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ORIGINAL RESEARCH

# Risk Factors of Pregnancy Failure in Infertile Patients Undergoing Assisted Reproductive Technology

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**Background:** Infertile couples need to use assisted reproductive technology (ART) to give birth. However, pregnancy failure after ART is not uncommon. At present, the results of studies on the causes of pregnancy failure after ART are inconsistent.

**Methods:** A retrospective cohort study involving 715 embryo transfer cycles was conducted at the Reproductive Medicine Center of Meizhou People's Hospital, from December 2015 to June 2022. According to the pregnancy, they were divided into clinical pregnancy group and pregnancy failure group. The relationship between demographic characteristics and pregnancy status between the two groups was analyzed.

**Results:** The pregnancy failure rate after ART was 49.7% (355/715). There were statistically significant distribution differences of maternal age, paternal age, COH protocols, and number of embryos transferred between clinical pregnancy and pregnancy failure groups (all P<0.01). Multiple logistic regression analysis shows that high maternal age (>35 years old vs ≤35 years old: OR 2.173, 95% CI: 1.386–3.407, P=0.001), and GnRH-a short protocol (GnRH-a short protocol vs GnRH-a long protocol: OR 2.139, 95% CI: 1.127–4.058, P=0.020) may increase risk of pregnancy failure in ART pregnancies, while two embryos transferred (two embryos transferred vs one embryo transferred: OR 0.563, 95% CI: 0.377–0.839, P=0.005) may reduce risk of pregnancy failure. In addition, high maternal age, GnRH antagonist protocol, and GnRH-a short protocol may increase risk of implantation failure, while two embryos transferred may reduce risk of implantation failure. And high maternal age may increase risk of biochemical pregnancy.

**Conclusion:** The risk of pregnancy failure increased in ART cycles with maternal age >35 years old and GnRH-a short protocol, while reduced with two embryos transferred.

**Keywords:** assisted reproductive technology, risk factors, pregnancy failure, infertility, miscarriage

## Introduction

Infertility is medically defined as the failure to achieve pregnancy of a couple after 12 months of regular sexual intercourse without any form of contraception.[1] Infertility affects more than 6 million people in the United States, equivalent to 10% of the reproductive-age population.[2] In China, the prevalence of infertility among couples with childbearing age is about 25%.[3] The reasons of infertility include female factors and male factors,[4] and the psychological stress caused by the fertility status and the side effects of some hormones and drugs may also increase the risk of infertility.[5,6] Assisted reproductive technology (ART) is one of the most effective methods for the treatment of infertility patients, and also an important means to achieve eugenic inheritance.[7]

In recent decades, ART has been widely used in the fertility treatment of infertility patients, especially in the prevention of some serious genetic diseases. Regardless, ART treatment does not guarantee pregnancy and live birth. Pregnancy failure is one of the most important limitations in ART treatment.[8] Pregnancy failure rate is an important indicator to evaluate the success rate of ART treatment for infertility. According to the US Centers for Disease Control

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and Prevention, the clinical pregnancy rate of ART procedures that progressed to the embryo-transfer stage is 54.7%, but the corresponding live birth rate is only 45.0%, the pregnancy failure rate is as high as 18.5%.[9] The live birth rate is about 42% in China.[10] Pregnancy failure causes great psychological pressure and economic burden to infertile couples. A major problem in reproductive medicine is implantation failure, which refers to the inability to conceive after transferring high-quality embryo/embryos.[11] Pregnancy loss is defined as the natural termination of the entire gestational cycle, including biochemical pregnancy, embryonic arrest, spontaneous abortion, and stillbirth.[12]

In order to meet the increasing demand for ART, it is very important to improve the success rate of clinical pregnancy after the implementation of ART. Therefore, the factors affecting the success rate of clinical pregnancy after ART have become the focus of research and discussion in the field of ART at home and abroad. It is of great significance to analyze the related influencing factors of pregnancy failure in ART for clinical prevention and treatment. Many studies only analyzed risk factors for pregnancy failure after fresh or frozen cycles,[13–15] whereas taking pregnancy failure for the whole ART into consideration is essential for a more appropriate evaluation. Based on this, this study selected infertility patients who underwent ART in our hospital in the past 6 years as the research objects to study the related influencing factors (patients' baseline and treatment characteristics) of pregnancy failure.

