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Research article

Multivariate analyses on male factors and construction of a nomogram for predicting low in vitro fertilization rate

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ABSTRACT

Low fertilization rate (LFR) and total fertilization failure (TFF) are often encountered in routine in vitro fertilization (IVF) procedure. To solve this problem, multivariate analyses on the relationship between male factors and in vitro fertilization rate were performed, and a nomogram for prediction of LFR was constructed. This retrospective study contained 2011 couples who received IVF treatment from January 2017 to December 2021. Man factors and in vitro fertilization rate were collected. Among these couples, 1347 cases had in vitro fertilization rates ≥30 % (control group), and 664 cases had in vitro fertilization rates <30 % (LFR group). Univariate analyses of male factors found that between the two groups there were significant differences ($p < 0.05$) in sperm progressive motility (SPR), sperm concentration (SC), total sperm number, normal sperm morphology rate (NSMR), DNA fragmentation index (DFI), sperm acrosin activity (SAA) and the clinical diagnosis of primary or secondary infertility. Multivariate logistic regression analyses showed that SPR, SAA, and SC were independent risk factors for LFR. An algorithm and a correspondent nomogram for predicting high LFR risk were constructed using data from the training cohort. The LFR nomogram exhibited an excellent discrimination power and a high fitting degree in both the training cohort (AUC = 0.90, 95 % CI: 0.88–0.92), (H-L: $x^2 = 5.43$, $p = 0.71$) and validation cohort (AUC = 0.89, 95 % CI:0.87–0.92), (H-L: $x^2 = 7.85$, $p = 0.45$), respectively. The decision curve analysis (DCA) demonstrated a high efficiency of the LFR nomogram for clinical utility. SPR, SAA, and SC are independent risk factors for LFR. The LFR nomogram established based on these factors could be a useful tool to predict high risk of LFR, and patients with high risk of LFR can be guided to direct ICSI procedure. Clinical application of the LFR nomogram may increase the in vitro fertilization rate by facilitating the decision making in IVF service.

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1. Introduction

Many studies have shown a worldwide trend for an increasing incidence of infertility in the past decades. According to data from European countries, the incidence rate of infertility in couples of childbearing age has risen from 7% to 8% in the 1960s to 15%–20 % in the 2010s [1,2]. It is estimated that in all infertility cases, the female factors account for 50 % of cases, and the male factors account for 30 % cases, with the rest 20 % being related to interactions between the two factors [3]. As a major technology of the assisted reproductive technology (ART) system, in vitro fertilization (IVF) is frequently applied to treat infertility patients with fallopian tube obstruction and/or male oligoasthenospermia. Intracytoplasmic sperm injection (ICSI) technique is used when routine IVF fails to achieve fertilization or in cases that laboratory tests show an obvious low number/viability of oocytes/sperms [4,5]. By reducing the requirements for gametes' quality and quantity required for fertilization under natural conditions, ICSI is effective for treating "refractory" infertility cases including those suffering severe oligoasthenospermia [6]. However, in clinical practice, the problem of low fertilization rate (LFR) and total fertilization failure (TFF) is often encountered in routine IVF procedure. The rescue-ICSI (re-ICSI) will be conducted in many centers of reproductive medicine. Unfortunately, the efficacy of re-ICSI is severely affected by the delayed decision and a reduced oocyte viability during the cell culture period of time in the initial in vitro fertilization attempt [7–9]. Indeed, it was reported that re-ICSI resulted in a much lower fertilization rate and pregnancy rate than direct ICSI (30.4 %, 0 % vs 50.1 %, 25.3 %) [9]. If we could anticipate the occurrence of LFR/TFF in routine IVF, a decision for direct ICSI would improve the efficiency of in vitro fertilization. Although some male factors are generally thought to be relevant to LFR/TFF, how to apply these parameters for TFF/LFR prediction and treatment decision making remains an issue to be solved.

