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The Sequel Of Advanced Maternal Age On Regulation Of Anti- Mullerian Hormone, Quality Of Oocytes, Embryo Competency And Pregnancy Outcomes In Intracytoplasmic Sperm Injection Cycles

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ABSTRACT

Advanced maternal age (AMA), defined as 35 years or older, significantly affects fertility due to diminished ovarian reserve (DOR), reduced oocyte quality, and decreased embryo competency. With a growing trend of delayed childbearing, AMA has become a key factor in the increasing demand for assisted reproductive technology (ART). This study aims to assess the impact of AMA on anti-Mullerian hormone (AMH) levels, oocyte quality, embryo competence, and pregnancy outcomes in intracytoplasmic sperm injection (ICSI) cycles. This retrospective study involved 240 women undergoing ICSI cycles at the Bliss Fertility Centre in Kottayam, Kerala, between January 2022 and August 2024. Participants were categorized into five age groups, and data were collected on AMH levels, oocyte quality, embryo grade, and pregnancy outcomes. AMH levels were measured using chemiluminescence immune assays, and oocyte morphology was assessed microscopically. Statistical analyses included logistic regression and chi-square tests. The mean AMH level across the cohort was 2.83 ± 2.05 ng/mL, with significantly lower levels observed in women aged >37 years. Good-quality oocytes were retrieved in 88.4% of women aged 25–31, but only 43.2% of those aged >37 ($p < 0.05$). Embryo competency, measured by the percentage of good-quality embryos, was not significantly different across age groups ($p = 0.808$). Pregnancy outcomes declined with increasing age; women aged 25–31 had a 68.4% positive outcome rate, compared to 27% in women aged >37 ($p < 0.01$). Logistic regression highlighted that younger maternal age significantly increased the odds of achieving positive outcomes (OR: 5.85; 95% CI: 2.51–13.61). This study highlights the urgent need for early fertility interventions and ART strategies for AMA patients to optimize reproductive outcomes.

Keywords: *Advanced maternal age, assisted reproductive technology, anti-Müllerian hormone, embryo quality, oocytes, pregnancy*

1. INTRODUCTION

Fertility, defined as the natural ability to conceive offspring, is influenced by several factors, where age is one of the most important. Fertility in women usually peaks in their early 20s and then steadily drops, with a significant decline in reproductive potential beyond age 35. The success of both natural and ART is affected by age-related decline in fertility, which is mainly triggered by a decrease in the number and quality of oocytes as well as a decreased ovarian reserve [1] [2]. The decrease in fertility is further complicated due to the menopause, the natural end of the reproductive years, classically happening in late 40s or early 50s in a woman [3]. The AMA, generally considered to be 35 years or older, is a vital factor in the increasing demand of ART, especially in in-vitro fertilization (IVF) and ICSI. With the increasing number of women delaying childbirth for social, economic, or personal reasons, AMA is now one of the most common factors contributing to infertility. Due to the age-related infertility problems, women in this age range often require ART to become pregnant [4].

To understand the reproductive potential, the regulation of AMH, a key indicator of ovarian reserve, plays a crucial role. The granulosa cells of the antral follicles produce AMH, and it remains stable throughout the menstrual cycle, making it a suitable indicator of ovarian function. AMH levels progressively decrease with age in women, indicating the decrease in ovarian reserve and antral follicle loss [5]. Age alone is a less accurate predictor of reproductive capacity, though, as there is considerable individual variability and some women may experience age-related reductions in AMH levels earlier or later than others [6]. A key factor in ART cycles is the quality of the oocytes, which regulates the chances of successful fertilization and the growth of the embryo. Oocytes from women who are older tend to have more chromosomal abnormalities, like

aneuploidy, which cause implantation failure or pregnancy loss. Furthermore, older women severely impair their embryos' capacity to reach the blastocyst stage, a crucial developmental milestone [7] [8].

Because of its potential to address age-related fertility issues, ICSI, a procedure designed to aid fertilization in cases of male infertility, has become more and more popular among women of AMA. However, studies demonstrated that the AMA possesses a negative impact on embryo quality [9]. Also, the ICSI has an impact on the epigenetic composition of embryos, potentially affecting their developmental outcomes [10] [11]. Women of reproductive age with DOR have lower fertility potential, especially if they have regular menstrual cycles but a poor ovarian response (POR) to gonadotropin stimulation. DOR differs from premature ovarian failures or menopause and is mainly attributed to the natural aging process. DOR leads to a lower number of oocytes retrieved during treatments like ART, affecting the success of IVF.

ART advances considerably due to ICSI, as it involves the direct injection of a single sperm into an oocyte, particularly in cases of male factor infertility. The use of frozen-thawed embryo transfer (FET) in conjunction with ICSI has become common practice. ART is commonly preferred to women with an age group over 35 [12]. Gene regulation, DNA methylation, and histone modification are some of the concerns associated with ICSI [13]. Increased DNA methylation is linked to advanced maternal age, which considerably raises the chance of blastocyst aneuploidy but has no effect on fertilization rates and embryo development up to the blastocyst stage. The molecular and physiological cause of infertility contributes to DOR, reduced oocyte and embryo quality [14]. However, this research aims to investigate the effect of AMA on the regulation of AMH, oocyte quality, embryo competence, and pregnancy outcomes in ICSI cycles. The major contributions of the study are as follows:

- Examine the impact of AMA on serum levels of AMH in women undergoing ICSI cycles.
- Evaluate the impact of maternal age on oocyte quality.
- Investigate the potential predictors of successful pregnancy in AMA patients undergoing ICSI.
- Analyze the pregnancy outcomes in relation to maternal age, oocyte quality, and embryo competence in ICSI cycles.

The study organizes the remaining portion as follows: Section 2 provides a comprehensive literature review. Section 3 details the materials and methods, outlining the research design, participant criteria, and analytical techniques used. Section 4 presents the results, including statistical analyses of AMH levels, oocyte quality, and pregnancy outcomes. Section 5 offers a discussion of the findings in relation to existing literature. Finally, Section 6 concludes with key findings and recommendations for future research.

2. RELATED WORKS

Aghajani et al. [15] investigated the relationship between maternal age and chromosomal aneuploidy in embryos produced from ICSI cycles. Aneuploidy was evaluated using fluorescence in situ hybridization techniques and polymerase chain reaction (PCR). The findings showed that while aneuploidy rates were not dramatically impacted, pregnancy outcomes drastically declined as maternal age increased. The application of outdated genetic methods limited the study, and interpretation was impacted by smaller sample sizes in older age groups. Wang et al. [16] explored the impact of female age at the time of pregnancy outcomes for patients experiencing their first elective single embryo transfer (eSET) in IVF cycles using 7089 patients from Henan Provincial People's Hospital. The study applied a generalized additive model and logistic regression to clearly prove that the pregnancy rate, both clinical and ongoing, was significantly diminished with age, significantly beyond the age of 34.