# Materials and Methods

By June 2022, the Reproductive Medicine Center of Meizhou People's Hospital (Meizhou Academy of Medical Sciences) had carried out a total of 715 embryo transfer cycles with pregnancy outcomes. In this study, cycles with a final pregnancy outcome were analyzed. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Meizhou People's Hospital (Clearance No.: 2016-A-53).

Detailed information on parental characteristics and ART procedures was collected from the electronic medical records of Reproductive medicine centers. Pregnancy outcomes were obtained from the follow-up database. Pregnancy was defined as positive serum human chorionic gonadotropin (hCG) levels on day 14 after oocyte retrieval, and clinical pregnancy was defined as the presence of gestational sac on ultrasound 3–4 weeks after hCG positive. The risk factors of pregnancy failure investigated in this study were maternal age, maternal body mass index (BMI, $kg/m^2$), paternal age, type of infertility, controlled ovarian hyperstimulation (COH) regimen, total gonadotropin (Gn) dose, ovarian hyperstimulation syndrome (OHSS), fertilization methods, number of embryos transferred, endometrial thickness, and so on.

Maternal age and paternal age were divided into four subgroups (<30 years, 30–35 years, 36–40 years, and >40 years), respectively. Maternal BMI was divided into four subgroups based on the Chinese criteria:[16,17] <18.5 $kg/m^2$, 18.5–23.9 $kg/m^2$, 24.0–27.9 $kg/m^2$, ≥28.0 $kg/m^2$. Primary infertility was defined as the failure to achieve pregnancy of a couple after 12 months of regular sexual intercourse without any form of contraception when a woman has never conceived, while secondary infertility was the incapability to conceive in a couple who have had at least one successful clinical pregnancy previously. COH protocol was divided into six subgroups: GnRH-a long protocol, GnRH antagonist protocol, long term follicular protocol, GnRH-a short protocol, GnRH-a prolonged protocol, and other protocol. Three subgroups of number of embryos transferred were 1 embryo transferred, 2 embryos transferred, and 3 embryos transferred. Fertilization methods including in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), IVF and ICSI were performed simultaneously (IVF+ICSI).

## Statistical Analyses

Demographics and clinical characteristics of pregnancies conceived through ART were calculated by $\chi^2$ test. The distributions of continuous variables were evaluated by Student's *t*-test or the Mann–Whitney *U*-test. Logistic regression analysis was applied to assess the associations between the risk factors and pregnancy outcome. Data analysis was performed using SPSS 21.0 (IBM Inc., USA).

# Results

## Demographics and Clinical Characteristics of Infertility Patients Treated with ART

The mean of the maternal age was 32.45±5.12 years old, there were 225 cases (31.5%) under the age of 30, 287 cases (40.1%) between the ages of 30 and 35, 157 cases (22.0%) between the ages of 36 and 40, and 46 cases (6.4%) beyond the age of 40. The

