Nomogram models to predict low fertilisation rate and total fertilisation failure in patients undergoing conventional IVF cycles

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ABSTRACT

Objectives To establish visualised prediction models of low fertilisation rate (LFR) and total fertilisation failure (TFF) for patients in conventional in vitro fertilisation (IVF) cycles.

Design A retrospective cohort study.

Setting Data from August 2017 to August 2021 were collected from the electronic records of a large obstetrics and gynaecology hospital in Sichuan, China.

Participants A total of 11,598 eligible patients who underwent the first IVF cycles were included. All patients were randomly divided into the training group (n=8129) and the validation group (n=3469) in a 7:3 ratio.

Primary outcome measure The incidence of LFR and TFF.

Results Logistic regressions showed that ovarian stimulation protocol, primary infertility and initial progressive sperm motility were the independent predictors of LFR, while serum luteinising hormone and P levels before human chorionic gonadotropin injection and number of oocytes retrieved were the critical predictors of TFF. And these indicators were incorporated into the nomogram models. According to the area under the curve values, the predictive ability for LFR and TFF were 0.640 and 0.899 in the training set and 0.661 and 0.876 in the validation set, respectively. The calibration curves also showed good concordance between the actual and predicted probabilities both in the training and validation group.

Conclusion The novel nomogram models provided effective methods for clinicians to predict LFR and TFF in traditional IVF cycles.

INTRODUCTION

In vitro fertilisation-embryo transfer (IVF-ET) is widely used for infertility treatment, but total fertilisation failure (TFF) and low fertilisation rate (LFR) are still inevitable in IVF cycles. It is reported that low or failed fertilisation affects 5–20% of IVF cycles. This leads to serious psychological and economic burdens for infertile couples.

In traditional IVF cycles, TFF still occurs in the absence of abnormal semen. This may suggest that it is not enough to predict it by only male parameters and combining some representative indicators of women...
seems to be necessary. Furthermore, it has been proved that performing intracytoplasmic sperm injection (ICSI) on some oocytes could increase the fertilisation rate compared with IVF. If there is a specific model to accurately predict low or failed fertilisation in the early stage of treatment to take remedial measures, the unnecessary loss of patients will be reduced. Therefore, this study aims to identify the critical predictors of LFR and TFF and establish nomogram models in patients treated with conventional IVF.

MATERIALS AND METHODS

Study design and patients

This was a retrospective cohort study conducted at Chengdu Xinan Gynaecology Hospital (China). The data of patients were collected from electronic medical records. All the first fresh IVF cycles performed in infertile couples from August 2017 to August 2021 were reviewed for possible inclusion. The reason for the restriction to the first cycle was to eliminate any bias that may arise from the clinical management of previous cycles over subsequent cycles. Those women with (1) ICSI, (2) aged ≥50 years old, (3) polycystic ovarian syndrome, (4) endometriosis, (5) none of the oocyte retrieved, (6) donor sperm and (7) missing data were excluded. Final analyses were limited to 11 598 patients with complete information. The data set of the patients was randomly divided into the training set and the validation set by the ‘set.seed ()’ command of R software. Among the 11 598 patients, 70% of the patients (n=8129) were grouped into the training set to develop the nomogram models for LFR and TFF, respectively. The remaining 30% of the patients (n=3469) were grouped into a validation set for internal model verification.

Couples would undergo fertility examinations before receiving IVF treatment. The information of baseline characteristics of age, body mass index (BMI), duration of infertility, infertility type, diagnosis, history of pregnancy, history of abortion, basal hormone levels and pretreatment semen parameters were collected before the IVF treatment. Patients with polycystic ovarian syndrome were diagnosed according to the Rotterdam criteria. For the diagnosis of endometriosis, women who had been diagnosed with endometriosis by laparoscopy were classified as endometriosis. For the remaining patients, endometriosis was diagnosed only after it was confirmed by transvaginal ultrasound or MRI based on the European Society of Human Reproduction and Embryology (ESHRE) guideline.