Gunasheela et al. [17] aimed to create age-specific percentile charts of Indian infertile women for AMH and antral follicle count (AFC), based on data from 5525 patients. The study found that over half of women under 35 had low ovarian reserve, with AMH and AFC declining significantly with age. Limitations included variability in AFC measurements, the lack of a fertile comparison group, and the cross-sectional design. Zhao et al. [18] assessed the impact of AMH on IVF outcomes based on data from 909 women from Shenzhen Maternity and Child Healthcare Hospital who underwent IVF. A polynomial least-squares regression model identified the 10^{th} centile of AMH as an independent predictor of IVF success and demonstrated that lower AMH levels predicted poorer outcomes, particularly in those less than 35 years. The study is limited by the potential bias in patient selection due to AMH availability.

Havrljenko et al. [19] explored the impact of AMA on IVF outcomes, analyzing data from 491 women aged ≥35 at Ferona Fertility Clinic between January 2020 and May 2021. The study found that age significantly decreases oocyte and embryo quality, leading to lower pregnancy and live birth rates, with women aged ≥42 showing the greatest decline. Limitations included the focus on autologous IVF without considering oocyte donation as an option for women with poor ovarian reserve. Fancsovits et al. [20] compared the efficiency of conventional IVF versus ICSI in patients with AMA, low oocyte number, and non-severe male factor infertility. The prospective randomized trial involved 336 IVF cycles and found no significant difference in embryo quality, implantation, clinical pregnancy, or live birth rates between the two groups, though conventional IVF showed a higher fertilization rate. The main constraint was the relatively small sample size, especially in subgroup analyses.

Lebovitz et al. [21] examined the differences in early embryonic development in women of young and AMA and to identify embryonic development with pregnancy outcomes. The data were analysed from 2021 oocytes produced in 364 ICSI cycles through time-lapse imaging, revealing faster embryonic development in younger women with embryos that result in pregnancy. Limitations of the study included its retrospective design and inclusion of cycles beyond the first ICSI cycle. Liu et al. [22] investigated the causes of chromosome abnormalities in offspring of older pregnant women, focusing on factors like spindle assembly checkpoint dysfunction, telomere shortening, and oxidative stress. The study explored how these age-related cellular changes contribute to increased aneuploidy in oocytes and embryos, ultimately affecting pregnancy outcomes. Limitations included the lack of empirical data from specific studies on screening strategies and treatment techniques for older women with elevated risks of chromosomal abnormalities.

Glick et al. [23] analysed the risks and management strategies for pregnancies in women of AMA, emphasizing maternal and foetal complications. The study highlighted risks like miscarriage, preterm labor, and chromosomal abnormalities and recommended interventions such as pre-conception counselling, low-dose aspirin, and non-invasive genetic testing. Kortekaas et al. [24] investigated the association between AMA and pregnancy outcomes using data from the Netherlands Perinatal Registry (1999-2010). The cohort included low-risk women with singleton pregnancies, and the results showed that AMA was linked to increased risks of both maternal and perinatal adverse outcomes, particularly in women aged ≥40. Limitations included the exclusion of women with comorbidities like hypertension and diabetes and potentially underestimating the effects of AMA.

While studies have examined individual factors such as AMH levels and oocyte quality, few have focused specifically on how AMA influences these elements within the context of ICSI, a widely used fertility treatment. Moreover, the complex interplay between AMH regulation, oocyte maturation, embryo quality, and pregnancy success in older women remains underexplored. This gap suggests a need for comprehensive studies that integrate these factors to provide a clearer understanding of how AMA affects reproductive outcomes in ICSI, potentially guiding better clinical strategies and personalized treatment approaches for women of advanced reproductive age.

3. MATERIALS AND METHODS

3.1 Study Population and sampling

This study involved 240 female patients undergoing stimulated ICSI cycles at the Bliss Fertility Centre in Kottayam, Kerala, between January 2022 and August 2024. To examine the influence of maternal age on AMH levels, oocyte quality, embryo competency, and pregnancy outcomes, the participants were divided into five different age groups. The age group between 25 and 31 comprised the greatest portion of the sample (39.6%), followed by the group between 35 and 37 (24.6%). Of the patients, 20.4% were between the ages of 32 and 34, 11.3% were between the ages of 38 and 40, and 4.2% were older than 40, showing a wide span of reproductive ages. The data regarding the age, causes of infertility, and important clinical factors like AMH levels, oocyte numbers and quality, and embryo grading were collected from patients by taking history, blood tests, and transvaginal scans. Understanding the effect of maternal age on reproductive outcomes in ICSI cycles is made possible by this age distribution. The fertility profile was categorized based on reproductive factors like DOR, tubal factors, unexplained infertility, etc., which influence pregnancy outcomes. Women with good physical and mental health, two ovaries, an intact uterus, and those who are undergoing the first or second ICSI cycles were chosen for the study. Women with endometriosis, poly cystic ovarian syndrome, and severe gynaecological and medical diseases were excluded from the study. Female partners with their male partners who are having azoospermia, oligoasthenotertozoospermia (OAT) or severe OAT, all cases of variation in spermatogenesis, including surgically and frozen retrieved sperm, were also omitted in the study.

3.2 Estimation of AMH in female partners of the study population

AMH serves as an indicator of the quality of remaining eggs in the ovaries, which is crucial for predicting fertility outcomes and determining the ovarian reserve. To evaluate the ovarian responsiveness to simulation, AMH levels were measured for all the patients undergoing ICSI using a fully auto-chemiluminescence immune assay analyzer (Maglums 800, Shenzhen New Industries Bio Medical Engineering Company Limited). This method is based on a sandwich chemiluminescence immune assay technique, which allows for precise and reliable detection of AMH levels in the blood. Unlike other reproductive hormones, AMH levels are stable throughout the cycle, making it suitable as a perfect biomarker for this study and declining with age, offering crucial data about a woman's ovarian reserve and potential for conception. Typically, women's AMH levels increase during adolescence, peak at age 25, and then progressively decrease as they become older. The values were measured in ng/mL, or nanograms per milliliter. The AMH reference range values for each condition are mentioned in Table 1.

Table 1. AMH reference values for each level of fertility.