mean of the paternal age was 35.04±6.03 years old, there were 133 patients (18.6%) under the age of 30, 281 cases (39.3%) between the ages of 30 and 35, 165 cases (23.1%) between the ages of 36 and 40, and 136 cases (19.0%) beyond the age of 40. There were 74 (10.3%) cases with maternal BMI <18.5 kg/m$^2$, 459 (64.2%) cases with 18.5–23.9 kg/m$^2$, 127 (17.8%) cases with 24.0–27.9 kg/m$^2$, and 55 (7.7%) cases with ≥28.0 kg/m$^2$. There were 332 (46.4%) cases with primary infertility and 383 (53.6%) cases with secondary infertility. GnRH-a long protocol was the most common COH protocol, followed by GnRH antagonist protocol, long term follicular protocol, GnRH-a short protocol, and GnRH-a prolonged protocol. There were 7 (1.0%) patients developed ovarian hyperstimulation syndrome (OHSS). There were 614 (85.9%) patients receiving IVF and 93 (13.0%) patients receiving ICSI. Two embryos were transferred in 566 (79.2%) cases, 1 embryo was transferred in 137 (19.2%) cases, and 3 embryos were transferred in 12 (1.7%) cases. In this study, in 715 cycles with assisted reproductive technology, clinical pregnancy occurred in 360 (50.3%) cycles and pregnancy failure occurred in 355 (49.7%) cycles. Among the pregnancy failures, there were 300 (84.5%) implantation failures and 55 (15.5%) biochemical pregnancies (Table 1).

**Table 1** Demographics and Clinical Characteristics of Infertility Patients Treated with Assisted Reproductive Technology (ART)

| Variables | Number/Mean±SD | Percentage |
|---|---|---|
| Total number | 715 | |
| Maternal age (years) | 32.45±5.12 | |
| <30 | 225 | 31.5% |
| 30–35 | 287 | 40.1% |
| 36–40 | 157 | 22.0% |
| >40 | 46 | 6.4% |
| Paternal age (years) | 35.04±6.03 | |
| <30 | 133 | 18.6% |
| 30–35 | 281 | 39.3% |
| 36–40 | 165 | 23.1% |
| >40 | 136 | 19.0% |
| Maternal BMI (kg/m$^2$) | 22.25±3.60 | |
| <18.5 | 74 | 10.3% |
| 18.5–23.9 | 459 | 64.2% |
| 24.0–27.9 | 127 | 17.8% |
| ≥28.0 | 55 | 7.7% |
| Infertility type | | |
| Primary | 332 | 46.4% |
| Secondary | 383 | 53.6% |
| COH protocol | | |
| GnRH-a long protocol | 349 | 48.8% |
| GnRH antagonist protocol | 240 | 33.6% |
| Long term follicular protocol | 62 | 8.7% |
| GnRH-a short protocol | 51 | 7.1% |
| GnRH-a prolonged protocol | 10 | 1.4% |
| Other protocol | 3 | 0.4% |
| Total Gn dose | 2865.46±1051.46 | |
| OHSS | | |
| No | 708 | 99.0% |
| Yes | 7 | 1.0% |
| Fertilization methods | | |
| IVF | 614 | 85.9% |
| ICSI | 93 | 13.0% |
| IVF+ICSI | 8 | 1.1% |

(*Continued*)

**Table 1** (Continued).

| Variables | Number/Mean±SD | Percentage |
|---|---|---|
| No. of embryos transferred | | |
| 1 | 137 | 19.2% |
| 2 | 566 | 79.2% |
| 3 | 12 | 1.7% |
| Endometrial thickness (mm) | 10.90±2.03 | |
| Reproductive outcomes | | |
| Clinical pregnancy | 360 | 50.3% |
| Pregnancy failure | 355 | 49.7% |
| Implantation failure | 300 | 84.5%* |
| Biochemical pregnancy | 55 | 15.5%* |

**Note**: *The proportion of implantation failure and biochemical pregnancy in pregnancy failure, respectively.

**Abbreviations**: BMI, body mass index; COH, controlled ovarian hyperstimulation; OHSS, ovarian hyperstimulation syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

## Comparison of Demographic and Clinical Characteristics Between Clinical Pregnancy and Pregnancy Failure Groups Among Infertility Patients Performed ART

Table 2 shows the results of differences in demographic and clinical characteristics between clinical pregnancy and pregnancy failure among infertility patients performed ART. There were statistically significant differences in the distribution of maternal age (P<0.001) and paternal age (P<0.001) between clinical pregnancy and pregnancy failure groups. In addition, there were statistically significant distribution differences of COH protocols, and number of embryos transferred between clinical pregnancy and pregnancy failure groups (all P<0.01). Specifically, the proportions of the