Numerous univariate analyses have been performed to analyze the relationship between male factors such as age, sperm quality, normal sperm morphology rate (NSMR), sperm acrosin activity (SAA) and sperm DNA fragmentation index (DFI) to determine their relationship and the success of in vitro fertilization [10–13]. Chapuis et al. reported that low sperm progressive motility (SPR), e.g., below 32 %, had a significant negative impact on IVF, leading to a decreased fertilization rate and a low number of viable embryos at day 2. Also, when the father was older than 51 years and the mother older than 37 years, the success rates for either routine IVF or ICSI were significantly lower than young couples [10]. A study by Chen et al. showed that patients with low NSMR (<4 %) had significantly higher TFF rates than patients with high NSMR (≥ 4 %) (2.8 % versus 1.2 %, $p = 0.01$) [11]. Tang et al. reported that sperm DFI closely correlated with LFR/TFF (OR: 1.19; 95 % CI: 1.03–1.36; $p = 0.014$) in 523 study subjects containing 116 men diagnosed as mild-to-moderate asthenozoospermia and 407 men diagnosed as normozoospermia [12]. It is important to point out that all these previous studies were based on univariate analyses, and the complicated interactions among multiple male factors were not considered, which may cause biased conclusion. Moreover, without a comprehensive and unified algorithm, the findings from univariate analyses are difficult to apply in decision making for routing IVF or direct ISCI. From this point of view, multivariate analyses and construction of a nomogram capable of qualitatively reflecting the contributions by multiple male factors is required.

In this study, we performed multivariate analyses and identified the independent male factors for a high LFR risk, built a LFR predicting nomogram, and determined the optimal cutoff value. The efficiency of LFR nomogram was validated retrospectively in clinical cases. It is expected that the nomogram would guide cases with high LFR risk to direct ICSI procedure. By avoiding the loss of oocyte viability during routing IVF in cases with high LFR risk, application of the LFR nomogram may lead to a more effective IVF service.

2. Materials and methods

2.1. Study subjects

All the 3875 cases receiving IVF treatment at the Center of Reproductive Medicine, Women's Hospital of Jiangnan University, from January 2017 to December 2021, were reviewed. Diagnosis of infertility was made following the European Association of Urology guidelines: "Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year" [14]. The inclusion criteria were: couples went through the first conventional IVF cycle; the wife's age $\leq$38 years with more than 5 oocytes retrieved. Exclusion criteria were: family history of hereditary diseases, diagnosis of malignancies in reproductive system, abnormalities of the testis/epididymis/vas deferens, and infection of reproductive tract. As a widely applied accepted standard [15] and the routine practice in this center, cases with fertilization rates $\geq$30 % were classified into the control group, and cases with fertilization rates $<$30 % were classified into the LFR group. This retrospective study was approved by the Ethics Committee of the Women's Hospital of Jiangnan University (No:2021-01-0927-27). All of the participants provided written informed consent before enrollment, and the study was conducted according to the principles of the Helsinki Declaration. After application of exclusion criteria, 2011 cases were included for the study.

2.2. Semen analyses and sperm morphological analyses

Semen samples were collected by masturbation after abstinence for 2–7 days. Following liquefaction at 37 °C, routine semen tests was performed on a computer-assisted semen analysis platform (Sperm Class Analyzer, MICROPTIC, Spain) according to the criteria of the 5th edition of "WHO laboratory manual for the examination and processing of human semen" [16]. The laboratory tests were conducted by experienced technicians in the Andrology Laboratory with the ISO15189 license. Sperm morphological analyses were carried out by two trained technicians independently, and average values were used for analyses.

2.3. Sperm acrosin activity (SAA)

SAA was determined with a commercial kit purchased from the BERD Life Science (Shenzhen, China). Briefly, sperms were collected by centrifuging the liquefied semen samples at 500 rpm for 15 min. After rinsing with washing buffer provided by the kit, sperms were counted, and 7×10^6 sperms were exposed to polytetrafluoroethylene (PTFE) membranes. Two hundred μL of reaction buffer containing the N-α-benzoyl-DL-arginine-*para*-nitroanilide HCl (BAPNA) substrate was added to sperm cells absorbed to PTFE membranes. The mixture was incubated at 24 °C for 1 h. Activated acrosin hydrolyzed BAPNA to produce chromophoric 4-nitroaniline, whose absorbance was detected at 405 nm on a SpectraMax M2 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, USA). A control experiment without addition of spermatozoa was performed in parallel. After subtraction of the values from control experiments, the final SAA results were presented as μIU/10^6 sperm.

2.4. Sperm DNA fragmentation index (DFI)

Sperm DFI was assessed with the sperm chromatin structure assay (SCSA). The SCSA kit was purchased from CellPro Biotech Co., Ltd. (Ningbo, China). The assay was performed according to the manufacturer's instructions. In brief, 15,000 sperms were treated for 30 s with 400 μl of cell membrane permearlizing solution (0.1 % Triton X-100, 0.15 mol/L NaCl, and 0.08 mol/L HCl, pH 1.2). Sperms were stained with 1.2 ml of staining solution (6 μg/ml acridine orange [AO], 37 mmol/L citric acid, 126 mmol/L Na2HPO4, 1 mmol/L disodium EDTA, 0.15 mol/L NaCl, pH 6.0) for 3 min. Samples were measured with the FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) that was interfaced with a data analysis software (DFIView 2010 Alpha11.15, CellPro Biotech, Ningbo, China). A minimum of 5000 cells from two aliquots of each sample were analyzed by FACS scan. DFI was calculated as a ratio of red vs green fluorescence. As a quality control, if a difference between the replicated DFI assays was larger than 5 %, the sample would be re-measured.