Semen samples were obtained by masturbation and analysed based on the fifth WHO criteria when patients underwent fertility examinations before the initial cycle, including concentration, morphology and motility. Ovulatory induction hormone levels and clinical parameters were also recorded in the process of ovarian stimulation.

Clinical protocols

Women in this study were administrated by follicular phase gonadotropin-releasing hormone (GnRH) agonist protocol, luteal phase GnRH agonist protocol, GnRH antagonist protocol or progesterone primed ovarian stimulation based on their baseline characteristics. For the follicular phase GnRH protocol, women were treated with an injection of 3.75 mg GnRH agonist (Dayfline, Beaufour Ipsen, France) on the 2nd to 5th day of menstrual cycle. Downregulation (follicle diameter ≤5 mm, estradiol (E2) <50 pg/mL, LH <5 mIU/mL) was confirmed after 28–37 days. For the luteal phase GnRH agonist protocol, 0.1 mg short-acting GnRH agonist (Triptorelin Acetate, Ferring GmbH, Germany) was injected subcutaneously from the mid-luteal phase of the menstrual cycle for 15–18 days until pituitary downregulation was confirmed (follicle diameter ≤5 mm, E2 <50 pg/mL, LH <5 mIU/mL). Then the gonadotropin (Gn) (recombinant follicle-stimulating hormone (FSH), Gonalfin, Merck Serono, Switzerland) with an initial dose of 100–225 IU was administered until the follicles matured. For the GnRH antagonist protocol, Gn (recombinant FSH, Gonalfin, Merck Serono, Switzerland), generally 100–300 IU per day, was administered on the 2nd to 4th day of the menstrual cycle. GnRH antagonist 0.25 mg (Cetroteide, Aeterna Zentaris, Canada) was injected subcutaneously every day when the dominant follicle diameter was ≥12–14 mm or on the 5th to 6th day of administration until the trigger day. For the progestin primed ovarian stimulation (PPPOS), medroxyprogesterone acetate (MPA, Xianju Pharmaceutical, Zhejiang, China) was taken orally for 6–10 mg/day on the 2nd to 5th days of menstrual cycle. FSH (Urofollitropin, Lizhu Pharmaceutical Group, Shanghai, China), 150–300 IU per day, was injected on the same day. During the ovarian stimulation, the follicle development and the levels of serum LH and E2 were monitored to adjust the dose of Gn. Human chorionic gonadotropin (HCG) (Merck Serono, Switzerland or Lizhu Pharmaceutical, China) and/or GnRH agonist (Ferring Pharmaceutical, Switzerland) injection was used as a trigger when at least three follicles were ≥17 mm in diameter, and oocyte retrieval was performed after 34–36 hours.

Outcome measures

The presence of two pronuclei (2PN) and two polar bodies (2PB) in an oocyte was considered as normal fertilisation. According to The Vienna Consensus, total fertilisation failure was defined as no oocytes showing signs of fertilisation, and low fertilisation rate referred to the proportion of fertilised oocytes with 2PN and 2PB <25%. The fertilisation rate—the number of oocytes with 2PN and 2PB/the number of cumulus-oocyte complexes (COC) inseminated×100%.

Statistical analysis

Descriptive statistics of quantitative and qualitative data were presented as mean (SD) and numbers (percentages), respectively. Corresponding differences in the
Characteristics between-group were compared using χ² test (categorical variables) or Student’s t-test (continuous variables) where appropriate.

Variables with p<0.05 in the univariate analyses were considered as candidate predictive factors to be included in the multivariate analysis. Multivariate logistic regression analyses with forward–backward stepwise selection in the training set were proceeded to identify the most predictive indicators with p<0.05. And then the nomograms visualised the prediction models structured by logistic regressions. The discrimination power of the models was assessed by the receiver operating characteristic (ROC) curve which calculated the area under the curve (AUC). The calibration curves with bootstrap method were plotted to evaluate the accuracy of the models based on the observed and predicted probabilities.

All statistical analyses were performed using R V.4.0.5. P values<0.05 were considered indicative of statistical significance.