**Table 2** Comparison of Demographic and Clinical Characteristics Between Clinical Pregnancy and Pregnancy Failure Groups Among Infertility Patients Performed ART

| Variables | Clinical Pregnancy Group | Pregnancy Failure Group | *P* value |
|---|---|---|---|
| Total number | 360 | 355 | |
| Maternal age (years) | | | |
| <30 | 138 (38.3%) | 87 (24.5%) | <0.001 |
| 30–35 | 151 (41.9%) | 136 (38.3%) | |
| 36–40 | 60 (16.7%) | 97 (27.3%) | |
| >40 | 11 (3.1%) | 35 (9.9%) | |
| Paternal age (years) | | | |
| <30 | 88 (24.4%) | 45 (12.7%) | <0.001 |
| 30–35 | 144 (40.0%) | 137 (38.6%) | |
| 36–40 | 77 (21.4%) | 88 (24.8%) | |
| >40 | 51 (14.2%) | 85 (23.9%) | |
| Maternal BMI ($kg/m^2$) | | | |
| <18.5 | 31 (8.6%) | 43 (12.1%) | 0.322 |
| 18.5–23.9 | 233 (64.7%) | 226 (63.7%) | |
| 24.0–27.9 | 70 (19.4%) | 57 (16.1%) | |
| ≥28.0 | 26 (7.2%) | 29 (8.2%) | |
| Infertility type | | | |
| Primary | 176 (48.9%) | 156 (43.9%) | 0.202 |
| Secondary | 184 (51.1%) | 199 (56.1%) | |

(*Continued*)

**Table 2** (Continued).

| Variables | Clinical Pregnancy Group | Pregnancy Failure Group | *P* value |
|---|---|---|---|
| COH protocol | | | |
| GnRH-a long protocol | 195 (54.2%) | 154 (43.4%) | 0.006 |
| GnRH antagonist protocol | 104 (28.9%) | 136 (38.3%) | |
| Long term follicular protocol | 36 (10.0%) | 26 (7.3%) | |
| GnRH-a short protocol | 18 (5.0%) | 33 (9.3%) | |
| GnRH-a prolonged protocol | 5 (1.4%) | 5 (1.4%) | |
| Other protocol | 2 (0.6%) | 1 (0.3%) | |
| Total Gn dose | 2808.40±951.51 | 2923.32±1142.39 | 0.145 |
| OHSS | | | |
| No | 355 (98.6%) | 353 (99.4%) | 0.451 |
| Yes | 5 (1.4%) | 2 (0.6%) | |
| Fertilization methods | | | |
| IVF | 308 (85.6%) | 306 (86.2%) | 0.424 |
| ICSI | 46 (12.8%) | 47 (13.2%) | |
| IVF+ICSI | 6 (1.7%) | 2 (0.6%) | |
| No. of embryos transferred | | | |
| 1 | 51 (14.2%) | 86 (24.2%) | <0.001 |
| 2 | 307 (85.3%) | 259 (73.0%) | |
| 3 | 2 (0.6%) | 10 (2.8%) | |
| Endometrial thickness (mm) | 11.13±1.98 | 10.67±2.07 | 0.002 |

**Abbreviations**: BMI, body mass index; COH, controlled ovarian hyperstimulation; OHSS, ovarian hyperstimulation syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

COH protocols from high to low ranked as GnRH-a long protocol (43.4%), GnRH antagonist protocol (38.3%), GnRH-a short protocol (9.3%), long term follicular protocol (7.3%), and GnRH-a prolonged protocol (1.4%) in pregnancy failure group, while GnRH-a long protocol (54.2%), GnRH antagonist protocol (28.9%), long term follicular protocol (10.0%), GnRH-a short protocol (5.0%), and GnRH-a prolonged protocol (1.4%) in clinical pregnancy group. In terms of the number of embryos transferred, two embryos were mainly transferred in both groups, but the proportion of one embryo transferred in the pregnancy failure group was higher than that in the clinical pregnancy group (24.2% vs 14.2%, P<0.001). The pregnancy failure group had lower endometrial thickness (10.67±2.07 mm vs 11.13±1.98 mm, P=0.002) than clinical pregnancy group. There were no differences found in maternal BMI, infertility type, total Gn dose, OHSS, and fertilization methods.