2.5. IVF laboratory procedures

All the technicians involved in this study had received an uniform training and were licensed for performing IVF procedures in the laboratory of reproductive medicine. High quality sperms were enriched with the use of density gradient centrifugation method. Three to 4 h after oocyte retrieval, for each fertilization assay, one oocyte was inseminated with 20,000 selected spermatozoa in a 100-mL microdroplet. After 4 h, the oocytes were picked and placed in another microdroplet to repeat the fertilization step. The fertilization status was checked 16–20 h after insemination. The presence of 2 pronuclei (2 PN) indicated a normal fertilization. On day 3, the outcomes of IVF were determined for each oocyte retrieval operation. Fertilization rate = number of fertilized oocytes/number of oocytes retrieved.

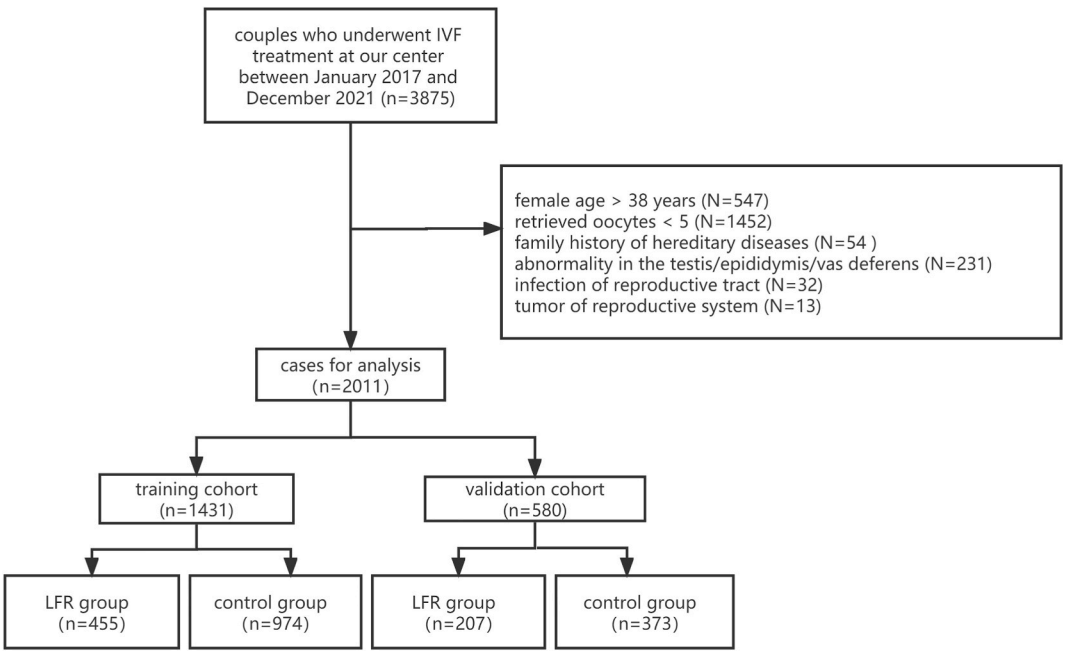


Fig. 1. Enrollment of study subjects. The total number of cases reviewed, time duration, exclusion, assignments of study subjects to the training and validation cohorts, and final numbers of cases in the control and LFR groups were documented. Note that"N"represents the number of cases excluded from the study by various reasons; "n" represents the number of cases enrolled to the study cohorts and groups.

2.6. Statistical analysis

Chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables were used in the univariate comparison of the variables between the LFR and normal groups as well as between the training and validation cohorts. The Shapiro-Wilk test was used for assessment of normal distribution. By the highly skewed distribution patterns of variables including the blood glucose level, body mass index (BMI), semen volume, SPR, sperm concentration (SC), total sperm number, NSMR, SAA, and DFI, natural logarithm transformation was applied before subsequent analyses. Univariate and multivariate logistic regression analyses were performed to identify factors significantly associated with LFR. The R software version 4.2.1 (http://www.rproject.org) was used for construction of the LFR nomogram. The discrimination power and optimal cutoff value of the nomogram were validated by the area under the receiver (AUC) of operating characteristic curve (ROC) as well as sensitivity/specificity in the validation cohort. The calibration plot was used to illustrate the correlation between the actual probability and the predicted probability of LFR. Clinical usefulness of the LFR nomogram was assessed with the decision curve analysis (DCA). Statistical analyses were conducted with the use of SPSS (version 18.0; SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Data collection and classification of study subjects

As illustrate in Fig. 1, applying the inclusion and exclusion criteria, 2011 cases entered study (Fig. 1). Among these cases, 1431 (70 %) cases were randomly assigned to training cohort and 580 (30 %) cases were for validation cohort.

