High Anti-Müllerian Hormone Level is Associated with A Lower Chromosomal Aberration Rate in Miscarried Conceptus From Women with Early Spontaneous Abortion

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Research

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Abstract

**Background:** Approximately 10-15% of clinically recognized pregnancies end in abortion. Most miscarriages occur as early spontaneous abortions. There are many factors leading to early spontaneous abortion, and 50–60% of such cases are associated with chromosomal abnormalities. The reason for this occurrence is not clear, but advanced age is a risk factor for chromosomal aberration in miscarried conceptus from women with early spontaneous abortion. As a marker of ovarian reserve, anti-Müllerian hormone (AMH) is negatively correlated with age. As women become older, AMH levels decrease. The objective of this study was to investigate whether different anti-Müllerian hormone (AMH) levels are associated with chromosomal aberration rate in miscarried conceptus from women who experience early spontaneous abortion.

**Methods:** We collected the clinical history and miscarried conceptus of 434 women with early spontaneous abortion from January 2016 to June 2019. The women were divided into three groups according to AMH level (Group 1: low AMH <1.1 ng/ml [N =13], Group 2: normal AMH 1.1–4.5 ng/ml [N = 138], and Group 3: high AMH≥ 4.5 ng/ml [N =283]). Clinical history included age, anti-Müllerian hormone (AMH) level, number of previous abortions, estradiol (E2) level, luteinizing hormone (LH) level, follicle-stimulating hormone (FSH) level, infertile years, body mass index (BMI) and infertility factors. The miscarried conceptus was submitted for chromosomal copy number variation (CNV) analysis in the gene testing laboratory of the Third Affiliated Hospital of Zhengzhou University.

**Results:** There were significant differences in age (39.5±4.3 vs. 33.0±5.3 vs. 29.7±3.9, P<0.001), E2 (187.9±513.4 vs. 92.9±160.6 vs. 66.5±139.3, P=0.019), LH (5.1±3.9 vs. 4.5±2.5 vs. 5.4±3.5, P=0.039), and FSH (10.7±5.6 vs. 7.3±2.6 vs. 6.4±2.1, P<0.001) in different AMH groups. There were no significant differences in infertility years, BMI and infertility factors among the three groups.

There was a significant difference in chromosomal aberration rate between different AMH groups (76.9% vs. 67.4% vs. 53.7%, Groups 2 vs. 3, respectively, P=0.008, OR 0.797, 95% CI 0.680-0.934). With the increase in AMH level, the chromosomal aberration rate in miscarried conceptus decreased gradually. After age stratification, the chromosomal aberration rate in miscarried conceptus was still significantly different among AMH groups, with a similar trend in women ≥35 years old (88.9% versus 76.0% versus 51.5%, P2 vs. 3 = 0.021, OR 0.678, 95% CI 0.470-0.977). There was the same trend in the younger group (<35 years), but there was no significant difference (88.9% vs. 76.0% vs. 51.5).

**Conclusions:** These findings indicate that high AMH level was associated with reduced risk of chromosomal aberration rate, especially in women of advanced age (≥35 years).

**Background**

Approximately 10-15% of clinically recognized pregnancies end in abortion, and approximately 1% of couples experience recurrent pregnancy loss[1]. Early miscarriage is defined as pregnancy loss before 12 weeks’ gestation after confirmation with ultrasound (gestational sac or fetal pole) or products of conception diagnosed histologically by patient report or medical records. Spontaneous abortion can cause serious physical and mental injuries to couples of childbearing age. Its etiology is complex, including genetic factors, anatomical factors, endocrine factors, infection factors, and prethrombotic state[2]. Most miscarriage occurs in early abortion, and 50–60% of early spontaneous abortions are associated with chromosomal abnormalities[3, 4].

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β superfamily[5]. In the male, AMH is produced by Sertoli cells during fetal sex differentiation, in which it represses the development of Müllerian ducts. In the female, it is a postnatal product of the granulosa cells from pre-antral and small antral follicles[6]. In addition, different reports have demonstrated that serum AMH concentrations correlate with the outcome of ovarian reserves. Therefore, serum AMH concentrations constitute a sensitive marker of the ovarian reserve[7, 8]. With the increase of women's age, the ovarian reserve capacity and AMH level decrease. Age is known to be a risk factor for chromosomal aberration in miscarried conceptus from women with early spontaneous abortion[9, 10].
The objective of the present study was to identify the association between serum AMH and the incidence of chromosomal abnormalities in miscarried conceptus from women with early spontaneous abortion. The detection of chromosomal copy number variations (CNVs) in aborted tissues, combined with related clinical history and AMH levels, is conducive to providing better genetic counseling in the clinic\textsuperscript{[11]}.

