Influencing factors of fertilization failure during in vitro fertilization

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Research Article

Keywords: in vitro fertilization, fertilization failure, influencing factors

Posted Date: May 29th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4422854/v1

Additional Declarations: No competing interests reported.

Abstract

Objective: To explore the influencing factors of fertilization failure (FF) during in vitro fertilization (IVF) to prevent and manage it in clinical practice and improve treatment efficiency.

Methods: IVF cycles were included and grouped according to the fertilization rate. There were 75 cycles with a fertilization rate of 0, i.e., complete FF, and 98 cycles with a fertilization rate of <30%, i.e., partial FF, and these cycles were included in the FF group; and there were 2301 cycles with a fertilization rate of ≥30%, and included in the normal fertilization(NF) group. Sperm quality of males, basic conditions of females, clinical ovulation induction and laboratory fertilization were compared between the two groups, and no differences were observed. Multivariate logistic regression analysis was performed using FF in the IVF process as the dependent variable, and the indicators with statistically significant differences in the univariate analysis as independent variables to screen the independent risk factors for FF in IVF.

Results: There were significant differences in female age, infertile duration, initial dose of Gn, Gn dose/egg, sperm concentration before treatment, sperm motility, percentage of normal sperm morphology, sperm concentration after treatment, and fertilization concentration(10,000 sperms/ml), and the differences were statistically significant(all $P<0.05$). Multivariate logistic regression analysis showed that a high percentage of primary infertility, a low percentage of tubal factors, a low percentage of normal sperm morphology, and low sperm concentration after treatment were independent risk factors for FF, and the differences were statistically significant (all $P<0.05$). Logistic binary regression fitting was used to construct a ROC curve prediction model for combined prediction of fertilization failure using various indicators, and the AUC was 74.6%.

Conclusion: A high percentage of primary infertility, a low percentage of tubal factors, a low percentage of normal sperm morphology, and low sperm concentration after treatment are independent risk factors for FF. The ROC curve model using combined indicators to predict FF constructed by logistic binary regression fitting is valuable in FF prediction.

Introduction

Complete FF (fertilization rate = 0) and low fertilization rate (fertilization rate < 30%) during in vitro fertilization (IVF) treatment are collectively referred to as FF, and their incidence is about 5–20% [1]. FF is associated with a significant reduction in oocyte utilization and even no available embryos, and the early treatment cost, time spent, and mental and emotional dedication are irreparable, which is a shattering blow to doctors and patients.

Most of the studies on FF focus on male sperm parameters, and it has been found that the FF rate increases significantly with the decrease in sperm parameters [2]. However, FF often occurs in patients with normal sperm parameters, and normal fertilization is observed in most patients with decreased sperm parameters in clinical practice, suggesting the presence of other factors affecting FF. Therefore, in addition to male sperm parameters, the whole treatment cycle of IVF, including female factors, ovulation

induction (OI) and laboratory fertilization, was analyzed in the present study to discover independent risk factors for FF in these processes and construct prediction curves based on these independent influencing factors to provide references for the fertilization method selection and reduce the incidence of FF.

Materials and Methods

Study subjects and grouping

Instead of the NF rate [3], a total FF of 30% was used as the cutoff point in the present study, considering that even abnormal fertilization also suggests the presence of fertilization ability of sperms and eggs, and the occurrence of abnormal fertilization is mainly related to the level of ovulation stimulating hormones [4] and the laboratory fertilization process [5].

Inclusion criteria

females with an age of ≤ 42 years old and a number of retrieved oocytes of ≥ 3 at the first oocyte retrieval cycle.

Exclusion criteria

males or females with abnormal chromosomes, and abnormal egg maturity(MII egg rate ≤ 50%). A total of 24 cycles with FF due to MII rate < 50% were excluded from the FF group.