3.2. Identification of factors associated with LFR

Since the results of Shapiro-Wilk test showed that the variables of blood glucose level, BMI, semen volume, SPR, SC, total sperm number, NSMR, SAA, and DFI did not conform with the normal distribution pattern, these values were transformed to the natural logarithm before univariate analysis. Patients were divided into ≤35 and > 35 groups. Initially, univariate analysis was performed, and the results (Table 1) indicated that compared to the control group, DFI and diagnosis pf primary infertility as significantly higher, and SPR, SC, total sperm number, NMRS, SAA and DFI were significantly lower in LFR group ($P < 0.05$). No significant difference was found in blood glucose level, BMI, and semen volume between the control and LFR groups.

3.3. Multivariate analyses, identification of independent factors for LFR and construction of algorithm

The eight significant variables found by univariate analysis were further analyzed with multivariate logistic regression method. Among these factors, SPR, SC, and SAA were identified as independent prognostic factors for LFR (Table 2). Multivariate analyses of all included factors reached the same conclusion on the independent nature of the 3 factors. An algorithm reflecting these 3 factors' contributions to LFR probability (LFRP) was derived based the data from training cohort with the use of logistic regression model: LFRP = 26.72–0.407ln(SC) - 3.658ln(SPR) - 3.157ln(SAA). Fig. 2 was the nomogram depicting the scoring system based on the 3 factors. As an example, the total points can be obtained for a case of male primary infertility with detailed SC, SPR, SAA values.

3.4. Validation and clinical utility of the LFR nomogram

Table 3 summarized the clinical characteristics and laboratory test results of all the study subjects, the training cohort and validation cohort. No significant difference was detected between the training and validation cohorts in all the studies variables ($p > 0.05$).

Table 1
Male variables for LFR risk as assessed by univariate analyses.

Factors		Control group (n = 974)	LFR group (n = 455)	Z/F	p
age	≤35	766(78.64 %)	340(74.73 %)	4.48	**0.03**
	> 35	209(21.36 %)	116(25.27 %)		
ln(Blood glucose level)		1.64(1.59,1.71)	1.64(1.59,1.70)	−1.16	0.24
ln(BMI)		3.19(3.11,3.29)	3.20(3.11,3.29)	−0.06	0.94
ln(Semen volume)		1.13(0.88,1.28)	1.13(0.83,1.31)	−0.15	0.87
ln(SPR)		4.00(3.84,4.18)	3.61(3.24,3.91)	−18.29	< **0.01**
ln(SC)		4.31 ± 0.68	3.93 ± 0.78	−18.29	< **0.01**
ln(total sperm number)		5.31(4.81,5.81)	4.97(4.40,5.62)	−6.16	< **0.01**
ln(NMRS)		2.48(2.14,2.71)	2.40(1.95,2.64)	−4.30	< **0.01**
ln(SAA)		4.08(3.81,4.25)	3.56(3.22,3.81)	−20.32	< **0.01**
ln(DFI)		2.49(2.02,2.90)	2.63(2.10,3.10)	−3.73	< **0.01**
Clinical diagnosis	Primary infertility	566(58.11 %)	302(66.37 %)	8.71	< **0.01**
	Secondary infertility	409(41.89 %)	154(33.63 %)		

The values of continuing variables are logarithm-transformed, and the numbers in brackets are lower and upper quartile values. Under the categories of age and clinical diagnosis, the numbers in brackets are composition percentages.

Table 2
Independent predictive factors for LFR identified by multivariate logistic regression analysis.