Fertility Level	AMH reference range values (in ng/mL)
High	> 6.8
Optimal	4.0 - 6.8
Satisfactory	2.2 - 4.0
Low	0.30 - 2.2
Very low	0.00 - 0.3

***Adapted from** Meczekalski et al., 2016 and Cedars, 2022 [25, 26].

High AMH indicates a strong ovarian reserve, commonly seen in younger women with high fertility potential, while optimal AMH suggests healthy ovarian reserve and excellent fertility potential. Satisfactory AMH reflects adequate ovarian reserve, but with some decline in fertility. Low AMH suggests reduced ovarian reserve, and very low AMH indicates minimal ovarian reserve and a significantly reduced chance of conception. This classification helps in evaluating the relationship between maternal age, ovarian reserve, and fertility outcomes in ICSI cycles.

3.3 Oocyte Quality, Embryo Grading, and Pregnancy Outcomes in ICSI Cycles

The study evaluated the quality of oocytes retrieved during ICSI in 240 patients. The number of oocytes retrieved from each case was noted soon after ovum aspiration. Oocyte quality was recorded through Samsung SDNR mounted on Olympus and processed with OPT scopes Olympus 1X73P2F at the time of ICSI. This setup allows high-resolution imaging of oocyte structure to identify any morphological abnormalities, such as irregular cytoplasm, dark cytoplasm, etc., that affect the oocytes and the development of the embryo. By examining these factors, the study aimed to correlate oocyte morphology with factors such as maternal age and AMH levels, which are known to influence oocyte quality and fertility potential.

One crucial component of oocyte quality is the maturity of the oocyte. For the oocyte to be fertilized by sperm, it should progress to the metaphase II (MII) stage of meiosis. The maturity of the oocyte is a crucial component of oocyte quality. For the oocyte to be fertilized by sperm, it must progress to the MII stage of meiosis. Oocytes at earlier phases of meiosis (such as the Germinal Vesicle (GV) or Metaphase I (MI)) are capable of fertilization, whereas MII oocytes are considered mature and ready for fertilization. Based on the proportion of oocytes that advanced to the MII stage, the study evaluated the oocytes' maturity. Because the percentage of mature oocytes retrieved after an ICSI cycle is a major factor in determining the likelihood of successful fertilization and embryo development. MII assessment is an essential component of the study since inaccurate or incomplete maturation can lower the chance of attaining pregnancy. Table 2 presents the details of oocyte maturity.

Table 2. Categorization of MII oocyte maturity and its associated fertility potential

Maturity Bracket	Description
<50% MII	Less than half of the oocytes were mature; low maturation rate and potentially reduced fertility.
50–74% MII	Majority of the oocytes were mature; satisfactory response and moderate fertility potential.
75–100% MII	Nearly all oocytes were mature; optimal response and higher likelihood of successful fertilization.

This classification of maturity helped in quantify oocyte development in response to stimulation, providing a measurable indicator of fertility readiness across age groups. After fertilization, on day 3, the embryos were observed and graded based on their morphology to predict their ability to implant and develop into a healthy pregnancy.

Table 3 represents the grading of the embryo. The success rate of IVF or ICSI treatments is directly linked with the ability of embryos to implant and develop after transfer. The Beta HCG (Human Chorionic Gonadotropin) reports were obtained 14 days after FET to evaluate the success of ART. Pregnancy was indicated by the presence of increased HCG levels.

Table 3. Embryo grading

Grade	Interpretation
Grade A	Good-quality embryos with optimal development, higher chance of successful implantation and pregnancy.
Grade B	Average-quality embryos with moderate development, lower chance of success compared to Grade A but still reasonable.
Grade C	Poor-quality embryos with compromised development, less likely to implant or develop into a viable pregnancy.

3.4 Statistical Analysis

Categorical and quantitative variables were stated as frequency (percentage) and mean ± SD, respectively. A chi-square test was employed to find associations between categorical variables. Receiver Operating Characteristic (ROC) graphs were plotted, and the area under the curve (AUC) was considered to measure the diagnostic accuracy of maternal age in predicting optimal fertility, good-quality embryos, and positive maternal outcomes in ICSI cycles and to assess the optimal cut-off scores. Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) and accuracy have been considered for the diagnostic accuracy of maternal age. Simple logistic regression was used to predict optimal fertility, good quality embryos, and positive maternal outcomes using maternal age in ICSI cycles of metabolic syndrome and renal dysfunction. A Z test for proportion was used to compare outcomes between ICSI and PIEZO ICSI. Statistical significance was defined as $p<0.05$ for all statistical interpretations. The statistical software program SPSS, version 20.0, was used to perform the statistical analyses.

4. RESULTS

The study aimed to evaluate the effects of maternal age on various factors influencing the outcomes of ICSI cycles. Table 4 represents the background characteristics of women who underwent ICSI. The study sample's age distribution shows that most women receiving ICSI were in their early to mid-30s. In addition, the participant's mean age was 33.1 ± 4.4 years; the cohort consisted mainly of women in their early to mid-30s, a crucial age range for fertility. The data highlights the difficulties associated with AMA in ART by demonstrating that the number of women in the older age groups declines as maternal age rises.

Table 4. Percentage distribution of the sample based on age

Age	Count	Percent
25 - 31	95	39.6
32 - 34	49	20.4
35 - 37	59	24.6
38 - 40	27	11.3
>40	10	4.2
Mean ± SD	33.1 ± 4.4	

The female factors contributing to infertility are provided in Table 5. The DOR was the most common factor, followed by unexplained infertility and tubal factor infertility. AMA accounted for 2.9%, while recurrent implantation failure and multiple IVF failures were rare. These findings suggest that DOR and unexplained infertility are the primary reasons women seek ICSI.

Table 5. Percentage distribution of the sample based on female factor

Female factors	Count	Percent
DOR	81	33.8
Sterilized	2	0.8

Poor Responder	19	7.9
Tubal factor	50	20.8
Unexplained	76	31.7
AMA	7	2.9
Recurrent implantation failure	2	0.8
Multiple IVF Failure	1	0.4
DOR, tubal factor	2	0.8

Table 6 illustrates the descriptive statistics regarding AMH in ICSI cycles. The descriptive statistics for AMH levels in the study sample show a mean of $2.83 \pm 2.05\ ng/mL$, indicating a moderate average AMH level across the cohort. The median AMH value was $2.12\ ng/mL$, with an interquartile range (IQR) of 1.16 to $4.7\ ng/mL$, suggesting that half of the participants had AMH levels between 1.16 and $4.7\ ng/mL$. The minimum AMH level recorded was $0.01\ ng/mL$, and the maximum was $9.00\ ng/mL$, reflecting a wide range of ovarian reserve in the study population. Figure 1 represents the boxplot for AMH in ICSI cycles.

