Predictive value of D-dimer in patients with unexplained recurrent implantation failure during freeze-thaw embryo transfer cycles

Xin Li
Peking University First Hospital
Cheng Zeng
Peking University First Hospital
Peili Wu
Peking University First Hospital
Jing Shang
Peking University First Hospital
Qing Xue (drxueqing@163.com)
Peking University First Hospital

Research Article

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Abstract

Background: This study aimed to evaluate whether D-dimer can predict the clinical outcomes of patients with unexplained recurrent implantation failure (URIF) during freeze-thaw embryo transfer (FET) cycles.

Methods: Our study was divided into two parts. The first part was a retrospective study that included 433 patients. Plasma D-dimer levels were monitored in all patients before FET, and the patients were classified into two groups according to whether they delivered at least one live infant or not. D-dimer was compared between groups, and receiver operating characteristic (ROC) curves were constructed to analyze the impact of D-dimer on live birth. The second part was a prospective study that included 113 patients who were categorized into high and low D-dimer groups based on the ROC curve analysis from the retrospective study. Clinical outcomes were compared between these two groups.

Results: In the first part, we found that plasma D-dimer levels in patients with live birth were significantly lower than those in patients without live birth. According to the ROC curve, 0.22 mg/L was the cutoff value for D-dimer in the prediction of the live birth rate (LBR) (AUC 0.806, 95% CI: 0.763, 0.848). The second part of the study confirmed that clinical pregnancy rate (50.98% vs. 32.26%, P=0.044) and LBR (41.18% vs. 22.58%, P=0.033) of patients with D-dimer ≤0.22 mg/L were all significantly higher than those of patients with D-dimer>0.22 mg/L.

Conclusions: Our study indicates that D-dimer >0.22 mg/L is a useful index for predicting URIF during FET cycles.

Background

Even after years of development, implantation failure in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI)–embryo transfer (ET) remains an impenetrable barrier to increasing successful pregnancy outcomes. In many cases, women who undergo repeated IVF/ICSI-ET attempts with good-quality embryos ultimately fail to become pregnant. As early as 1983, the term recurrent implantation failure (RIF) was used to describe embryo implantation failure following IVF/ICSI. To the best of our knowledge, there is still a lack of a specific standard that clearly defines RIF, which include the number of failed cycles, the type of transfer cycle (fresh embryo transfer cycle or freeze-thaw embryo transfer cycle) and the total number of transferred embryos that have not been successfully implanted [1–2]. In previous research, Polanski proposed two conditions that can be used to determine the occurrence of RIF: at least two fresh or freeze-thaw embryo transfer implantation failures and either at least 4 high-quality cleavage-stage embryos transferred or at least 2 blastocysts transferred [3].

Implantation is a complex physiological process involving the embryo and the endometrium that involves adhesion, nidation and invasion of the trophoblast within the endometrial tissues. Current studies have found that many factors can affect implantation failure. However, for most of these factors, the corresponding pathogenesis has not been established. It has been proven that natural prothrombotic events are caused by physiological changes during pregnancy [4]. The hemostatic balance in placental

vessels may be altered during pregnancy. These events may cause hypercoagulable states, which may in turn lead to inadequate fetomaternal circulation due to reduced placental perfusion [5]. Previous studies have suggested that some cases of recurrent pregnancy loss are a consequence of an exaggerated hemostatic response [6–8]. Although the association between thrombophilia and recurrent pregnancy loss is well known, there is limited research on the association between thrombophilia and RIF. Similar to recurrent miscarriage, thrombophilia plays an important role in RIF through various mechanisms, which has also drawn the attention of several researchers. During implantation, upregulated expression of tissue factors such as thrombin generation can promote human endometrial stromal cell hemostasis by protecting against bleeding due to endometrial capillaries being invaded by implanting cytotrophoblasts [9]. It has been hypothesized that the invasion of maternal vessels by syncytiotrophoblasts can be affected by localized thrombosis at the implantation site, leading to IVF failure [10]. Foad Azem et al. claimed that inherited thrombophilia had some impact on the etiology of RIF [11]. In their case-control study, they found that hereditary thrombophilia was more prevalent in patients with a history of RIF than in healthy women. In recent years, increasing evidence has implied that correlations exist between RIF and thrombophilia (both inherited and acquired) [12–13]. However, the exact effect of thrombophilia on URIF is still unclear.

Early detection of the coagulation cascade's progressive activation in thromboembolic diseases can enable management of the problem. D-dimer is currently considered a useful biochemical marker in thromboembolism [14]. It is a product of increases in fibrin degradation during acute thrombotic processes due to secondary activation of the fibrinolytic system. The level of plasma D-dimer may reflect the coagulation status in URIF patients.