Factors	B	SE	Walds x^2	p	OR (95 % CI)
ln(SPR)	−3.66	0.29	156.27	0.02	0.03(0.02 ~ 0.05)
ln(SAA)	−3.16	0.24	176.11	0.01	0.04(0.03 ~ 0.07)
ln(SC)	−0.41	0.20	4.06	0.04	0.67(0.45 ~ 0.99)

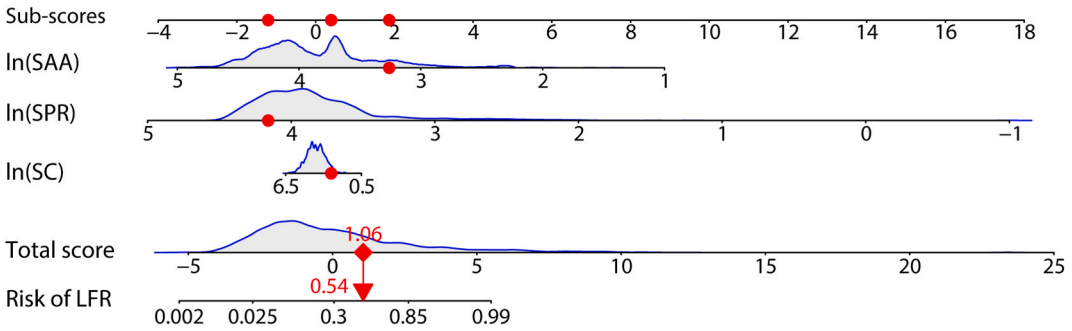


Fig. 2. The LFR nomogram and its usage. As an example, a 29-year-old male diagnosed with primary infertility, the results of laboratory tests showed: SC = 18.1 × 10^6/ml; SPR = 64.1 % and SAA = 26 uIU/10^6. The correspondent sub-scores (0.43, −1.32, 1.95) contributed by these 3 factors were found from the scale line on the top. A total score (1.06) was reached by addition of the 3 sub-scores. An estimated risk of LFR (0.54) was obtained by finding the value codependent to the total score on the scale line at bottom. This score higher than the cutoff value (0.31) predicts a high LFR risk for the patient.

Table 3
Characteristics between the training cohort and validation cohort.

