

# Nomogram to predict pregnancy outcomes of emergency oocyte freeze-thaw cycles

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## Abstract

**Background:** Existing clinical prediction models for *in vitro* fertilization are based on the fresh oocyte cycle, and there is no prediction model to evaluate the probability of successful thawing of cryopreserved mature oocytes. This research aims to identify and study the characteristics of pre-oocyte-retrieval patients that can affect the pregnancy outcomes of emergency oocyte freeze-thaw cycles.

**Methods:** Data were collected from the Reproductive Center, Peking University Third Hospital of China. Multivariable logistic regression model was used to derive the nomogram. Nomogram model performance was assessed by examining the discrimination and calibration in the development and validation cohorts. Discriminatory ability was assessed using the area under the receiver operating characteristic curve (AUC), and calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test and calibration plots.

**Results:** The predictors in the model of “no transferable embryo cycles” are female age (odds ratio [OR] = 1.099, 95% confidence interval [CI] = 1.003–1.205,  $P = 0.0440$ ), duration of infertility (OR = 1.140, 95% CI = 1.018–1.276,  $P = 0.0240$ ), basal follicle-stimulating hormone (FSH) level (OR = 1.205, 95% CI = 1.051–1.382,  $P = 0.0084$ ), basal estradiol (E2) level (OR = 1.006, 95% CI = 1.001–1.010,  $P = 0.0120$ ), and sperm from microdissection testicular sperm extraction (MESA) (OR = 7.741, 95% CI = 2.905–20.632,  $P < 0.0010$ ). Upon assessing predictive ability, the AUC for the “no transferable embryo cycles” model was 0.799 (95% CI: 0.722–0.875,  $P < 0.0010$ ). The Hosmer–Lemeshow test ( $P = 0.7210$ ) and calibration curve showed good calibration for the prediction of no transferable embryo cycles. The predictors in the cumulative live birth were the number of follicles on the day of human chorionic gonadotropin (hCG) administration (OR = 1.088, 95% CI = 1.030–1.149,  $P = 0.0020$ ) and endometriosis (OR = 0.172, 95% CI = 0.035–0.853,  $P = 0.0310$ ). The AUC for the “cumulative live birth” model was 0.724 (95% CI: 0.647–0.801,  $P < 0.0010$ ). The Hosmer–Lemeshow test ( $P = 0.5620$ ) and calibration curve showed good calibration for the prediction of cumulative live birth.

**Conclusions:** The predictors in the final multivariate logistic regression models found to be significantly associated with poor pregnancy outcomes were increasing female age, duration of infertility, high basal FSH and E2 level, endometriosis, sperm from MESA, and low number of follicles with a diameter >10 mm on the day of hCG administration.

**Keywords:** Nomogram; Oocyte freeze-thaw; *In vitro* fertilization; Pregnancy outcome

## Introduction

As early as 1986, researchers had applied frozen oocytes for *in vitro* fertilization (IVF) and achieved a successful pregnancy.<sup>[1]</sup> With the development of intracytoplasmic sperm injection (ICSI) and the improvement of cryogenic freezing technology, the recovery rate, fertilization rate, and pregnancy rate of oocytes frozen cycle were significantly improved. The American Society for Repro-

ductive Medicine also proposed in 2013 that the freezing of mature oocytes could be expanded beyond the experimental stage and widely applied in clinical practice.<sup>[2]</sup>

At present, the clinical research on oocyte cryopreservation mainly focuses on the social and economic benefit for patients or fertility preservation of malignant tumor patients. However, we have identified another common scenario in our daily clinical practice: patients who planned to receive assisted reproductive technology

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(ART) on the day of oocyte retrieval where there is an unexpected sperm collection failure, such as situations wherein the partner or sperm donor is unable to get to the hospital for sperm collection due to sudden illness or accident, fails to perform masturbation, or fails to appear for operation (microdissection testicular sperm extraction [MESA]/testicular sperm aspiration [TESA]), or due to other various and sundry reasons. If these patients give up oocyte retrieval, the cost and time of treatment in previous ovulation induction process would be wasted, and the risk of ovarian hyperstimulation syndrome would be significantly increased due to the excessive physiological dose of estrogen in the body. However, if the oocytes are harvested as planned, the damage caused by freezing and thawing, and the risk of injury caused by the operation itself, will also cause physical and psychological damage to the patients. As there is no unified evaluation framework or reference standard for this scenario to guide doctors in their daily clinical work, doctors often advise patients to give up oocyte retrieval or oocyte cryopreservation based on their own experience, or leave patients to choose completely by themselves.

IVF cannot guarantee 100% success; between 38% and 49% of couples who start IVF will remain childless, even after undergoing up to six IVF cycles.<sup>[3]</sup> To manage the expectations of the infertile couples, several clinical prediction models for IVF have been developed over the last three decades. However, all of those models are based on fresh oocyte cycles, and no prediction model exists to evaluate the probability to successfully thaw cryopreserved mature oocytes. Our reproductive center, in 2007, began to cryopreserve mature oocytes. Eighty percent of cryopreserved mature oocytes are frozen because the partner or sperm donor cannot come to the hospital on the day of oocyte retrieval. In this study, clinical data of emergency oocyte cryopreservation for male reason from 2007 to 2019 were retrospectively analyzed.

The prediction model of pregnancy outcomes using cryopreserved mature oocytes has been established and validated using clinical characteristics and laboratory values. We hope this model could provide individualized and targeted suggestions to patients for decision-making.

## Methods

### Ethical approval

This study was approved by the Ethics Committee of Peking University Third Hospital (No. 2019SZ-092). Patients provided written consent for the information to be used in the analyses, editing, and publications.