**Patient and public involvement**

No patients or members of the public were involved in the design, conduct or reporting of this study. The study results were not disseminated to study participants.

## RESULTS

### Patient characteristics

There were 318 women (2.7%) in the LFR group and 360 women (3.1%) in the TFF group. The mean age of these subjects was 31.8 years. Baseline characteristics, ovarian stimulation parameters and sperm parameters were listed in tables 1 and 2 respectively.

| Table 1 | Comparisons of patient baseline characteristics according to the fertilisation rate |
|---------|----------------------------------------------------------------------------------|
| **Baseline characteristics** | **Total (n=11598)** | **Control (n=10920)** | **LFR (n=318)** | **TFF (n=360)** | **P value (LFR vs control)** | **P value (TFF vs control)** |
| **Age, mean (SD)** | 31.8 (4.7) | 31.7 (4.7) | 30.7 (4.4) | 34.5 (5.4) | <0.001 | <0.001 |
| **BMI (kg/m²), mean (SD)** | 22.0 (3.2) | 22.0 (3.2) | 22.3 (3.2) | 22.4 (3.0) | 0.093 | 0.038 |
| **Infertility years, mean (SD)** | 3.8 (3.2) | 3.8 (3.1) | 3.8 (3.1) | 4.7 (3.7) | 0.776 | <0.001 |
| **Infertility diagnosis, n (%)** |  |  |  |  |  |  |
| Tubal factor | 8276 (71.4) | 7849 (71.9) | 234 (73.6) | 193 (53.6) | 0.001 | <0.001 |
| Diminished ovarian reserve | 1237 (10.7) | 1094 (10.0) | 15 (4.7) | 128 (35.6) |  |  |
| Ovulatory dysfunction | 236 (2.0) | 217 (2.0) | 13 (4.1) | 6 (1.7) |  |  |
| Uterine factor | 335 (2.9) | 323 (3.0) | 5 (1.6) | 7 (1.9) |  |  |
| Sperm factor | 741 (6.4) | 702 (6.4) | 26 (8.2) | 13 (3.6) |  |  |
| Unexplained infertility | 773 (6.7) | 735 (6.7) | 25 (7.9) | 13 (3.6) |  |  |
| Primary infertility, n (%) | 6538 (56.4) | 6193 (56.7) | 147 (46.2) | 198 (55.0) | <0.001 | 0.555 |
| **Gravidity, n (%)** | 0.002 | 0.510 |
| 0 | 4554 (39.3) | 4263 (39.0) | 153 (48.1) | 138 (38.3) |  |  |
| 1 | 2997 (25.8) | 2831 (25.9) | 80 (25.2) | 86 (23.9) |  |  |
| ≥2 | 4047 (34.9) | 3826 (35.0) | 85 (26.7) | 136 (37.8) |  |  |
| **Parity, n (%)** | 0.210 | 0.061 |
| 0 | 9779 (84.3) | 9212 (84.4) | 277 (87.1) | 290 (80.6) |  |  |
| ≥1 | 1819 (15.7) | 1708 (15.6) | 41 (12.9) | 70 (19.4) |  |  |
| **Induced abortion, n (%)** | 3770 (32.5) | 3548 (32.5) | 95 (29.9) | 127 (35.3) | 0.357 | 0.292 |
| **Miscarriage, n (%)** | 1659 (14.3) | 1569 (14.4) | 38 (11.9) | 52 (14.4) | 0.257 | 1.000 |
| Basal FSH (mIU/mL), mean (SD) | 8.30 (3.4) | 8.23 (3.3) | 7.69 (2.8) | 10.8 (5.3) | <0.001 | <0.001 |
| Basal LH (mIU/mL), mean (SD) | 4.5 (3.5) | 4.5 (3.4) | 4.3 (2.8) | 4.8 (4.1) | 0.225 | 0.146 |
| AMH (ng/mL), mean (SD) | 3.2 (2.4) | 3.2 (2.4) | 3.5 (2.1) | 1.4 (1.6) | 0.036 | <0.001 |
| AFC, mean (SD) | 13.4 (7.7) | 13.6 (7.7) | 15.0 (7.5) | 7.0 (5.3) | <0.001 | <0.001 |

Continuous variables are expressed as mean (SD), categorical variables as absolute frequencies, n (%).

AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicular-stimulating hormone; LFR, low fertilisation rate; LH, luteinising hormone; TFF, total fertilisation failure.
Women with low fertilisation rates were younger than controls but more suffered from primary infertility so they were less likely to be pregnant. Sperm abnormality was the main cause of infertility second to tubal factor. They had lower FSH levels, higher anti-Müllerian hormone (AMH) levels, and more antral follicle count (AFC) than controls. After ovarian stimulation, their mean concentration of LH on trigger day was significantly lower but other hormones and Gn dose were not different compared with the controls. However, women with LFR underwent a longer duration of stimulation and got more oocytes retrieved. Moreover, the sperm concentration and motility of their spouses at the initial semen analysis were significantly decreased.

Women with TFF displayed significantly higher age, BMI, FSH level and longer duration of infertility compared with the controls. More women were diagnosed with diminished ovarian reserve than controls. But the history of pregnancy and miscarriage were comparable between the two groups. Furthermore, the indicators of ovarian reserve (AMH and AFC) in patients with TFF were significantly lower than in controls (p<0.001). They were more likely to employ the PPOS protocol (p<0.001). The responses to ovarian stimulation which were reflected by the concentrations of progesterone (P), LH, E2 and FSH before HCG injection were also significantly different. The less Gn dose, shorter duration of stimulation and fewer oocytes retrieval were observed in the TFF group (p<0.001). Their partner’s initial sperm motility decreased significantly.

We also compared the characteristics of patients between the training set (n=8115) and the validation set (n=3483) (tables 3 and 4). There were no statistically significant differences between the two cohorts generally (except for infertility years).

### Logistic regression analyses for LFR and TFF

Multivariate logistic regression analyses revealed that follicular phase GnRH agonist protocol and primary infertility were independently associated with a higher risk of LFR, whereas PPOS and initial progressive sperm motility were independently associated with decreased

| Table 2 Comparisons of ovarian stimulation and semen characteristics according to the fertilisation rate |
|--------------------------------------------------------------------------------------------------|
|                                                                                                                         |
|                                                                                                                         |
| **Ovarian stimulation**                                                                                                   |
| **Ovarian stimulation protocol, n (%)**                                                                                   |
| GnRH antagonist                                                                                                          |
| 4387 (37.8) 4229 (38.7) 103 (32.4) 55 (15.3)                                                                             |
| Follicular phase GnRH agonist                                                                                             |
| 3887 (33.5) 3683 (33.7) 159 (50.0) 45 (12.5)                                                                             |
| Luteal phase GnRH agonist                                                                                                 |
| 1067 (9.2) 1012 (9.3) 32 (10.1) 23 (6.4)                                                                               |
| Progesterone primed ovarian stimulation                                                                                   |
| 2257 (19.5) 1996 (18.3) 24 (7.5) 237 (65.8)                                                                             |
| FSH on HCG day (mIU/mL), mean (SD)                                                                                       |
| 15.6 (5.6) 15.5 (5.6) 14.9 (5.7) 18.4 (6.3) 0.050 <0.001                                                             |
| LH on HCG day (mIU/mL), mean (SD)                                                                                       |
| 2.1 (2.2) 2.1 (2.1) 1.8 (1.9) 3.7 (4.5) 0.007 <0.001                                                                |
| E2 on HCG day (pg/mL), mean (SD)                                                                                         |
| 2790 (1850) 2840 (1850) 2800 (1700) 1170 (956) 0.700 <0.001                                                            |
| P on HCG day (ng/mL), mean (SD)                                                                                          |
| 1.1 (0.7) 1.1 (0.7) 1.0 (0.8) 0.9 (1.0) 0.543 <0.001                                                                |
| Total dose of Gn (IU), mean (SD)                                                                                         |
| 1980 (680) 1980 (673) 2040 (690) 1790 (843) 0.108 <0.001                                                            |
| Duration of stimulation (days), mean (SD)                                                                               |
| 10.4 (2.6) 10.4 (2.6) 11.1 (2.4) 9.0 (3.7) <0.001 <0.001                                                           |
| Number of retrieved oocytes, mean (SD)                                                                                   |
| 9.8 (6.4) 10.0 (6.4) 11.1 (5.7) 2.6 (2.7) <0.001 <0.001                                                            |
| **Pretreatment semen analysis**                                                                                          |
| Initial sperm concentration (million/mL), mean (SD)                                                               |
| 74.6 (56.3) 74.6 (56.3) 68.6 (47.9) 80.9 (60.3) 0.029 0.053                                                        |
| Initial normal sperm morphology, mean (SD)                                                                                 |
| 4.9 (2.8) 4.9 (2.7) 5.1 (3.1) 5.6 (4.8) 0.273 0.009                                                               |
| Initial progressive sperm motility, mean (SD)                                                                               |
| 40.8 (14.5) 41.0 (14.4) 37.3 (14.1) 38.1 (14.8) <0.001 <0.001                                                      |

