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Independent predictors and thresholds of in vitro fertilization outcomes in patients with diminished ovarian reserve

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This study aimed to identify the independent predictors of in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) outcomes among patients with diminished ovarian reserve (DOR), focusing on factors that predict the retrieval of oocytes (cumulus–oocyte complexes [COC]), day 3 (D3) available cleavage-stage embryos, clinical pregnancy during the IVF/ICSI fresh embryo transfer cycle, and viable blastocyst formation. We retrospectively analyzed 1,403 IVF/ICSI cycles involving 1,039 patients diagnosed with DOR, of which 441 cycles underwent fresh embryo transfer. Patients were categorized into groups based on their IVF/ICSI outcomes, which included oocyte retrieval, obtaining D3-available cleavage-stage embryos, clinical pregnancies, and viable blastocyst formation. Univariate and multivariate logistic regression analyses were performed to identify factors influencing IVF/ICSI outcomes. The predictive model incorporated the receiver operating characteristic curve to evaluate the predictive performance of the identified factors for IVF/ICSI outcomes. Anti-Mullerian hormone (AMH) was identified as a more effective independent predictor for oocyte retrieval than antral follicle count (AFC) and basal follicle-stimulating hormone (FSH), whereas AFC demonstrated superior predictive accuracy for obtaining D3-available cleavage-stage embryos, with prediction thresholds of 0.345 ng/mL and 3.5, respectively. D3 top-quality cleavage-stage embryos were a more reliable independent predictor of clinical pregnancy than age for patients aged below 40 years, whereas age showed greater predictive reliability in those aged 40 years or above. Additionally, D3-available cleavage-stage embryos were the sole predictor of viable blastocyst formation. In conclusion, AMH and AFC were more reliable than basal FSH at predicting the retrieval of oocytes and D3-available cleavage-stage embryos. Acquiring a D3 top-quality cleavage-stage embryo suggests potential for clinical pregnancy, but for patients aged 40 years or older, even access to such embryos does not mitigate the significant effects of age on clinical pregnancy outcomes. If none of the three to four D3 available cleavage-stage embryos are of top quality, culturing them to the blastocyst stage may improve clinical outcomes by yielding viable blastocysts.

Keywords Diminished ovarian reserve, IVF/ICSI outcome, Clinical pregnancy, Viable blastocyst, Predictor

According to the report by the US Centers for Disease Control and Prevention, which was based on the National Survey of Family Growth conducted between 2015 and 2019, 13.1% of women aged 15–49 years in the US suffered from impaired fecundity, whereas 12.7% had utilized some form of infertility services[1]. Assisted reproductive technology (ART) has long been used to address infertility, accounting for 1.7–4% of all pregnancies[2]. Diminished ovarian reserve (DOR) denotes a decrease in the quantity and quality of oocytes, affecting women of reproductive age who, despite having regular menstrual cycles, exhibit reduced fecundity relative to their peers[3]. The prevalence of DOR has increased from 19 to 26% between 2004 and 2011 [4], signifying

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a notable rise in DOR diagnoses among women redeciding ART. Approximately 31% of patients with DOR seek the services of reproductive centers for ART[5]. The application of ART has provided numerous women suffering from infertility the opportunity to conceive. However, DOR patients have exhibited low success rates and adverse outcomes with ART[6].

Patients with DOR who suffer from infertility frequently exhibit poor response to controlled ovarian stimulation (COS), necessitating increased gonadotropin usage, which increased cancellation rates and lower ART success rates[7]. The high costs and side effects associated with ART impose a significant burden on patients; therefore, the selection and counseling of DOR patients regarding prognosis are crucial prior to initiating ART. Ovarian reserve tests (ORTs) and age have been frequently utilized to assess oocyte reserve and quality, as well as evaluate their capacity to predict IVF/ICSI outcomes, such as oocyte yield, embryo formation, and pregnancy onset, in patients suffering from infertility[8]. Subfertility rates increase as female age, with estimates showing that 6%, 9%, 15%, 30%, and 64% of those aged 20–24, 25–29, 30–34, 35–39, and 40–44 years suffer from subfertility[9]. Aging has also been found to be accompanied by a decline in both the number of ovarian follicles and oocyte quality[10]. Consequently, IVF/ICSI is not fully capable of addressing the challenges associated with age-related DOR[11]. In recent years, a trend toward increasing incidence rates of DOR and a lowering of the average age of affected patients has been observed. Approximately 10% of women suffer from infertility at a younger age due to early-onset DOR[12]. However, it remains unclear whether differences in the adverse outcomes of DOR exist between young and older patients. Accordingly, Tarek El-Toukhy et al. posited that youthful age does not safeguard against the adverse effects of DOR[13]. However, Morin et al. suggested that younger patients with DOR might be less susceptible to its adverse effects given their age-related characteristics[14]. The challenge lies in distinguishing between DOR patients with favorable and unfavorable prognoses, despite similar chronological age. ORTs often predict the likelihood of pregnancy and response to gonadotropin in patients with DOR[8]. Anti-Mullerian hormone (AMH) has demonstrated a predictive capacity for ovarian reserve and response that is nearly equivalent to that of antral follicle count (AFC) but exceeds that of age and basal follicle-stimulating hormone (FSH)[15]. Although AMH and AFC are comparable predictors of the number of available embryos, they provide minimal clinical value for predicting pregnancy outcomes[16,17], with basal FSH being similarly ineffective in predicting nonpregnancy[18]. The use of AMH to predict oocyte quality or clinical pregnancy remains controversial. Although some studies have identified a correlation between oocyte quality and AMH levels[19,20], others have supported the opposite view[21,22]. To enhance IVF success rate, numerous centers have recently opted for blastocyst culture and transfer[23]. The extension of cleavage-stage embryo culture to the blastocyst stage for transfer has been adopted to eliminate embryos with poor developmental potential, thereby improving clinical pregnancy and live birth rates[24]. However, blastocyst culture is not without risks, with reported blastocyst formation rates ranging from 40 to 60% [25]. Furthermore, patients with DOR are at increased risk of obtaining no viable blastocysts following sequential embryo culture, highlighting the paramount importance of investigating factors influencing blastocyst formation. Nonetheless, research identifying predictors of blastocyst formation in DOR patients has been scant[26,27]. In summary, DOR patients are susceptible to a poor ovarian response (POR), thereby complicating the capacity of predictive markers to accurately evaluate their IVF prognosis, especially the blastocyst formation and clinical pregnancy after fresh embryo transfer.

To boost IVF success rates among patients with DOR, we sought to identify predictors of IVF/ICSI outcomes and their thresholds. A predictive model based on these factors can help clinicians assess the fertility of DOR patients before starting an IVF/ICSI cycle, which could guide them in selecting the most appropriate reproductive treatments, prevent unnecessary IVF procedures among couples struggling with infertility, and avoid misguided denials of IVF.

Methods

Patients

We retrospectively reviewed 1,039 patients with DOR who underwent 1,403 IVF/ICSI cycles at the Department of Reproductive Medicine, West China Second University Hospital, Sichuan University, between January 1, 2021, and December 31, 2021. In accordance with the diagnostic criteria for DOR, patients who met at least two of the following three criteria were included[3]: (1) AMH < 1.1 ng/mL; (2) AFC < 7 follicles; and (3) basal FSH ≥ 10 IU/L. Patients who presented with any of the following conditions were excluded: (1) polycystic ovary or polycystic ovary syndrome; (2) chronic diseases, such as hypertension, cardiovascular disease, autoimmune disease, and abnormal hepatic and renal function; (3) other endocrine diseases, such as hypogonadotropin hypogonadism and hyperprolactinemia; (4) uterine malformation; (5) chromosomal abnormalities; (6) a history of recurrent spontaneous abortion; and (7) an active period of infectious diseases. Prior to study participation, written informed consent was obtained from all participants in compliance with national legislation and institutional requirements. The study protocol was approved by the Ethics Committee of West China Second University Hospital of Sichuan University (Approval No. 2023278).

Grouping method

A total of 1,403 IVF/ICSI cycles were included in this study, of which 1,352 cycles proceeded to oocyte retrieval. Cycles were then categorized into distinct groups. First, cycles were divided based on oocyte (cumulus–oocyte complex [COC]) retrieval, resulting in the oocyte retrieval group and the no oocyte retrieval group. Second, cycles were stratified based on the D3-available cleavage-stage embryos obtained, creating the D3-available cleavage-stage embryos group and the non-D3-available cleavage-stage embryos group. Third, DOR patients in fresh cleavage-stage embryo transfer cycles were segregated into the clinical pregnancy group and nonpregnant group (Fig. 1). The remaining cleavage-stage embryos were cultured to the blastocyst stage and subsequently classified into two categories: viable and nonviable blastocysts.