Clinical data of IVF-assisted pregnancy in our hospital between January 2018 and December 2023 were retrospectively analyzed. A total of 2,474 cycles were included and divided according to the fertilization rate. There were 75 cycles with a fertilization rate of 0, i.e., complete FF, and 98 cycles with a fertilization rate of < 30%, i.e., partial FF, and these cycles were included in the FF group; and there were 2301 cycles with a fertilization rate of ≥ 30%, and included in the NF group. Because some patients in our center used short-term fertilization combined with rescue intracytoplasmic sperm injection (RICSI), FF in the RICSI cycles was determined based on the IVF fertilization rate before the RICSI procedure, and cycles with an IVF fertilization rate of < 30% were included in the FF group. Basic data, OI and laboratory fertilization were compared between the two groups. Multivariate logistic regression analysis was performed with FF in IVF as the dependent variable and the indicators with statistically significant differences in the univariate analysis as independent variables to screen independent risk factors for FF in IVF. A ROC curve prediction model for FF using combined indicators was constructed by logistic binary regression fitting.

Methods

OI and egg retrieval

An appropriate OI program was developed according to the age, ovarian reserve and other conditions of the patients.

Semen collection and treatment

Males abstained for 2–7 days, and semen was collected by masturbation. Semen was treated by density gradient centrifugation combined with swim-up methods. Briefly, 1ml 80% high gradient solution was added to the bottom of a 15ml conical centrifuge tube, and 1ml 40% low gradient solution was gently placed on the top. The liquefied semen was gently added to the top layer and centrifuged at 100g for 20 mins. The supernatant was removed, and the precipitate was transferred to a new centrifuge tube containing 3ml fertilization medium, mixed well, and centrifuged at 300g for 5 mins. The supernatant was removed, and the precipitate was mixed and added to the bottom of a 5ml round-bottom centrifuge tube for swim-up. The volume of the swim-up solution in the round-bottom centrifuge tube was adjusted according to the amount of recovered precipitate, and 0.5ml of fertilization solution was usually used as the swim-up solution. The mixture was incubated in an incubator with 6% CO_2. The gradient solution and culture medium used were Sage products(CooperSurgical, USA).

Fertilization methods (1) Long-term fertilization: 39-40hrs after hCG injection, the cumulus-oocyte complex was mixed with sperms and fertilized in a center well culture dish. The final fertilization concentration was adjusted to 100,000-300,000 sperms/ml according to the number of eggs in the dish, with no more than 12 eggs per dish. The granulosa cells were removed after 18–20 hours of culture, and the fertilization of oocytes was observed. (2) Short-term fertilization combined with RICSI: 39 -40hrs after hCG injection, the cumulus-oocyte complex was mixed with sperms. The granulosa cells were removed 5 hrs after fertilization, and the second polar body was observed in a new dish. If the ratio of oocytes with second polar body to mature oocytes (2pb rate) was > 30%, no intervention was needed. If the 2pb rate was < 30%, observation was performed again 1hr later. If the 2pb rate increased to > 30%, no intervention was needed, and if the rate remained < 30%, RICSI was performed on the oocytes with one polar body.

Determination of fertilization and calculation of fertilization rate

The pronucleus was observed 18-20hrs after hCG injection. The number of fertilized eggs was calculated as the sum of 2PN + 1PN + multiple PN + 0PN, and the fertilization rate was calculated as the number of fertilized eggs/total eggs.

Outcome measures

(1) Univariate analysis of FF during IVF. (2) Multivariate logistic regression analysis of FF during IVF. Factors with statistically significant differences in univariate analysis were included in the multivariate logistic regression analysis model to screen the independent risk factors for FF in IVF. (3) Logistic binary regression fitting was used to construct a ROC curve model of combined indicators to predict FF, and the area under the curve (AUC) was calculated.

Statistical analysis

SPSS23.0 software was used for statistical analysis. Measurement data with normal distributions were presented as ($\bar{x} \pm s$), and the independent sample T test was used for comparison between groups. Enumeration data were presented as percentages, and the chi-square test was used for comparison between groups. Risk factors were screened using multivariate logistic regression analysis. Differences with a p value of < 0.05 were considered statistically significant.

Results

Univariate analysis

Univariate analysis showed no significant differences in male age, female BMI, basal FSH, basal LH, basal PROL, AMH, blood glucose, TSH, FT4, Gn days, total Gn, concentrations of E2, LH, and P on hCG day, total number of eggs, and sperm concentration/egg (x10,000 sperms/egg) between the FF group and the NF group ($P > 0.05$ for all comparisons). However, there were significant differences in female age, infertile duration, initial dose of Gn, Gn dose/egg, sperm concentration before treatment, sperm motility, percentage of normal sperm morphology, sperm concentration after treatment, and fertilization concentration (10,000 sperms/ml), and all differences were statistically significant(all $P < 0.05$)(Table 1).