Continuous variables are expressed as mean (SD), categorical variables as absolute frequencies, n (%). BMI, body mass index; E2, estradiol; FSH, follicular-stimulating hormone; Gn, gonadotropin; GnRH, gonadotropin-releasing hormone; HCG, human chorionic gonadotropin; LFR, low fertilisation rate; LH, luteinising hormone; P, progesterone; TFF, total fertilisation failure.
odds of LFR. The ORs and CIs were 1.566 (95% CI: 1.124 to 2.195), 1.494 (95% CI: 1.152 to 1.940), 0.579 (95% CI: 0.345 to 0.935) and 0.984 (95% CI: 0.975 to 0.992), respectively (table 5). In addition, table 6 showed that the concentration of LH (OR: 1.042, 95% CI: 1.004 to 1.080) and P (OR: 1.213, 95% CI: 1.015 to 1.451) on HCG day and the number of oocytes obtained (OR: 0.560, 95% CI: 0.518 to 0.606) were statistically related to the risk of TFF. Specifically, women with lower levels of LH and P and more eggs were less likely to develop TFF.

**Development and validation of nomograms for LFR and TFF**

The independent predictors (p<0.05) in logistic regression of LFR or TFF were used to generate the nomograms (figure 1). To use the nomogram, first, we drew a vertical line from the appropriate point for each selected variable to the ‘Points’ scale. And then the point values for each variable were summed together to generate the total point value. Finally, the patient’s probability of suffering LFR or TFF was read down from the ‘Total Points’ scale. ROC analysis showed that the AUC (ie, the C-index) of the prediction model was 0.640 (95% CI: 0.605 to 0.675) for LFR and 0.899 (95% CI: 0.880 to 0.917) for TFF in the training group, which indicated an acceptable and sufficient discrimination capacity, respectively (figure 2A). Then the models were validated in the validation set with an AUC of 0.661 (95% CI: 0.611 to 0.712) for LFR and 0.876 (95% CI: 0.845 to 0.908) for TFF (figure 2B). These results were similar to the C-indexes obtained in the training set. Moreover, the calibration curves (figure 3, figure 4) showed satisfactory agreement between the actual and predicted probabilities both in the training and validation set.