Table 6. Descriptive statistics regarding AMH in ICSI Cycles

Mean ± SD	2.83 ± 2.05
Median (IQR)	2.12 (1.16 - 4.7)
Minimum	0.01
Maximum	9.00

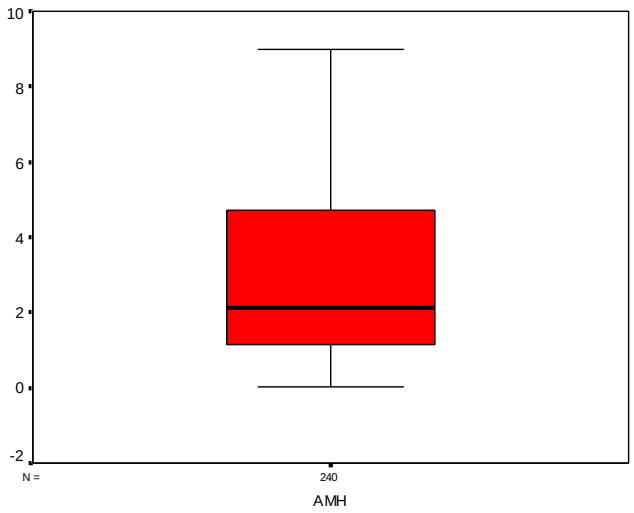

Figure 1. Box plot for AMH in ICSI Cycles

Table 7 demonstrates the percentage distribution of the sample based on AMH. The majority of the participants exhibited low fertility, indicating DOR, when classified by reproductive potential based on AMH levels. A significant percentage of women had adequate ovarian reserve, indicating optimal fertility. A very small percentage had high levels of AMH, indicating excellent ovarian reserve, whereas a smaller group had satisfactory fertility, indicating moderate ovarian reserve. On the other hand, only a small number of women were categorized as having extremely low fertility, indicating a significantly higher DOR that has an adverse effect on the quality of oocytes and overall fertility potential.

Table 7. Percentage distribution of the sample according to AMH

AMH	Count	Percent
Very low fertility	5	2.1
Low fertility	124	51.7
Satisfactory Fertility	40	16.7
Optimal Fertility	65	27.1
High level	6	2.5

Table 8 provides the percentage distribution of the sample according to the oocyte obtained. It reveals varying responses to ovarian stimulation during the ICSI cycles. The majority of participants retrieved between 1 and 10 oocytes, with a significant portion of women obtaining 6 to 10 oocytes. The mean number of oocytes retrieved was 8.5, with a standard deviation of 5.2, indicating some variation in ovarian response across the sample. These findings suggest that while many women were able to retrieve a moderate number of oocytes, there were cases with poor ovarian response, which could be influenced by factors such as ovarian reserve, age, and underlying fertility conditions.

Table 8. Percentage distribution of the sample according to oocyte obtained

Oocyte obtained	Count	Percent
0	2	0.8
$1 - 5$	76	31.7
$6 - 10$	91	37.9
$11 - 15$	45	18.8
> 15	26	10.8
Mean $\pm$ SD	8.5 ± 5.2	

Table 9 shows the percentage distribution of the sample according to the percentage of MII oocyte among the total oocytes obtained. According to the study, a significant percentage of the retrieved oocytes are MII, with most patients having 75–100% of their oocytes reach the MII stage, which is optimal for fertilization.

Table 9. Percentage distribution of the sample according to percentage of MII oocyte among total oocyte obtained

Percentage of MII oocyte among total oocytes obtained	Count	Percent
0	1	0.4
< 50	16	6.7
$50 - 74$	62	26.1
$75 - 100$	159	66.8
Mean ± SD	79.6 ± 20.1	

Table 10 shows the percentage distribution of the sample according to oocyte quality. A majority of oocytes were classified as “Good” with 72.9% of the retrieved oocytes showing normal morphology, while a small percentage presents abnormalities like large perivitelline space (PVS), fragile cytoplasm, dark cytoplasm, and fragmented polar bodies, which negatively affect the fertilization potential. The presence of these abnormalities in the remaining oocytes reduces their ability to develop into healthy embryos.

Table 10. Percentage distribution of the sample according to oocyte quality

Oocyte quality	Count	Percent
Good	175	72.9
Thick zona	3	1.3
large PVS	13	5.4
Irregular cytoplasm	2	0.8
Dark cytoplasm	7	2.9
debris in PVS	4	1.7
Fragmented polar body	7	2.9
Fragile cytoplasm	13	5.4
Central pit	2	0.8
Thick zona	1	0.4
vacuolated cytoplasm	3	1.3
Large PB	3	1.3
Granular cytoplasm	7	2.9
Not Good	**65**	**27.0**
Good	**175**	**72.9**

Table 11 demonstrates the percentage distribution of the sample according to embryos obtained. The majority of samples yielded between 1 and 3 embryos, while a significant number produced between 6 and 10 embryos. With a standard deviation of 3.6 and a mean of 5.2 embryos produced, the data showed considerable variation.

Table 11. Percentage distribution of the sample according to embryos obtained

Embryos obtained	Count	Percent
0	11	4.6
1 - 3	83	34.6
4 - 5	58	24.2
6 - 10	70	29.2
>10	18	7.5
Mean ± SD	5.2 ± 3.6	

Table 12 shows the percentage distribution of the sample according to the outcome in ICSI cycles. More than half cases had a favorable pregnancy outcome, indicating the success rate for the procedure in the study population. 42.9% of the cases had negative results, demonstrating the difficulties that fertility treatments can provide, particularly due to AMA or DOR. These findings highlight the possibility of getting pregnant using ICSI.

Table 12. Percentage distribution of the sample according to outcome

Outcome	Count	Percent
NA	10	4.2

Negative	103	42.9
Positive	127	52.9

1.1 Effect of Maternal Age on AMH

The study analyzed the effect of maternal age on AMH. Table 13 illustrates the distribution of AMH levels by age groups and fertility status. Table 14 shows the diagnostic accuracy, which highlights maternal age as a predictor of optimal fertility, evaluated through AMH levels.

Table 13. Distribution of AMH Levels by Age Group and Fertility Status

Age	**AMH**		
	Optimal Fertility	**Not Optimal Fertility**	**Total**
≤30	58	2	60
>30	7	173	180
Total	65	175	240

Table 14. Diagnostic accuracy of prediction of optimal fertility assessed using AMH using maternal age

Evaluation metrics	**Values (%)**
Sensitivity	89.2
Specificity	98.9
False negative (FN)	10.8
False positive (FP)	1.1
Negative predictive value (NPV)	96.1
Positive predictive value (PPV)	96.7
Negative likelihood ratio	0.1
Positive likelihood ratio	78.1
Accuracy	96.3

Women aged ≤30 were correctly identified with optimal fertility in 58 out of 60 cases, resulting in a sensitivity of 89.2%. Similarly, among those aged >30, 173 out of 180 were accurately classified as having non-optimal fertility, yielding a specificity of 98.9%. The PPV indicates that 96.7% of women identified as having optimal fertility were correctly classified. The NPV reflects that 96.1% of women identified as having non-optimal fertility were correctly classified. The FN rate was 10.8%, and the FP rate was only 1.1%, showcasing minimal misclassification. With an overall accuracy of 96.3%, this data reinforces maternal age as a highly reliable predictor of fertility outcomes when assessed via AMH levels.