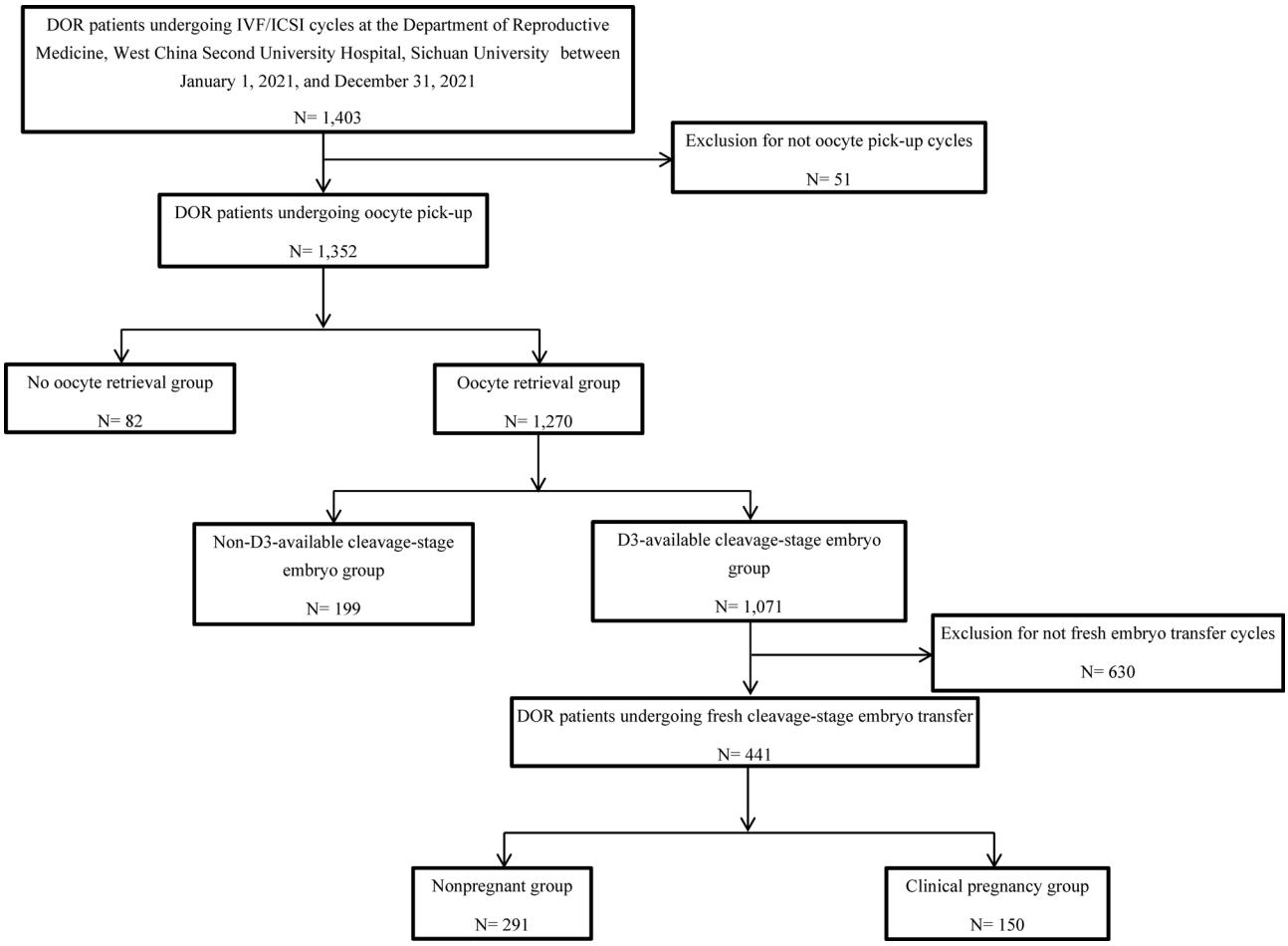

Fig. 1. Flow chart of patient selection and group.

IVF/ICSI treatment

Each patient received individualized COS, employing various protocols, including the gonadotropin-releasing hormone (GnRH) antagonist protocol, long GnRH agonist protocol, mild stimulation protocol, progestin-primed ovarian stimulation protocol (PPOS), and natural cycle. Oocyte maturation was triggered when two dominant follicles reached a mean diameter of 18 mm using urinary-derived human chorionic gonadotropin (Qingdao Guanlong Biopharm Co.) at 5000–10,000 IU or recombinant human chorionic gonadotropin (Ovidrel, Serono) at 250 μg, and/or short-acting GnRH agonists (Daphylline, Ipsen; or Decapeptyl, Ferring; or Triprolen, Changchun Jinsai Pharmaceutical Co.) at 0.1–0.2 mg. COCs were retrieved via transvaginal ultrasound-guided follicle aspiration 36–38 h after hCG injection. Subsequent fertilization was performed using routine IVF or ICSI, depending on gamete quality[28]. Luteal support was initiated on the day of oocyte retrieval[29]. During routine IVF procedures, metaphase II (MII) oocytes are expected to complete fertilization 16–18 h after insemination, with normal fertilization being characterized by the presence of two distinct pronuclei (2PN) in the zygote. During ICSI procedures, fertilization involves initially removing cumulus/corona cells, subsequently assessing oocyte nuclear maturity, and extruding the first polar body under an inverted microscope; fertilization is then observed 16 h after sperm injection. Zygotes were cultured in cleavage medium. All cleavage-stage embryos were evaluated for quality on the morning of days 2 and 3 after oocyte retrieval[30]. Embryos with ≥ 6 blastomeres and ≤ 25% cell fragmentation on day 3 after normal fertilization were classified as available cleavage-stage embryos[31]. Among these embryos, those with 7–9 blastomeres (i.e., appropriate size for developmental stage), ≤ 10% cell fragmentation, and no multinucleation were considered top-quality cleavage-stage embryos (grade I and II embryos)[32]. On the third day following oocyte retrieval, one or two highest-grade D3 cleavage-stage embryos were selected for transfer. For other protocols, such as PPOS, when accumulating embryos for later transfer is desired or under other circumstances unsuitable for fresh embryo transfer (e.g., elevated progesterone levels or endometrial thickness ≤ 7 mm), the remaining D3-available cleavage-stage embryos were either cryopreserved or cultured to the blastocyst stage for subsequent freeze–thaw embryo transfer cycles. Blastocysts were evaluated and graded using the Gardner blastocyst grading system, which assesses blastocyst expansion (grades 1–6), trophectoderm (grades A–C), and inner cell mass (grades A–C). Blastocysts graded 4BC, 4CB, or higher were cryopreserved[33]. Pregnancies were confirmed through a positive serum hCG test conducted 14 days after embryo

transfer. Clinical pregnancies were ascertained through the visualization of a gestational sac via transvaginal ultrasonography between 28 and 35 days after embryo transfer.

Statistical analysis

Statistical analyses were conducted using SPSS (IBM Crop, Armonk, NY, USA) and R software (https://cran.r-project.org/doc/FAQ/R-FAQ.html#Citing-R). Baseline characteristics were compared across four distinct groups: oocyte retrieval versus no oocyte retrieval groups, D3-available cleavage-stage embryo versus non-D3-available cleavage-stage embryo groups, clinical pregnancy versus nonpregnant groups, and viable blastocyst versus nonviable blastocyst groups. Descriptive statistics for continuous variables were presented as mean and standard deviation, whereas categorical variables are presented as numbers and percentages. For variables adhering to a normal distribution, we employed t-tests or one-way analysis of variance to compare differences between groups. Chi-square tests or Kruskal–Wallis H rank sum tests were utilized to determine whether differences in categorical and non-normally distributed variables were statistically significant, respectively. Univariate and multivariate binary logistic regression analyses were performed to identify factors correlated with IVF/ICSI outcomes. Odds ratios (ORs) and 95% confidence intervals (CIs) were based on univariate analysis to demonstrate the level of association. The adjusted ORs (AORs) were calculated by multiple logistic regression. The predictive performance of the predictors was evaluated using the receiver operating characteristic (ROC) curve analysis, with the area under the ROC curve (AUC) ranging from 0.5 (indicating no discrimination) to 1 (indicating perfect discrimination). The Youden index was calculated, employing the maximum value as the predictive threshold. Additionally, the AUC was constructed to compare the predictive capabilities of the number of D3 top-quality cleavage-stage embryos and age on clinical pregnancy across three age strata: <35, 35–40, and ≥ 40 years. A P value of 0.05 indicated statistical significance.

Results

A total of 1,039 patients with DOR underwent 1,403 IVF/ICSI cycles. Accordingly, oocytes were retrieved in 1,270 cycles following oocyte pick-up, embryos were obtained in 1,071 cycles post-oocyte collection, and only 150 patients achieved pregnancy in their IVF/ICSI fresh embryo transfer cycles, with the remaining patients failing to do so. Among the 379 embryo cycles, 280 resulted in viable blastocysts following blastocyst culture.