**Materials And Methods**

**Participants**

We collected 434 early spontaneous abortion women from January 2016 to June 2019. The age of the patients was 10-45 years old, and the gestational age was 6-12 weeks. We excluded abnormal chromosome karyotype for either member of each couple. The clinical history of the patients included age, anti-Müllerian hormone (AMH) level, number of previous abortions, infertile years, estradiol (E\textsubscript{2}) level, luteinizing hormone (LH) level, follicle-stimulating hormone (FSH) level, and body mass index (BMI). Moreover, for the 434 women, their etiology of infertility was evaluated, including unknown reason infertility, ovulation dysfunction, tubal factor, male factor infertility and other factors. Data in this study were from our Center for Reproduction Medicine, The Third Affiliated Hospital of Zhengzhou University, and the miscarried conceptus were undergoing CNVs in the gene testing laboratory.

**Methods**

**Sample collection and DNA extraction**

Spontaneous miscarriage was defined as the absence of fetal cardiac pulsation in the uterine cavity after confirmation of clinical pregnancy. Once carefully diagnosed, curettage was performed, and 5-10 g of villi were taken for gene analysis in the gene laboratory of the scientific research center to determine the cause of this adverse reproductive outcome. Each sample was rinsed in normal saline solution three times. Then, 10 mg of the tissue was submitted for genomic DNA extraction using a DNA extraction kit (QIAamp DNA Blood Midi Kit QIAGEN, USA).

**Chromosomal copy number analysis by NGS**

Chromosomal copy number variations (CNVs) can be used for the detection of aneuploidy of 22 pairs of autosomes and sex chromosomes, as well as chromosome copy number variations and low proportion chimera detection of more than 100 kb\textsuperscript{[10]}.

Next-generation sequencing technology (NGS) first shears the genomic DNA extracted under sterile conditions into small fragments of nucleic acids, constructs a DNA fragment library, and carries out on-line sequencing. The sequencing instrument used was the NextSeq CN500 high-throughput sequencer. Finally, RUPA (rapid unique parallel alignment) software was used to compare and analyze the sequencing results to determine whether there was abnormality in the chromosome segment.

**Statistical analysis**

Data were analyzed using SPSS 21.0 statistical software. The categorical variables were analyzed using the chi-square test, while continuous variables are expressed as the means ± SD. $P < 0.05$ was considered statistically significant. Logistic regression analysis was performed to adjust the confounders, including age, E\textsubscript{2}, LH, and FSH.

We further applied a two-piecewise linear regression model to examine the threshold effect of AMH on the chromosomal aberration rate using a smoothing function (Fig. 1). The threshold level (i.e., turning point) was determined using trial and error, including selection of turning points along a predefined interval and then choosing the turning point that gave the maximum model likelihood. Data were analyzed using Empower(R) (www.empowerstats.com, X&Y solutions, Inc. Boston MA) and R (http://www.R-project.org).

**Results**
A total of 434 miscarried conceptus from women with early spontaneous abortion were recruited to this study. Groups were divided into three groups according to AMH level (Group 1: low AMH < 1.1 ng/ml [N = 13], Group 2: normal AMH 1.1–4.5 ng/ml [N = 138], and Group 3: high AMH ≥ 4.5 ng/ml [N = 283]).

There were significant differences among the three groups in age [39.5 ± 4.3 vs. 33.0 ± 5.3, P < 0.001], E2 level [187.9 ± 513.4 vs. 92.9 ± 160.6 vs. 66.5 ± 139.3, P < 0.019], LH [5.1 ± 3.9 vs. 4.5 ± 2.5 vs. 5.4 ± 3.5, P < 0.039], and FSH [10.7 ± 5.6 vs. 7.3 ± 2.6 vs. 6.4 ± 2.1, P < 0.001]. There were no significant differences in the number of previous abortions, infertile years, BMI and infertility factors between the three groups [Table 1].

The chromosomal aberration rates in the miscarried conceptus were 76.9% versus 67.4% versus 53.7% among the three groups. The chromosomal aberration rate in the miscarried conceptus in group 3 was significantly lower compared with that in group 2 (P3VS2 = 0.008, OR 0.797, 95% CI 0.680–0.934). Group 1 was comparable to group 2 (P1VS2 = 0.200, OR 1.256, 95% CI 0.969–1.627) [Table 2, Fig. 1].

