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The impact of advanced maternal age on fertility outcomes in IVF cycles in Al amal IVF Center-Misrata

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Abstract

Background: A delay in childbearing to later n life has increase the number of women of advance maternal age (AMA) opting for assisted reproductive, women should be made aware that age-related changes to fertility including decline in Oocyte reserve and quality in addition to increase in the number of Oocyte chromosomal aberration.

Objective: To evaluate the effect of maternal age on the outcomes of in vitro fertilization (IVF) and embryo transfer (ET) in fresh and frozen embryo cycles.

Materials and Methods: This is a retrospective observational cohort study from a last 1000 patients aged 40-48 years in sequence without exclusion, who underwent IVF with intra-cytoplasmic sperm injection in Al Amal IVF Center – Misrata. The study assess the embryo transfer rate, clinical pregnancy and miscarriage rates.

Results: The study includes last 1000 cases aged above 40 years old underwent ICSI/IMSI cycle. In fresh embryo cycle 500 cases and in frozen embryo cycle 500 cases, four groups of different maternal age periods were compared, (40-42) years old group, (42-44) years old group, (44-46) years old group and (46-48) years old group. The retrieved Oocyte number and clinical pregnancy rate were significantly lower in groups of higher maternal age, while the miscarriage rate was significantly higher in groups of good quality embryo.

Conclusion: Patient with higher maternal age had low IVF outcomes and patients over 40 years old have poor IVF outcome and high miscarriage rate, clinicians should be very cautious in counseling of this age group and give the expected incidence.

Keywords: Frozen embryo; In vitro fertilization; Embryo transfer

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Cohort and Case-Control Study

Introduction

Advanced maternal age (AMA) is a critical social and clinical issue. Women over the age of 35 are generally considered to be of advanced maternal age. Age is an important factor in infertility, and since women's fertility declines with age, assisted reproductive technology (ART) has become a preferred option for many women in this age group(1).To enhance the success rates of fertilization and high-quality embryo development in advanced maternal age, it has been recommended to prioritize the use of intra-cytoplasmic insemination(ICSI)over conventional insemination CI, with the rationale that ICSI can help mitigate challenges related to sperm-oocyte interaction and sperm penetration (2).The frequency of ICSI was 45% in 1997 and increased up to 70% in Europe in 2007; however, it shows a large variation worldwide (3,4).

Despite improvements in assisted reproduction technology procedures, live birth rates (LBRs) are still suboptimal, particularly for women of advanced maternal age. The lack of effectiveness in ART could be due to a variety of factors, but since 1990s, age-related embryo aneuploidy has been regarded as the most important factor influencing cycle outcomes (5, 6, 7).The aneuploidy rate in mature oocytes of women nearing 40 years of age is approximately 75% (8). In particular, the likelihood of generating a chromosomally normal blastocyst in women older than 43 year might be even lower than 5% (9,10). This can be attributed, on the one hand to the gradual depletion of the ovarian reserve, and on the other hand to the progressive decrease in oocyte/embryo competence, defined as the ability to produce a live birth (11,12).

In the United States of America, only 3.5% of births occur in women aged 40 years or older (13). A possible reason for this decrease in fertility can be the decline in ovarian reserves combined with age-related decreased endometrial receptivity and increasing aneuploidy rates in oocytes (14).

Conventionally, assisted reproductive technology (ART) involves fresh embryo transfer (ET) immediately following ovarian stimulation, but the mother may still have high estrogen levels from medications to increase egg production. This means her uterus may not be receptive to embryo implantation, risking miscarriage. However, with advancements in technology in recent years, there has been a notable increase in the use of thawed frozen embryo transfers (FETs).This approach allows for the use of frozen embryo at a later time, when the ovaries are not stimulated, thereby avoiding the non-physiological endometrium. This strategy reduces the risk of ovarian hyper-stimulation syndrome (OHSS),enhances implantation rates and pregnancy outcomes (15,16). Since delayed ET may facilitate a more receptive endometrium in the subsequent cycle, it could serve to mitigate the negative effects of controlled ovarian Hyper-stimulation (COH), as suboptimal endometrial development can reduce the likelihood of embryo implantation and result in lower pregnancy rates (17).
However, the outcomes of the ET strategy for euploid embryos require further investigation in AMA patients. In this study, our aim is to evaluate the effect of maternal age on in vitro fertilization (IVF) outcomes and embryo transfer (ET) in fresh and frozen embryo cycles.

