

## Original Article

# Risk factors of pregnancy failure in elderly infertility patients undergoing human assisted reproductive technology

Shu Peng, Hong Sun, Jie Zheng, Ni Zeng, Fangxin Peng

*Reproductive Medicine, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei Province, China*

Received February 20, 2021; Accepted March 28, 2021; Epub June 15, 2021; Published June 30, 2021

**Abstract:** Objective: To investigate the risk factors of pregnancy failure in elderly infertile patients undergoing human assisted reproductive technology (ART). Methods: A total of 565 infertile patients undergoing ART were selected and divided into failed pregnancy group (127 cases) and continued pregnancy group (438 cases). Their clinical data were collected, and the influencing factors of pregnancy failure were assessed and compared by univariate and multivariate analysis. Results: The success and failure rates of in vitro fertilization-embryo transfer (IVF-ET) in pregnant women were 79.44% and 20.56%, while those of intracytoplasmic sperm injection (ICSI) were 75.96% and 24.04%, respectively. There was no remarkable difference between them (all  $P > 0.05$ ). Women's age, numbers of embryos transferred and previous abortion history in the failed pregnancy group were higher than those in the continued pregnancy group, while the number of high-quality embryos, BMI and endometrial thickness (EMT) on human chorionic gonadotropin (hCG) day in the former were lower (all  $P < 0.05$ ). Conclusion: The risk factors of ART pregnancy failure in elderly infertility patients are related to woman's age, numbers of embryos transferred, previous pregnancy abortion history, numbers of high-quality embryos and EMT on hCG day.

**Keywords:** Infertility, assisted reproductive technology, pregnancy failure, risk factors

## Introduction

Infertile patients are defined as those who have not been pregnant for one year or more under normal sexual life without taking any contraceptive measures. The two major causes are male and female infertility [1, 2]. IVF-ET is called in vitro fertilization and embryo transfer (IVF-ET) technology in medicine; It can also be called "the first generation of IVF", which is an artificial fertility skill [3, 4]. ICSI is a single sperm microinjection technology, which is characterized by injecting a single sperm into the egg with the help of a micromanipulation system to fertilize the egg [5]. When women of childbearing age are older than 35, their fertility begins to decline; It will lead to many birth complications with low pregnancy and high abortion rate [6, 7]. In serious cases, it will bring about infertility. Qi Quan et al. pointed out that the elderly infertile women had different clinical pregnancy outcomes after IVF-ET, among which the elderly and high hCG were not conducive to

clinical pregnancy [8]. The above research only makes statistical comparison on female factors. Therefore, this research analyzed male factors of female infertility statistically. In addition, elderly infertility patients were treated by human assisted reproductive technology (ART), in order to explore the risk factors of pregnancy failure.

## Materials and methods

### Materials

A total of 565 female infertility patients who underwent ART in our hospital from July 2018 to July 2020 were divided into failed pregnancy group (127 cases) and continued pregnancy group (438 cases). All of them were female. Patients in the failed pregnancy group were 36-42 years old, ( $39.0 \pm 2.4$ ) on average. While those in the continued pregnancy group were 38-45 years old, ( $41.5 \pm 2.8$ ) on average. Among them, 253 cases were treated by IVF-ET tech-

## Risk factors of elderly infertility assisted reproductive technology

nique and 312 were treated by ICSI. The research was approved by the Medical Ethics Committee of our hospital, and all included patients signed informed consent forms.

Inclusion criteria were as follows: (1) Age  $\geq 35$  years old; (2) All patients underwent ART for the first time.

Exclusion criteria were as follows: (1) Those with heart, liver, kidney and other organ dysfunction; (2) Poor compliance; (3) Patients undergoing ART for the second time or above.