After age stratification, the chromosomal aberration rate was still significantly different among AMH groups, with a similar trend in women ≥ 35 years old (88.9% versus 76.0% versus 51.5%; P1VS2 = 0.390, OR 1.170, 95% CI 0.885–1.545; P2VS3 = 0.021, OR 0.678, 95% CI 0.470–0.977), but not in young women < 35 years old (75.0% versus 62.5% versus 54.0%; P1VS2 = 0.612, OR 1.200, 95% CI 0.666–2.162; P2 vs. 3 = 0.167, OR 0.864 95% CI 0.709–1.053) [Table 3].

Discussion

In this study, it was found that the level of AMH is correlated with the chromosomal aberration rate in miscarried conceptus. As the level of AMH increases, the abnormal rate gradually decreases and tends to stabilize when it reaches a certain threshold. It can be seen that a high AMH level was associated with reduced risk of chromosomal aberration in miscarried conceptus from women with early spontaneous abortion.

To our knowledge, Jiang Xiao et al. showed a lower chromosomal aberration rate in patients with high AMH levels compared with patients with normal AMH levels in women with early spontaneous abortion. This finding was most significant in patients aged ≥ 38 years [12]. Research by Zhang Bingqian et al. showed that in women with differences between age and AMH levels, ovarian response was positively correlated with serum AMH levels[13]. The current study indicates that chromosomal aberration risk was mainly due to advanced age and related changes in oocytes and not to the isolated quantitative reserve.

Use of serum AMH level is a recently introduced method for the assessment of ovarian reserve. AMH and AFC are more reliable markers of ovarian reserve than FSH. The current findings regarding the age-related selective AMH correlation with egg quality have never been described. Interestingly, Al-Edani et al.[14] reported a similar age-selectivity effect on AMH expression in human cumulus cells. Expression of AMH and other proteins involved in the TGF-β signaling pathways were significantly reduced among patients older than 37 compared with younger ages[15]. Several studies have found that there is a significant positive correlation between AMH levels and the quality[5] and quantity[16] of oocytes, although the value of AMH in predicting oocyte quality is controversial[17,18].

With the high chromosomal aberration rate in miscarried conceptus from women with early spontaneous abortion [19–21], the only indisputable risk factor was advanced maternal age[22]. The causes of age-related aneuploidy are related to the following assumptions: spindle assembly checkpoints deteriorate with age, errors occur in exchange frequency or position reorganization, and the main hypothesis is premature loss of centromere cohesion[23]. Therefore, in the meiosis of human females, incorrect chromosome segregation will lead to abnormal chromosome number in eggs. During fertilization, these eggs combine with sperm to form embryos with abnormal chromosomes[9].

Chromosomal abnormalities are observed in embryos in connection with decreased quality of the oocyte, which has been reported as a reason for miscarriage in 35–75% of all cases[11]. Most of such cases involve autosomal trisomy, followed by
monosomy, triploid and other abnormalities. As in previous studies, the chromosomal aberration in miscarried conceptus from women with early spontaneous abortion in different groups in this study were mostly autosomal trisomy, among which 16-trisomy, 18-trisomy, 21-trisomy and 22-trisomy were more common. Advanced age is an important risk factor leading to an increase in the chromosomal aberration rate in miscarried conceptus from women with early spontaneous abortion. However, according to current data, the chromosomal aberration rate in miscarried conceptus from women with high ovarian response decreases, especially in older women. The study suggests that high levels of AMH are protective factors. There are also limitations in this study. First, the sample size is small. Second, the study is retrospective. Third, the study is not aimed at the detection of preimplantation embryos, which are affected by maternal and environmental factors. Further research is needed to clarify the results.

**Conclusion**

In conclusion, AMH is generally considered to be an excellent predictor of ovarian response and is related to the age of women. Current research shows that AMH is associated with the rate of chromosomal aberrations, but the prediction of these results is generally weak. Therefore, AMH may have certain clinical uses in consultations with women undergoing fertility treatment.