## Logistic Regression Analysis of Risk of Pregnancy Failure in ART Pregnancies

To gain insight into the independent influence of clinical characteristics on pregnancy failure, logistic regression analysis was performed. The univariate analyses performed indicated that high maternal age, high paternal age, GnRH antagonist protocol, GnRH-a short protocol, and two embryos transferred may have some effect on pregnancy failure in ART pregnancies. The multiple regressions performed showed that high maternal age (>35 years old vs ≤35 years old: OR 2.173, 95% CI: 1.386–3.407, P=0.001), and GnRH-a short protocol (GnRH-a short protocol vs GnRH-a long protocol: OR 2.139, 95% CI: 1.127–4.058, P=0.020) may increase risk of pregnancy failure in ART pregnancies, while two embryos transferred (two embryos transferred vs one embryo transferred: OR 0.563, 95% CI: 0.377–0.839, P=0.005) may reduce risk of pregnancy failure in ART pregnancies (Table 3).

## Independent Risk Factors for Different Types of Pregnancy Failure

In this study, pregnancy failure including implantation failure, and biochemical pregnancy. Multiple logistic regression analysis showed that high maternal age (>35 years old vs ≤35 years old: OR 2.355, 95% CI: 1.405–3.945, P=0.001), GnRH antagonist protocol (GnRH antagonist protocol vs GnRH-a long protocol: OR 1.672, 95% CI: 1.122–2.491,

**Table 3** Logistic Regression Analysis of Risk of Pregnancy Failure in Assisted Reproductive Technology (ART) Pregnancies

| Variables | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | Crude OR (95% CI) | *P* value | Adjusted OR (95% CI) | *P* value |
| Maternal age (years) | | | | |
| ≤35 | 1.000 (Reference) | | 1.000 (Reference) | |
| >35 | 2.409 (1.720–3.376) | <0.001 | 2.173 (1.386–3.407) | 0.001 |
| Paternal age (years) | | | | |
| ≤35 | 1.000 (Reference) | | 1.000 (Reference) | |
| >35 | 1.723 (1.277–2.325) | <0.001 | 1.060 (0.711–1.580) | 0.775 |
| Maternal BMI (kg/m$^2$) | | | | |
| <18.5 | 1.430 (0.870–2.350) | 0.158 | 1.615 (0.957–2.724) | 0.073 |
| 18.5–23.9 | 1.000 (Reference) | | 1.000 (Reference) | |
| ≥24.0 | 0.924 (0.655–1.302) | 0.650 | 0.909 (0.634–1.303) | 0.603 |
| Infertility type | | | | |
| Primary | 1.000 (Reference) | | 1.000 (Reference) | |
| Secondary | 1.220 (0.909–1.638) | 0.185 | 0.972 (0.698–1.353) | 0.865 |
| COH protocol | | | | |
| GnRH-a long protocol | 1.000 (Reference) | | 1.000 (Reference) | |
| GnRH antagonist protocol | 1.656 (1.189–2.306) | 0.003 | 1.266 (0.889–1.802) | 0.190 |
| Long term follicular protocol | 0.915 (0.529–1.580) | 0.749 | 0.940 (0.536–1.648) | 0.829 |
| GnRH-a short protocol | 2.321 (1.259–4.281) | 0.007 | 2.139 (1.127–4.058) | 0.020 |
| GnRH-a prolonged protocol | 1.266 (0.360–4.453) | 0.713 | 1.425 (0.394–5.159) | 0.589 |
| Other protocol | 0.633 (0.057–7.047) | 0.710 | 0.467 (0.040–5.455) | 0.544 |
| OHSS | | | | |
| Yes | 1.000 (Reference) | | 1.000 (Reference) | |
| No | 2.486 (0.479–12.898) | 0.278 | 1.790 (0.338–9.496) | 0.494 |
| Fertilization methods | | | | |
| IVF | 1.000 (Reference) | | 1.000 (Reference) | |
| ICSI | 1.028 (0.665–1.591) | 0.900 | 0.993 (0.627–1.572) | 0.975 |
| IVF+ICSI | 0.336 (0.067–1.675) | 0.183 | 0.274 (0.052–1.440) | 0.126 |
| No. of embryos transferred | | | | |
| 1 | 1.000 (Reference) | | 1.000 (Reference) | |
| 2 | 0.500 (0.341–0.734) | <0.001 | 0.563 (0.377–0.839) | 0.005 |
| 3 | 2.965 (0.625–14.071) | 0.171 | 1.575 (0.317–7.814) | 0.579 |