In this study, we determined the association between plasma D-dimer levels and clinical outcomes in patients with URIF during FET cycles. Moreover, we aimed to evaluate whether plasma D-dimer levels can be used to predict the pregnancy outcomes of URIF patients in clinical practice.

Materials And Methods

Patients and Clinical Data

This research was conducted in a single IVF center at Peking University First Hospital from January 2017 to December 2020. The inclusion criteria of patients were as follows: age < 38 years, body mass index (BMI) < 28, history of failure for at least two implantation cycles (either at least 4 high-quality cleavage-stage embryos transferred or at least 2 blastocysts transferred [3]), and normal uterine cavity (assessed by hysteroscopy or hysterosalpingography). The exclusion criteria included the following: hormonal disorders, hydrosalpinx, a lack of high-quality cleavage stage embryos or high-quality blastocysts for transfer, positive autoantibodies (including antinuclear antibodies, anticardiolipin antibody, lupus anticoagulant, anti-β2 glycoprotein-1 IgG, and anti-β2 glycoprotein-1 IgM), inherited coagulation disorders (including mutations of factor V Leiden, prothrombin gene, methylenetetrahydrofolate reductase gene,

and antithrombin III, protein C, and protein S) and abnormal karyotyping. Couples were recruited for one FET cycle only.

The patient demographic characteristics collected included age, body mass index (BMI) (kg/m^2), infertility type, infertility duration, the number of previous failed embryo transfer cycles, endometrial thickness (mm) on the day of embryo transfer, the number of embryos transferred, the number of high-quality embryos transferred and the type of embryos transferred (cleavage or blastocyst embryos).

Clinical Management

The FET cycle protocol was based on patient characteristics. All patients underwent either a natural cycle or a substituted cycle.

(1) Natural cycle with luteal progestin (P) supplementation: This protocol was used on patients with regular menses (21–37 days). After spontaneous menses, a vaginal ultrasound examination was performed on the 10th–12th day of the cycle to detect the leading follicle. Based on the ultrasound examination, patients had their plasma levels of luteinizing hormone (LH), estradiol (E_2) and P checked. ET was scheduled between 3 and 6 days after ovulation upon ultrasound examination. Luteal phase support was commenced on the day of ET using dydrogesterone (Abbott Healthcare Products B.V., Netherlands) at a dose of 10 mg twice daily for 14 days. If the pregnancy test was positive, luteal phase support was continued until the 10th gestational week.

(2) This protocol was used in patients with irregular menses (> 37 days). Progynova (Delpharm Lille S.A.S., France) was orally administered on the 1st-5th day of the cycle at a dose of 2–3 mg, twic a day, to support endometrial proliferation and suppress follicle growth. After 14 days, a vaginal ultrasound examination was performed to confirm whether a dominant follicle had emerged and to measure the endometrial thickness. When the endometrial thickness reached more than 8 mm, 10 mg dydrogesterone (Abbott Biologicals B.V., Holland), twice daily, along with 90 mg vaginal micronized progesterone (Fleet Laboratories Ltd, US), once daily, was used, and ET was planned. If the endometrium remained at less than 8 mm, the FET cycle was canceled. The supplementation was continued until a pregnancy test was performed. In the case of a positive test, the patients were advised to maintain luteal support until the 10th gestational week.

From the day of FET, all patients were experimentally administered low molecular weight heparin (LMWH) (Nadroparin Calcium Injection, 4100 anti-Xa IU, Glaxo Wellcome Production, Britain) subcutaneously (SC) at a standard dose of 0.4 mL per day. The patients self-administered the LMWH. If the pregnancy test 12 to 14 days after ET was positive, LMWH was continued until the 10th week of pregnancy. Otherwise, it was stopped.

Laboratory Tests

The plasma D-dimer and other coagulation indictors of the patients on the day of embryo transferred were monitored. The levels of D-dimer and other coagulation indictors were detected using an ACL-Advance automatic coagulation analyzer (ACL-TOP-700, Werfen Inc, Spain). Total platelet count in blood samples was measured using a Sysmex XN-9100 Hematology Analyzer (Sysmex, Kobe, Japan)

Study Period And Groups

The study was divided into two parts. First, enrolled patients were collected from January 2017 to January 2020 and divided into two groups according to clinical pregnancy outcomes. Patients with at least one live birth (LB) were classified as LB (+), while the others were classified as LB (-). We compared clinical data, especially plasma D-dimer, between these groups. Additionally, the simultaneous relationship of multiple prognostic factors for pregnancy outcome was assessed using binary logistic regression analysis. Moreover, we adopted receiver operating characteristic (ROC) curve analysis to determine the cutoff value of plasma D-dimer for the live birth rate (LBR).