Materials and Methods

This study was structured as a single-center cohort investigation. Its retrospective observational study from the last 1000 patients aged 40-48years in sequence without exclusion, who underwent IVF with intra-cytoplasmic sperm injection in Al amal IVF center-Misrata. These patients were treated with three different protocols (antagonist, short agonist and minimal protocol), with half of the cases undergoing a fresh embryo transfer and the other half undergoing a frozen- thawed embryo transfer.
The study assesses the clinical pregnancy rate and miscarriage rate across embryo transfer methods.

Inclusion criteria:

- Women aged 40-48 years who underwent IVF treatment during the study period.
- Women who were treated with one of the three protocols (antagonist, short agonist, or minimal protocol).
- Women who underwent either a fresh embryo transfer or a frozen-thawed embryo transfer.
- All patients with complete medical records for data collection.

Exclusion criteria:

- No exclusions, as the study aims to include all patients in the age group.

Ethical approval: The Misrata University Ethics Committee granted approval for this research. The study was conducted in compliance with guidelines established by the ethical policy throughout the research process. Informed consent was secured from each participant included in the study.

Statistical Analysis

The statistical analysis was conducted using **SPSS version 24**, which facilitated the execution of the various tests and provided a comprehensive overview of the data. A range of statistical methods was employed in this research, including:

1. **Chi-Square Test**: This was used to compare pregnancy and miscarriage rates between frozen and fresh embryos across different age groups. The significance level was set at $p < 0.05$ for acceptance or rejection of the null hypothesis.
2. **Logistic Regression Analysis**: This was utilized to explore the impact of age and type of embryo transfer on the occurrence of clinical pregnancy. The p-value was also used to assess the significance of the predictors in the model.
3. **Descriptive Statistics**: This was applied to calculate means and standard deviations for demographic and treatment data.

Sample Size

The study included a sample of **1000 cases (500 underwent the Frozen cycle and 500 underwent the Fresh cycle)** aged between **40 and 48**, categorized into specific age groups (40-42, 42-44, 44-46, and 46-48).

Results

Table 1: Distribution of patients (Frozen cycle and Fresh cycle) Across Age Groups

Age	40-42	42-44	44-46	46-48
No of cases on frozen cycle	360	85	35	20
No of cases on fresh cycle	405	60	30	5

Table (1) illustrates the distribution of patients across different age groups, categorized into two conditions: **frozen embryo cycle** and **fresh embryo cycle**. The following observations can be made:

In the **40-42 age group**, the number of fresh embryo cycle exceeds frozen embryo cycle (405 vs. 360), which may indicate a preference for transferring fresh embryos when available. As age progresses, the number of cases in both categories declines; however, the decline in fresh embryo cycle occurs at a faster rate. This is evident in the **46-48 age group**, where the number of fresh embryo cycle (5) is significantly lower than frozen embryo cycle (20).This trend may reflect an increasing reliance on embryo freezing technology with advancing age, especially since fresh embryos become less available or may exhibit lower quality in older age groups.

The **dependence on frozen embryo cycle increases with age**, as the likelihood of obtaining high-quality fresh embryo cycle declines. In **older age groups (44-48), the difference becomes more pronounced in favor of frozen embryo cycle**, emphasizing the importance of cryopreservation techniques in preserving future pregnancy opportunities.

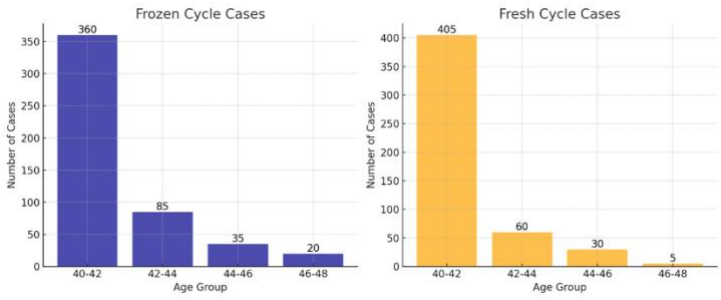

Figure 1 Distribution of patients (Frozen cycle and Fresh cycle) Across Age Groups

Table 2: Compares the average number of oocytes retrieved for each age group.