Patients' general and cycle characteristics

Patients with DOR were classified based on the retrieval of oocytes and D3-available cleavage-stage embryos, as well as the achievement of clinical pregnancies during IVF/ICSI fresh embryo transfer cycles. Table 1 presents the baseline and stimulation cycle characteristics. Significant differences were observed in basal AMH, AFC, FSH, luteinizing hormone (LH), estradiol (E2), the mean initial gonadotropin doses, total gonadotropin doses, duration of stimulation days, trigger day E2, and LH value between the oocyte retrieval and no oocyte retrieval groups ($P < 0.05$; Table 1, Comparison 1). Age; levels of basal AMH, AFC, and FSH; initial gonadotropin doses; total gonadotropin doses; duration of stimulation days; trigger day E2, progesterone, and LH values; and the number of retrieved oocytes, MII oocytes, 2PN embryos, and normal cleavage embryos varied significantly between the D3-available cleavage-stage embryos and the non-D3-available cleavage-stage embryo groups ($P < 0.05$; Table 1, Comparison 2). Within the oocyte/embryo-retrieved group, the most frequently utilized COS regimen was the GnRH antagonist protocol, followed by the PPOS and mild stimulation protocols. Significant differences in age, basal AFC, mean initial gonadotropin doses, total gonadotropin doses, trigger day E2 values, number of retrieved oocytes, MII oocytes, 2PN embryos, normal cleavage embryos, D3-available cleavage-stage embryos, D3 top-quality cleavage-stage embryos, and embryo transferred at the cleavage stage were noted between the clinical pregnancy and nonpregnant groups ($P < 0.05$; Table 1, Comparison 3). Aside from utilizing D3-available cleavage-stage embryos for fresh transfer, the remaining embryos were either cryopreserved or cultured to the blastocyst stage. Table 2 summarizes the features associated with blastocyst culture. Significant differences in age, body mass index (BMI), mean levels of E2 and LH on the trigger day, number of retrieved oocytes, MII oocytes, 2PN embryos, normal cleavage embryos, D3-available cleavage-stage embryos, D3 top-quality cleavage-stage embryos, and the proportions of 2PN and D3 top-quality cleavage-stage embryos were observed between the two groups ($P < 0.05$; Table 2).

Predictive model 1 for oocytes retrieval in DOR patients

DOR patients presenting higher AMH levels (0.63 ± 0.42 vs. 0.39 ± 0.33, $P < 0.001$), higher AFC (3.67 ± 1.9 vs. 2.41 ± 1.49, $P < 0.001$), and lower basal FSH levels (11.55 ± 5.96 vs. 14.77 ± 8.94, $P < 0.001$) exhibited improved ovarian response and oocyte retrieval after COS (Table 1, Comparison 1). Univariate analysis revealed that AMH, AFC, and basal FSH were significantly correlated with the number of oocytes retrieved. Multivariate logistic regression analysis identified AMH levels (AOR: 4.043, 95% CI: 1.810–9.684, $P = 0.001$), AFC (AOR: 1.335, 95% CI: 1.152–1.555, $P < 0.001$), and basal FSH levels (AOR: 0.968, 95% CI: 0.945–0.993, $P = 0.008$) as independent predictors of oocyte retrieval in patients with DOR (Table 3, Model 1). Mode 1 constructed the AUC utilizing AMH levels, AFC, and basal FSH levels (Table 4, Model 1), which was then employed to assess the predictive efficacy of AMH, AFC, and basal FSH for oocyte retrieval in patients with DOR. Notably, the AUC for AMH (0.679) exceeded that for AFC and basal FSH (0.696 and 0.630, respectively; $P < 0.001$). AMH showed superior predictive performance in predicting oocyte retrieval than did AFC and basal FSH, with a sensitivity and specificity of 56.1% and 73.15%, respectively, based on a cut-off value of 0.345 ng/mL (Fig. 2A). The threshold value for AFC was 3.5, accompanied by a sensitivity of 81.71% and a specificity of 53.46% (Fig. 2B). The threshold level for basal FSH was 12.85 IU/L, with a sensitivity and specificity of 53.66% and 72.6%, respectively (Fig. 2C).

	Comparison 1			Comparison 2			Comparison 3		
	Oocyte retrieval	No oocyte retrieval	*P*	D3-available cleavage-stage embryo	Non-D3-available cleavage-stage embryo	*P*	Clinical pregnancy	Nonpregnant	*P*
Number	990	78		888	85		150	271	
Cycle	1270	82		1071	199		150	291	
Age (years)	35.38 ± 5.33	35.91 ± 5.60	0.573	35.13 ± 5.19	36.73 ± 5.89	< 0.001*	33.24 ± 4.35	35.24 ± 5.09	< 0.001*
Duration of infertility (year)	3.62 ± 3.33	2.97 ± 2.61	0.085	3.59 ± 3.27	3.82 ± 3.65	0.766	3.51 ± 2.9	3.75 ± 3.37	0.785
Cause of infertility (1/2/3/4)			0.703			0.430			0.111
Female factor (1)	1071 (84.33%)	72 (87.8%)		908 (84.78%)	163 (81.91%)		123 (82%)	253 (86.94%)	
Male factor (2)	14 (1.1%)	0 (0%)		10 (0.93%)	4 (2.01%)		1 (0.67%)	4 (1.37%)	
Mixed factor (3)	183 (14.41%)	10 (12.2%)		151 (14.1%)	32 (16.08%)		24 (16%)	34 (11.68%)	
Unexplained factor (4)	2 (0.16%)	0 (0%)		2 (0.19%)	0 (0%)		2 (1.33%)	0 (0%)	
Anti-Mullerian hormone (ng/ml)	0.63 ± 0.42	0.39 ± 0.33	< 0.001*	0.65 ± 0.42	0.48 ± 0.37	< 0.001*	0.77 ± 0.42	0.7 ± 0.39	0.127
Antral follicle count	3.67 ± 1.9	2.41 ± 1.49	< 0.001*	3.82 ± 1.88	2.87 ± 1.82	< 0.001*	4.69 ± 1.73	4.15 ± 1.8	0.005*
Basal follicle-stimulating hormone (IU/L)	11.55 ± 5.96	14.77 ± 8.94	< 0.001*	11.24 ± 4.99	13.19 ± 9.49	< 0.001*	10.28 ± 3.92	10.75 ± 4.56	0.524
Basal luteinizing hormone (IU/L)	3.8 ± 2.19	4.62 ± 3.04	0.006*	3.77 ± 2.22	3.99 ± 2.05	0.090	3.3 ± 1.62	3.7 ± 2.22	0.093
Basal estradiol (pg/ml)	45.58 ± 29.45	51.75 ± 44.39	< 0.001*	44.81 ± 27.16	49.73 ± 39.38	0.821	43.94 ± 22.23	42.49 ± 22.76	0.253
Basal progesterone (ng/ml)	0.61 ± 0.36	0.61 ± 0.35	0.933	0.6 ± 0.35	0.67 ± 0.41	0.068	0.57 ± 0.23	0.57 ± 0.35	0.107
Body-mass index (kg/m2)	22.26 ± 2.96	22.32 ± 2.7	0.601	22.31 ± 2.96	21.99 ± 2.95	0.174	22.11 ± 3.14	22.33 ± 2.97	0.347
Controlled ovarian stimulation protocol (1/2/3/4/5)			< 0.001*			< 0.001*			0.072
GnRH antagonist protocol (1)	863 (67.95%)	37 (45.12%)		745 (69.56%)	118 (59.3%)		138 (92%)	265 (91.07%)	
GnRH-agonist long protocol (2)	16 (1.26%)	0 (0%)		15 (1.4%)	1 (0.5%)		6 (4%)	3 (1.03%)	
Mild stimulation protocol (3)	171 (13.46%)	22 (26.83%)		141 (13.17%)	30 (15.08%)		6 (4%)	21 (7.22%)	
Progestin-primed ovarian stimulation protocol (4)	184 (14.49%)	9 (10.98%)		150 (14.01%)	34 (17.09%)		0 (0%)	0 (0%)	
Natural cycle (5)	36 (2.83%)	14 (17.07%)		20 (1.87%)	16 (8.04%)		0 (0%)	2 (0.69%)	
Initial gonadotropin doses (IU)	238.88 ± 75.47	179.27 ± 94.84	< 0.001*	244.16 ± 71.68	210.43 ± 88.16	< 0.001*	280.83 ± 41.71	262.63 ± 61.81	0.005*
Total gonadotropin doses (IU)	2219.69 ± 993.09	1399.39 ± 929.25	< 0.001*	2309.45 ± 885.37	2093.95 ± 1182.26	< 0.001*	2609.42 ± 716.88	2485.11 ± 843.74	0.027*
Duration of stimulation days (d)	8.93 ± 2.84	6.6 ± 3.77	< 0.001*	9.22 ± 2.3	8.84 ± 3.32	0.003*	9.49 ± 1.65	9.41 ± 2.15	0.494
Trigger day E2 value (pg/ml)	1023.68 ± 732.67	425.88 ± 334.16	< 0.001*	1081.07 ± 746.61	683.94 ± 556.52	< 0.001*	1196.71 ± 696.37	1085.04 ± 771.0	0.040*
Trigger day P value (ng/ml)	0.82 ± 1.16	0.98 ± 2.2	0.211	0.83 ± 1.21	0.72 ± 0.88	0.007*	0.7 ± 0.27	0.69 ± 0.27	0.791
Trigger day LH value (IU/L)	4.68 ± 5.86	8.71 ± 7.81	< 0.001*	4.19 ± 4.76	7.13 ± 9.48	< 0.001*	2.9 ± 2.83	3.75 ± 5.81	0.182
No. of retrieved oocytes				3.53 ± 2.19	1.71 ± 0.98	< 0.001*	4.43 ± 2.03	3.81 ± 2.13	< 0.001*
No. of mature (MII) oocytes				3.03 ± 1.92	1.07 ± 0.87	< 0.001*	3.77 ± 1.8	3.22 ± 1.87	< 0.001*
No. of 2PN embryos				2.28 ± 1.59	0.42 ± 0.64	< 0.001*	2.79 ± 1.56	2.35 ± 1.62	< 0.001*
No. of normal cleavage embryos				2.25 ± 1.57	0.38 ± 0.61	< 0.001*	2.78 ± 1.55	2.34 ± 1.6	0.001*
No. of D3-available cleavage-stage embryos							2.78 ± 1.3	2.32 ± 1.49	< 0.001*
No. of D3 top-quality cleavage-stage embryos							1.57 ± 1.17	1.12 ± 1.17	< 0.001*
Endometrial thickness at transfer day (double layer) (cm)							1.18 ± 0.73	1.16 ± 0.89	0.242
No. of embryo transferred at cleavage stage (1/2)									< 0.001*
Single embryo transfer (1)							38 (25.33)	147 (50.52)	
Double embryos transfer (2)							112 (74.67)	144 (49.48)	