### Study design and participants

From August 2007 to December 2019, 418 women who had undergone oocyte cryopreservation in the Reproductive Center, Peking University Third Hospital, China, were identified. Infertile couples who received IVF and conducted emergency oocyte cryopreservation due to issues with the sperm donor were enrolled [Figure 1]. Issues with the sperm donor on the day of oocyte retrieval includes: the sperm donor cannot come to the hospital for sperm collection due

to sudden illness or accident, fails to perform masturbation or fails to appear for operation (MESA, TESA), or fails to obtain enough sperm, as well as other unexpected sperm collection failures. Data used in the investigation data includes: female age, body mass index, duration of infertility, primary/secondary infertility, causes of infertility, previous history of gestation, basal hormone levels, semen quality, gonadotropin (Gn) dosage and duration totally applied, number of follicles with a diameter >10 mm and hormone levels on the day of human chorionic gonadotropin (hCG) administration, oocyte storage duration, and other relevant parameters. The final date of follow-up was May 31, 2020. The final date of follow-up was May 31, 2020. The study utilized the TRIPOD (Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) score<sup>[4]</sup> to establish and validate the models.

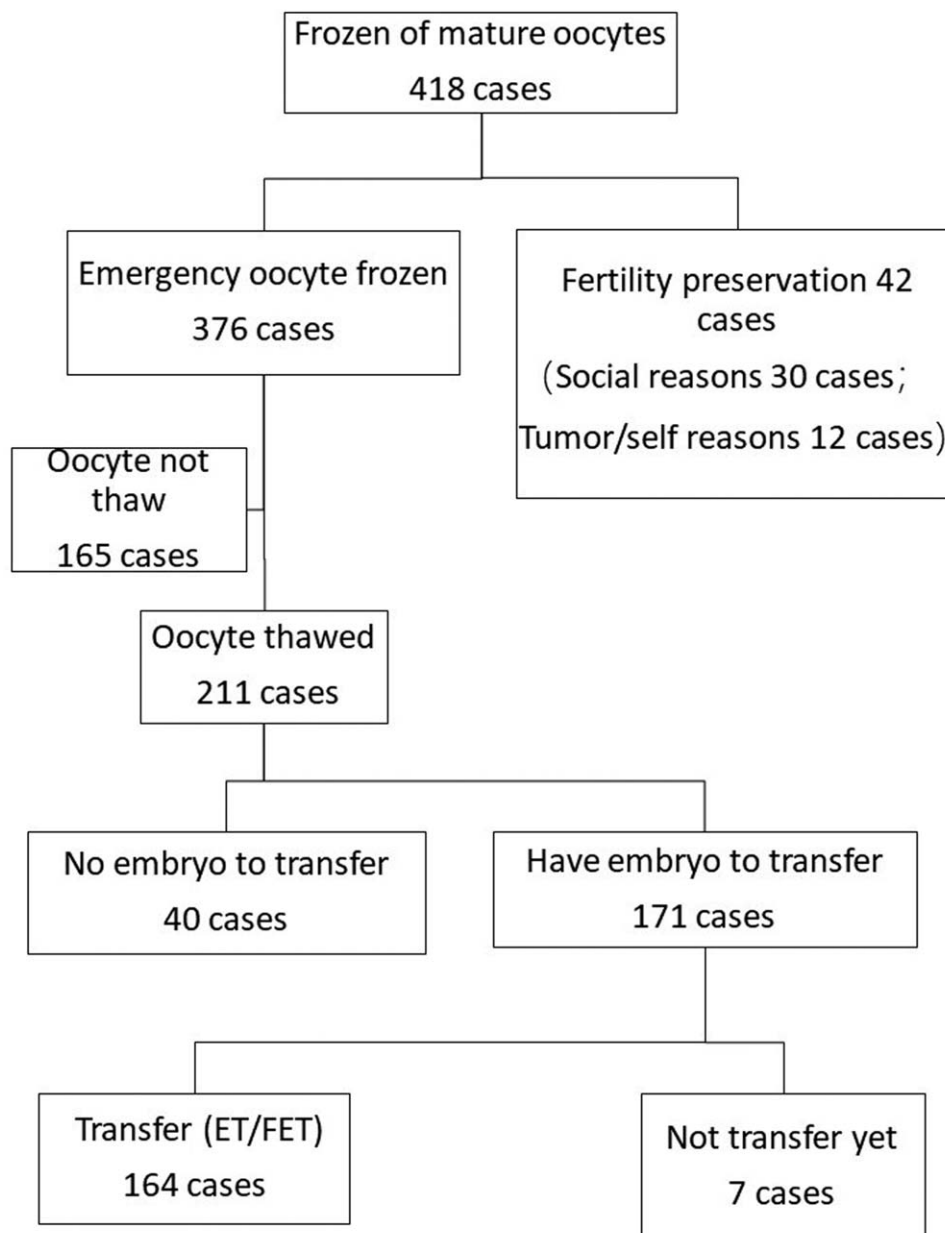
### Procedures

The initial dose of Gn applied to ovulation promotion was selected according to the age of the patients, the level of basal hormone, and other ovarian reserve situation. And the Gn was adjusted based on the growth of follicles. The trigger time was decided based on the diameter of follicles and the level of serum hormone. When the diameter of two or more follicles is  $\geq 18$  mm, recombinant human chorionic gonadotropin (r-hCG [Ezer, Merck Serono S.p.A., Modugno, Italy], 250  $\mu$ g) was administered to the patients. The oocyte retrieval was conducted 34–38 h later.

Mature oocytes were vitrified and thawed as previously described.<sup>[5]</sup> Briefly, oocytes were first equilibrated in a 7.50% (v/v) ethylene glycol (EG) and 7.5% dimethyl sulfoxide (DMSO) solution for 5 min at room temperature. These oocytes were then transferred into the vitrification solutions composed of 15% (v/v) EG + 15% (v/v) DMSO + 0.5 mol/L sucrose for <1 min at room temperature. Finally, these oocytes were loaded on the sterile vitric straw immediately and transferred directly into liquid nitrogen for storage. Thawing of the frozen oocytes was carried out step by step using different concentrations of sucrose solution. After recovery, only an oocyte with intact membrane and uniform cytoplasm was considered as having survived. Following ICSI, all embryos were further cultured for 3 days; the quality of the embryo was evaluated by experienced embryologists. The embryo which could be transferred was then transferred back to the uterus or frozen. Patients with regular menstruation and normal ovulation were grouped in natural cycles, while patients with irregular menstruation or anovulation were given artificial cycles to prepare the endometrium. Luteal support was provided to all patients after embryo transfer (ET).