### *Treatment methods*

**IVF-ET pregnancy method:** Patients were treated with long-term plan for superovulation, and GnRH was injected on the 8th day of menstruation. Gn was added to induce ovulation on the 2nd day after menstruation, and 10000 IU human chorionic gonadotropin (HCG) could be injected into the muscle of patients when the diameter of 1-2 follicles was  $\geq 18$  mm. Afterwards, vaginal B-ultrasound guided puncture was performed to collect eggs two days later, and then embryos were incubated with laser and transplanted after three days. Two weeks after ET, blood samples were collected from patients and the serum hCG concentration was detected, and the biochemical pregnancy was determined by hCG concentration.

**ICSI pregnancy method:** All patients were treated with long- or short-term ovarian hyperstimulation. GnRH agonist was used 8 days before menstruation, and then Gn was added to promote ovulation the next day after that. When the follicle diameter was  $\geq 18$  mm, 10,000 IU HCG was injected into the muscle of patients. Thirty-six hours later, eggs were collected by vaginal B-ultrasound guided puncture. At 3 h after eggs were obtained, sperm was added according to the ratio of 10,000 sperm/follicular cells to incubate for 3 h. After 42 h, cleavage was observed. While after 72 h, high-quality embryos were selected and implanted into uterus via vagina under the guidance of B-ultrasound, and the embryos were transplanted for two weeks. Blood and serum hCG concentration of patients were tested; Whether biochemical pregnancy was determined by hCG concentration.

**Endometrial thickness (EMT) on hCG day:** Patients were examined by GE E9 color ultra-

sound system, and the probe was the one with transvaginal ultrasound with frequency of 5-9 MHz. They were placed in lithotomy position. Routine disinfection was completed, and coupling agent was smeared and condom was slowly inserted into the cervix under the examination of probe. In the morning of hCG injection day, the EMT was measured by transvaginal ultrasound.

### *Variable selection*

The risk factors of pregnancy failure in elderly infertility patients undergoing ART were set as follows.

**Dependent variable:** The general data of 565 patients were collected: IVF-ET pregnancy succeeded, IVF-ET failed, ICSI succeeded and ICSI failed.

**Independent variable:** Variable setting: The number of ET.  $\geq 2$  is 1, and that  $< 2$  is 0; Age  $\geq 35$  years old is 1, and that  $< 35$  is 0; The number of antral follicles  $\geq 4$  is 1, and that  $< 4$  is 0; The number of mature eggs  $\geq 1$  is 1, and that  $< 1$  is 0; BMI  $\leq 25$  kg/m<sup>2</sup> is 1, and that  $> 25$  kg/m<sup>2</sup> is 0.

**Evaluation criteria of high-quality embryos:** After normal fertilization, the oocyte could normally divide the oosphere. If the number of cleaved oocytes is  $\geq 4$  on the second day and  $\geq 6$  on the third day, and the fragment is  $< 20\%$ , it can be defined as a high-quality embryo.

**Evaluation of pregnancy results:** Two weeks after ET., patients' blood was tested after they returned to the hospital; The serum hCG concentration was measured to determine whether they were biochemical pregnancy. When hCG  $> 20$  U/L, the biochemical pregnancy was confirmed. When confirmed as biochemical pregnancy, patients returned to the hospital 28 days after ET. to use ultrasonic test to determine whether it was clinical pregnancy. The pregnancy sac was confirmed as clinical pregnancy by ultrasonic test.

**Standard of pregnancy loss:** Pregnancy loss after ART can be defined as the natural termination of pregnancy in the whole pregnancy cycle, including embryo abortion and stillbirth. Biochemical pregnancy failure means that the serum hCG is positive 2 weeks after embryo transfer, and no gestational sac is found by ultrasound one month later. Early abortion

## Risk factors of elderly infertility assisted reproductive technology

**Table 1.** Comparison of success and failure rates of ART treatment methods performed (n, %)

ART treatment method	Case (n)	Success	Failure
IVF-ET pregnancy	253	201 (79.45)	52 (20.55)
ICSI pregnancy	312	237 (75.96)	75 (24.04)
$\chi^2$		0.956	0.973
P		0.305	0.323

Note: ART: assisted reproductive technology; IVF-ET: in vitro fertilization-embryo transfer; ICSI: intracytoplasmic sperm injection.

means that the existence of sac can be detected by ultrasound 28 days after embryo transfer, but symptoms such as abortion or stillbirth appear 3 months ago. Biochemical pregnancy, spontaneous abortion and ectopic pregnancy are all regarded as pregnancy failure.