AGE	40-42	42-44	44-46	46-48
NO OF OOCYTES ON FROZEN CYCLE	(2-7)	(2-5)	(1-3)	(1-2)
NO OF OOCYTES ON FRESH CYCLE	(2-8)	(2-5)	(1-3)	(1-2)

Table (2) clear compares the average number of oocytes retrieved for each age group. It illustrates the general trend of declining average oocyte count with increasing age, which aligns with research confirming the decrease in ovarian reserve and ovulation efficiency as age advances.

A gradual decline in the number of retrieved oocytes is observed with increasing age, with the highest average in the 40-42 age group, followed by a significant decrease in older age groups. This decline reflects the biological impact of aging on ovulatory efficiency and oocyte quality.

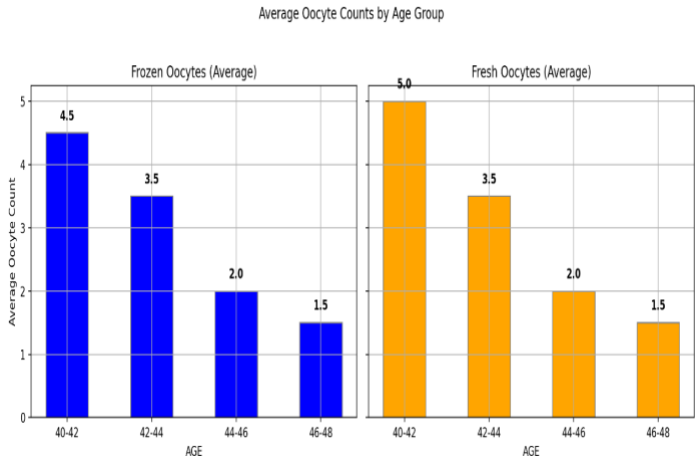

Figure 2. Compares the average number of oocytes retrieved for each age group.

Table 3: Comparison of Clinical Pregnancy Rates Between Frozen and Fresh Embryo Transfers Across Age Groups

Age	40-42	42-44	44-46	46-48
Clinical pregnancy frozen cycle	30	5	0	0
Clinical pregnancy fresh cycle	35	5	0	0

Table (3) illustrates the clinical pregnancy outcomes for frozen and fresh embryo transfers across different maternal age groups. The data reveal the following key insights:

1. **Age Group 40-42:** The clinical pregnancy rate was higher for fresh embryo transfers (35 cases) compared to frozen embryo transfers (30 cases).This suggests a potential advantage of fresh embryo transfers in this age group, although the difference is relatively small.
2. **Age Group 42-44:** Both embryo transfer methods resulted in an equal number of clinical pregnancies (5 cases each).This indicates that the choice between fresh and frozen embryo transfer may not significantly affect pregnancy success rates in this age bracket.
3. **Age Groups 44-46 and 46-48:** No clinical pregnancies were recorded for either method, suggesting a significant decline in success rates beyond the age of 44. This aligns with existing literature on reduced fertility and implantation potential in older maternal age groups.

The findings suggest that fresh embryo transfers may offer a slight advantage in younger women (40-42 years), while both methods yield similar outcomes in women aged 42-44. However, clinical pregnancy rates decline sharply beyond the age of 44, regardless of the embryo transfer method used. Further research with a larger sample size and controlled clinical trials may help clarify the relative efficacy of these approaches in older reproductive age groups.

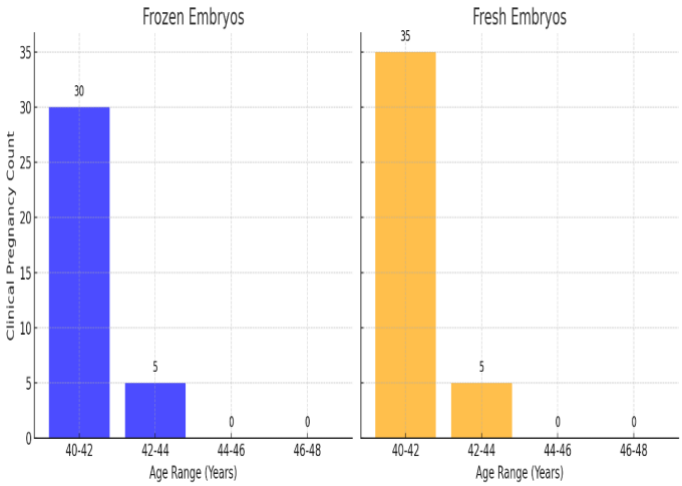

Figure 3. Comparison of Clinical Pregnancy Rates Between Frozen and Fresh Embryo Transfers Across Age Groups