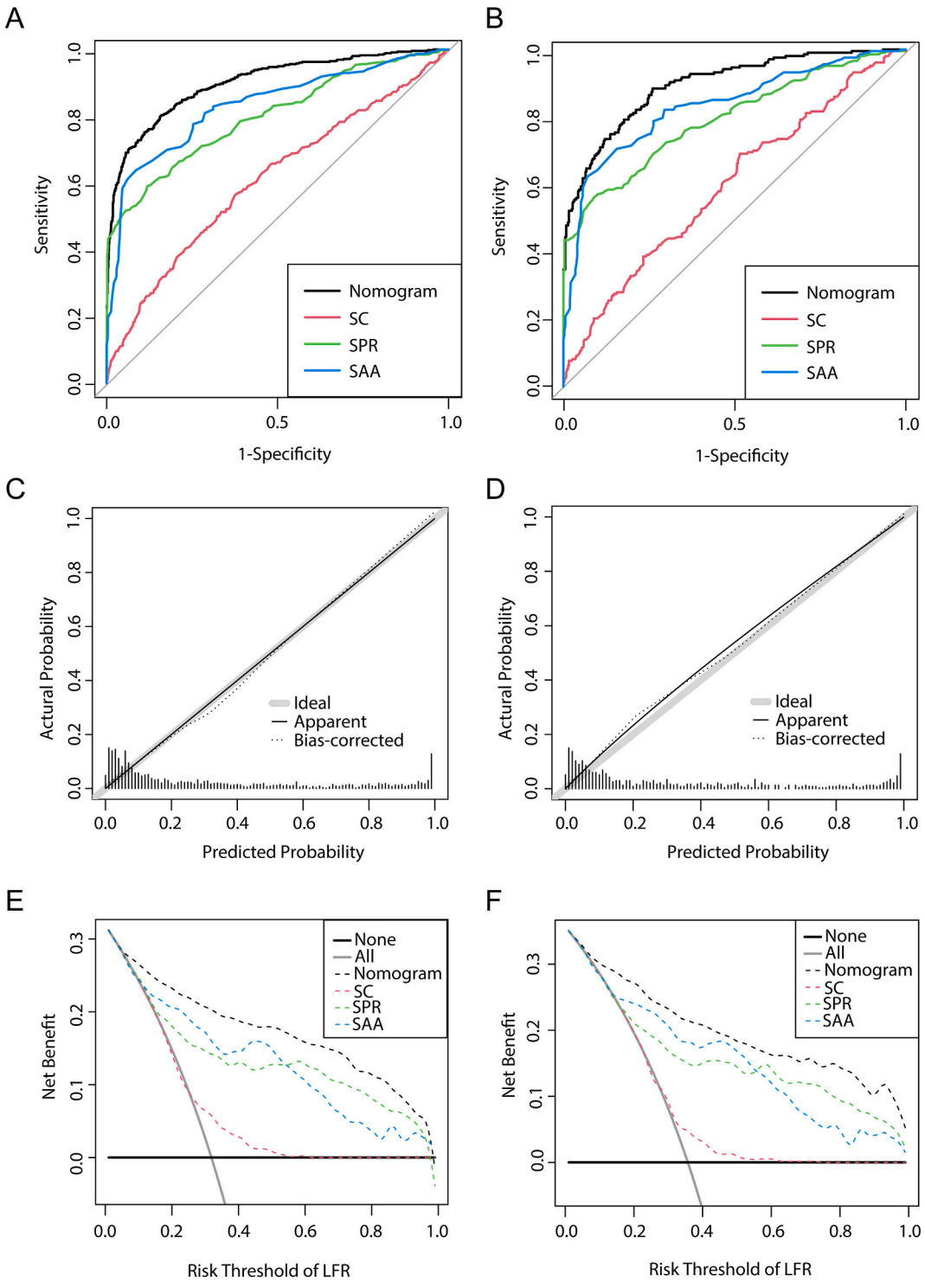
Characteristic		Total study subjects (n = 2011)	Training cohort (n = 1431)	Validation cohort (n = 580)	P
Age (years)	≤35	1558(77.47 %)	1106 (77.29 %)	452 (77.93 %)	0.37
	> 35	453(22.53 %)	325 (22.71 %)	128 (22.07 %)	
Blood glucose level (mmol/L)		5.21(4.92; 5.54)	5.23 (4.81; 5.57)	5.12 (4.83; 5.54)	0.23
BMI		24.43(22.41; 26.72)	24.41 (22.31; 26.70)	24.8 (22.42; 26.73)	0.902
Semen volume (ml)		3.11 (2.42; 3.60)	3.09 (2.31; 3.60)	3.14 (2.40; 3.60)	0.96
SPR (%)		50.32 (40.02; 61.81)	50.25 (40.22; 62.11)	50.67 (38.28; 60.89)	0.68
SC (10^6/ml)		62.4 (38.1; 101.7)	61.9 (37.8; 101.9)	63.3 (39.5; 101.1)	0.61
Total sperm number		186.1 (108.8; 312.3)	189.1 (108.2; 318.6)	183.5 (111.1; 307.3)	0.97
NSMR (%)		11.51 (8.23; 14.12)	12.12 (8.11; 14.55)	10.75 (7.81; 14.01)	0.24
SAA (μIU/10^6)		52.20 (39.22; 66.3)	53.32 (39.37; 66.28)	50.15 (38.85; 67.32)	0.35
DFI (%)		12.61 (7.83; 19.31)	12.51 (7.74; 19.25)	12.76 (7.89; 19.61)	0.66
Clinical diagnosis	Primary infertility	1231 (61.21 %)	868 (70.51 %)	363 (29.49 %)	0.42
	Secondary infertility	780 (38.79 %)	563 (72.17 %)	217 (27.73 %)	

For continuing variables, the numbers in brackets are lower and upper quartile values. Under the categories of age and clinical diagnosis, the numbers in brackets are composition percentages.

The ROC of the nomogram were plotted based on data from the training and validation cohorts. The AUC in the training and validation cohort were 0.90 (95 % CI: 0.88–0.92) and 0.89 (95 % CI:0.87–0.92), respectively, indicating a favorable discrimination ability of the nomogram for estimation of LFR probability. Moreover, as demonstrated by Fig. 3A, the AUC by the LFR nomogram was larger than any AUC by a single predictor (SAA, SC, SPR) in either training cohort or validation cohort (Fig. 3B). An optimal cutoff value of 0.31 for the best usage of nomogram was derived from the ROC curve of the training cohort. With this cutoff value, the sensitivity, specificity and overall accuracy of the LFR nomogram reached 0.84, 0.83 and 0.86, respectively. There was a high agreement between LFR probability predicted by the LFR nomogram and actual LFR in both the training cohort (H-L: $x^2 = 5.431$, p = 0.711, Fig. 3C) and the validation cohort ($x^2 = 7.85$, p = 0.45, Fig. 3D). DCA was performed to evaluate the clinical utility of the LFR nomogram based on the net benefits at different threshold probabilities. Compared with the usage of SAA, SC, SPR alone, the increased net benefit the LFR nomogram was the largest in both the training cohort (Fig. 3E) and validation cohort (Fig. 3F), indicating that the nomogram was a reliable clinical tool for predicting the LFR outcome.