### Statistical processing

All the data were analyzed and processed through SPSS 20.0 statistical software. The measurement data were described by mean and standard deviation ( $\bar{x} \pm sd$ ), and those between the two groups were compared by independent-samples t-test. The counting data were expressed in n (%), and those between groups were compared by Chi-square analysis. The influencing factors were analyzed by Logistic regression model. A P value lower than 0.05 has statistical significance.

### Results

#### Comparison of success and failure rates of ART therapy

The success and failure rates of IVF-ET pregnancy patients were 79.44%, 20.56%, while those of ICSI pregnancy patients were 75.96%, 24.04%, respectively. There was no remarkable difference between them ( $P > 0.05$ , **Table 1**).

#### Comparison of risk factors between failed pregnancy group and continued pregnancy group

The female age and numbers of embryos transferred in the failed pregnancy group were higher than those in the continued pregnancy group, while the numbers of embryos transferred, previous abortion history, high-quality embryos and EMT on hCG day in the former were lower ( $P < 0.05$ ). There was no marked difference in

BMI, male age, infertility years, number of antral follicles, mature eggs, and eggs obtained, amount of Gn and basic endocrine levels ( $P > 0.05$ , **Table 2**).

#### Multivariate logistic regression analysis of ART pregnancy failure in elderly infertility patients

Multivariate Logistic regression analysis of ART pregnancy failure in elderly infertile patients manifested that the female age, numbers of embryos transferred and the EMT on hCG day were the risk factors (**Table 3**).

#### ROC curves of advanced age, numbers of embryos transferred, EMT on hCG day and pregnancy failure

The risk factors of pregnancy failure of elderly infertile patients undergoing ART were evaluated by ROC curve. After that, the number of ET and EMT on hCG day was shown in **Table 4** and **Figure 1**.

### Discussion

IVF-ET is a reproductive technology that takes out eggs and sperm separately, puts them in a petri dish to fertilize eggs, and then transplants fertilized eggs back to the uterus [9-14]. Although this method can effectively treat infertile patients, the failure rate of IVF-ET pregnancy is very high. In the face of high cost and low success rate, it is urgent to improve its experimental technology. Mu Xin et al. found that in the elderly patients with IVF-ET pregnancy, age, the proportion of blastocyst transfer and HCG day membrane were the risk factors for pregnancy failure [15]. If there are functional problems in sperm provided by men, IVF-ET cannot be carried out. While ICSI breaks the high standard requirements of IVF-ET for sperm provided by men [16, 17]. Therefore, ICSI has made great achievements in male infertility treatment [8, 18]. ICSI and IVF-ET pregnancy were performed in elderly infertile patients. Huang Ying et al. discovered that the effect of ICSI was similar to that of IVF-ET in infertile patients with advanced age, low number of eggs and non-male factors [19]. In this research, elderly infertility patients were treated by IVF-ET and ICSI. The failure rates were 20.55% and 24.04%, respectively, with no marked difference.