In the second part, we prospectively included patients from January 2020 to December 2020. We used the cutoff value of plasma D-dimer from the retrospective study to classify the included patients into two groups, i.e., a low D-dimer group and a high D-dimer group, and compared their clinical outcomes.

Definition Of Outcomes

The LBR and clinical pregnancy rate (CPR) were the major outcomes of this study. A clinical pregnancy is defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs. The CPR is defined as the percentage of clinical pregnancies occurring in every 100 ET cycles. The LBR is defined as the percentage of deliveries that resulted in at least one live birth, expressed per 100 ET cycles. The following clinical outcome was also included: implantation rate, which was defined as the number of gestational sacs detected divided by the number of embryos transferred.

Statistical Analysis

All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL). The significance of differences in proportions was tested using chi-square and Fisher's exact statistics. Differences in continuous variables were analyzed using Student's t-test and the Mann-Whitney U test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Binary logistic regression analysis was used to assess whether the pregnancy outcomes were affected by maternal age, BMI (kg/m^2), infertility type (primary infertility or secondary infertility), endometrial thickness, the number of embryos transferred, and the type of embryos transferred (cleavage stage embryo or blastocyst). An ROC curve was drawn to obtain the plasma D-dimer threshold, and the area

under the curve (AUC) was calculated. Values of $P < 0.05$ were considered statistically significant unless otherwise noted.

Results

Outcomes of Patients in the Retrospective Study

Figure 1 summarizes the selection of study participants. A total of 433 patients underwent FET cycles during the retrospective study period. The average level of plasma D-dimer was 0.23 (0.13, 0.45) mg/L. Of these patients, 154 women had live births, and the others did not. Table 1 compares the demographic characteristics and cycle parameters of patients in the LB (+) group and the LB (-) group. Overall, there were no differences between the two groups in terms of age, BMI, infertility duration, infertility type, the number of previous failed embryo transfer cycles, clinical management, endometrial thickness, the number of embryos transferred, the number of high-quality embryos transferred or the type of embryos transferred (Table 1). The levels of activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), platelet aggregation in response to adenosine diphosphate (ADP) and arachidonic acid (Ara) were not significantly different between the LB (+) group and LB (-) group. However, the level of D-dimer (0.13[0.09,0.20] mg/L vs. 0.34[0.21,0.50] mg/L, $P \leq 0.001$), antithrombin III (AT-III) (104[98,109] % vs. 101[95,108] %, $P = 0.014$) and platelet (PLT) counts (256[215,286] $\times 10^3$ /ml vs. 265[240,290] $\times 10^3$ /ml, $P = 0.011$) in the LB (-) group was significantly higher than that in the LB (+) group, and the difference was statistically significant.

Table 1
Clinical parameters and coagulation markers of LB (+) group and LB (-) group

	LB(+)	LB(-)	Test value	P value
N	154	279		
maternal age (year)	33(30,35)	33(30,36)	z =-0.187	P = 0.852
BMI (kg/m^2)	22.43(20.56,25.10)	23.04(21.00,25.39)	z =-1.358	P = 0.174
primary infertility	73.38% (113/154)	75.63% (211/279)	χ^2 = 0.267	P = 0.605
infertility duration (year)	3(2,5)	4(2,6)	z =-0.662	P = 0.508
the number of previous failed embryos transfer cycles	3 (2,3)	3 (2,3)	z =-0.678	P = 0.498
endometrial thickness (mm)	9.30(8.35,11.00)	9.00(8.00,11.00)	z =-0.338	P = 0.735
natural cycles	60.39% (93/154)	58.78% (164/279)	χ^2 = 0.106	P = 0.744
the number of embryos transfer	1.89 ± 0.48	1.91 ± 0.47	t = 0.504	P = 0.614
the type of embryos transferred (cleavage stage embryos)	66.23% (102/154)	65.23% (182/279)	χ^2 = 0.026	P = 0.872
the number of high-quality embryos transferred	1.05 ± 0.66	1.04 ± 0.63	t =-0.246	P = 0.806
APTT (s)	31.50(29.3,33.05)	30.90(29.30,33.30)	z =-0.816	P = 0.414
PT (s)	11.30(10.80,11.90)	11.30(10.70,11.90)	z =-0.537	P = 0.591
TT (s)	14.10(13.20,14.90)	14.10(13.10,15.00)	z =-0.110	P = 0.912
FIB (g/L)	2.92(2.58,3,34)	2.96(2.58,3.39)	z =-0.572	P = 0.568

Abbreviations: LB, live birth; BMI, body mass index; APTT, thromboplastin time; PT, prothrombin time; TT, thrombin time; FIB, fibrinogen; AT-III, antithrombin III; ADP, platelet aggregation in response to adenosine diphosphate; Ara, platelet aggregation in response to arachidonic acid; PLT, platelet. All data was recorded as mean ± standard, median (1st quartile-3rd quartile), or coefficient of variation (%). The significance of differences in proportions was tested using chi-square statistics. Differences in continuous variables were analyzed using Student's t-test and the Mann-Whitney U test.