### Outcomes

“No transferable embryo” and “cumulative live birth” are the two key outcomes. “No transferable embryo” means that after thawing the oocyte and formation of the embryo by ICIS, there was no available embryo to transfer back to the uterus. Cumulative live birth was defined as at least one live birth from the oocyte cryopreservation cycle as of May 2020 due to either the thawing fresh ET cycle or the following frozen ET cycle.



**Figure 1:** Flowchart of the study. ET/FET: Embryo transfer/fresh & frozen embryo transfer.

## Statistical analysis

### Primary statistical analysis

For the quantitative data, the Kolmogorov-Smirnov method was used to test the normality distribution. The quantitative elements were expressed as mean  $\pm$  std or median (p25, p75) according to whether it conformed to the normal distribution. For the qualitative data, the  $n$  (%) was used to express the data. Statistical tests were done with R software (version 3.6.0; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 25.0; Chicago, IL, USA). Statistical significance was set at two-sided  $P$  values  $< 0.0500$ .

### Model development

Univariable logistic regression analyses were performed to assess the association of each of the predictive factors with

“cumulative live birth” and “no transferable embryo cycles.” A multivariable logistic regression model was used to derive the nomogram. The predictors included in the multivariable model were selected based on the result of univariable logistic regression analyses ( $P < 0.1000$ ). The backward procedure for variable selection was applied for the multivariable logistic regression model. Regression coefficients were used to generate a nomogram.

### Missing data

The entire dataset contained 211 women, and data entry was complete for all variables. There is no missing data.

### Predictive ability

Nomogram model performance was assessed by examining discrimination and calibration in the development and

validation cohorts.<sup>[6]</sup> The discrimination was assessed by the area under the receiver-operator characteristic and area under curve (AUC) and its 95% confidence interval [CI]. The calibration was constructed to examine the agreement between the predicted probabilities with the observed outcome, which was assessed by the Hosmer–Lemeshow goodness-of-fit test and calibration plots. The calibration plot was calculated by the 400 repetitions bootstrap resampling.

## Results

### Basic characteristics

A total of 211 patients with 215 cycles of freeze-thaw oocytes participated in this study. Four patients received two

freeze-thaw oocytes cycles. Forty patients with 43 cycles did not have embryos to transfer. Seven patients conducted oocyte thawing and had embryo to transfer but have not completed transfer. The number of patients that received IVF-ET/fresh & frozen ET amounted to 164. Figure 1 shows how the study established the eligible cohort of oocyte freeze-thaw treatment cycles. Table 1 shows the baseline characteristics of the cohort. In total, there were 2546 oocytes that were thawed. The average recovery rate of oocytes was  $75.42\% \pm 24.04\%$ , the fertilization rate was  $69.54\% \pm 26.07\%$  and the cleavage rate was  $95.05\% \pm 12.53\%$ . The overall rate of cumulative live birth from the whole dataset was 39.63% (65/164), the rate of no transferable embryo cycles was 20.00% (43/215) and the live birth rate per frozen oocyte was 2.55% (65/2546).

**Table 1: Baseline characteristics of the women who received oocyte cryopreservation.**

Characteristics	“No transferable embryo” cohort (n = 215)	Live birth rate (n = 164)
Female age (years)	29.91 ± 4.95	29.41 ± 4.85
BMI (kg/m <sup>2</sup> )	22.32 ± 3.56	22.41 ± 3.61
Duration of infertility (years)	3.44 ± 3.24	3.13 ± 2.68
Types of infertility		
Primary infertility	174 (80.93)	131 (79.88)
Secondary infertility	41 (19.07)	33 (20.12)
Gravidity (times)	0 (0, 5)	0 (0, 5)
Delivery (times)	0 (0, 2)	0 (0, 2)
Endometriosis	17 (7.91)	14 (8.54)
PCOS	24 (11.16)	18 (10.98)
POR	22 (10.23)	13 (7.93)
Tubal factor	28 (13.02)	22 (13.41)
IVF failure history	21 (9.77)	16 (9.76)
Basal FSH (MIU/mL)	6.11 ± 2.82	5.86 ± 2.38
Basal LH (MIU/mL)	3.64 ± 1.99	3.64 ± 1.95
Basal E2 (mmol/L)	164.58 ± 88.24	157.86 ± 58.30
AFC	14.55 ± 6.86	14.85 ± 6.69
Duration of Gn applied (days)	11.47 ± 2.59	11.47 ± 2.53
Total Gn applied (units)	2505.62 ± 1140.04	2428.80 ± 1106.91
LH on the day of hCG (mIU/mL)	1.73 ± 2.40	1.63 ± 2.17
E2 on the day of hCG (mmol/L)	11,036.42 ± 7532.33	11,622.27 ± 7552.50
Progesterone on the day of hCG (pmol/L)	2.85 ± 1.86	2.89 ± 1.59
The number of follicles with a diameter >10 mm on the day of hCG (number)	16.08 ± 6.88	16.76 ± 6.81
Male age (years)	31.20 ± 6.21	30.70 ± 5.99
Semen quality		
Azoospermia	158 (73.49)	123 (75.00)
Oligozoospermia	21 (9.77)	14 (8.54)
Normal semen	36 (16.74)	27 (16.46)
Semen source		
AID	104 (48.37)	90 (54.88)
TESA/PESA	10 (4.65)	7 (4.27)
MESA	42 (19.53)	22 (13.41)
Masturbation	59 (27.44)	45 (27.44)
Duration of oocyte frozen (month)	8.23 ± 8.94	8.51 ± 9.12
Oocyte retrieval time grouped by year		
2007–2011	24 (11.16)	23 (14.02)
2012–2015	76 (35.35)	54 (32.93)
2016–2019	115 (53.49)	87 (53.05)