## Risk factors of elderly infertility assisted reproductive technology

**Table 2.** Comparison of factors affecting two groups of patients ( $\bar{x}\pm sd$ )

Influence factors	Pregnancy failure group (n=127)	Pregnancy persistence group (n=438)	t	P
Age of female partner (year)	39.0±2.5	41.5±2.8	9.075	<0.001
Number of embryos transferred (n)	2.78±0.31	2.19±0.49	3.025	0.002
History of previous pregnancy miscarriage (times)	4.32±1.52	3.00±1.02	11.381	<0.001
Number of quality embryos (n)	4.16±0.95	3.15±0.76	12.431	<0.001
BMI (kg/m <sup>2</sup> )	25.59±4.31	25.79±4.37	0.865	0.817
Endometrial thickness on hCG day	9.85±2.01	10.61±2.29	3.381	0.001
Age of male partner (year)	39.0±2.41	39.5±2.80	1.826	0.068
Years of infertility (year)	6.57±3.56	6.58±3.48	0.028	0.977
Number of sinus follicles (n)	4.59±3.21	4.56±3.28	0.091	0.927
Number of mature oocytes (n)	1.23±0.59	1.24±0.58	0.170	0.864
Number of eggs obtained (n)	9.56±4.58	9.54±4.53	0.043	0.965
Gn dosage (IU/L)	2856.31±458.26	2856.33±456.52	0.001	0.999
Basal endocrine leve (IU/L)	7.02±2.59	7.02±1.98	0.000	0.999

**Table 3.** Multifactorial logistic regression analysis of pregnancy failure in advanced infertility patients undergoing ART reproductive technology

Risk factor	Beta	SE	Wald	P value	OR value	95% CI
Age of female partner (year)	1.569	0.589	9.987	0.001	6.528	1.259-10.137
Number of embryos transferred	1.758	0.687	10.958	0.001	6.128	1.285-8.016
hCG day endometrial thickness	1.658	0.398	5.213	0.001	6.1278	1.106-10.598

**Table 4.** Analysis of predictive value of advanced age, number of embryos transferred, endometrial thickness on hCG day and pregnancy failure

	AUC	Cut-off value	Sensitivity	Specificity
Advanced age	0.691	35.00	65.89	84.15
Number of embryos transferred	0.582	2.56	68.59	75.63
hCG day endometrial thickness	0.726	9.23	72.25	76.21
P	0.001	0.035	0.013	0.001

Note: hCG: human chorionic gonadotropin.

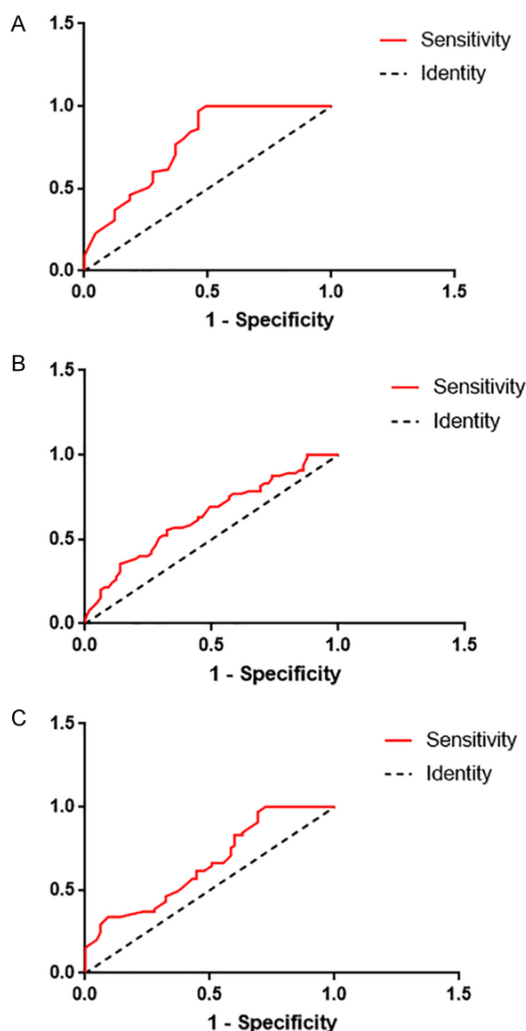
A large number of scholars believe that women's fertility will gradually weaken with the increase of age, which is mainly due to the gradual degradation of their genitals and the decrease of endometrial receptivity. As each year goes by, their ovarian function becomes low, the number of eggs obtained decreases, and that of high-quality embryos available for transplantation also reduces. All these can directly or indirectly affect the decline of women's clinical pregnancy rate [20]. He Hua et al. found that EMT and previous pregnancy history were the major risk factors for infertility patients undergoing ART [21]. Yang Xia et al.

also pointed out that the female age, EMT on hCG day and previous abortion history were the risk factors for female infertility with ART [22]. This research verified that the age of the elderly infertility patients was the risk factor for ART pregnancy failure. Univariate and multivariate analysis demonstrated that the older the patients, the higher the failure rate is, which

was consistent with the results of Yang Xia et al. [22].