Figure 2 represents the scatter diagram showing the correlation between maternal age and AMH. AMH levels decrease with increasing maternal age, indicating a negative correlation with $r = -0.611, p < 0.01$. The fitted regression line in the scatter plots demonstrates the inverse relation. This decrease demonstrates the importance of age in the fertility evaluation, which highlights the natural reduction in ovarian reserve.

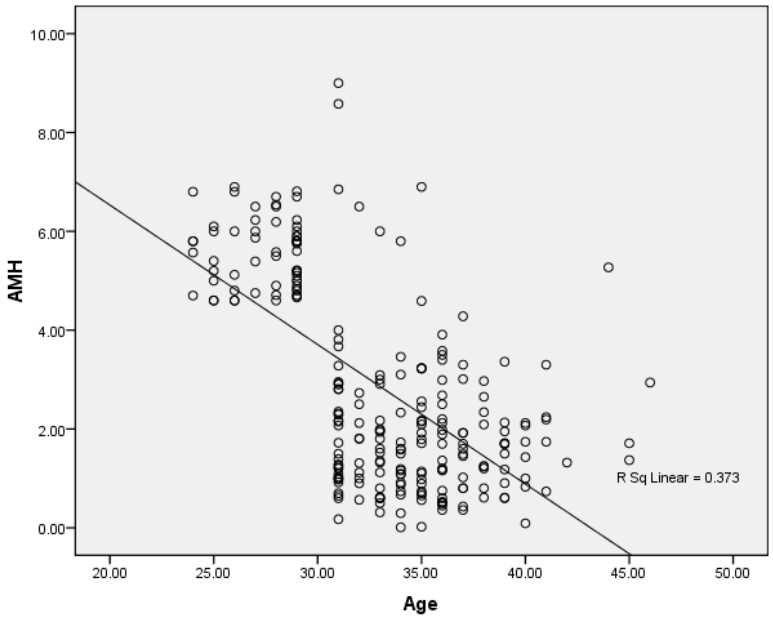

Figure 2. Scatter diagram Showing correlation between maternal age and AMH

Figure 3 shows the ROC curve showing the predictive accuracy of maternal age for optimal fertility based on AMH levels. The AUC was 0.944 (0.900 – 0.987), $p < 0.01$, demonstrating the accurate predictive capability. According to the analysis, the ideal cutoff for differentiating between women with optimal and non-optimal fertility is 30. The high AUC suggests maternal age as a strong predictor of fertility outcome.

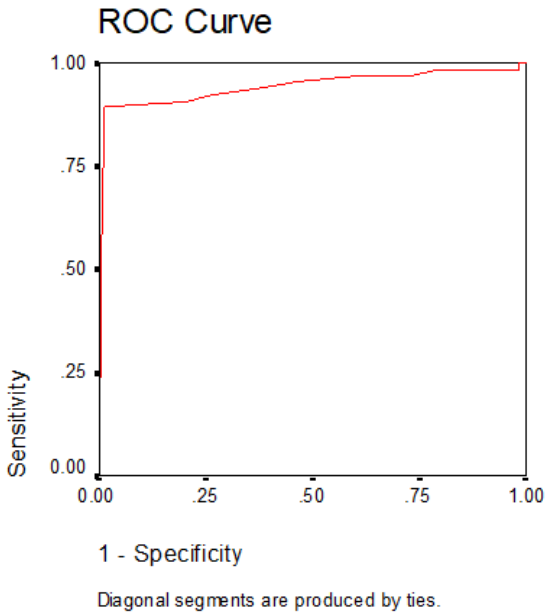

Figure 3. ROC Curve

Table 15 shows the association of maternal age and optimal fertility assessed by AMH and was highly significant ($\chi^2 = 97.81, p < 0.01$). Women aged between 25 and 31 showed the highest proportion of optimal fertility by maintaining optimal AMH levels of 62.1%. 96.6% of women aged between 35 and 37 and 97.3% aged greater than 37 were found to be having non-optimal fertility as it gradually increased with age. This trend highlights a critical age threshold, as fertility appears to decline significantly after 31 years.

Table 15. Association of maternal age and optimal fertility assessed by AMH

Age	AMH				χ^2	p
	Not Optimal Fertility		Optimal Fertility			
	Count	Percent	Count	Percent		
25 - 31	36	37.9	59	62.1	97.81	p<0.01
32 - 34	46	93.9	3	6.1		
35 - 37	57	96.6	2	3.4		
>37	36	97.3	1	2.7		

The impact of maternal age on fertility is quantified using the logistic regression model as given in Table 16. Women aged 25-31 had higher odds of maintaining optimal fertility than those of the reference group (>37). This finding demonstrates that women in this younger age group are significantly more likely to sustain optimal fertility.

Table 16. Prediction of optimal fertility assessed by AMH using maternal age using logistic regression

Age	B	Std. Error	P	Odds (95% CI)
25 - 31	4.08	1.04	0.000	58.99 (7.75 - 448.95)
32 - 34	0.85	1.18	0.468	2.35 (0.23 - 23.52)
35 - 37	0.23	1.24	0.851	1.26 (0.11 - 14.44)

However, the odds ratios for women aged 32–34 and 35–37 were not statistically significant, indicating a steep decline in fertility after 31 years. This finding emphasizes the critical role of younger maternal age in maintaining fertility potential. The analysis provides strong evidence that maternal age is a key determinant of fertility, as reflected by AMH levels.

4.1 Effect of Maternal Age on Oocyte Quality

The study analysed the outcome of maternal age on oocyte quality as shown in Table 17. The table highlights a significant association between maternal age and oocyte quality ($\chi^2 = 29.69, p < 0.01$). Conversely, women over 37 exhibited the poorest outcomes, with only 43.2% having good-quality oocytes. This trend suggests a decline in oocyte quality with increasing maternal ages, emphasizing the detrimental effect of age on reproductive potential.