Table 4: Comparison of Abortion Rates Between Frozen and Fresh Embryo Transfers Across Age Groups

Age	40-42	42-44	44-46	46-48
Abortion infrozen cycle	20	5	0	0
Abortion in fresh cycle	25	5	0	0

Table (4) clear presents a comparative analysis of clinical abortion rates between frozen and fresh embryo transfers across different maternal age groups. The data indicate that in the 40-42 age group, the clinical abortion count was 20 for frozen embryo transfers and 25 for fresh embryo transfers, suggesting a slightly higher abortion rate in fresh transfers. In the 42-44 age groups, both methods resulted in an equal count of 5 clinical abortions. No recorded clinical abortions were observed in the older age groups (44-46 and 46-48), indicating a potential decline in pregnancy occurrences or clinical abortions in these age brackets. These findings may highlight differences in embryo viability and implantation success rates between the two methods, particularly in older maternal age groups

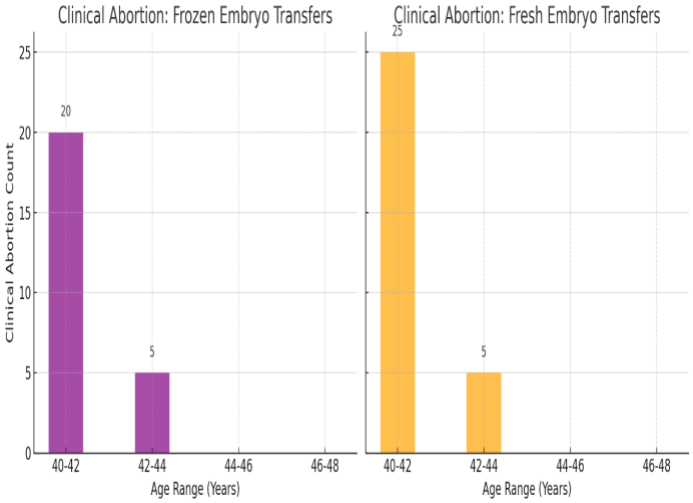

Figure 4. Comparison of Abortion Rates Between Frozen and Fresh Embryo Transfers Across Age Groups

Table 5: Clinical Pregnancy and Miscarriage Rates Between Frozen and Fresh Embryo Transfers Across Age Groups

Age group	Clinical pregnancy rate - fresh (%)	Miscarriage rate - fresh (%)	Clinical pregnancy rate - frozen (%)	Miscarriage rate - frozen (%)
40-42	8.64	71.43	8.33	66.67
42-44	8.33	100.00	5.88	100.00
44-46	0.00	-	0.00	-
46-48	0.00	-	0.00	-

Table (5) clear Clinical pregnancy rates are low across all age groups, especially after the age of 44. The miscarriage rate is very high, reaching 100% in some age groups, indicating a poor likelihood of pregnancy continuation with age progression.

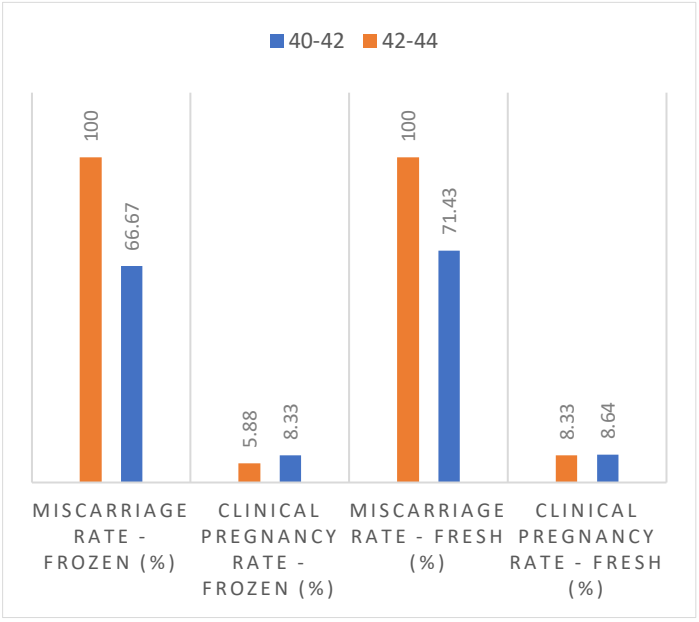

Figure 5. Clinical Pregnancy and Miscarriage Rates Between Frozen and Fresh Embryo Transfers

Table 6: Descriptive Statistics for Age and Number of Embryos Transferred

Statistic	Age (Years)
Mean	41.7
Median	41.0
Standard Deviation	1.42

Table (6) clear average age falls within the age group of 40-42 years. The low standard deviation (1.42) indicates that most cases are close in age.