Table 1
Univariate analysis of three fertilization methods for female primary infertility, with measurement data presented as $\bar{x} \pm s$ and enumeration data as n(%).

Items	The FF group (n = 173)	The NF group (n = 2301)	P
Female age	31.05 ± 3.85	31.86 ± 4.21	0.013
Male age	32.34 ± 4.69	32.86 ± 4.65	0.157
Infertile duration(years)	4.37 ± 2.56	3.61 ± 2.70*	0.000
Female BMI	24.45 ± 4.40	24.50 ± 4.30	0.882
Basal FSH	5.87 ± 1.76	6.20 ± 2.14	0.052
Basal LH	6.67 ± 6.25	6.62 ± 6.64	0.919
Basal PROL	374.97 ± 217.37	354.26 ± 201.50	0.203
AMH	3.32 ± 3.45	3.93 ± 3.32	0.139
Blood glucose	5.39 ± 4.30	5.44 ± 0.52	0.190
TSH	2.20 ± 1.06	2.15 ± 1.08	0.563
FT4	15.49 ± 3.95	15.89 ± 3.62	0.159
Percentage of primary infertility	119(68.79%)	991(43.07%)*	0.000
Percentage of insulin resistance	2(1.16)	41(1.78%)	0.544
Percentage of PCOS	38(21.97%)	475(20.64%)	0.679
Percentage of endometriosis	9(5.20%)	90(3.91%)	0.403
Percentage of hypothyroidism	6(3.47%)	83(3.61%)	0.925
Percentage of tubal factors	85(49.13)	1614(70.14%)*	0.000
Initial dose of Gn	218.79 ± 64.10	232.53 ± 65.10	0.007
Gn days	11.81 ± 2.15	11.64 ± 2.16	0.316
Total Gn	2679.00 ± 880.73	2787.95 ± 973.05	0.153
Gn dose/egg	249.03 ± 183.97	294.59 ± 245.83	0.017
E2 on hCG day	14295.83 ± 8982.83	14948.76 ± 10255.93	0.484
LH on hCG day	2.51 ± 3.60	2.26 ± 2.15	0.173
P on hCG day	3.32 ± 2.12	3.43 ± 3.61	0.691
Total number of eggs	14.32 ± 6.78	13.75 ± 7.45	0.327
Sperm concentration	65.38 ± 66.63	84.14 ± 71.54	0.001

Items	The FF group (n = 173)	The NF group (n = 2301)	P
Sperm motility	29.33 ± 16.71	36.53 ± 17.41*	0.000
Percentage of normal sperm morphology	4.18 ± 1.76	5.33 ± 2.57	0.000
Sperm concentration after treatment	9.34 ± 5.75	12.00 ± 6.00*	0.000
Fertilization concentration	23.29 ± 3.32	22.61 ± 3.51*	0.014
Sperm concentration/egg	2.06 ± 1.16	2.17 ± 1.27	0.256

Multivariate Logistic regression analysis

Multivariate logistic regression analysis was performed using FF in IVF-embryo transfer cycles as the dependent variable, and indicators with statistically significant differences in univariate analysis as independent variables. The results showed that a high percentage of primary infertility, a low percentage of tubal factors, a low percentage of normal sperm morphology, and low sperm concentration after treatment were independent risk factors for FF, and the differences were statistically significant (all $P < 0.05$) (Table 2).