Table 17. Association of maternal age and oocyte quality

Age	Oocyte quality				χ^2	p
	Not Good		Good			
	Count	Percent	Count	Percent		
25 - 31	11	11.6	84	88.4	29.69	$p < 0.01$
32 - 34	18	36.7	31	63.3		
35 - 37	18	30.5	41	69.5		
>37	21	56.8	16	43.2		

Figure 4 shows different morphologies of oocytes where there is a clear differentiation of normal and abnormal oocytes. Patients below 30 years with optimal AMH obtained MII oocytes with good quality, 31-37 years with suboptimal AMH obtained MII oocytes with morphological abnormalities like irregular cytoplasm, debris in PVS, and dark cytoplasm and patients with age 37 years and above mostly failed to obtain good oocytes. It was also seen that the number of metaphase II oocytes dropped as age increased.

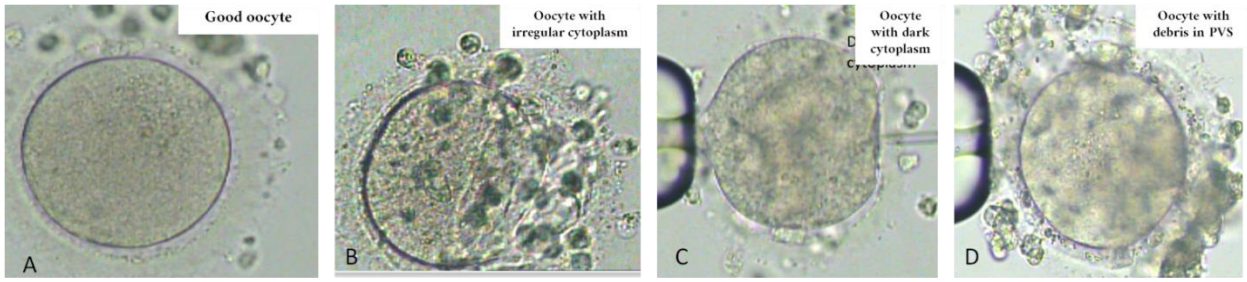

Figure 4. Oocyte Morphology (A) Good oocyte; (B) Oocyte with irregular cytoplasm; (C) Oocyte with dark cytoplasm; (D) Oocyte with debris in perivitelline space (PVS).

The impact of maternal age on oocyte quality is further confirmed by logistic regression analysis. Table 18 shows that the women aged 25-31 had a significantly increased chance of producing high-quality oocytes with an odds ratio of 10.02; $p < 0.001$ compared to women over 37. These findings highlight the advantage in oocyte quality among women under age 31.

Table 18. Prediction of oocyte quality by maternal age using logistic regression

Age	B	Std. Error	P	Odds (95% CI)
25 - 31	2.30	0.46	0.000	10.02 (4.06 - 24.76)
32 - 34	0.82	0.44	0.067	2.26 (0.95 - 5.41)
35 - 37	1.10	0.44	0.012	2.99 (1.27 - 7.03)

The relationship between maternal age and the proportion of MII oocytes among the total number of oocytes obtained is shown in Table 19. The percentage of MII oocytes in the 25–31 age group was 67.4% and was statistically significant ($p = 0.043$). The percentage of MII oocytes declined for older age groups, suggesting that oocyte quality decreases with maternal age. The percentage of MII oocytes and maternal age are significantly correlated, according to the statistical analysis ($\chi^2 = 8.17$), underscoring the effect of age on oocyte quality, especially in the older age groups.

Table 19. Association of maternal age and percentage of MII oocyte among total oocyte obtained

Age	Percentage of MII oocyte among total oocytes obtained				χ^2	p
	<75		75 - 100			
	Count	Percent	Count	Percent		
25 - 31	31	32.6	64	67.4	8.17*	0.043
32 - 34	22	44.9	27	55.1		
35 - 37	12	20.3	47	79.7		
>37	14	40.0	21	60.0		

Logistic regression analysis revealed a significant association between maternal age and the percentage of MII oocytes among total oocytes obtained, as in Table 20. Women aged 35–37 had significantly higher odds of achieving a favorable percentage of MII oocytes compared to those over 37. However, the associations for women aged 25–31 and 32–34 were not statistically significant. These results suggest that while age impacts oocyte quality and maturity, women in their mid-30s still exhibit relatively favorable outcomes compared to those over 37.

Table 20. Prediction of good percentage of MII oocyte among total oocyte obtained by maternal age using logistic regression

Age	B	Std. Error	P	Odds (95% CI)
25 - 31	0.32	0.41	0.434	1.38 (0.62 - 3.07)
32 - 34	-0.20	0.45	0.655	0.82 (0.34 - 1.97)
35 - 37	0.96	0.47	0.042	2.61 (1.03 - 6.6)

4.2 Effect of Maternal Age on Embryo Competency

The analysis explores the effect of maternal age on the percentage of good-quality embryos (defined as ≥ 50% of embryos being classified as good quality). The ROC curve analysis for predicting sufficient embryo quality based on maternal age shows an AUC of 0.534 and a p −value of 0.425, as shown in Figure 5. This indicates that maternal age is not a strong predictor of good-quality embryos, as the AUC is close to 0.5, which suggests no better predictive value than random chance. The best cut-off point for maternal age to predict good-quality embryos was found to be 27.5 years, however, this threshold does not appear to significantly improve prediction.

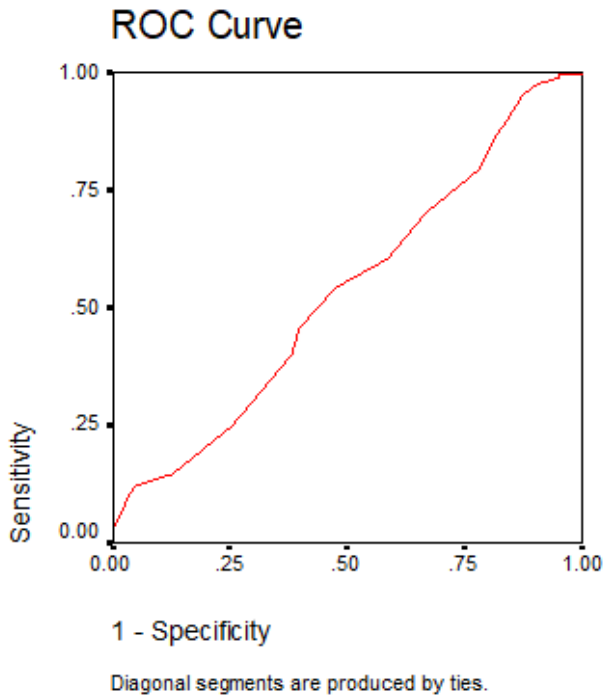

Figure 5. ROC Curve

Table 21 shows the association between maternal age and the percentage of good quality embryos, with no significant differences across age groups ($p > 0.05$). Women aged 25-31 produced good-quality embryos. The chi-square test (χ^2 =0.97) and the p value of 0.808 indicate that maternal age does not significantly influence the proportion of good-quality embryos, suggesting that other factors play a more crucial role in embryo quality than maternal age alone. Figure 6 shows the embryo grading.