Table 7: Comparing Results between Types of Embryo Transfer (Fresh and Frozen)

Age Group	Clinical Pregnancy - Fresh	Clinical Pregnancy - Frozen
40-42	35	30
42-44	5	5

Chi-Square Value: 0.0
P-value: 1.0 (no significant difference between the types of transfer)

Table (7) clear There is no statistically significant difference between clinical pregnancy rates resulting from fresh and frozen embryo transfers. The P-value of 1.0 suggests that any notable differences in the outcomes may be attributed to chance, indicating a lack of clear impact of the type of embryo transfer on pregnancy success.

1. **Similarity in Results**: Despite the differences in numbers between fresh and frozen embryos in the 40-42 age group, the test showed no significant difference, reinforcing the hypothesis that the type of transfer does not affect the outcomes.
2. **Age Distribution**: In the 42-44 age group, the numbers were equal between fresh and frozen embryos, supporting the conclusion that the type of transfer does not influence pregnancy rates.
3. **Clinical Implications**: These results highlight the necessity to reevaluate embryo transfer practices, as it is likely that both fresh and frozen embryos have equal effects on pregnancy rates. This underscores the need for further research to explore factors that may contribute to pregnancy success.

The findings suggest that the choice of embryo transfer type, whether fresh or frozen, may not significantly affect clinical pregnancy rates. This emphasizes the importance of considering other factors that may play a role in pregnancy success, warranting future studies to confirm these results and investigate the factors influencing clinical outcomes in greater detail.

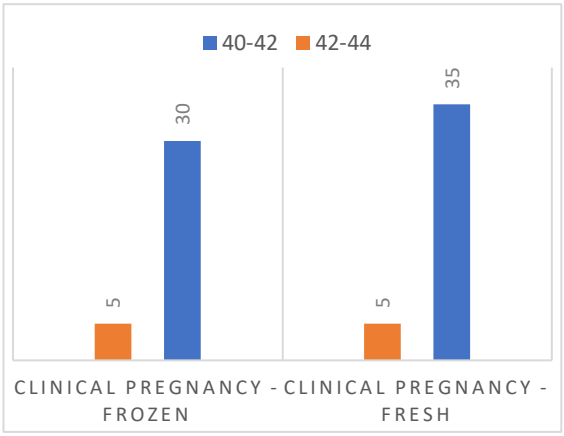

Figure 6. Comparing Results Between Types of Embryo Transfer (Fresh and Frozen)

Table 8: Logistic regression analysis of the Impact of Age and Type of Embryo Transfer on Results

Variable	Coefficient	Standard Error	P-Value
Constant	390.90	63061.16	0.995
Age	-9.58	1538.08	0.995
Type of Transfer (1=Fresh, 0=Frozen)	-0.56	0.25	0.022

Table (8) There is no significant effect of age on clinical pregnancy (p = 0.995). However, there is a significant effect of the type of transfer, with frozen transfers associated with higher pregnancy chances compared to fresh transfers (p = 0.022). Nonetheless, the clinical pregnancy rate is low across all age groups, especially after the age of 44 and the miscarriage rate is very high, reflecting poor chances of pregnancy continuity with advancing age. However, the Age did not show a significant effect on pregnancy chances, but the type of transfer had a significant effect, with frozen transfer results being better

The results indicated that clinical pregnancy rates were significantly higher for fresh embryos in the age group 40-42, with 35 pregnancies recorded compared to 30 for frozen embryos. However, in the age group 42-44, no statistically significant differences were observed between the two types, as both fresh and frozen embryos recorded 5 pregnancies. Furthermore, there were no recorded clinical pregnancies in the age groups 44-46 and 46-48, suggesting a sharp decline in success rates after the age of 44.A clear decrease in the number of embryos (both frozen and fresh) was observed with advancing age.