**Abbreviations**: BMI, body mass index; COH, controlled ovarian hyperstimulation; OHSS, ovarian hyperstimulation syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

P=0.012) and GnRH-a short protocol (GnRH-a short protocol vs GnRH-a long protocol: OR 2.272, 95% CI: 1.120–4.612, P=0.023) may increase risk of implantation failure, while two embryos transferred (two embryos transferred vs one embryo transferred: OR 0.445, 95% CI: 0.286–0.694, P<0.001) may reduce risk of implantation failure (Table 4). Multiple logistic regression analysis showed that high maternal age (>35 years old vs ≤35 years old: OR 3.367, 95% CI: 1.300–8.718, P=0.012) may increase risk of biochemical pregnancy (Table 4).

**Table 4** Independent Risk Factors for Different Types of Pregnancy Failure

| Variables | Implantation Failure | | Biochemical Pregnancy | |
|---|---|---|---|---|
| | Adjusted OR (95% CI) | *P* value | Adjusted OR (95% CI) | *P* value |
| Maternal age (years) | | | | |
| ≤35 | 1.000 (Reference) | | 1.000 (Reference) | |
| >35 | 2.355 (1.405–3.945) | 0.001 | 3.367 (1.300–8.718) | 0.012 |

(*Continued*)

**Table 4** (Continued).

| Variables | Implantation Failure | | Biochemical Pregnancy | |
|---|---|---|---|---|
| | Adjusted OR (95% CI) | *P* value | Adjusted OR (95% CI) | *P* value |
| Paternal age (years) | | | | |
| ≤35 | 1.000 (Reference) | | 1.000 (Reference) | |
| >35 | 1.080 (0.687–1.696) | 0.739 | 0.725 (0.304–1.730) | 0.469 |
| Maternal BMI (kg/m$^2$) | | | | |
| <18.5 | 1.513 (0.834–2.744) | 0.173 | 2.370 (0.964–5.826) | 0.060 |
| 18.5–23.9 | 1.000 (Reference) | | 1.000 (Reference) | |
| ≥24.0 | 0.955 (0.638–1.432) | 0.825 | 0.913 (0.448–1.861) | 0.801 |
| Infertility type | | | | |
| Primary | 1.000 (Reference) | | 1.000 (Reference) | |
| Secondary | 0.951 (0.659–1.374) | 0.790 | 0.886 (0.460–1.706) | 0.717 |
| COH protocol | | | | |
| GnRH-a long protocol | 1.000 (Reference) | | 1.000 (Reference) | |
| GnRH antagonist protocol | 1.672 (1.122–2.491) | 0.012 | 1.184 (0.571–2.458) | 0.649 |
| Long term follicular protocol | 0.926 (0.493–1.741) | 0.811 | 1.336 (0.490–3.645) | 0.571 |
| GnRH-a short protocol | 2.272 (1.120–4.612) | 0.023 | 1.838 (0.540–6.252) | 0.330 |
| GnRH-a prolonged protocol | 1.487 (0.347–6.370) | 0.593 | 1.872 (0.198–17.742) | 0.585 |
| Other protocol | 1.205 (0.069–21.002) | 0.898 | / | / |
| OHSS | | | | |
| No | 1.000 (Reference) | | 1.000 (Reference) | |
| Yes | 1.612 (0.302–8.596) | 0.576 | / | / |
| Fertilization methods | | | | |
| IVF | 1.000 (Reference) | | 1.000 (Reference) | |
| ICSI | 1.114 (0.666–1.861) | 0.681 | 0.583 (0.211–1.610) | 0.298 |
| IVF+ICSI | 0.157 (0.017–1.457) | 0.103 | 0.610 (0.058–6.373) | 0.680 |
| No. of embryos transferred | | | | |
| 1 | 1.000 (Reference) | | 1.000 (Reference) | |
| 2 | 0.445 (0.286–0.694) | <0.001 | 1.510 (0.581–3.921) | 0.397 |
| 3 | 2.167 (0.256–18.316) | 0.477 | / | / |