Table 21. Association of maternal age and percentage of good quality embryos

Age	Percentage of Good Quality Embryos				χ^2	p
	<50 %		≥50%			
	Count	Percent	Count	Percent		
25 - 31	24	25.3	71	74.7	0.97	0.808
32 - 34	13	26.5	36	73.5		
35 - 37	14	23.7	45	76.3		
>37	12	32.4	25	67.6		

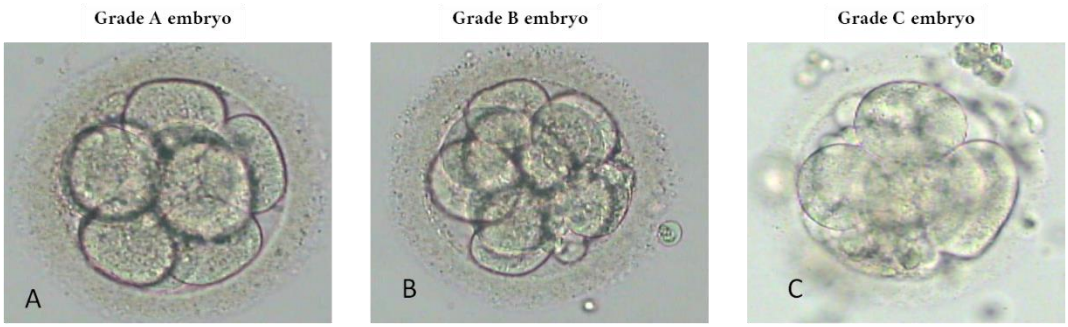

Figure 6. Embryo Grading. (A) good-quality embryo is graded as grade a; (b) average-quality embryo is graded as grade b; (c) poor-quality embryo is graded as grade c

1.2 Effect Of Maternal Age on Pregnancy Outcome

The study analyzed the effect of maternal age on pregnancy outcome. Figure 7 shows a ROC curve for prediction of positive pregnancy outcome using maternal age. Accordingly, maternal age has a predictive ability for positive pregnancy outcomes, with an AUC of 0.674. The optimal cutoff age was found to be 32.5, demonstrating that the maternal age below this threshold is associated with a higher chance of successful pregnancy.

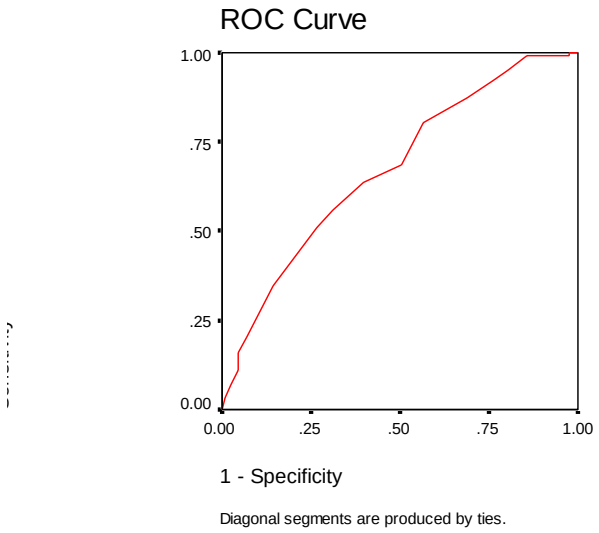

Figure 7. ROC curve for prediction of positive pregnancy outcome using maternal age

Table 22 and Table 23 illustrate the distribution of predicting pregnancy outcome and its diagnostic accuracy, respectively, using maternal age. A cut-off age of 32.5 years offers moderate pregnancy outcomes based on the maternal age. For women

aged >32.5, 56 had positive outcomes and 78 had negative outcomes, showing a decline in positive outcomes with increasing age. Positive and negative cases were identified with moderate accuracy with 55.9% sensitivity and 69.0% specificity. While the NPV showed lower reliability for predicting negative outcomes in older women, the PPV indicated a moderate probability of positive results for women ≤32.5. Additionally, the positive and negative likelihood ratio reflected moderate odds variations with an accuracy of 62.1%.

Table 22. Distribution of predicting pregnancy outcomes

Age	**Pregnancy outcome**		
	Positive	**Negative**	**Total**
<=32.5	71	35	106
>32.5	56	78	134
Total	127	113	240

Table 23. Diagnostic accuracy of prediction of pregnancy outcome using maternal age

Sensitivity	55.9
Specificity	69.0
False positive	31.0
False Negative	44.1
Negative predictive value	58.2
Positive predictive value	67.0
Negative likelihood ratio	0.6
Positive likelihood ratio	1.8
Accuracy	62.1

Maternal age and pregnancy outcomes were found to be significantly correlated by the chi-square analysis ($\chi^2 = 20.49, p < 0.01$) as given in Table 24. Positive pregnancy outcomes were most common among women aged 25–31, followed by those aged 32–34 and 35–37. However, just 27.0% of women over 37 had favourable results. The negative impact of increased mother age on the success of pregnancies, especially after the age of 37, is further supported by this finding.

Table 24. Association of maternal age and pregnancy outcome

Age	**Pregnancy outcome**				χ^2	**p**
	Negative		**Positive**			
	Count	**Percent**	**Count**	**Percent**		
25 - 31	30	31.6	65	68.4	20.49	p<0.01
32 - 34	27	55.1	22	44.9		
35 - 37	29	49.2	30	50.8		
>37	27	73.0	10	27.0		

Pregnancy outcomes are highly impacted by maternal age, according to the logistic regression analysis given in Table 25.

The odds of a positive outcome were almost six times higher for women aged 25–31 than for those over 37 ($p < 0.001$). Additionally, the odds of successful outcomes were considerably greater for women aged 35–37, although this correlation was not statistically significant for those aged 32–34 ($p = 0.092$). These findings highlight the crucial role of younger maternal age in increasing the likelihood of successful pregnancies.

Table 25. Prediction of pregnancy outcome using maternal age using logistic regression

Age	B	Std. Error	P	Odds (95% CI)
25 - 31	1.77	0.43	0.000	5.85 (2.51 - 13.61)
32 - 34	0.79	0.47	0.092	2.2 (0.88 - 5.51)
35 - 37	1.03	0.45	0.023	2.79 (1.15 - 6.78)

4.3 Comparison of ICSI Cycles and PIEZO ICSI Outcomes

The study evaluates the outcomes of conventional ICSI and PIEZO ICSI procedures in terms of embryo yield, embryo quality (>50%), and positive pregnancy rates among women with at least one MII oocyte, using the Z-test to assess statistical significance. Table 26 demonstrates the comparison of at least one embryo obtained among those who have at least one MII oocyte between ICSI Cycles and PIEZO ICSI. The comparison between embryo yield between ICSI cycles and PIEZO ICSI proved that 96.6% of patients in ICSI cycles and 100% in PIEZO ICSI recovered at least one embryo. Although the success rate was slightly higher for PIEZO ICSI, there was no statistical significance. The results demonstrate that both techniques were excellent at obtaining embryos, and with at least one MII oocyte available, piezo ICSI has the higher advantage.