4. Discussion

Low fertilization rate and total failure of fertilization occur in 5–20 % of conventional IVF cycles [17–19]. Not only disappointing for patients and physicians, LFR/TFF and repeated IVF cycles impose a heavy health and financial burden to patients. Although



(caption on next page)

Fig. 3. Validation and clinical utility of the LFR nomogram. The ROCs were plotted by sensitivities and specificities of the LFR nomogram as well as individual factors of SPR, SC and SAA, using data from the training (A) or validation (B) cohorts. The calibration curves of the LFR nomogram were plotted with data from the training (C) or validation (D) cohorts. There was a high fitting degree between the predicted and actual probability (H–L: $x^2 = 5.43$, $p = 0.71$ in training cohort and $x^2 = 7.85$, $p = 0.45$ in validation cohort). DCA of LFR nomogram in the training (E) and validation (F) cohorts. The x-axis and the y-axis are the threshold probability and the net benefit, respectively. Solid black line: assuming no patient will have LFR; solid gray line: assuming all patients will have LFR. Dotted black line: decision based on the LFR nomogram; dotted red line: binary decision rule based on SC alone; dotted green line: binary decision rule based on SPR alone; dotted blue line: binary decision rule based on SAA alone. Decisions by the LFR nomogram accomplish the most benefit in comparison with decisions by any single factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rescue-ICSI provides another fertilization opportunity and the procedure has been broadly used in many reproductive medicine centers, the procedure achieves a low fertilization rate [7,8]. Observation of our reproductive medicine center is consistent with this conclusion (data not shown). It was also reported that the developmental potentials of the embryos from rescue-ICSI are often compromised in comparison to those from direct ISCI [8,9]. The low fertilization rate of rescue-ICSI is mostly caused by the continuous deterioration of oocyte's viability during the conventional IVF stage. Direct application of ICSI to cases with high LFR/TFF risk may improve the fertilization outcome. Without comprehensive consideration, the findings of univariate analyses on individual factors are difficult to apply in medical practice. Thus, construction of a nomogram capable of predicting the high LFR/TFF risk afford a convenient tool for treatment decision. Take an example of a 29-year-old patient diagnosed with primary infertility. The results of semen analysis are as following: semen volume: 4.2 ml; SC: 18.1×10^6/ml; SPR: 64.1 %; total sperm number: 75.6×10^6; NMSR: 14 %; DFI 54.2 %; SAA: 26 uIU/10^6. While the former 5 test results are in normal range, the later 2 test results are abnormal, leaving the physician in charge perplexed to make a treatment decision. As shown in Fig. 2, in this case the LFR nomogram gives a total score of 0.54, which has surpassed the cutoff value of 0.31. With a high risk of LFR/TFF, the couple may benefit most from a decision for direct ICSI.

The identification of SPR, SC, SAA as independent predictive factors for a high LFR risk is not a total surprise. SPR and SC are considered defining parameters for sperm quantity and viability. Several studies have shown decreased SAA levels in infertile men [20,21]. Xu et al. investigated the relationship between SAA and IVF fertilization rate, and found that patients with low SAA levels (<25 uIU/10^6) had a significantly lower fertilization rate than those with relatively high SAA levels (≥25 uIU/10^6) [13]. Acrosin represents a series of trypsin-like serine proteinases within the acrosome of human spermatozoa [22,23]. Activated acrosin lyses the zona pellucida (ZP) of oocyte, facilitating the sperm to penetrate the ZP to achieve fertilization [23,24]. The observation that the total sperm number was not an independent predictive factor for LFR was partially due to the pre-selection of patients for conventional IVF. In this and many other reproductive medicine centers, only patients with sufficient total sperm numbers are considered for conventional IVF. Kastrop et al. proposed a minimum count of 1 million motile spermatozoa as a criterion for conventional IVF [25]. In this center, patients with total sperm numbers higher than 2 millions will receive conventional IVF treatment. Patients with total sperm numbers below 2 millions will be treated with direct ICSI. Thus, the negative result regarding the total sperm number should be limited to the selected patient population in this study.

Literature review found controversial observations on the clinical significance of DFI for fertilization. In this study, univariate analysis showed that DFI was significantly higher in LFR group than control group, a reminiscence of positive observations from several studies [12,26]. On the other side, some studies showed that DFI may not be a critical parameter. Lin et al. reported that among the high, moderate, and low DFI groups, there were no significant difference in IVF rate, and ICSI fertilization rate, good embryo rate, and pregnancy rate [27]. A meta-analysis covering data from 11 studies found that between the high and low DFI groups there was no significant difference in IVF fertilization rate (RR = 0.94, 95 % CI: 0.77–1.14, P = 0.61), pregnancy rate (RR = 0.83, 95 % CI: 0.57–1.21, P = 0.32), and live birth rate (RR = 0.53, 95 % CI: 0.16–1.80, P = 0.31) [28]. More studies with better control of confounding factors and application of multivariate analysis method are required to reach a firm conclusion.