Table 1. Baseline and cycle characteristics in DOR patients undergoing IVF/ICSI fresh embryo transfer. Continuous variables are presented as mean ± standard deviation. Categorical variables are displayed as n (%). T tests or one-way analysis of variance were used for normally distributed data, and Chi-square tests or Kruskal–Wallis H rank sum tests were used for non-normally distributed data. DOR = diminished ovarian reserve, IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, MII = metaphase II, 2PN = 2 pronuclei, D3 = Day 3. * $P < 0.05$.

	Viable blastocyst	Nonviable blastocyst	*P*
Number	275	97	
Cycle	280	99	
Age (years)	33.7 ± 4.51	35.57 ± 5.15	0.002*
Duration of infertility (year)	3.1 ± 2.83	3.34 ± 3.16	0.838
Type of infertility (%) (1/2)			0.218
Primary infertility (1)	138 (49.29)	41 (41.41)	
Secondary infertility (2)	142 (50.71)	58 (58.59)	
Cause of infertility (%) (1/2/3/4)			0.673
Female factor (1)	232 (82.86)	85 (85.86)	
Male factor (2)	2 (0.71)	0 (0)	
Mixed factor (3)	45 (16.07)	14 (14.14)	
Unexplained factor (4)	1 (0.36)	0 (0)	
Anti-Mullerian hormone (ng/ml)	0.8 ± 0.46	0.72 ± 0.3	0.268
Antral follicle count	4.56 ± 1.7	4.31 ± 1.8	0.278
Basal follicle-stimulating hormone (IU/L)	10.0 ± 3.51	10.92 ± 5.26	0.220
Basal luteinizing hormone (IU/L)	3.6 ± 1.54	3.45 ± 1.95	0.166
Basal estradiol (pg/ml)	43.76 ± 24.31	41.5 ± 21.89	0.147
Basal progesterone (ng/ml)	0.62 ± 0.44	0.57 ± 0.25	0.638
Body-mass index (kg/m2)	21.83 ± 2.92	22.63 ± 3.29	0.029*
Controlled ovarian stimulation protocol (%) (1/2/3/4/5)			0.096
GnRH antagonist protocol (**1**)	222 (79.29)	64 (64.65)	
GnRH-agonist long protocol (**2**)	6 (2.14)	1 (1.01)	
Mild stimulation protocol (3)	17 (6.07)	12 (12.12)	
Progestin-primed ovarian stimulation protocol (4)	35 (12.5)	17 (17.17)	
Natural cycle (5)	0 (0)	0 (0)	
Initial gonadotropin doses (IU)	267.41 ± 50.87	257.83 ± 60.68	0.237
Total gonadotropin dose (IU)	2565.27 ± 671.62	2517.42 ± 925.42	0.285
Duration of stimulation days (d)	9.85 ± 1.8	9.63 ± 2.16	0.237
Trigger day E2 value (pg/ml)	1600.94 ± 831.6	1321.58 ± 698.52	0.004*
Trigger day P value (ng/ml)	0.9 ± 0.74	0.88 ± 0.98	0.157
Trigger day LH value (IU/L)	3.0 ± 2.66	3.75 ± 3.24	0.030*
No. of retrieved oocytes	5.56 ± 2.3	4.23 ± 1.71	< 0.001*
No. of mature (MII) oocytes	4.97 ± 2.07	3.68 ± 1.32	< 0.001*
No. of 2PN embryos	3.98 ± 1.7	2.65 ± 1.28	< 0.001*
No. of normal cleavage embryos	3.94 ± 1.66	2.62 ± 1.29	< 0.001*
No. of D3-available cleavage-stage embryos	3.97 ± 1.4	2.87 ± 1.03	< 0.001*
No. of D3 top-quality cleavage-stage embryos	2.15 ± 1.3	1.35 ± 1.02	< 0.001*
MII oocyte rate (%)	1392/1556 (89.46)	364/419 (86.87)	0.920
2PN rate (%)	1114/1556 (71.59)	262/419 (62.53)	0.013*
D3 top-quality cleavage stage-embryo rate (%)	601/1316 (45.67)	134/331 (40.48)	0.039*

Table 2. Baseline and cycle characteristics in DOR patients undergoing blastocyst culture. Continuous variables are presented as mean ± standard deviation. Categorical variables are displayed as n (%). T tests or one-way analysis of variance were used for normally distributed data, and Chi-square tests or Kruskal–Wallis H rank sum tests were used for non-normally distributed data. DOR = diminished ovarian reserve, MII = metaphase II, 2PN = 2 pronuclei, D3 = Day 3. * $P < 0.05$.

Predictive model 2 for obtaining D3-available cleavage-stage embryos in DOR patients

Younger age (35.13 ± 5.19 vs. 36.73 ± 5.89, $P < 0.001$), higher AMH levels (0.65 ± 0.42 vs. 0.48 ± 0.37, $P < 0.001$), higher AFC (3.82 ± 1.88 vs. 2.87 ± 1.82, $P < 0.001$), and lower basal FSH levels (11.24 ± 4.99 vs. 13.19 ± 9.49, $P < 0.001$) in DOR patients were associated with improved embryo formation and quality post-fertilization (Table 1, Comparison 2). Age, AMH, AFC, and basal FSH were correlated with the retrieval of D3-available cleavage-stage embryos. However, after multivariate logistic regression analysis to adjust for confounding factors, only AMH (AOR: 2.263, 95% CI: 1.392–3.804, $P = 0.001$), AFC (AOR: 1.217, 95% CI: 1.109–1.339, $P < 0.001$), and basal FSH (AOR: 0.973, 95% CI: 0.951–0.995, $P = 0.017$) emerged as independent predictors for the retrieval of D3-available cleavage-stage embryos in patients with DOR (Table 3, Model 2). The AUC for model 2 was derived from the AMH levels, AFC, and basal FSH levels (Table 4, Model 2), with AUC values of