Dou Qian et al. explained that under the same condition of the number of ET, EMT on hCG day, infertile patients aged 35 or more were dangerous when performing ART, indicating that the three were the risk factors for ART [23]. In this research, apart from patients' age, numbers of ET, high-quality embryos, and the EMT on hCG day were all risk factors for pregnancy failure in ART. Univariate and multivariate analysis manifested that the more the number of ET, the lower the pregnancy failure rate of ART was;

## Risk factors of elderly infertility assisted reproductive technology



**Figure 1.** ROC plots of advanced age, number of embryos transferred, endometrial thickness on hCG day and pregnancy failure. A: ROC plots of advanced age and pregnancy failure; B: ROC plots of number of embryos transferred and pregnancy failure; C: ROC plots of membrane thickness on hCG day and pregnancy failure; hCG: human chorionic gonadotropin.

The results were the same as those of Dou Qian et al. Univariate and multivariate analysis of previous pregnancy abortion history signified that the more times of previous pregnancy abortion history, the higher the pregnancy failure rate of ART was. Univariate and multivariate analysis of numbers of high-quality embryos showed that the more high-quality embryos obtained, the lower the failure rate of ART. Univariate and multivariate analysis of EMT on hCG day manifested that the higher EMT on hCG day, the lower the failure rate of ART pregnancy was.

It was found that the male age, infertility years, antral follicle count, numbers of mature and other eggs, Gn dosage and basic endocrine levels of the elderly infertile women were not relevant to pregnancy failure; This indicated that the failure of ART pregnancy of the elderly infertile women had nothing to do with the man.

To sum up, the risk factors of pregnancy failure rate of elderly infertile patients undergoing ART are relevant to their advanced age, fewer ET and increased EMT on hCG day. Hence, infertile patients should do ART as soon as possible. In this research, few cases were selected, and the distribution of ART failure of the elderly infertile women was not targeted. The next step is to increase the research sample size and make a detailed statistical comparison of ART failure distribution of the elderly infertile women.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Shu Peng, Reproductive Medicine, Maternal and Child Health Hospital of Hubei Province, No. 745 Jiedaokou, Wuluo Road, Hongshan District, Wuhan 430070, Hubei Province, China. Tel: +86-18627942201; E-mail: Pengshu08-01@163.com

### References

- [1] Vander Borgh M and Wyns C. Fertility and infertility: definition and epidemiology. *Clin Biochem* 2018; 62: 2-10.
- [2] Sharma RS, Saxena R and Singh R. Infertility & assisted reproduction: a historical & modern scientific perspective. *Indian J Med Res* 2018; 148 Suppl: S10-S14.
- [3] Zhang L, Xu WH, Fu XH, Huang QX, Guo XY, Zhang L, Li SS, Zhu J and Shu J. Therapeutic role of granulocyte colony-stimulating factor (G-CSF) for infertile women under in vitro fertilization and embryo transfer (ivf-et) treatment: a meta-analysis. *Arch Gynecol Obstet* 2018; 298: 861-871.
- [4] Li J, Luo H and Long L. A qualitative investigation of the experience of participation in mindfulness-based intervention for IVF-ET (MBII) with Chinese women undergoing first IVF-ET. *Nursing Open* 2019; 6: 493-503.
- [5] O'Neill CL, Chow S, Rosenwaks Z and Palermo GD. Development of ICSI. *Reproduction* 2018; 156: F51-F58.