Data are presented as n (%), median (min, max) or mean ± standard deviation. AFC: Antral follicle count; AID: Artificial insemination by donor; BMI: Body mass index; E2: Estradiol; FSH: Follicle stimulating hormone; Gn: Gonadotropin; hCG: Human chorionic gonadotropin; IVF: *In vitro* fertilization; LH: Luteinizing hormone; MESA: Microdissection testicular sperm extraction; PCOS: Polycystic ovary syndrome; PESA: Percutaneous epididymal sperm aspiration; POR: Poor ovarian response diagnosis according to Bologna diagnosis criteria; TESA: Testicular sperm aspiration.

**Development and validation of a nomogram for predicting no transferable embryo cycles**

The univariate associations of the potential predictors and multivariable logistic regression model for “no transferable embryo cycles” are shown in Table 2. Predictors included in the multivariable logistic regression were as follows: female age, antral follicle count (AFC), basal luteinizing hormone level, Gn dosage, number of follicles on the day of hCG administration, endometriosis, semen quality, sperm source, and storage duration of oocytes. The variables that showed a statistically significant increment in odds ratio (OR) of “no transferable embryo cycles” in the final model were: female age (OR = 1.099, 95% CI = 1.003–1.205, P = 0.0440), duration of infertility (OR = 1.140, 95%

CI = 1.018–1.276, P = 0.0240), basal follicle-stimulating hormone (FSH) (OR = 1.205, 95% CI = 1.051–1.382, P = 0.0084), and estradiol (E2) (OR = 1.006, 95% CI = 1.001–1.010, P = 0.0120) level. As for the source of sperm, compared with masturbation and percutaneous epididymal sperm aspiration, sperm from MESA significantly increased the risk of “no transferable embryo cycles” (OR = 7.741, 95% CI = 2.905–20.632, P < 0.0010).

The nomogram was derived from a multivariable logistic regression model. The model showed an AUC of 0.799 (95% CI: 0.722–0.875, P < 0.0010), which denotes good discrimination. The Hosmer–Lemeshow goodness-of-fit test, and the calibration curve, showed good calibration of nomogram in the internal validation cohort [Figure 2].

**Table 2: Potential predictors and multivariable logistic regression model for no transferable embryo cycles.**

Predictor	Univariate analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Female age (years)	1.098 (1.028, 1.172)	0.0050*	1.099 (1.003, 1.205)	0.0440*
BMI (kg/m <sup>2</sup> )	0.975 (0.885, 1.075)	0.6160		
Duration of infertility (years)	1.149 (1.047, 1.262)	0.0040*	1.140 (1.018, 1.276)	0.0240*
Secondary infertility	1.575 (0.616, 4.029)	0.3430		
Gravidity (times)	0.881 (0.568, 1.367)	0.5730		
Delivery (times)	0.842 (0.126, 5.603)	0.8590		
Endometriosis	1.181 (0.324, 4.310)	0.8010		
PCOS	0.944 (0.331, 2.690)	0.9140		
POR	0.309 (0.122, 0.781)	0.0130*		
Tubal factor	1.518 (0.518, 4.825)	0.4210		
IVF failure history	1.069 (0.341, 3.358)	0.9090		
Basal FSH (MIU/mL)	1.156 (1.029, 1.299)	0.0140*	1.205 (1.051, 1.382)	0.0080*
Basal LH (MIU/mL)	1.014 (0.858, 1.198)	0.8710		
Basal E2 (mmol/L)	1.003 (1.000, 1.007)	0.0590	1.006 (1.001, 1.010)	0.0120*
AFC	0.960 (0.912, 1.010)	0.1150		
Duration of Gn applied (days)	0.994 (0.873, 1.131)	0.9260		
Total Gn applied (units)	1.003 (1.000, 1.001)	0.0950		
LH on the day of hCG (MIU/mL)	1.066 (0.944, 1.203)	0.3020		
E2 on the day of hCG (mmol/L)	1.000 (1.000, 1.000)	0.2040		
Progesterone on the day of hCG (pmol/L)	0.972 (0.804, 1.174)	0.7650		
Number of follicles with a diameter >10 mm on the day of hCG	0.950 (0.901, 1.001)	0.0560		
Male age (years)	1.066 (1.014, 1.121)	0.0120*		
Semen quality				
Azoospermia	1	0.5160		
Oligozoospermia	1.779 (0.636, 4.978)	0.2720		
Normal semen	1.271 (0.526, 3.073)	0.5950		
Semen source				
AID	1	0.0010*	1	<0.0010*
TESA/PESA	4.029 (0.898, 18.081)	0.0690	2.180 (0.383, 12.403)	0.3800
MESA	6.392 (2.607, 15.675)	<0.0010*	7.741 (2.905, 20.632)	<0.0010*
Masturbation	2.675 (1.084, 6.512)	0.0330	1.399 (0.486, 4.034)	0.5340
Duration of oocyte frozen (month)	0.978 (0.936, 1.022)	0.3230		
Oocyte retrieval time grouped by year				
2007–2011	1	0.1900		
2012–2015	6.133 (0.769, 48.931)	0.0870		
2016–2019	6.719 (0.866, 52.154)	0.0680		

\* P < 0.05. AFC: Antral follicle count; AID: Artificial insemination by donor; BMI: Body mass index; CI: Confidence interval; E2: Estradiol; FSH: Follicle stimulating hormone; Gn: Gonadotropin; hCG: Human chorionic gonadotropin; IVF: *In vitro* fertilization; LH: Luteinizing hormone; MESA: Microdissection testicular sperm extraction; OR: Odds ratio; PCOS: Polycystic ovary syndrome; PESA: Percutaneous epididymal sperm aspiration; POR: Poor ovarian response diagnosis according to Bologna diagnosis criteria; TESA: Testicular sperm aspiration.