	Univariate		Multivariate	
	OR (95% CI)	*P* value	AOR (95% CI)	*P* (a) value
Model 1				
Age (years)	0.981 (0.941–1.024)	0.384	1.022 (0.980–1.067)	0.301
Anti-Mullerian hormone (ng/ml)	8.834 (4.053–20.29)	< 0.001*	4.043 (1.810–9.684)	0.001*
Antral follicle count	1.487 (1.301–1.710)	< 0.001*	1.335 (1.152–1.555)	< 0.001*
Basal follicle-stimulating hormone (IU/L)	0.952 (0.924–0.977)	< 0.001*	0.968 (0.945–0.993)	0.008*
Model 2				
Age (years)	0.945 (0.918–0.972)	< 0.001*	0.973 (0.944–1.002)	0.070
Anti-Mullerian hormone (ng/ml)	4.117 (2.542–6.811)	< 0.001*	2.263 (1.392–3.804)	0.001*
Antral follicle count	1.330 (1.220–1.453)	< 0.001*	1.217 (1.109–1.339)	< 0.001*
Basal follicle-stimulating hormone (IU/L)	0.954 (0.930–0.977)	< 0.001*	0.973 (0.951–0.995)	0.017*
Model 3				
Age (years)	0.917 (0.878–0.956)	< 0.001*	0.928 (0.887–0.969)	< 0.001*
Anti-Mullerian hormone (ng/ml)	1.467 (0.905–2.407)	0.120	0.927 (0.539–1.580)	0.781
Antral follicle count	1.187 (1.061–1.332)	0.003*	1.130 (0.999–1.280)	0.054
Basal follicle-stimulating hormone (IU/L)	0.974 (0.926–1.021)	0.292	1.000 (0.950–1.050)	0.963
D3-available cleavage stage embryos	1.240 (1.080–1.420)	0.001*	1.010 (0.836–1.230)	0.886
D3 top-quality cleavage stage embryos	1.377 (1.160–1.63)	< 0.001*	1.310 (1.040–1.650)	0.023*
Model 4				
Age(years)	0.92 (0.875–0.966)	< 0.001*	0.959 (0.907–1.014)	0.142
Body-mass index(kg/m2)	0.92 (0.854–0.99)	0.026*	0.935 (0.859–1.017)	0.115
Retrieved oocytes	1.413 (1.231–1.622)	< 0.001*	0.965 (0.640–1.453)	0.863
Mature (MII) oocytes	1.572 (1.333–1.853)	< 0.001*	0.897 (0.599–1.342)	0.596
2PN embryos	1.818 (1.512–2.187)	< 0.001*	1.452 (0.304–6.931)	0.640
Normal cleavage embryos	1.832 (1.520–2.208)	< 0.001*	1.019 (0.233–4.45)	0.980
D3-available cleavage-stage embryos	2.264 (1.757–2.917)	< 0.001*	1.755 (1.123–2.742)	0.014*
D3 top-quality cleavage-stage embryos	1.766 (1.422–2.194)	< 0.001*	1.049 (0.545–2.016)	0.887
2PN rate (%)	3.826 (1.494–9.800)	0.005*	0.735 (0.060–9.071)	0.811
D3 top-quality cleavage stage embryo rate (%)	2.297 (1.030–5.122)	0.042*	1.439 (0.173–11.958)	0.736

Table 3. Clinical prediction model for IVF/ICSI outcomes constructed from univariate and multivariate logistic regression analysis. The odds ratios (ORs), 95% confidence intervals (CIs), and P values presented herein are derived from univariate analysis. Adjusted ORs (AORs), 95% CIs, and adjusted P values were computed using a multiple logistic regression model, where P(a) denotes the adjusted P value. Model 1 included factors associated with oocyte retrieval; Model 2 included factors associated with obtaining D3-available cleavage-stage embryos; Model 3 included factors associated with clinical pregnancy in fresh embryo transfer cycles; Model 4 included factors associated with viable blastocyst formation. IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, DOR = diminished ovarian reserve, MII = metaphase II, 2PN = 2 pronuclei, D3 = Day 3. *$P < 0.05$.

0.649, 0.655, and 0.588 for obtaining D3-available cleavage-stage embryos in patients with DOR, respectively. As can be observed, the AUC for AFC (0.655) was significantly higher than those for AMH and basal FSH (0.649 and 0.588, respectively; $P < 0.001$). AFC was identified as a more effective predictor than AMH and basal FSH for obtaining D3-available cleavage-stage embryos, with a sensitivity and specificity of 69.35% and 57.7%, respectively, at a threshold of 3.5 (Fig. 3B). At a cutoff value of 0.395 ng/mL, AMH exhibited a sensitivity and specificity of 53.27% and 72.83%, respectively (Fig. 3A). At a threshold of 10.95 IU/L, basal FSH level showed a sensitivity of 59.8% and a specificity of 56.36% (Fig. 3C).

Predictive model 3 for clinical pregnancy in DOR patients undergoing fresh embryo transfer

DOR patients who were younger (33.24 ± 4.35 vs. 35.24 ± 5.09, $P < 0.001$), had higher AFC (4.69 ± 1.73 vs. 4.15 ± 1.8, $P = 0.005$), more D3-available cleavage-stage embryos (2.78 ± 1.3 vs. 2.32 ± 1.49, $P < 0.001$), and more D3 top-quality cleavage-stage embryos (1.57 ± 1.17 vs. 1.12 ± 1.17, $P < 0.001$) were significantly more likely to achieve clinical pregnancy following fresh embryo transfer (Table 1, Comparison 3). Age, AFC, the number of D3-available cleavage-stage embryos, and the number of D3 top-quality cleavage-stage embryos were correlated with clinical pregnancy; however, subsequent multivariate logistic regression analysis identified only female age (AOR: 0.928, 95% CI: 0.887–0.969, $P < 0.001$) and number of D3 top-quality cleavage-stage embryos (AOR: 1.310, 95% CI: 1.040–1.650, $P = 0.023$) as independent predictors of clinical pregnancy in patients with DOR undergoing fresh embryo transfer (Table 3, Model 3). The AUC for model 3 was constructed from age and D3 top-quality cleavage-stage embryos (Table 4, Model 3), which was then utilized to evaluate their predictive efficacy for clinical pregnancy in patients with DOR undergoing IVF fresh embryo transfer. Notably, the AUC

	Predictors	AUC	*P* value	Cutoff value	Sensitivity (%)	Specificity (%)
Model 1	Anti-Mullerian hormone (ng/ml)	0.697 (0.637–0.756)	< 0.001*	0.345	56.1	73.15
	Antral follicle count	0.696 (0.643–0.748)	< 0.001*	3.5	81.71	53.46
	Basal follicle-stimulating hormone (IU/L)	0.630 (0.560–0.699)	< 0.001*	12.85	53.66	72.6
Model 2	Anti-Mullerian hormone (ng/ml)	0.649 (0.607–0.691)	< 0.001*	0.395	53.27	72.83
	Antral follicle count	0.655 (0.614–0.696)	< 0.001*	3.5	69.35	57.7
	Basal follicle-stimulating hormone (IU/L)	0.588 (0.543–0.632)	< 0.001*	10.95	59.8	56.36
Model 3	Age (years)	0.613 (0.559–0.666)	< 0.001*	35.5	45.36	72.67
	D3 top-quality cleavage-stage embryos	0.619 (0.564–0.673)	< 0.001*	0.5	35.74	81.88
Model 4	D3-available cleavage-stage embryos	0.721 (0.667–0.776)	< 0.001*	3.5	77.78	57.5

Table 4. Performance comparison of predictors for IVF/ICSI outcomes in DOR patients. The receiver operating characteristic (ROC) curve was employed to assess the predictive efficacy of various factors on IVF/ICSI outcomes among DOR patients. The area under the ROC curve (AUC) was determined to quantify the predictive accuracy of these factors in predicting IVF/ICSI success. The Youden index, calculated as sensitivity plus specificity minus 1, was utilized to identify the optimal cut-off points, with the highest Youden index value selected for this purpose. Specifically, Model 1 assessed predictors for oocyte retrieval; Model 2 assessed predictors for obtaining D3-available cleavage-stage embryos; Model 3 assessed predictors for clinical pregnancy in fresh embryo transfer cycles; Model 4 assessed predictors for viable blastocyst formation. IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, DOR = diminished ovarian reserve, D3 = Day 3. $P < 0.05$.

values for age (0.613) and D3 top-quality cleavage-stage embryos (0.619) in predicting clinical pregnancy were statistically significant ($P < 0.001$). Age, as a predictor for clinical pregnancy, showed a sensitivity and specificity of 45.36% and 72.67% at a threshold of 35.5 years, respectively (Fig. 4A). D3 top-quality cleavage-stage embryos, as predictors of clinical pregnancy, exhibited a sensitivity and specificity of 35.74% and 81.88% at a threshold of 0.5, respectively (Fig. 4B). Table 5 presents the AUC values for age and number of D3 top-quality cleavage-stage embryos across different age strata: <35, 35–40, and ≥ 40 years. Among DOR patients younger than 40 years, the number of D3 top-quality cleavage-stage embryos showed superior predictive accuracy for clinical pregnancy than did age (AUC for < 35 years: 0.674 vs. 0.48; AUC for 35–40 years: 0.667 vs. 0.565, $P < 0.001$). Conversely, age exhibited an increased predictive value for clinical pregnancy in DOR patients aged 40 years or above (≥ 40 years, AUC: 0.7 vs. 0.608, $P = 0.004$).

Predictive model 4 for viable blastocyst formation in DOR patients

A model incorporating age, BMI, number of retrieved oocytes, MII oocytes, 2PN embryos, normal cleavage embryos, D3-available cleavage-stage embryos, D3 top-quality cleavage-stage embryos, and the rates of 2PN and D3 top-quality cleavage-stage embryos was developed to predict the formation of viable blastocysts in patients with DOR (Table 3, Model 4). Notably, we found that the aforementioned variables were correlated with the formation of viable blastocysts; however, a subsequent multivariate logistic regression analysis identified only D3-available cleavage-stage embryos (AOR: 1.755, 95% CI: 1.123–2.742, $P = 0.014$) as an independent predictor of viable blastocyst formation in patients with DOR. The AUC for model 4 was derived from D3-available cleavage-stage embryos (Table 4, Model 4). The AUC of D3-available cleavage-stage embryos (0.721) indicated a statistically significant predictive value for the formation of viable blastocysts ($P < 0.001$). Utilizing a threshold of 3.5, D3-available cleavage-stage embryos exhibited a sensitivity and specificity of 77.78% and 57.5%, respectively (Fig. 5A).