Table 2
Multivariate logistic regression analysis

Items	B	SE	Wald	Degree of freedom	Significance	Exp(B)
Female age	0.028	0.025	1.299	1	0.254	1.028
Infertile duration(years)	-0.061	0.032	3.607	1	0.058	0.941
Presence of primary infertility	0.785	0.201	15.296	1	0.000	2.192
Presence of tubal factors	-0.508	0.182	7.735	1	0.005	0.602
Gn dose/egg	0.001	0.001	1.055	1	0.304	1.001
Initial dose of Gn	0.001	0.002	.227	1	0.634	1.001
Sperm concentration	0.002	0.002	1.042	1	0.307	1.002
Sperm motility	0.009	0.006	2.554	1	0.110	1.009
Percentage of normal sperm morphology	0.209	0.057	13.154	1	0.000	1.232
Sperm concentration after treatment	0.063	0.018	11.587	1	0.001	1.065
Fertilization concentration	-0.013	0.029	.214	1	0.643	0.987
Constant	-0.292	1.052	.077	1	0.781	0.747

Logistic binary regression analysis

Logistic binary regression fitting was used to construct a ROC curve prediction model for the combined prediction of FF using various indicators, and the AUC was 74.6%(Fig. 1).

Discussion

A total of 2,474 cycles were included in this study, of which 173 cycles were included in the FF group(75 cycles with complete FF and 98 cycles with partial FF), with a FF rate of 7%, which was slightly lower than that reported previously [1]. This was possibly explained by the fact that only cycles with a number of eggs ≥ 3 and a female age ≤ 42 years old were selected, and cycles with abnormal egg maturation were excluded.

In patient factor analysis, in addition to general factors such as age, infertile duration, BMI, ovarian function parameters, and presence of primary infertility, the endocrine factors [6] that may affect egg quality were also analyzed, including blood glucose related to islet function [7], thyroid function [8], polycystic ovary syndrome(PCOS) [9], and endometriosis [10], and no differences were observed

between the two groups. It is notable that age and infertile duration were not independent influencing factors of FF in the present study.

The process of OI affects the quality of eggs [11]. Comparisons of the parameters such as Gn dose, Gn days, concentration of E2, LH, and P on hCG day, and total number of eggs revealed no differences. Factors with significant differences in univariate analysis, including the initial dose of Gn and Gn dose/egg, were not independent influencing factors in logistic regression analysis. Due to the complexity of the OI process, many individualized procedures are involved. Different study and grouping methods may lead to different conclusions. The absence of differences did not necessarily indicate that the OI process has no effect on fertilization, and future studies on this topic are needed.

Although significant differences in sperm concentration before treatment and sperm motility were noted in univariate analysis, they were not independent influencing factors of FF in logistic binary regression analysis, which may be explained by the fact that although the quality of sperm from each male was different, the final fertilization concentration can be adjusted in laboratory by increasing the amount of sperm to control the final fertilization concentration (10,000 sperms/egg, 10,000 sperms/ml) in a certain range [12].

In addition, there were differences in different semen analysis software and analysis methods [13], and unified external quality control was absent. The present study showed that the percentage of normal sperm morphology is an independent influencing factor of FF, which was consistent with the findings of other studies [14]. Therefore, the percentage of normal sperm morphology is of important value in the evaluation of male fertility.

In terms of laboratory factors, a difference in the sperm recovery rate is noted when different treatment methods and sperm separation solutions are used [15]. Density gradient centrifugation combined with swim-up was used in our hospital. Semen samples with different qualities were treated using the optimal treatment method in 5ml round bottom centrifuge tube with 0.5 ml culture medium, and no difference in sperm motility was found after treatment. Furthermore, the selection of fertilization methods is basically based on the quality of sperm processed on the same day. Therefore, the concentration of motile sperms in the swim-up solution is more representative of the total number of forward moving sperms, and can serve as an independent influencing factor of FF, which was consistent with the results of other studies [16].

In the selection of fertilization methods, due to the different sperm quality standards for implementing ICSI [16, 17], it is inevitable that changes in the percentage of ICSI may affect the incidence of FF in IVF because different sperm quality standards are used when ICSI procedure is performed, which is also one of the reasons for the significant difference in FF rates reported. In our center, the sperm quality standard for ICSI was a swim-up sperm concentration of $\leq 2x10^6$/ml, or a total number of motile sperm of $\leq 1x10^6$. If the cutoff value increases, the percentage of ICSI as well as the consequent risk will increase [18], and if the cutoff value decreases, the incidence of FF will increase.

In addition to the clinical characteristics of patients, complete FF may also be induced by genetic defects. NF can be achieved in some cycles through ICSI [19], and some cycles, however, require ICSI combined with artificial oocyte activation [20], which was not discussed in the present study.