**Development and validation of a nomogram for predicting cumulative live birth**

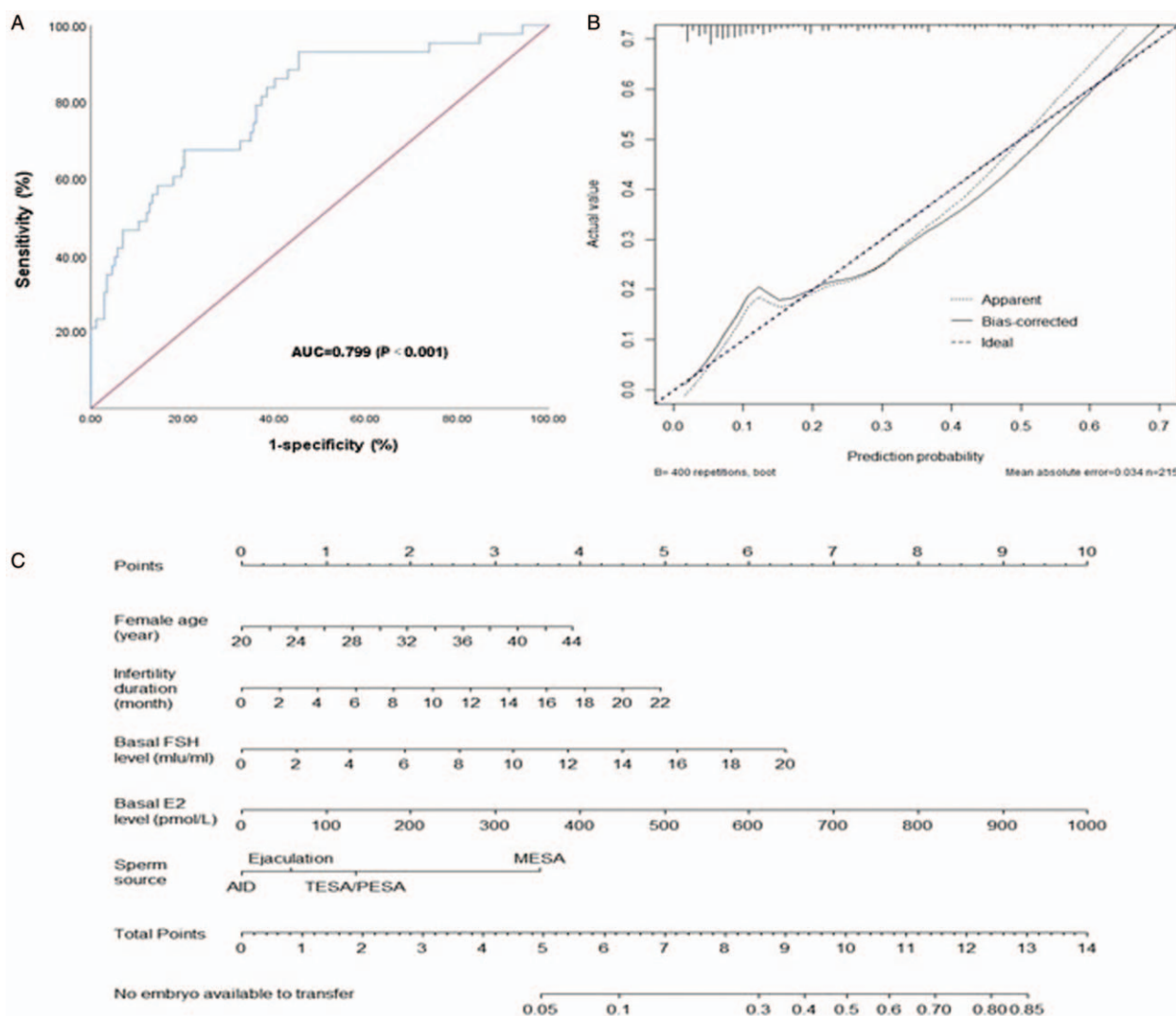
The univariate associations of the potential predictors and multivariable logistic regression model for the cumulative live birth of freeze-thaw oocytes are shown in Table 3. Predictors included in the multivariable logistic regression were as follows: age of female and male, duration of infertility, basal FSH and E2 level, Gn dosage, number of follicles on the day of hCG administration, poor ovarian response, and sperm source. The model shows that the OR of a successful live birth decreases with the number of follicles on the day of hCG administration (OR = 1.088, 95% CI = 1.030–1.149,  $P = 0.0020$ ) and endometriosis (OR = 0.172, 95% CI = 0.035–0.853,  $P = 0.0310$ ).

The nomogram was derived from the multivariable logistic regression model. The model showed an AUC of 0.724 (95% CI: 0.647–0.801,  $P < 0.0010$ ) and indicate a moderate discrimination. The Hosmer–Lemeshow goodness-of-fit test, and the calibration curve, showed good

calibration of nomogram in the internal validation cohort [Figure 3].