**Table 3** Baseline characteristics of the training and validation group

|                          | Training group (n=8129) | Validation group (n=3469) | P value |
|--------------------------|-------------------------|---------------------------|---------|
| Outcomes                 |                         |                           | 0.610   |
| LFR                      | 230 (2.8)               | 88 (2.5)                  |         |
| TFF                      | 256 (3.1)               | 104 (3.0)                 |         |
| Baseline characteristics  |                         |                           |         |
| Age, mean (SD)           | 31.8 (4.7)              | 31.8 (4.7)                | 0.688   |
| BMI (kg/m²), mean (SD)   | 22.0 (3.5)              | 22.1 (3.1)                | 0.387   |
| Infertility years, mean (SD) | 3.8 (3.1)              | 3.9 (3.2)                | 0.013   |
| Infertility diagnosis, n (%) |                       |                           |         |
| Tubal factor             | 5804 (71.4)             | 2472 (71.3)               | 0.050   |
| Diminished ovarian reserve | 854 (10.5)             | 383 (11.0)                |         |
| Ovulatory dysfunction    | 158 (1.9)               | 78 (2.2)                  |         |
| Uterine factor           | 226 (2.8)               | 109 (3.1)                 |         |
| Sperm factor             | 554 (6.8)               | 187 (5.4)                 |         |
| Unexplained infertility  | 533 (6.6)               | 240 (6.9)                 |         |
| Primary infertility, n (%) | 3582 (44.1)            | 1478 (42.6)               | 0.147   |
| Gravidity, n (%)         |                         |                           | 0.297   |
| 0                        | 3220 (39.6)             | 1334 (38.5)               |         |
| ≥1                       | 2069 (25.5)             | 928 (26.8)                |         |
| ≥2                       | 2840 (34.9)             | 1207 (34.8)               |         |
| Parity, n (%)            |                         |                           | 0.131   |
| 0                        | 6827 (84.0)             | 2952 (85.1)               |         |
| ≥1                       | 1302 (16.0)             | 517 (14.9)                |         |
| Induced abortion, n (%)  | 2630 (32.4)             | 1140 (32.9)               | 0.592   |
| Miscarriage, n (%)       | 1157 (14.2)             | 502 (14.5)                | 0.737   |
| Basal FSH (mIU/mL), mean (SD) | 8.3 (3.3)             | 8.4 (3.6)                | 0.241   |
| Basal LH (mIU/mL), mean (SD) | 4.5 (3.3)              | 4.6 (3.5)                | 0.261   |
| AMH (ng/mL), mean (SD)   | 3.2 (2.4)               | 3.2 (2.4)                 | 0.951   |
| AFC, mean (SD)           | 13.5 (7.7)              | 13.3 (7.7)                | 0.350   |

Continuous variables are expressed as mean (SD), categorical variables as absolute frequencies, n (%). AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicular-stimulating hormone; LFR, low fertilisation rate; LH, luteinising hormone; TFF, total fertilisation failure.
DISCUSSION

Fertilisation failure and poor fertilisation are still challenges and it is difficult to predict well in IVF cycles. In the present study, we constructed satisfactory nomogram models to predict LFR with indicators of ovarian stimulation protocol, primary infertility and initial progressive sperm motility and to predict TFF with indicators of levels of LH and P on the HCG day and numbers of oocytes obtained.

Primary infertility has been proven to be the cause of most infertility and to decrease the clinical pregnancy rate. In our study, most LFR patients also embraced primary infertility. Some studies have found that the rate of chromosome abnormality in patients with primary infertility is higher than that in other patients, and new zona pellucida glycoprotein gene mutation (ZPI, ZP2) can lead to zona pellucida abnormality. It is suggested that the quality of oocytes in patients with primary infertility is inherently poor due to molecular abnormalities.

We found that patients with follicular phase GnRH agonists have a higher risk of developing LFR than patients with GnRH antagonists, while patients with PPOS have a lower risk of developing LFR. Previous studies have shown that women who employed the GnRH agonist protocol

Table 4  Ovarian stimulation and semen characteristics between the training and the validation group