The results revealed a gradual decrease in the number of retrieved oocytes with advancing age, with the highest average in the age group 40-42. In contrast, the number declined in the age groups 44-46 and 46-48 to 2.0 and 1.5 respectively, reflecting the biological impact of aging on ovarian function and oocyte quality.

The data showed that miscarriage rates were slightly higher for fresh embryos in the age group 40-42 (25 cases) compared to frozen embryos (20 cases). In the age group 42-44, rates were equal (5 cases each), while no miscarriages were recorded in the older age groups (44-46 and 46-48), which may indicate a decline in pregnancy opportunities or an increase in miscarriage rates in these cohorts.

The logistic regression analysis demonstrated no statistically significant effect of age on the likelihood of pregnancy (p = 0.995). However, the type of transfer had a significant effect, with results indicating that frozen transfers were associated with higher pregnancy chances *compared to fresh transfers (p = 0.022). These findings highlight the* importance of considering the type of embryos within the context of reproductive treatments.

Pregnancy rates were generally low across all age groups, particularly after the age of 44, where miscarriage rates reached 100% in some cohorts, indicating poor chances of pregnancy continuation with advancing age.

These results emphasize the need for effective embryo transfer strategies and the impact of age and embryo type on pregnancy outcomes. They also call for further research to understand the factors influencing pregnancy success in women undergoing in vitro fertilization.

Discussion:

This study aimed to explore the influence of advanced maternal age on fertility outcomes and the role of fresh and frozen embryo transfers in clinical pregnancy rates and miscarriage rates. Our finding suggest that while the clinical pregnancy rates was significantly higher for fresh embryo transfer, the miscarriage rate was also slightly higher in this age group. Additionally, no statistically

significant relationship was found between maternal age and the likelihood of pregnancy, while the type of embryo transfer had a significant effect, with frozen embryo transfers resulting in higher pregnancy rates.

Our results align with previous research indicating that fresh embryo transfers tend to yield higher clinical pregnancy rates but also slightly higher miscarriage rate compared to frozen embryo transfer, particularly in younger women. For example, studies often attributed to the immediate synchronization between the embryo and the endometrial environment during fresh cycle (Shapiro et al.,2011;cakmak& decherney,2013) (18,19) increase risk of pregnancy loss (Macklon& Fauser,2012; Coughlan,C.,et al. 2014) (20,21) this suggests that while fresh embryo transfers might be more successful in initiating pregnancies, the quality of the embryo or the uterine environment may influence the viability of the pregnancy.

On the other hand, frozen embryo transfer showed significantly higher pregnancy rate in this study. This aligns with previous studies suggesting that FET may provide better outcomes, possibly due to the ability to synchronize the embryo transfer with an optimally prepared endometrium (Li et al., 2015). (22). The improved outcomes with FET might also reflect the potential for better embryo selection and the decrease risk of OHSS, which is a concern with fresh Cycles. (20),Freeze-only transfer protocols are associated with statistically significantly higher ongoing implantation and pregnancy rates compared with fresh transfer cycles.(Ange Wang,M.D. et al., 2017) (23)

In this study, the advanced maternal age does not have a statistically significant impact on the likelihood of pregnancy, which is an interesting. this result may attributed to several factors, including improvement in IVf technology , such as egg retrieved techniques , embryo culture system, and the use of genetic screening methods, all of which may reduce age-related infertility challenges.

It is important to recognize that while frozen embryo transfers show promise in terms of higher pregnancy rates in older women, the process also carries logistical and financial challenges, as well as the need for carful long-term storage (Zegers-Hochschild et al., 2017)(24) . Future research could explore the long-term outcomes of fresh vs. frozen cycles, particularly with respect to the health of both the mother and child.

Conclusion:

In summary, our study suggests that while fresh embryo transfers result in higher clinical pregnancy rates, they are also associated with slightly higher miscarriage rates. Frozen embryo transfers, however, appear to offer better pregnancy outcomes, especially for women of advanced maternal age. The lack of a significant effect of age on the likelihood of pregnancy may indicate that the quality of the embryo and the transfer method play a more significant role than maternal age itself. These findings highlight the complexity of IVF outcomes and suggest that personalized treatment strategies based on embryo quality and transfer method may improve the chances of successful pregnancies in women with advanced maternal age.

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