**Abbreviations**

AMH: anti-Mullerian hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; BMI: body mass index; CNV: chromosomal copy number variations.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of The Third Affiliated Hospital of Zhengzhou University.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The data that support the findings of this study are available from the Center for Reproduction Medicine, The Third Affiliated Hospital of Zhengzhou University and the gene testing laboratory. However, restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

GYC and MMK designed the study and selected the populations to be included and excluded. SSM, ZW, YSH, YC and ZRW were involved in data extraction and analysis. ZLL, GJS, LJ and WXL reviewed the data. MMK was responsible for drafting the article. All authors approved the final version of the manuscript.
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Tables
Table 1 Comparison of parameters between patients with different AMH levels

| Group | Group1 | Group2 | Group3 | P-value | P₁VS₂ | P₁VS₃ | P₂VS₃ |
|-------|--------|--------|--------|---------|-------|-------|-------|
| Age   | 39.5±4.3 | 33.0±5.3 | 29.7±3.9 | 0.001   | 0.001 | 0.001 | 0.001 |
| Infertile years | 2.6±2.8 | 2.9±2.5 | 3.4±2.3 | NS      | NS    | NS    | NS    |
| Number of previous abortions | 1.3±0.6 | 1.4±0.7 | 1.1±0.8 | NS      | NS    | NS    | NS    |
| E₂ (pg/ml) | 187.9±513.4 | 92.9±160.6 | 66.5±139.3 | 0.019   | NS    | NS    | NS    |
| LH (IU/l)      | 5.1±3.9 | 4.5±2.5 | 5.4±3.5 | 0.039   | NS    | NS    | 0.011 |
| FSH (IU/l)     | 10.7±5.6 | 7.3±2.6 | 6.4±2.1 | 0.001   | 0.001 | 0.001 | 0.001 |
| BMI (kg/m²)    | 22.9±2.0 | 23.1±3.2 | 23.4±3.3 | NS      | NS    | NS    | NS    |
| Infertility factors (% of patients) | Unknown | 1(7.7) | 28(20.3) | 62(21.9) | NS    | NS    | NS    |
|        | Ovulation dysfunction | 3(23.1) | 21(15.2) | 84(29.7) |       |       |       |
|        | Tubal factor | 5(38.4) | 47(34.1) | 49(17.3) |       |       |       |
|        | Male factor | 3(23.1) | 23(16.7) | 48(17.0) |       |       |       |
|        | Other | 1(7.7) | 19(13.7) | 40(14.1) |       |       |       |

Notes: Group 1: low AMH <1.1ng/ml [N =13], Group2: normal AMH 1.1–4.5ng/ml [N = 138], and Group 3: high AMH≥4.5ng/ml [N =283]). NS = not statistically significant; P < 0.05 was statistically significant.

Table 2 Logistic analysis of AMH related to chromosomal aberration rate

| Group | normal chromosome | abnormal chromosome | chromosomal aberration rate (%) | P-value | OR (95%CI) |
|-------|-------------------|---------------------|---------------------------------|---------|------------|
|       |                   |                     |                                 |         |            |
| Group1 | 2      | 11                 | 76.9                            | 0.2     | 1.256(0.969-1.627) |
| Group2 | 45     | 93                 | 67.4                            | Ref     | 1          |
| Group3 | 131    | 152                | 53.7                            | 0.008   | 0.797(0.680-0.934) |

Notes: Group 1: low AMH <1.1ng/ml, Group2: normal AMH 1.1–4.5ng/ml, and Group 3: high AMH≥4.5ng/ml). P < 0.05 was statistically significant.

Table 3 Logistic analysis of AMH related to chromosome aberration rate after age stratification

| Age | Group | normal chromosome | abnormal chromosome | chromosomal aberration rate (%) | P-value | OR (95%CI) |
|-----|-------|-------------------|---------------------|---------------------------------|---------|------------|
|     |       |                   |                     |                                 |         |            |

Notes: Group 1: low AMH <1.1ng/ml, Group2: normal AMH 1.1–4.5ng/ml, and Group 3: high AMH≥4.5ng/ml). P < 0.05 was statistically significant.
| Age Group | Group | N  | AMH (ng/ml) | Chromosomal Aberration Rate (%) | P Value | 95% CI |
|-----------|-------|----|-------------|-------------------------------|---------|--------|
| 35 years  | Group 1 | 1  | 3 | 75 | 0.612 | 1.200(0.666-2.162) |
|           | Group 2 | 33 | 55 | 62.5 | Ref | 1 |
|           | Group 3 | 115 | 135 | 54 | 0.167 | 0.864(0.709-1.053) |
| ≥35 years | Group 1 | 1  | 8 | 88.9 | 0.39 | 1.170(0.885-1.545) |
|           | Group 2 | 12 | 38 | 76 | Ref | 1 |
|           | Group 3 | 16 | 17 | 51.5 | 0.021 | 0.678(0.470-0.977) |

Notes: Group 1: low AMH <1.1ng/ml, Group 2: normal AMH 1.1-4.5ng/ml, and Group 3: high AMH≥4.5ng/ml. P < 0.05 was statistically significant.

Figures

Figure 1

AMH and chromosomal aberration rate relationship.