Discussion

Patients with DOR who undergo IVF/ICSI experience reduced reproductive prognosis, with clinical pregnancy rates ranging from 10 to 30% [34], highlighting the significant challenge posed by DOR in reproductive medicine[35]. A large sample size is imperative for drawing more precise conclusions and for guiding IVF/ICSI treatment in patients with DOR. Concurrently, constructing a multivariate predictive model provides valuable recommendations for DOR patients regarding their specific needs for ART therapies before initiating an IVF/ICSI cycle. Therefore, our group decided to conduct a recent large-scale retrospective cohort study in patients with DOR undergoing IVF/ICSI treatments.

AMH, but not age, was an independent and superior predictor of retrieved oocytes in patients with DOR

COS is a principal step during IVF treatment, aiming to produce sufficient oocytes and embryos to achieve pregnancy. The selection of an appropriate COS protocol is critical, as it may enhance ovarian response and improve oocyte retrieval outcomes. In the present study, the most frequently utilized COS regimens in the oocyte/embryo-retrieved group were the GnRH antagonist protocol, followed by the PPOS and mild stimulation protocols. The GnRH antagonist protocol has been considered optimal in terms of clinical pregnancy rate per initiating cycle, low risk of cycle cancellation, and number of oocytes retrieved[36]. The PPOS protocol has been shown to achieve a higher number of oocytes retrieved, optimal embryo rates, and a lower cycle cancellation

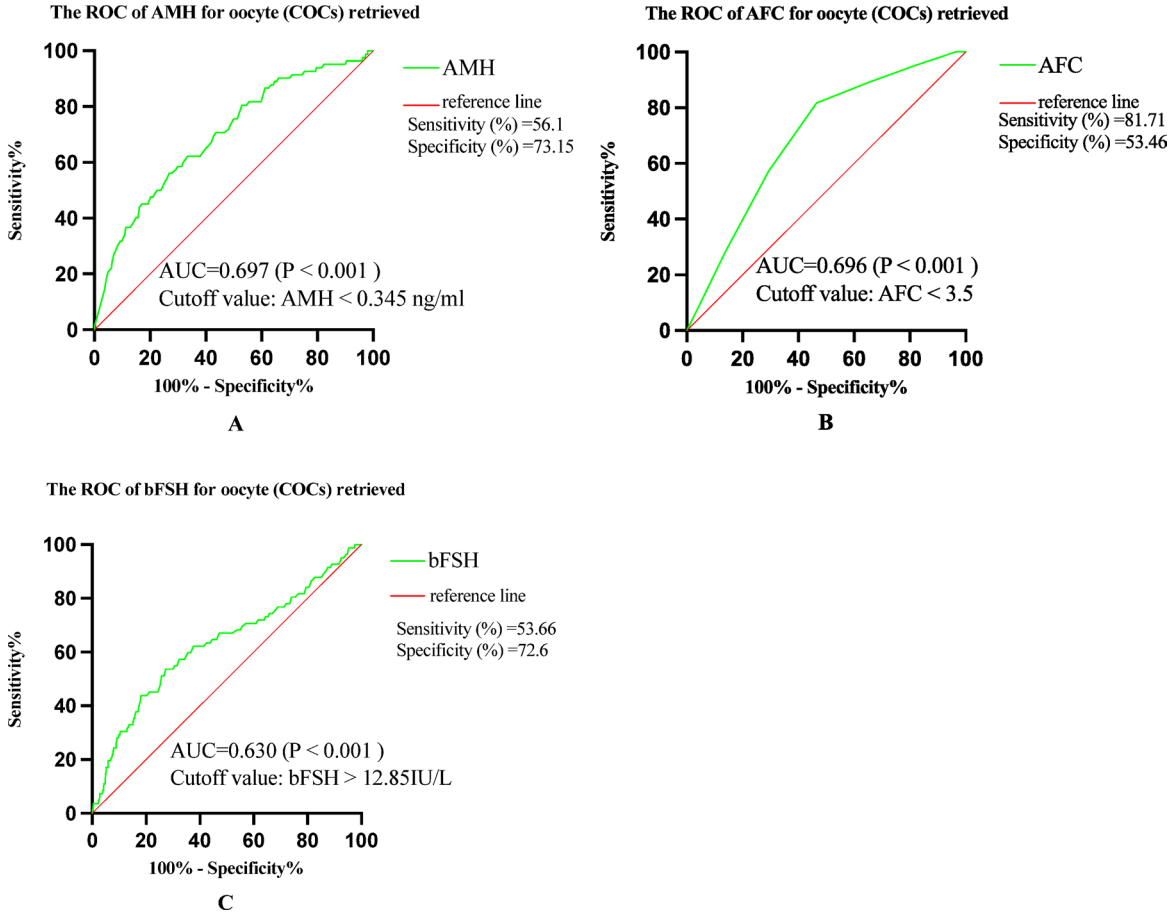

Fig. 2. Comparison of the predictive performance of AMH, AFC, and basal FSH for oocyte retrieval among DOR patients undergoing IVF/ICSI cycles. (**A**). The ROC curve and the corresponding AUC of the predictive utility of AMH for oocyte retrieval. (**B**). The ROC curve and the corresponding AUC of the predictive utility of AFC for oocyte retrieval. (**C**). The ROC curve and the corresponding AUC of the predictive utility of basal FSH for oocyte retrieval. COCs = cumulus-oocyte complexes, DOR = diminished ovarian reserve, IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, AMH = anti-Mullerian hormone, AFC = antral follicle count, FSH = follicle-stimulating hormone, ROC = receiver operating characteristic, AUC = area under the ROC curve.

rate[37]. Mild stimulation protocol has the advantage of reducing costs and the risk of ovarian hyperstimulation syndrome, and it has acceptable fresh and cumulative live birth rates[38]. Unfortunately, patients with DOR often present with POR during COS, and the retrieval of a limited number of oocytes is often inevitable, regardless of the COS regimen selected. Moreover, determining the best protocol for patients with DOR is challenging due to significant individual variability. Hence, an accurate assessment of ovarian reserve and response in patients with DOR can potentially increase the retrieval of oocytes. However, current ovarian reserve and response predictors have shown controversial and limited accuracy, particularly in patients with DOR. As women age, their ovarian reserve and response decline, significantly reducing the number of retrieved oocytes[39,40]. However, our study found that age had no predictive value for oocyte retrieval in DOR patients. We believe that age is a critical factor in determining the quality and quantity of oocytes. Nevertheless, patients with DOR have a diminished oocyte count, which does not necessarily accompany a decrease in oocyte quality. Consequently, the ovaries can still respond to gonadotropin stimulation and potentially yield oocytes regardless of age, provided that the follicular pool is not depleted. Serum AMH levels have been found to exhibit significantly less variability throughout the menstrual cycle, and their measurement is less operator-dependent[41]. The AFC is the sum of the number of antral follicles in each of the ovaries as observed through transvaginal ultrasound during the early follicular phase[42]. DOR patients have elevated levels of basal FSH from days two to four of the menstrual cycle, but the reliability of a single FSH value is limited due to inter- and intracycle variability[43]. Serum AMH levels and AFC have been widely recognized as robust biomarkers for assessing ovarian reserve and response[44,45]. Abnormally elevated basal FSH levels can be considered a late indicator of ovarian reserve depletion[46]. Numerous studies have demonstrated that AMH and AFC are more sensitive measures of ovarian reserve than is FSH[47,48]. However, several studies have been confirmed that AFC is inferior to AMH in predicting poor ovarian response within IVF cycles[49,45]. Our study revealed that AMH, AFC, and basal FSH all significantly predicted oocyte retrieval in patients with DOR, with AMH demonstrating superior predictive accuracy than did AFC and basal FSH. Given