It is noteworthy that while our variate analyses detected significant differences between the LFR and control groups in as many as 8 male factors including DFI, age, SPR, SC, total sperm number, NMRS, SAA, and clinical diagnosis of primary or secondary infertility, the mutiunivaraite analyses only identified SPR, SC, and SAA as independent predictive factors for high LFR risk. This seemingly paradoxical situation may reflect the divergence between the two statistical methods and the potential advantage of mutivariate analyses. The results of univariate analyses indicate the importance of a single factor with an assumption that this factor operates by itself, without considering the interactions with other relevant factors. For this reason, by the multi-factoral nature and strong intrinsic interactions of various male factors related to fertilization failure, results of univariate analyses may fall short to reach a subjective conclusion. Multivariate analyses can largely eliminate this limitation. For example, age by itself may be an important contributing factor to LFR, but by its strong interactions with SPR, SC, and SAA, its effects could be readily represented by these independent factors. The comprehensive nature and the convenience for clinical application constitute 2 major advantages of the multivariate analyses as well as the LFR nomogram.

In both the training and validation cohorts, the AUC of the LFR nomogram was higher than 0.85 and the calibration curves were close to the ideal 45° line, which demonstrated an excellent discrimination and calibration of the nomogram. The DCA curves revealed a greater net benefit of the LFR nomogram than that of any single factor. The usefulness of the LFR nomogram comes from its efficiency for predicting a high possibility of LFR, a key to the improvement of overall fertilization outcome. Its clinical application will direct some high-score individuals to direct ICSI treatment, instead of going through the conventional IVF and rescue-ICSI that may drag down the total success rate. Although the 83 % sensitivity and 84 % specificity are relatively high, usage of the LFR nomogram may lead to an increased cost, especially for the 16 % patients that would be mis-prognosed for LFR. Although the direct ICSI procedure

does not constitute a health concern, and the increased cost of ICSI is moderate considering whole cost of IVF process including examination, operation preparation, oocyte retrieval, and in vitro fertilization, but the possibility of extra-cost should be fully explained to patients, and an informed consent is necessary.

One shortage of the current study is that limited by the data availability, the model construction was merely based the male factors. It is known that female factors such as the oocyte quality and quantity, age, and hormone levels can all affect the IVF outcome of in vitro fertilization. Wang et al. reported that a nomogram model combined both male parameters and female reproductive indicators was constructed to produce convenient prediction models of poor fertilization and fertilization failure [29]. The effects of technique, equipment and skill levels of IVF performers were not considered either. Future studies with these factors included should be conducted for the improvement of the prediction model. Another limitation of the current study is the lack of an actual validation. Since the application of LFR nomogram will change the downstream treatment procedures, the model validation based on retrospective data was unable to replace a prospective validation. The real efficacy of the LFR nomogram should be determined by comparing the total fertilization rate of conventional IVF (fertilization rate from routine IVF plus the fertilization rate from rescue-ISCI) with the total fertilization rate applying the LFR nomogram (fertilization rate from routine IVF by the cases of low LFR risk plus the fertilization rate from direct ICSI by the cases of high LFR risk). Also, a multi-center trial with better representation of total male infertility cases need to be performed for extrapolation purpose.

5. Conclusions

Through multivariate logistic regression analyses, we found that SC, SPR and SAA are independent factors for high LFR risk. Based on these factors, a nomogram capable of quantitative and conveniently predicting an individual couple's LFR risk was built. In spite of its limitations, the study efforts represents the first attempt to establish a predictive model, and upon actual validation, the LFR nomogram may facilitate the decision making in clinical practice, and ultimately, leads to the improvement of IVF service.

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Data availability

The data associated with our study has not been deposited into any publicly available repository. The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Mengyuan Lin: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Yuwei Zhang:** Writing – original draft, Data curation, Conceptualization. **Honghua Wang:** Methodology, Investigation, Conceptualization. **Yan Wang:** Writing – original draft, Conceptualization. **Yang Wang:** Project administration, Methodology, Investigation. **Ninghan Feng:** Writing – review & editing, Writing – original draft, Formal analysis. **Qingwen He:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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