| Ovarian stimulation                          | Training group (n=8129) | Validation group (n=3469) | P value |
|----------------------------------------------|------------------------|---------------------------|---------|
| Ovarian stimulation protocol, n (%)          |                        |                           |         |
| GnRH antagonist                              | 3023 (37.2)            | 1364 (39.3)               | 0.167   |
| Follicular phase GnRH agonist                | 2764 (34.0)            | 1123 (32.4)               |         |
| Luteal phase GnRH agonist                    | 752 (9.3)              | 315 (9.1)                 |         |
| Progesterone primed ovarian stimulation      | 1590 (19.6)            | 667 (19.2)                |         |
| FSH on HCG day (mIU/mL), mean (SD)           | 15.6 (5.6)             | 15.5 (5.7)                | 0.666   |
| LH on HCG day (mIU/mL), mean (SD)            | 2.1 (2.2)              | 2.2 (2.3)                 | 0.131   |
| E2 on HCG day (pg/mL), mean (SD)             | 2790 (1860)            | 2770 (1820)               | 0.539   |
| P on HCG day (ng/mL), mean (SD)              | 1.1 (0.7)              | 1.1 (0.8)                 | 0.071   |
| Total dose of Gn (IU), mean (SD)             | 1980 (678)             | 1980 (685)                | 0.639   |
| Duration of stimulation (days), mean (SD)    | 10.4 (2.5)             | 10.4 (2.8)                | 0.654   |
| Number of retrieved oocytes, mean (SD)       | 9.9 (6.5)              | 9.7 (6.3)                 | 0.343   |
| Pretreatment semen analysis                  |                        |                           |         |
| Initial sperm concentration (million/mL), mean (SD) | 74.7 (56.3)            | 74.4 (56.2)               | 0.782   |
| Initial normal sperm morphology, mean (SD)   | 4.9 (2.8)              | 4.9 (2.7)                 | 0.672   |
| Initial progressive sperm motility, mean (SD)| 40.9 (14.5)            | 40.6 (14.4)               | 0.453   |

Continuous variables are expressed as mean (SD), categorical variables as absolute frequencies, n (%).

Table 5  Multivariate logistic regression analysis of predictive factors associated with LFR

|                          | B     | OR   | 95% CI          | P value |
|--------------------------|-------|------|-----------------|---------|
| Primary infertility      |       |      |                 |         |
| No                       | Reference |     |                 |         |
| Yes                      | 0.401 | 1.494| 1.152 to 1.940  | 0.002   |
| Ovarian stimulation protocol |       |      |                 |         |
| GnRH antagonist          | Reference |     |                 |         |
| Follicular phase GnRH agonist | 0.448 | 1.566| 1.124 to 2.195  | 0.009   |
| Luteal phase GnRH agonist | 0.165 | 1.180| 0.714 to 1.889  | 0.503   |
| Progesterone primed ovarian stimulation | −0.546 | 0.579| 0.343 to 0.935  | 0.032   |
| Duration of stimulation (days) | 0.046 | 1.047| 0.984 to 1.107  | 0.131   |
| Initial progressive sperm motility | −0.016 | 0.984| 0.975 to 0.992  | <0.001  |

B, regression coefficient; GnRH, gonadotropin-releasing hormone; LFR, low fertilisation rate.
have a lower 2PN fertilisation rate\textsuperscript{17} and high-quality embryo rate.\textsuperscript{18} In addition, the diagnosis of male factor infertility increased slightly in this protocol.\textsuperscript{18,19} Studies by Tang \textit{et al}\textsuperscript{1} also showed that patients with asthenospermia were more likely to use agonist regimens. However, the PPOS regimen increased the normal fertilisation rate compared with the GnRH antagonist protocol.\textsuperscript{20–22} The above results may prove that in addition to the effects of different ovarian stimulation regimens on the quality of oocytes, there is also a deviation in sperm quality in different protocols. In the present study, sperm abnormalities were a secondary cause of infertility in LFR patients and sperm motility was even a predictor of LFR. Previous studies have demonstrated that reduced sperm motility is associated with poor assisted reproductive outcomes.\textsuperscript{23–25} Thus, a combination of reduced oocyte and sperm quality leads to poor fertilisation.