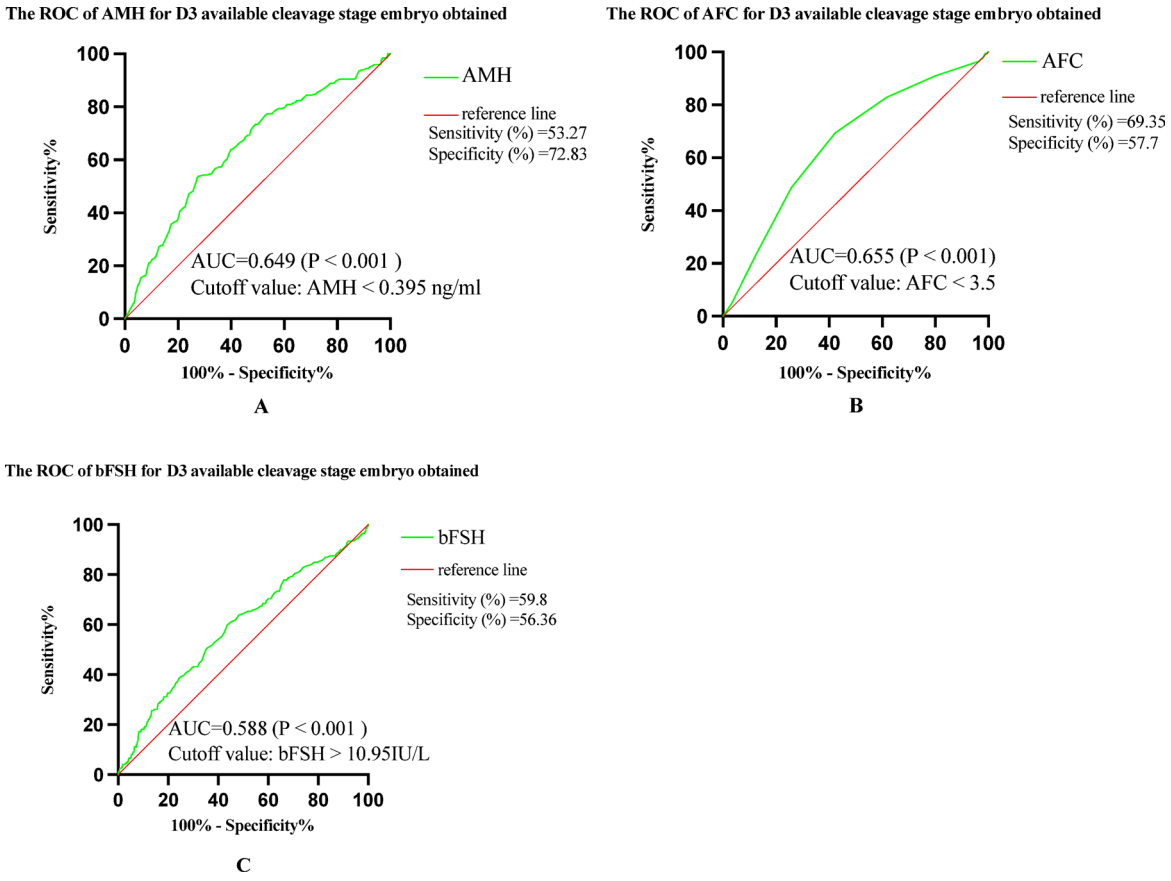

Fig. 3. Comparison of the predictive performance of AMH, AFC, and basal FSH for obtaining D3-available cleavage-stage embryos among DOR patients undergoing IVF/ICSI cycles. (**A**). The ROC curve and the corresponding AUC of the predictive utility of AMH for obtaining D3-available cleavage-stage embryos. (**B**). The ROC curve and the corresponding AUC of the predictive utility of AFC for obtaining D3-available cleavage-stage embryos. (**C**). The ROC curve and the corresponding AUC of the predictive utility of basal FSH for obtaining D3-available cleavage-stage embryos. D3 = Day 3, DOR = diminished ovarian reserve, IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, AMH = anti-Mullerian hormone, AFC = antral follicle count, FSH = follicle stimulating hormone, ROC = receiver operating characteristic, AUC = area under the ROC curve.

our findings showing that a serum AMH threshold of 0.345 ng/mL could predict oocyte retrieval, we concluded that measuring AMH levels is a reliable and robust method for assessing ovarian reserve and response in patients with DOR. Overall, oocyte retrieval through COS is feasible regardless of age and follicle count, provided that serum AMH levels exceed 0.345 ng/mL.

AFC outperformed AMH and basal FSH in predicting d3-available cleavage-stage embryos obtained, whereas age showed no predictive value

As they age, women exhibit a progressive decline in both the quantity and quality of oocytes, with oocyte quality being a critical factor for embryo formation following IVF[50]. DOR has also been found to affect both the quantity and quality of oocytes[51]. However, other studies have suggested that it does not inevitably diminish oocyte quality and that a comparable potential for their development into viable embryos during the ART cycle remains despite the reduced number of retrieved oocytes[52]. Our study further demonstrated that age was not an independent risk factor for the number of D3-available cleavage-stage embryos obtained in patients with DOR. Dai X et al. reported that AMH reflected the quantity of oocytes but not their quality, noting that embryo formation potential after retrieval was similar across various AMH levels[53]. Conversely, Silberstein et al. found that AMH levels may predict ovarian reserve and embryo morphology[54]. AFC has been correlated with the number of retrieved oocytes but has not been demonstrated to predict embryo formation[55]. However, a meta-analysis indicated that AFC may be correlated with both quantitative and qualitative aspects of ovarian function among patients with DOR[56]. Elevated basal FSH levels reflect ovarian aging and impact both the quality and quantity of oocytes[57]. However, elevated FSH levels in patients under 40 years old have not been demonstrated to affect embryo quality[58]. Our study demonstrated that AMH levels, AFC, and basal FSH levels can predict the number of D3-available cleavage-stage embryos obtained in DOR patients undergoing IVF/ICSI. Furthermore, we found that AFC at a threshold of 3.5 was a superior predictor of retrieved embryos than were AMH and basal

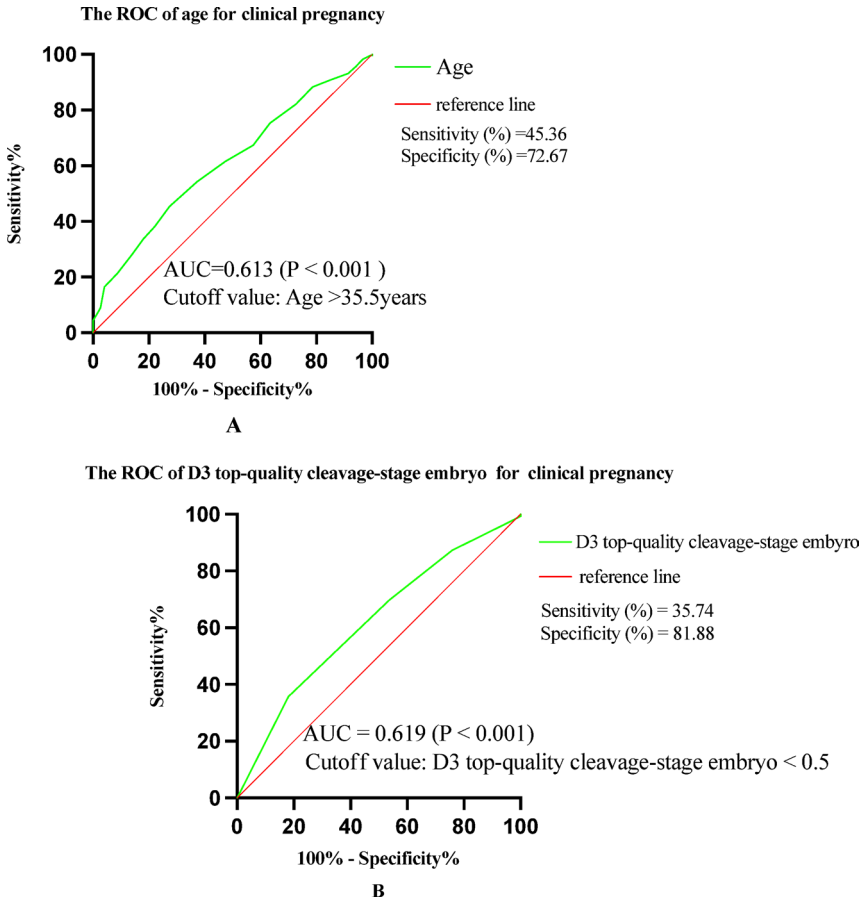

Fig. 4. Comparison of the predictive performance of age and D3 top-quality cleavage-stage embryo for clinical pregnancy among DOR patients undergoing IVF/ICSI-FET cycles. (**A**) The ROC curve and the corresponding AUC of the predictive utility of age for clinical pregnancy. (**B**) The ROC curve and the corresponding AUC of the predictive utility of D3 top-quality cleavage-stage embryo for clinical pregnancy. D3 = Day 3, DOR = diminished ovarian reserve, IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, FET = fresh embryo transfer, ROC = receiver operating characteristic, AUC = area under the ROC curve.

	Age < 35		35 ≤ Age < 40		Age ≥ 40	
Predictors	**AUC**	**P value**	**AUC**	**P value**	**AUC**	**P value**
D3 top-quality cleavage-stage embryos	0.674 (0.620–0.728)	< 0.001*	0.667 (0.589–0.747)	< 0.001*	0.608(0.456–0.759)	0.163
Age	0.480 (0.416–0.544)	0.533	0.565 (0.472–0.658)	0.170	0.700(0.561–0.838)	0.004*

Table 5. Age-stratified analysis of AUCs for predicting clinical pregnancy in DOR patients. The area under the receiver operating characteristic curves (AUCs) were calculated to assess and compare the predictive performance of D3 top-quality cleavage-stage embryos and age as predictors of clinical pregnancy within three distinct age strata among DOR patients: <35, 35–40, and ≥ 40 years. DOR = diminished ovarian reserve, D3 = Day 3. *$P < 0.05$.

FSH. This finding may be attributed to the fact that low AFC reflects a decrease in both quantity and quality of oocytes and that AFC has low intracycle variability and high interobserver reliability in experienced centers. Even among DOR patients with an AFC as low as 3.5, ovarian stimulation can still be attempted to accumulate embryos.