## Risk factors of elderly infertility assisted reproductive technology

- [6] Bila JS, Vidakovic S, Radjenovic SS, Dokic M, Surlan L and Sparic R. Predictors of IVF/ICSI success following treatment of endometriosis as the cause of primary infertility. *Ginekol Pol* 2018; 89: 240-248.
- [7] Roque M, Haahr T, Geber S, Esteves SC and Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* 2019; 25: 2-14.
- [8] Qi Q, Huang L, Li ZT, Yi YH and Liu FH. Factors related to clinical pregnancy outcomes of in-vitro fertilization-embryo transfer with GnRHAntagonist protocol in elderly women. *Chin J Woman Child Health Res* 2017; 28: 1695-1698.
- [9] Mol BW, Tjon-Kon-Fat R, Kamphuis E and van Wely M. Unexplained infertility: is it over-diagnosed and over-treated? *Best Pract Res Clin Obstet Gynaecol* 2018; 53: 20-29.
- [10] Bellver J and Donnez J. Introduction: infertility etiology and offspring health. *Fertil Steril* 2019; 111: 1033-1035.
- [11] Starc A, Trampuš M, Pavan Jukić D, Rotim C, Jukić T and Polona Mivšek A. Infertility and sexual dysfunctions: a systematic literature review. *Acta Clin Croat* 2019; 58: 508-515.
- [12] Infertility Workup for the Women's Health Specialist: ACOG Committee Opinion, Number 781. *Obstet Gynecol* 2019; 133: e377-e384.
- [13] Dong J, Ma L, Xia M, Meng H, Zhang Y, Cai LB and Liu JY. Impact of insemination time on clinical outcomes of ivf-et in women aged 38 years and below. *J Reprod Med* 2020; 29: 882-886.
- [14] Deng LL, Song XR, Yang HP and Huang JH. Correlation between vaginal micro-ecology and infertility as well as pregnancy outcome of IVF-ET. *J Reprod Med* 2020; 29: 972-977.
- [15] Mu X, Zhang N, Wang T and Shi JZ. Multivariate analysis of clinical pregnancy outcomes of fresh IVF cycle in women with advanced age. *J Reprod Med* 2020; 29: 336-343.
- [16] Huang HY. Analysis of IVF/ICSI pregnancy outcomes and related factors in elderly women. *Guangxi Med Univ* 2018.
- [17] Zhang J, Jiang H, Ni F and Liu Y. MPA is effective for pituitary down-regulation on the women over 40 years in vitro fertilization. *Prog Obstet Gynecol* 2016; 25: 406-410.
- [18] Zhang WB, Sun YJ, Chen JL, Li L and Sun XX. Analysis of clinical outcome of different ovarian hyperstimulation protocols in advanced patients undergoing IVF-ET. *J Reprod Med* 2017; 26: 503-510.
- [19] Huang Y and Qin AP. Application effects of two insemination methods for in vitro fertilization-embryo transfer in advanced age patients with fewer oocytes retrieved: a comparative study. *Guangxi Med J* 2020; 42: 1981-1984, 1994.
- [20] Zhou LY, Li S, Lin HY, Qiu Q, Xie YQ and Zhang QX. Comparison of pregnancy outcome of different assisted reproductive protocols for non-tubal infertility in women with advanced age. *Chin J Reprod Contracept* 2017; 37: 129-133.
- [21] He Y, Chen Y and Yang XJ. Analysis of the risk factors for ectopic pregnancy after assisted reproductive technology. *Chin J Front Med Sci* 2019; 11: 78-80.
- [22] Yang X, Yue F and Sun XY. Analysis of risk factors for pregnancy loss in advanced infertility patients treated with assisted reproductive technology. *Matern Child Health Care Chin* 2018; 33: 631-633.
- [23] Dou Q, Tan L, Ma LY, Zhao DM and Mao GH. Analysis of risk factors for early pregnancy loss in advanced infertility patients with assisted reproductive techniques. *Prog Obstet Gynecol* 2017; 26: 380-381, 384.