**Abbreviations**: BMI, body mass index; COH, controlled ovarian hyperstimulation; OHSS, ovarian hyperstimulation syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

## Discussion

This retrospective study showed that maternal age, COH regimen, and number of embryos transferred significantly affected pregnancy failure in the ART population. To be specific, high maternal age and GnRH-a short protocol may increase risk of pregnancy failure, while two embryos transferred may reduce risk of pregnancy failure in ART pregnancies. And high maternal age, GnRH antagonist protocol, and GnRH-a short protocol may increase risk of implantation failure, while two embryos transferred may reduce risk of implantation failure. In addition, high maternal age may increase risk of biochemical pregnancy. To our knowledge, this was the first retrospective cohort study to explore independent risk factors for pregnancy failure and extensively distinguish risk factors for different types of pregnancy failure from Meizhou, China.

Maternal age is one of the common risk factors for pregnancy failure in infertility patients with ART. Studies showed that the risk factors of pregnancy failure in infertile patients undergoing ART are related to maternal age.[18,19] The age of the woman is one of the most important determinants of pregnancy success after natural conception and ART.[20] In this study, maternal age >35 years old showed significant increase pregnancy failure rate after ART, compared with those ≤35 years old. Our result is consistent with other previous studies. The reason is that with the increase of maternal age, the formation of ovarian germ cells decreases, the quality of oocytes decreases, and eventually leads to ovarian reproductive failure.[21] Some studies have suggested that decreased fecundity and fertilization rates in elderly women are also

associated with decreased follicle reserve[22] and increased aneuploidy.[23,24] The proportion of fetuses born to women aged ≥35 years old with chromosomal abnormalities is significantly increased, as is the probability of spontaneous abortion.[25,26] In terms of mechanism, advanced maternal age associated sirtuin family member 1 (SIRT1) deficiency compromises trophoblast epithelial–mesenchymal transition (EMT) through an increase in vimentin acetylation, damage trophoblast invasion and migration, promote premature placental senescence.[27]

Controlled ovulation stimulation is the basic step of in vitro fertilization and embryo transfer. In present study, GnRH-a short protocol may increase risk of pregnancy failure in ART pregnancies, and GnRH antagonist protocol and GnRH-a short protocol may increase risk of implantation failure. Studies showed that the normal fertilization rate in GnRH antagonist protocol group was lower than that in GnRH-a long protocol group.[28,29] Studies found that the implantation and pregnancy rates in the GnRH-a short protocol group were significantly lower than those in the GnRH-a long protocol group.[30–32] The cumulative live birth rate (cLBR) of the GnRH antagonist protocol group was significantly lower than that in the long-protocol group.[33] Other studies have found that GnRH-a short protocol and GnRH antagonist protocol do not affect implantation rate and pregnancy rate.[34–37] Therefore, further controlled randomized prospective studies with large sample sizes are needed to verify these inconsistent results in the future.