**Discussion**

In the early 20th century, scientists began to preserve gametes and embryos at low temperatures. In 1999, Kuleshova *et al*<sup>[7]</sup> first reported the case of successful pregnancy and delivery after oocyte cryopreservation, which marked a breakthrough in oocyte cryopreservation. Presently, thousands of children have been born and benefited from using this technique. Although new techniques are emerging and existing ones are always evolving, the freeze-thaw process can cause damage and changes of spindles, genetic material, organelles, and epigenetics in the oocyte.<sup>[8]</sup> It remains unclear whether or not these alterations could produce long-term negative health effects. Existing studies have shown that the clinical pregnancy rate and live birth rate of mature oocytes after freezing and thawing are similar to those of fresh oocytes, with evidence from oocyte donation cycles. However, for



**Figure 2:** Discrimination and calibration of a model to predict no transferable embryo cycles of emergency oocyte frozen-thawed cycles (A) AUC, (B) calibration curve, (C) nomogram. AUC: Area under the ROC curve; AID: Artificial insemination by donor; E2: Estradiol; FSH: Follicle stimulating hormone; MESA: Microdissection testicular sperm extraction; PESA: Percutaneous epididymal sperm aspiration; ROC: Receiver-operator characteristic; TESA: Testicular sperm aspiration.

**Table 3: Potential predictors and multivariable logistic regression model for live birth rate.**

Predictor	Univariate analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Female age (years)	0.934 (0.872, 1.001)	0.0550		
BMI (kg/m <sup>2</sup> )	0.976 (0.894, 1.066)	0.5930		
Duration of infertility (years)	0.928 (0.817, 1.055)	0.2550		
Secondary infertility	1.991 (0.859, 4.615)	0.1080		
Gravidity (times)	0.795 (0.523, 1.211)	0.2860		
Delivery (times)	1.842 (0.386, 8.799)	0.4440		
Endometriosis	4.345 (0.939, 20.099)	0.0600	0.172 (0.035, 0.853)	0.0310*
PCOS	0.622 (0.233, 1.662)	0.3440		
POR	0.431 (0.114, 1.629)	0.2150		
Tubal factor	1.174 (0.462, 2.978)	0.7360		
IVF failure history	1.105 (0.381, 3.203)	0.8540		
Basal FSH (MIU/mL)	0.904 (0.790, 1.034)	0.1410		
Basal LH (MIU/mL)	0.865 (0.731, 1.025)	0.0950		
Basal E2 (mmol/L)	0.998 (0.992, 1.003)	0.3930		
AFC	1.046 (0.997, 1.097)	0.0660		
Duration of Gn applied (days)	0.911 (0.801, 1.037)	0.1580		
Total Gn applied (units)	1.000 (0.999, 1.000)	0.0340*		
LH on the day of hCG (MIU/mL)	1.036 (0.898, 1.194)	0.6300		
E2 on the day of hCG (mmol/L)	1.000 (1.000, 1.000)	0.2560		
P on the day of hCG (pmol/L)	0.861 (0.699, 1.061)	0.1610		
The number of follicles with a diameter >10 mm on the day of hCG (number)	1.071 (1.019, 1.125)	0.0060*	1.088 (1.030, 1.149)	0.0020*
Male age (years)	0.955 (0.901, 1.011)	0.1130		
Semen quality				
Azoospermia	1	0.0510		
Oligozoospermia	0.687 (0.218, 2.168)	0.5220		
Normal semen	0.281 (0.100, 0.790)	0.0160		
Semen source				
AID	1	0.0640		
TESA/PESA	0.228 (0.026, 1.974)	0.1790		
MESA	1.977 (0.767, 5.097)	0.1590		
Masturbation	0.556 (0.258, 1.199)	0.1340		
Duration of oocyte frozen (month)	1.028 (0.992, 1.065)	0.1300		
Oocyte retrieval time grouped by year				
2007–2011	1	0.6530		
2012–2015	1.500 (0.545, 4.127)	0.4320		
2016–2019	1.146 (0.438, 2.996)	0.7810		

\*  $P < 0.05$ . AFC: Antral follicle count; AID: Artificial insemination by donor; BMI: Body mass index; CI: Confidence interval; Gn: Gonadotropin; E2: Estradiol; FSH: Follicle stimulating hormone; hCG: Human chorionic gonadotropin; IVF: *In vitro* fertilization; LH: Luteinizing hormone; MESA: Microdissection testicular sperm extraction; OR: Odds ratio; PCOS: Polycystic ovary syndrome; PESA: Percutaneous epididymal sperm aspiration; POR: Poor ovarian response diagnosis according to Bologna diagnosis criteria; TESA: Testicular sperm aspiration.

patients with poor ovarian reserve function or a low expected number of oocytes, doctors and patients are still worried that no embryo may be available to be transferred after oocyte thawing. The data from our center indicates that nearly one-fifth of the patients (40/211, 18.96%) have no embryos to transfer after oocyte thawing. For those patients, if we can inform them of the probability of no embryos to transfer before oocyte retrieval, it may allow them to make an informed decision to reduce the economic loss and the risks of the operation.

### Main findings

At present, there is no predictive model of pregnancy outcome after emergency cryopreservation of oocytes. Based on the clinical data and laboratory results of

emergency oocyte cryopreservation, prediction models of pregnancy outcomes were developed to fill the gap. All the indicators in the model are available before oocyte retrieval. The internal verification of the model was also conducted. The key predictors which had significant effects on the result of the model of “no transferable embryo cycles” are: female age, duration of infertility, basal FSH, basal E2, and the source of semen, while for the model of “cumulative live birth,” the key predictors are: the number of follicles with a diameter >10 mm and endometriosis.