Table 26. Comparison of at least 1 embryo obtained among those who have at least 1 MII oocyte between ICSI Cycles and PIEZO ICSI

At least 1 embryo obtained	Group				*Z*	*p*
	ICSI Cycles		PIEZO ICSI			
	Count	Percent	Count	Percent		
Yes	228	96.6	15	100.0	0.726	0.4716
No	8	3.4	0	0.0		

Of patients that retrieved at least one MII oocyte, 74.6% in ICSI and 86.77% in PIEZO ICSI had more than 50% good-quality embryos, as shown in Table 27. The good-quality embryos proportion was considerably higher in the PIEZO ICSI; however, the difference was not statistically significant ($Z = 1.062, p = 0.2892$). This implies that both methods have a high capacity to obtain good-quality embryos and that the only benefit of slight improvements in embryo quality is given by PIEZO ICSI.

Table 27. Comparison of percentage of good quality embryo obtained among those who have at least 1 MII oocyte between ICSI Cycles and PIEZO ICSI

Percentage of good-quality embryo	Group				*Z*	*p*
	ICSI Cycles		PIEZO ICSI			
	Count	Percent	Count	Percent		
>50 %	176	74.6	13	86.77	1.062	0.2892
<50 %	60	25.4	2	13.23		

For patients who had ICSI cycles, as given in Table 28, the percentage of pregnancies achieved was 55.5%, and in the group

under PIEZO ICSI, it was 53.3%. In both cases, the differences were very small and not statistically significant ($Z = 0.166, p = 0.8728$). Therefore, there was an affirmation that both methods were equal to each other in success rate regarding positive outcomes regarding pregnancy.

Table 28. Comparison of percentage of positive outcome among those who have at least 1 embryo obtained between ICSI Cycles and PIEZO ICSI

Outcome	Group				Z	p
	ICSI Cycles		PIEZO ICSI			
	Count	Percent	Count	Percent		
Positive	127	55.5	8	53.3	0.166	0.8728
Negative	102	44.5	7	46.7		

5. DISCUSSION

The findings of this study are important descriptions of how AMA affects outcomes in the ICSI cycle, in particular in terms of levels of AMH, quality of oocytes, competency of the embryo, and likelihood of pregnancy. In agreement with Gunasheela et al., this study found significant declines in AMH levels with increasing maternal age, signifying decreased ovarian reserve in women older than 35 years of age [17]. The data underscore the predictive accuracy of maternal age for fertility potential using AMH with a specificity and sensitivity of 98.9% and 89.2%, respectively. Chi-square analysis revealed a precipitous decline in live birth rates among women aged over 37 years. This is consistent with previous studies pointing to AMA as adversely affecting implantation and pregnancy maintenance. AMH thus serves as a potential biomarker, reflecting the depleted follicular pool with aging. High AMH levels were maintained among younger women, while those over 37 years showed drastically depleted reserves, which was observed by Zhao et al.; this group has proved that low AMH is predictive of poor outcomes of ART [18]. This further proves the role of AMH as an indicator of fertility in women seeking ICSI.

Maternal age was strongly associated with declining oocyte quality, as shown by the lower proportion of MII oocytes and increased morphological abnormalities in older women. Similar findings were reported by Havrljenko et al., where women aged over 42 displayed the greatest decline in oocyte quality. This study supports the conclusion that the biological aging of oocytes significantly impairs ART outcomes [19]. Liu et al. emphasized that factors such as oxidative stress and spindle assembly dysfunction exacerbate chromosomal abnormalities in the oocytes of AMA patients, further diminishing their competence [20].

Although the correlation between maternal age and embryo quality was not statistically significant in this study, previous research suggests otherwise. Aghajani et al. found that chromosomal aneuploidy rates increased with maternal age, leading to reduced blastocyst formation [15]. Similarly, Lebovitz et al. demonstrated that embryos derived from younger women exhibited faster developmental kinetics, correlating with higher implantation success. These discrepancies suggest that factors beyond maternal age, such as laboratory techniques or embryo culture conditions, influence outcomes [21]. The decline in pregnancy success with increasing age is a consistent finding in ART studies. Wang et al. showed that clinical and ongoing pregnancy rates dropped significantly after age 34, corroborating the present study's findings of reduced positive outcomes in women over 37 [16]. Furthermore, Kortekaas et al. reported increased risks of adverse pregnancy outcomes, such as miscarriage and preterm labor, in AMA patients [24]. These data underscore the need for early intervention to improve reproductive outcomes in this population.

The findings of the study agree with the emerging perspective that individualized fertility treatment is important for older women. Developing ovarian stimulation protocols based on AMH levels, as suggested by Zhao et al., could optimize oocyte retrieval [18]. Additionally, preimplantation genetic testing, as recommended by Aghajani et al. [15], improves embryo selection and implantation success rates in AMA populations. Future work should include prospective multicenter trials to study the long-term effects of AMA on ART outcomes. Furthermore, exploring the potential benefits of novel ART techniques, such as PIEZO ICSI, could provide additional avenues for improving embryo quality and implantation rates.

6. CONCLUSION

This study shows the profound impact of AMI on ICSI outcomes. Women over 35 years experiences a decline in AMH levels, oocyte quality, and positive pregnancy rates, emphasizing the challenges posed by age-related fertility decline. The findings revealed that AMH levels, a key marker of ovarian reserve, diminish significantly in women aged >37, aligning

with poorer oocyte quality and reduced pregnancy outcomes. Women aged 25–31 demonstrated the highest rates of good-quality oocytes (88.4%) and positive pregnancy outcomes (68.4%), while those aged >37 had substantially lower success rates (43.2% and 27%, respectively). These results underscore the personalized fertility strategies for AMA patients, such as optimizing ovarian stimulation protocols and integrating genetic screening to enhance embryo selection and implantation success. Given the global trend of delayed parenthood, this study offers critical insights for improving ART outcomes and guiding clinical practices. By prioritizing early fertility evaluations and individualized treatment approaches, healthcare providers improve the reproductive prospects of older women, ultimately addressing the increasing demand for ART in this high-risk population.

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