer. Fertil Steril 2002;77:725-32.

24. de Bruin JP, Dorland M, Spek ER, Posthuma G, van Haften M, Looman CW, et al. Age-related changes in the ultrastructure of the resting follicle pool in human ovaries. Biol Reprod 2004;70:419-24.

25. Majumder K, Gelbaya TA, Laing I, Nardo LG. The use of anti-Mullerian hormone and antral follicle count to predict the potential of oocytes and embryos. Eur J Obstet Gynecol Reprod Biol 2010;150:166-70.

26. Honnma H, Baba T, Sasaki M, Hashiba Y, Oguri H, Fukunaga T, et al. Serum anti-Mullerian hormone levels affect the rate of ongoing pregnancy after in vitro fertilization. Reprod Sci 2013;20:51-9.

27. Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. Cochrane Database Syst Rev 2006;(1):CD005291.