### Strengths and weaknesses

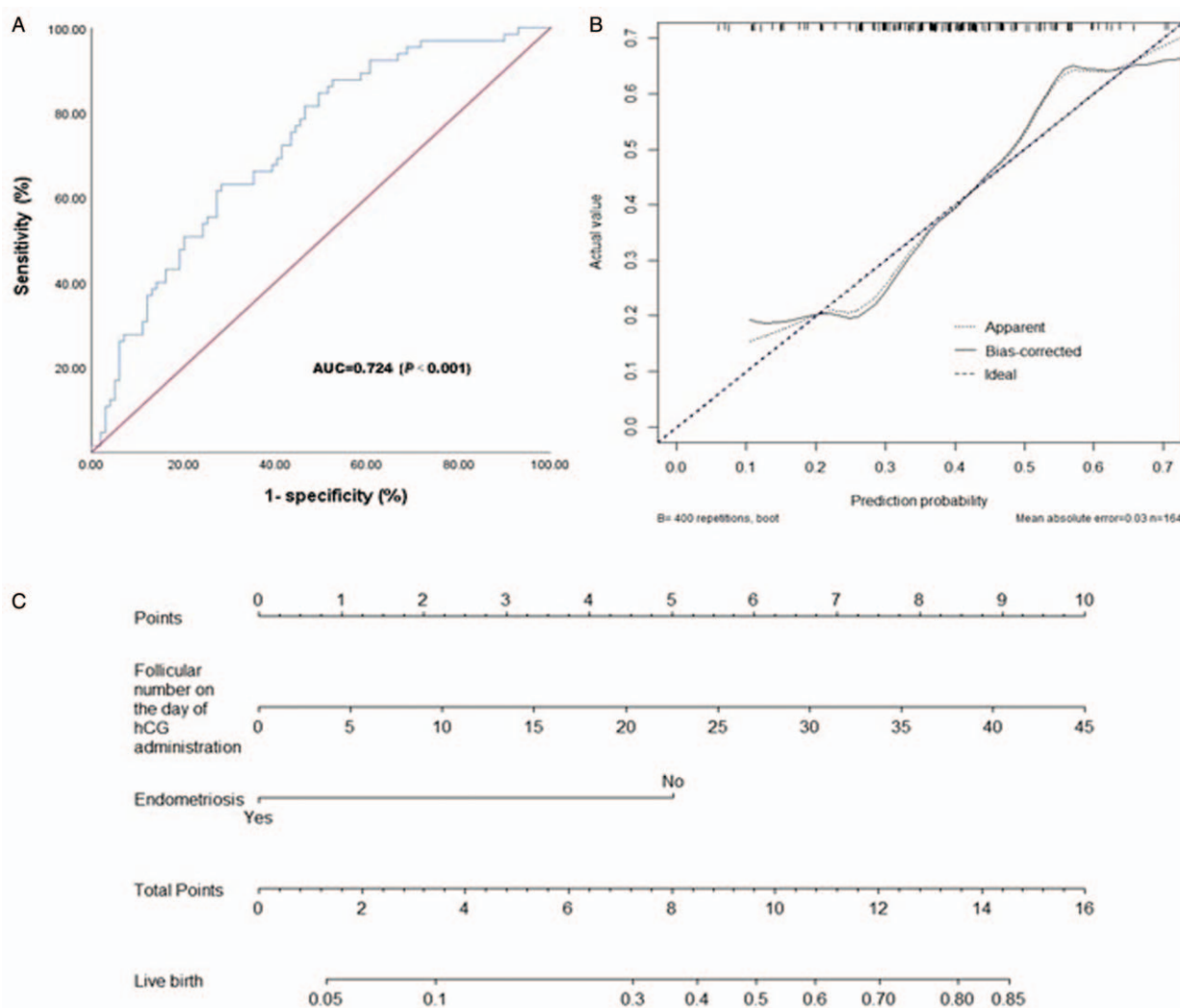
Ratna *et al*<sup>[9]</sup> suggested that a high-quality prediction model article should meet the following three criteria: (1) a TRIPOD<sup>[4]</sup> score >80%, (2) external validation, and

(3) the model had acceptable discrimination (c-statistic >0.7).<sup>[10]</sup> Thirty-five prediction models of IVF success have been published across 23 articles. These 35 models met between 29% to 95% of the items included in the TRIPOD checklist.<sup>[11]</sup> Only 21% of studies met at least 80% of the checklist items, and the highest achieved a TRIPOD score of 95%. Only four models<sup>[12-15]</sup> had conducted external validation (4/23 [17.39%]), and almost all of the indicators in the models have missing values or do not describe missing values. The range of c-statistic was between 0.55 and 0.77.

From research design to manuscript drafting, we strictly followed the TRIPOD list. The self-evaluation TRIPOD score is 90.91%. The AUC of the “no transferable embryo cycles” model is 0.799 (95% CI: 0.722–0.875,  $P < 0.0010$ ), and the AUC of the “cumulative live birth” model is 0.724 (95% CI: 0.647–0.801,  $P < 0.0010$ ), which are both >0.7. The accuracy of the prediction model is at the forefront of the existing models. On the one hand, it benefits from the guidance of TRIPOD, but on the other hand, it is closely related to the fact that this study covers

almost all the prediction indicators related to the pregnancy outcome of IVF and there is no missing value.

The main categories of predictors included in developed models are the following: couple factors, gender, embryo, and treatment. At present, several better prediction models recommended in the field of reproductive medicine mostly come from multicenter or national databases. Although the sample size is large, the number of prediction indicators included is limited.<sup>[16]</sup> The median number of predictors included in the existing models was 7 (range 3–14). Our model includes 26 forecast indicators. In addition to the most frequently used predictors such as female age, duration of infertility, endometriosis, and other relevant parameters,<sup>[17]</sup> we also included basic hormone levels, AFC, male age, and semen quality and hormone levels on the day of hCG injection. The information about the embryos could not be obtained due to the pre-treatment model. However, while other pre-treatment models exclude oocyte evaluation, this model uses the number of follicles with a diameter of >10 mm on hCG administration day to estimate the number of oocytes.



**Figure 3:** Discrimination and calibration of a model to predict cumulative live birth of emergency oocyte frozen-thawed cycles (A) AUC, (B) calibration curve, (C) nomogram. AUC: Area under the ROC curve; hCG: Human chorionic gonadotropin; ROC: Receiver-operator characteristic.



Moreover, the sources of the semen were also taken into consideration to further improve the accuracy of the prediction model.