However, the occurrence of TFF seemed to be more related to the number of oocytes. Moreover, we revealed that patients with TFF were significantly older and had poor ovarian reserves (\textit{tables 1 and 2}). As we all know, the number of retrieved oocytes reflects their ovarian function, that is, the poor ovarian reserve will result in a small number of eggs. Meanwhile, the quality of oocytes and ovarian reserves decreased with ageing. Previous studies have shown that fertilisation failure is related to the few numbers of oocytes retrieved in IVF cycles,\textsuperscript{3,26} which is consistent with our results. Jaswa \textit{et al}\textsuperscript{27} reported that the aneuploidy rate of blastocysts decreased in patients with reduced ovarian reserve, and further proposed that the quality of oocytes decreased with quantity. Vermey \textit{et al}\textsuperscript{28} also proved that the group with the largest number of eggs had the best quality. And the cumulative live birth rate increased with the oocyte number.\textsuperscript{29–31} Hence, few oocytes retrieved indicated the occurrence of TFF to a great extent.

Our study suggested that increased LH and P levels before HCG injection were independent indicators

| Table 6  | Multivariate logistic regression analysis of predictive factors associated with TFF |
|----------|----------------------------------------------------------------------------------|
|          | B   | OR      | 95% CI          | P value |
| AMH (ng/mL) | 0.085 | 1.089 | 0.985 to 1.203 | 0.097   |
| LH on HCG day (mIU/mL) | 0.041 | 1.042 | 1.004 to 1.080 | 0.027   |
| P on HCG day (ng/mL) | 0.193 | 1.213 | 1.015 to 1.451 | 0.034   |
| Number of retrieved oocytes | −0.580 | 0.560 | 0.518 to 0.606 | <0.001  |
| Initial progressive sperm motility | −0.008 | 0.992 | 0.983 to 1.001 | 0.082   |

AMH, anti-Müllerian hormone; B, regression coefficient; HCG, human chorionic gonadotropin; LH, luteinising hormone; P, progesterone; TFF, total fertilisation failure.
and the female parameters were not included in their analysis. Furthermore, Tang et al. found that the DNA fragmentation index (DFI) could predict the LFR or TFF with an AUC of 0.772 in men with mild-to-moderate asthenozoospermia, but the DFI was not an effective predictor in patients with normozoospermia. Recently, one study in China constructed a prediction model for LFR or TFF in IVF/ICSI cycles. However, they did not establish different models for LFR and TFF, respectively. In addition, their model was not visualised, which may not be convenient for clinical practice. In this study, we analysed as many potential influencing factors as possible and created both effective and convenient nomogram models.

Compared with previous studies, the advantage of this study is that both male and female indicators are included, which makes the results more reliable. Furthermore, since the causes of LFR and TFF are not completely the same, we established prediction models for these two cases respectively. However, this study has several limitations. First, we failed to provide semen parameters before oocytes aspiration. The reported sperm motility may not reflect the quality of the sperm collected before fertilisation. However, a previous study showed that the ability of total motile sperm count during fertility examination and at the time of ovum pickup to predict TFF was comparable. Therefore, the ability of sperm motility before treatment to predict fertilisation failure may not be completely denied. Second, limited to the retrospective cohort study, the data are obtained from a single centre, resulting in the absence of external validation of the models from other institutes. But the sample size in the present study included more than 10000 couples, second only to the latest study in China, which is much larger than the previous surveys. Thus, the population of our study is representative to some extent. Of course, we still need to verify the nomogram models by more well-designed prospective studies in other reproductive centres in our future works.

In conclusion, we identified the most predictive factors of LFR and TFF. Subsequently, visual prediction models were developed for patients in the first routine IVF cycles based on the selected factors. The model verification analyses showed that the two models, particularly the TFF model, had good prediction ability both in the training and validation set. Our nomograms would help to consult with the fertilisation method before carrying out the conventional fertilisation procedure.

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Contributors XT and YD designed the study and interpreted the data. QF, XL, MC, YQ and YY collected and checked the data. TL, XM and LG provided clinical guidance and advice. QWang performed the statistical analysis. QWang, XB and QWan collectively drafted the manuscript. ZZ further revised the manuscript. The final manuscript was approved by all coauthors. YD is the guarantor accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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Data availability statement Data are available upon reasonable request. Data are available upon reasonable request. Original data are available on request by emailing the corresponding author, who will delete the personal identification information of patients.

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