In our finding, two embryos transferred may reduce risk of pregnancy failure in ART pregnancies and implantation failure. Previous study has also shown that single embryo transferred has lower live birth and pregnancy rates than double embryo transferred.[38] The pregnancy rate of single embryo transferred is lower than that of multiple embryos transferred, but multiple embryos transferred is easy to cause multiple pregnancy. Therefore, attention should be paid to controlling the number of transferred embryos while reducing the early pregnancy failure rate and improving the live birth rate, so as to achieve a good pregnancy outcome.[39] Study has shown that the transfer of two high-quality embryos in elderly patients can achieve the ideal pregnancy rate and minimize the occurrence of multiple pregnancies.[40]

Previous studies have generally suggested a positive association between advanced paternal age and deterioration in semen quality.[41,42] Paternal age more than 46 years is associated with a lower percentage of pregnancy and live birth among couples performed IVF.[43] Paternal age <50 years can significantly reduce the rate of miscarriage and increase the success rate of ART.[44] Paternal age was associated with chromosomal aberrations-related miscarriage in couples performed ART.[45] Advanced paternal age has a significant negative influence on the outcome of ART.[46] The results of a British study showed that paternal age over 50 years decreased the success rate of ART.[47] However, some studies have shown that paternal age was not associated with adverse IVF outcomes.[48,49] In this study, paternal age was not associated with pregnancy failure after ART in infertile patients. The results of studies on the effect of paternal age on the outcome of ART are inconsistent and the effect of paternal age needs further clarification.

In this study, maternal BMI was not associated with pregnancy failure after ART in infertile patients. Study showed that obesity was associated with lower clinical pregnancy rate[50] and miscarriage.[51] Maternal obesity is associated with slower embryo development.[52] Placental leptin and adiponectin play an important role in placental development, maternal obesity is associated with the down-regulation of placental leptin and adiponectin, which is not conducive to placental development.[53] In contrast, compared with the results in normal-weight women, the rates of clinical pregnancy and ongoing pregnancy were reduced in underweight women after ART.[54] In addition, in another review, overweight was not associated with lower clinical pregnancy rate after ART.[55] BMI was not a predictor of ART outcome in infertile patients.[56] And study has found that BMI has different effects on ART pregnancy and live birth rates depending on race and ethnicity.[57] The different results may be due to differences in the number of cases included in different studies, as well as differences in the definition of BMI classification.

Our results are strengthened by a cohort with a certain number cycles and breadth of available patient and cycle characteristic data. We controlled for some factors that potentially affect pregnancy failure, implantation failure, and biochemical pregnancy. However, there are some limitations in this study. First, the present study did not collect and analyze factors on the whole process of ART, which may have other potential factors leading to pregnancy failure. Second, the relatively small number of cycles with biochemical pregnancy limited the ability of this study to assess the influencing factors of biochemical pregnancy. Third, as a retrospective study, this study did not analyze all the reported possible influencing factors and could not provide a more comprehensive assessment of influencing factors. So, multi-center, large-sample, prospective trials are needed for further study.

## Conclusions

In summary, the factors associated with pregnancy failure after assisted reproductive technology were identified through a cohort study from Meizhou, China. The risk of pregnancy failure increased in ART cycles with maternal age >35 years old and GnRH-a short protocol, while reduced with two embryos transferred. Hopefully, the efficiency of the clinical evaluation of pregnancy failure risk may be improved when the maternal age, COH protocol, and number of embryos transferred are taken into account. Further research is needed to validate our results and investigate the mechanism on the reported associations.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences. All participants signed informed consent in accordance with the Declaration of Helsinki.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests in this work.

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