One of the greatest strengths of our model is that it has highlighted the semen source as a key predictor for IVF success. Semen source is a factor that has never been used in any previous prediction models. Studies have indicated that non-azoospermia (NOA) patients could produce increased numbers of cytogenetically abnormal testicular spermatozoa despite their normal somatic karyotype, and were at increased risk to produce aneuploid gametes and of transmitting chromosome aneuploidy to the zygote,<sup>[18,19]</sup> which may lead to a reduced developmental potential of embryos. Geng An *et al*<sup>[20]</sup> had compared 150 NOA patients who underwent micro-TESE with 174 OA patients who underwent TESA and found that developmental competence of the embryo was greatest among couples using sperm obtained by TESA rather than micro-TESE, and was not dependent on whether vitrified or fresh oocytes were utilized. Capelouto *et al*<sup>[21]</sup> showed that the quality and source of sperm did not affect the clinical pregnancy rate and live birth rate in vitrified oocyte donation IVF model. On the contrary, the results of our study suggest that the source of semen is an important factor leading to no transferable embryo cycles in freeze-thaw oocyte cycles. If the sperm comes from MESA, the risk of no transferable embryo cycles will be increased by 7.74 times.

Female age and duration of infertility are two important predictors of the pregnancy/live birth chances after IVF.<sup>[17]</sup> They both have negative associations with treatment outcomes. Our results suggest that in terms of pregnancy outcome of oocyte cryopreservation, female age and duration of infertility also play a negative role. For every 1-year increase in female age, the risk of no transferable embryo cycles increases by 1.099 times. The risk of no transferable embryo cycles increases by 1.14 times for every 1-year extension of infertility years.

Basal FSH and E2 levels are important indexes for the evaluation of ovarian reserve function. A high level reflects a reduced ovarian reserve and is associated with poor IVF treatment outcome.<sup>[22]</sup> Some studies also suggested that the FSH level on cycle day 3 was a better indicator of IVF outcome than female age.<sup>[23]</sup> High levels of basal E2 level were associated with low oocyte yields, low pregnancy rates, and higher cancellation rate independent of FSH levels.<sup>[24,25]</sup> Our results are consistent with the above studies. In the multiple logistic regression equation, the effect of basal FSH and basal E2 on the adverse outcome of no transferable embryo cycles is greater than the effect of female age.

Endometriosis is one of the important factors leading to female infertility. Fifty-seven percent (20/35) of the prediction models take endometriosis as an important indicator to evaluate the success rate of IVF. After balancing many other prediction indicators, only the number of follicles >10 mm on the day of hCG administration and endometriosis entered the final equation, which shows that the expected number of retrieved oocytes and endometriosis are closely related to the outcome of oocytes cryopreservation.

One of the weaknesses of our model is insufficient external validation. This is because there are a limited number of patients which require emergency oocyte cryopreservation. In one of the largest assisted reproductive centers in China, in over 10 years, only 211 patients received emergency oocyte cryopreservation and returned to the hospital for follow-up treatment. The number is expected to be lower in other relatively smaller scale assisted reproductive centers. Therefore, it is difficult to carry out external verification at this moment. In the future, we will collect more data for external verification to verify the accuracy of the prediction model. In addition, the sample size used to derive this prediction model is small, and all of them are from a single center. The question of whether the research results can be extended to other races and regions remains to be further verified.

Given the complexities of ART, many other confounders can have an effect at different points in time. Although we can use the expected retrieved number of eggs and sperm sources to make a preliminary assessment of the embryo, our model is for pre-treatment counseling only. We appreciate that IVF success rates depend on more than the factors in this model alone. Therefore, when using the model, it is important for clinicians to ensure that their patients understand the probability of having a successful outcome will invariably change as they progress through their treatment and thus should be interpreted as a baseline prediction only.

### Comparison to existing models

The existing prediction models of IVF success rate are all for fresh oocyte; there is no available model for frozen oocyte. Therefore, there is no comparability of clinical indicators and prediction accuracy between this model and existing models. Unlike the published clinical model of assisted reproduction, a nomogram was applied in this research to display the prediction model, which is more practical and intuitive. The nomogram makes it convenient for clinicians and patients to calculate the benefits of oocytes cryopreservation in each treatment cycle according to their own conditions. This can help set expectations for both doctors and patients, reducing psychological pressure, and making decision-making easier. It may also reduce the economic and psychological pressure on patients when facing no transferable embryo cycles. We believe this prediction tool is an important and valuable addition in the counseling process for patients at this critical decision-making point in their journey.

Our results show that for oocyte cryopreservation cycles, similarly to fresh oocyte cycle, the risk of “no transferable embryo” increases and “live birth” decreases with the following clinical characteristics of the mother: increased age, longer duration of infertility, decreased ovarian reserve function, and endometriosis. We have illustrated not only the clinical utility of this model but also how a couple's characteristics might affect their prognosis. This model provides a personalized approach to counseling and estimates the chance of success based on a patients' individual characteristics. This can be applied by clinicians when counseling couples before emergency oocyte cryopreservation.

For example, take the case of an infertile patient and sperm donor where the woman is 32 years old with 3 years of infertile history, basal FSH, 7.5 MIU/mL, basal E2:131 mmol/L. Their IVF indicators are involved: endometriosis and severe oligozoospermia. The number of follicles with a diameter >10 mm on the day of hCG administration is eight. The sperm donor could not come to the hospital due to an emergency on the day of oocyte retrieval. If the man can obtain sperm by masturbation, the possibility of no transferable embryo cycles is 25.30%. If it is necessary to extract sperm by MESA, the possibility of no transferable embryo cycles is 48.90%, and the possibility of live birth is 39.39%. The results from our model might assist the couples to decide whether to freeze or give up oocyte retrieval.

The next step for this model is to further validate the research findings by performing external validation in other assisted reproduction centers in China and worldwide. Furthermore, this model may be developed into both a user-friendly web-based decision aid platform and as a mobile application to assist both clinicians and patients.

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### Conflicts of interest

None.

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