Conclusions

In conclusion, the present study showed that a high percentage of primary infertility, a low percentage of tubal factors, a low percentage of normal sperm morphology, and low sperm concentration after treatment are independent risk factors for FF. The ROC curve model using combined indicators to predict FF constructed by logistic binary regression fitting is valuable in FF prediction.

However, this study also comes with limitation of a small number of samples. In view of this, more samples should be included in future studies to further validate the findings of this study.

Declarations

Acknowledgements

We are very grateful to the patients who agreed to provide clinical data to us.

Authors' Contributions

HZ Shicarried out the studies, participated in collecting data, and drafted the manuscript, and are responsible and accountable for the accuracy or integrity of the work; JJ Liu and RR Liu performed the statistical analysis and participated in its design; C Li and Q Songperformed the statistical analysis and prepared figures 1. All authors reviewed the manuscript.

Funding

This study is supported by 2023 Qinhuangdao Municipal Science and Technology Research and Development Plan(NO.202301A046).

Availability of data and materials

Not applicable.

Ethical approval and consent to participate

The study was approved by the Institutional Ethics Committee of Maternity & Child Care Center of Qinhuangdao(No.20230310), and written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

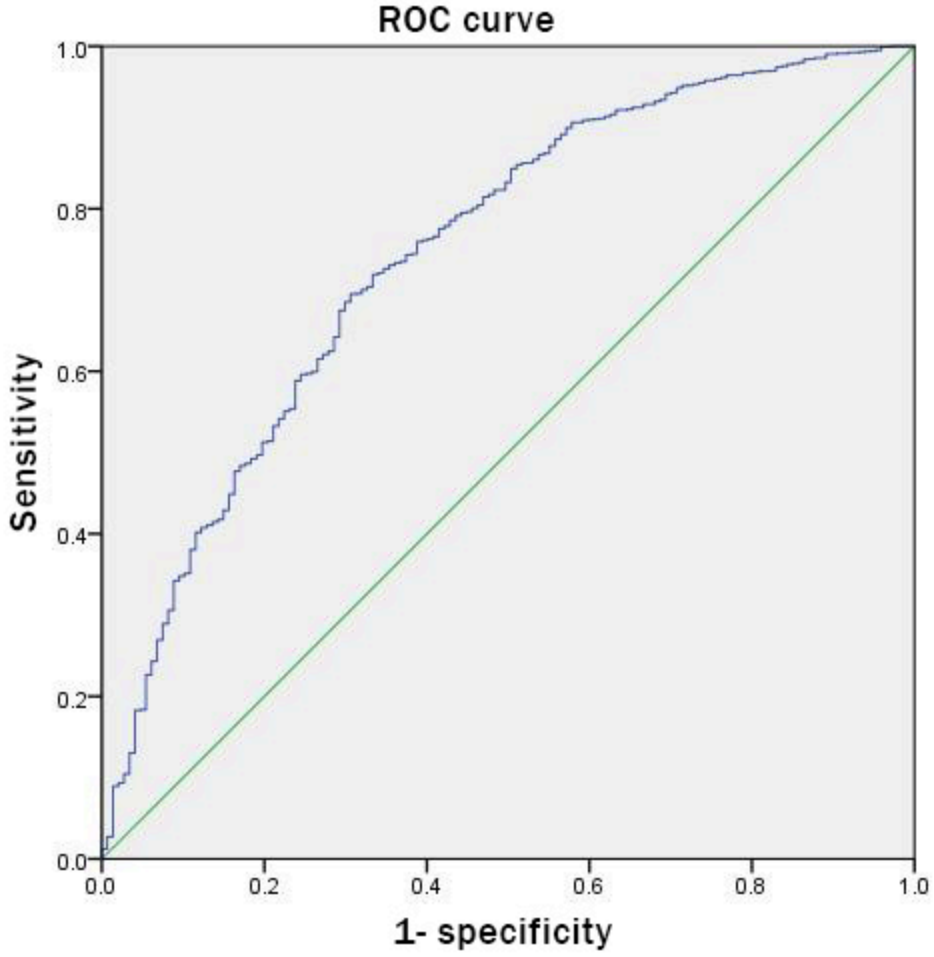

Figure 1

Comparative analysis of the non-recurrence rate in the 2 groups