In patients with DOR, Age and D3 cleavage-stage embryo predicted clinical pregnancy following fresh embryo transfer and viable blastocyst formation

AMH, AFC, and basal FSH have limited predictive reliability for clinical pregnancy[17,18]. Consistent with these findings, our study demonstrated that neither AMH, AFC, nor basal FSH independently predicted clinical pregnancy. Numerous studies have documented the impact of age on female fertility and IVF/ICSI outcomes[59,39]. Generally, ovarian function is negatively correlated with age, with female reproductive capacity

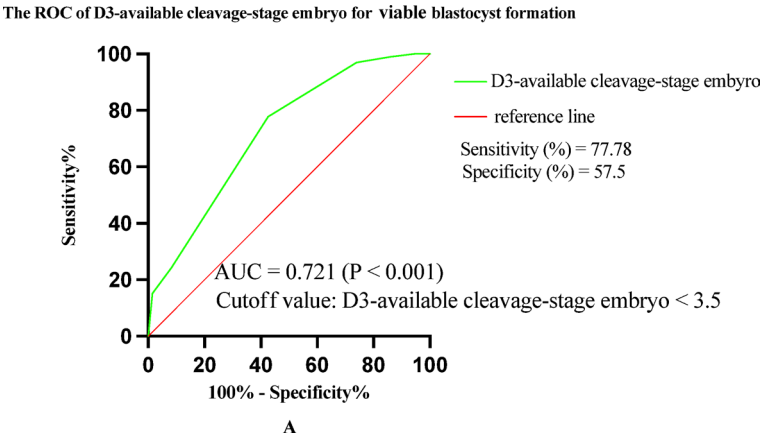

Fig. 5. Comparison of the predictive performance of D3-available cleavage-stage embryos for viable blastocyst formation among DOR patients undergoing blastocyst culture. (**A**). The ROC curve and the corresponding AUC of the predictive utility of D3 available cleavage-stage embryos for viable blastocyst formation. D3 = Day 3, DOR = diminished ovarian reserve, ROC = receiver operating characteristic, AUC = area under the ROC curve.

declining gradually starting from 30 years of age and more rapidly after 35 years of age[7]. As anticipated, our study identified age as a significant predictor of clinical pregnancy in IVF/ICSI fresh embryo transfer for patients with DOR, noting a marked decrease in the clinical pregnancy rates after the age of 35.5 years. Earlier research has found that young women with severe DOR who have high-quality embryos exhibit a comparable chance for pregnancy to those of the same age without DOR[60]. Our study also determined that D3 top-quality cleavage-stage embryos were significant predictors of clinical pregnancy in patients with DOR. Given the superior developmental potential of these top-quality embryos, patients with DOR may anticipate achieving pregnancy if they have at least one D3 top-quality cleavage-stage embryo. To mitigate the potential bias arising from an overrepresentation of younger patients with DOR in our dataset, we stratified our DOR patients into three distinct age groups, namely those aged < 35, 35–40, and ≥ 40 years, and then compared the predictive value of age and D3 top-quality cleavage-stage embryos for clinical pregnancy within each age stratum. Zhu S et al. reported that young women with DOR can still achieve favorable pregnancy outcomes once they have high-quality embryos available for transfer[61]. Similar findings emerged in our study, which showed that D3 top-quality cleavage-stage embryos were more reliable predictors of clinical pregnancy than was age in DOR patients under 40 years of age. This finding may be attributed to the limited decline in oocyte and embryo quality among younger patients with DOR, despite having retrieved fewer oocytes and embryos. Consequently, the acquisition of D3 top-quality cleavage-stage embryos suggests a favorable prognosis for clinical outcomes. However, our study found that top-quality embryos alone were not sufficient to achieve pregnancy following fresh embryo transfer, particularly for patients with DOR aged 40 years. The increasing predictive value of age for clinical pregnancy in patients with DOR may be attributed to the age-related decline in both the quantity and quality of embryos.

Blastocyst culture undergoes a developmental process involving cell fusion, formation, and expansion of the blastocoel, which selects embryos with higher developmental potential for implantation[24]. Extending cleavage-stage embryo culture to the blastocyst stage enhances synchrony between endometrial and embryo development, which increases the likelihood of achieving a live delivery when compared to cleavage-stage embryo transfer[62,63]. However, blastocyst culture is not suitable for all patients, particularly those with DOR, who may have fewer oocytes and viable embryos. Failure of a blastocyst formation by day 6 increases the risk of cycle cancelation, even with the presence of viable embryos on day 3. The decision to perform blastocyst culture in patients with DOR is critical, highlighting the importance of identifying factors influencing the formation of viable blastocysts to optimize treatment strategies. La Marca A et al. suggested that age was a significant determinant of IVF outcomes, including blastocyst formation[64]. In contrast, another study showed no statistical difference in blastocyst formation rates across different age groups[65]. Some studies have confirmed that high-quality embryos have a higher developmental potential to reach the blastocyst stage[66,27]. Interestingly, our study found that neither age nor D3 top-quality cleavage-stage embryos were determinants of viable blastocyst formation. Instead, the number of D3-available cleavage-stage embryos emerged as the only independent predictor of viable blastocyst formation. Although the effects of age and D3 top-quality cleavage-stage embryos on clinical pregnancy in patients with DOR are undeniable, these factors do not influence the formation of viable blastocysts. If none of the three to four D3-available cleavage-stage embryos are of top quality, culturing them to the blastocyst stage may improve clinical outcomes by yielding viable blastocysts.

Ultimately, age is the most important predictor of pregnancy outcomes. Patients with DOR who are over 35.5 years of age should promptly pursue ART interventions. Clinicians should consider age as the paramount factor when formulating clinical decisions for patients with DOR, particularly those aged 40 years or older. It is crucial that patients are thoroughly informed about their condition to prevent unnecessary financial, physical, and psychological strain. Nevertheless, patients with DOR under 40 years of age require rigorous assessment

of embryo quality before devising a strategy for transferring top-quality embryos to enhance the likelihood of clinical pregnancy. Regardless of whether top-quality embryos are not initially obtained in patients with DOR, performing blastocyst culture to identify those with developmental potential may optimally improve clinical outcomes provided that a sufficient number of D3-available cleavage-stage embryos are retrieved.

In the absence of established markers, the current study sought to identify predictors of IVF/ICSI outcomes by retrospectively analyzing 1,039 patients with DOR who underwent IVF/ICSI with fresh embryo transfer and blastocyst culture. The prediction models constructed from these factors demonstrated robust predictive value and clinical utility, offering a foundation for clinical prognostication and the development of personalized treatment plans. This approach may enhance the cost-effectiveness of healthcare and mitigate the adverse effects of prolonged, expensive procedures for patients with DOR. Several limitations of the current study need to be acknowledged. First, the retrospective design and single-center nature of this study preclude the adoption of prospective or interventional methodologies; therefore, our findings need to be validated through prospective, multicenter studies. Second, our study exclusively focused on fresh cleavage-stage embryo transfer on day 3 and did not include fresh blastocyst transfer on day 5. This limitation failed to stratify the analysis by embryo transfer day and overlooked the impact of the implantation potential of embryos at different developmental stages on the clinical pregnancy rate. Third, our data were exclusively applied to fresh embryo transfer cycles, necessitating further research to elucidate the impact of frozen–thawed embryo transfer on IVF/ICSI outcomes. We aim to address the aforementioned limitations in subsequent studies.

Data availability

The datasets utilized and/or analyzed in this study are available from the corresponding author upon reasonable request. The data are not publicly accessible due to privacy and ethical constraints.

Received: 12 June 2024; Accepted: 9 May 2025
Published online: 29 May 2025

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Author contributions

Yanru Zeng and Tian Tang made substantial contributions to the conception and design of the study, as well as the acquisition and analysis of data. Yanru Zeng also made significant contributions to the interpretation of data and the drafting of the manuscript. Zhan Dao, Yanru Zeng, and Qi Cao made contributions to the analysis of the data. Ying Zhang and Yanru Zeng contributed to the acquisition of data. Tian Tang and Qi Cao were involved in critically revising the manuscript fo significant intellectual content. All authors approved the final version of the manuscript. All authors have sufficiently participated in the work to assume public responsibility for appropriate sections of the content and have agreed to be accountable for all aspects of the work, ensuring that ant questions regarding the accuracy or integrity of the work are investigated and resolved appropriatedly.

Funding

This study was supported by the K-Fund Project of West China Second University Hospital (Grant No. KS402), the Horizontal Research Project of China Health Promotion Foundation (Grant No. 20H1089), and the Beijing Health Promotion Association (Grant No. 22H0819, 22H0686). The funding bodies had no involvement in the study design, data analysis, interpretation, or manuscript preparation.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Second University Hospital of Sichuan University (Approval No. 2023278). Written informed consent was obtained from all participants in this study, in compliance with national legislation and institutional requirements. The study was conducted in accordance with relevant guidelines and regulations.

Additional information

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