	LB(+)	LB(-)	Test value	P value
D-dimer (mg/L)	0.13(0.09,0.20)	0.34(0.21,0.50)	z =-10.538	P ≤ 0.001
AT-III (%)	104(98,109)	101(95,108)	z =-2.466	P = 0.014
ADP (%)	67.17(55.41,72.96)	67.00(54.60,72.21)	z =-0.999	P = 0.318
Ara (%)	74.27(66.51,79.26)	73.43(65.15,78.13)	z =-1.057	P = 0.290
PLT counts ($\times 10^3$/ml)	256(215,286)	265(240,290)	z =-2.552	P = 0.011

Abbreviations: LB, live birth; BMI, body mass index; APTT, thromboplastin time; PT, prothrombin time; TT, thrombin time; FIB, fibrinogen; AT-III, antithrombin III; ADP, platelet aggregation in response to adenosine diphosphate; Ara, platelet aggregation in response to arachidonic acid; PLT, platelet. All data was recorded as mean ± standard, median (1st quartile-3rd quartile), or coefficient of variation (%). The significance of differences in proportions was tested using chi-square statistics. Differences in continuous variables were analyzed using Student's t-test and the Mann-Whitney U test.

To determine whether the level of pre-embryo implantation plasma D-dimer or other coagulation markers could predict pregnancy outcomes in women with URIF, a binary logistic regression analysis was performed. In the logistic regression analysis, all potential prognostic factors were included. The levels of APTT, PT, TT, FIB, ADP and Ara were excluded from this analysis owing to stepwise screening. Table 2 shows the considered clinical variables, including maternal age, BMI, infertility type, endometrial thickness, the number of embryos transfers, the type of embryos transferred, the level of AT-III and platelet counts, none of which had a significant association with the LBR (OR: 1.011, 95%, CI: 0.989–1.035, P = 0.327; OR: 0.996, 95%, CI: 0.991–1.001, P = 0.083). The baseline D-dimer level was associated with the LBR (OR: 0.824, 95%, CI: 0.652–0.995, $P \leq 0.001$), as is shown in Table 2.

Table 2
Binary logistic regression analysis to account for confounding variables in the prediction of live birth rate

	B	S.E.	Wald	Df	*P* value	OR	95% CI for OR	
							Lower limit	Upper limit
maternal age	-0.021	0.029	0.524	1	0.469	0.979	0.924	1.037
BMI	-0.036	0.032	1.292	1	0.256	0.965	0.907	1.026
infertility type	-0.050	0.272	0.034	1	0.853	0.951	0.558	1.620
endometrial thickness	0.005	0.061	0.006	1	0.938	1.005	0.891	1.133
the number of embryos transferred	0.046	0.277	0.028	1	0.867	1.047	0.609	1.803
the type of embryos transferred	0.104	0.271	0.148	1	0.701	1.110	0.653	1.887
D-Dimer	-0.006	0.001	55.063	1	0.000	0.824	0.652	0.995
AT-III	0.011	0.012	0.961	1	0.327	1.011	0.989	1.035
PLT counts	-0.004	0.003	3.008	1	0.083	0.996	0.991	1.001

CI, Confidence interval; OR, Odds ratio; BMI, body mass index; AT-III, antithrombin III; PLT, platelet.

To determine the normal plasma D-dimer concentration threshold to apply in our trial, we used ROC curve analysis to analyze all included patients, as is shown in Fig. 2. The AUC of D-dimer vs. the LBR was 0.806, 95% CI: 0.763–0.848. The diagnostic threshold was 0.22 mg/L, and the sensitivity and specificity of D-dimer were 71.3% and 81.2%, respectively. We defined a plasma D-dimer level of more than 0.22 mg/L as abnormal.

Outcomes Of Patients In The Prospective Study

113 patients underwent freeze-thaw cycles during the prospective study period. Among these patients, no one was lost to follow up. We analyzed the characteristics and pregnancy outcomes of these patients and categorized them into low and high D-dimer groups (> 0.22 mg/L and ≤ 0.22 mg/L, respectively) based on the ROC curve results from the retrospective study. Table 3 compares the characteristics and cycle parameters of the patients in the two groups. Within the two groups, no significant differences appeared in age, BMI, infertility type, infertility duration, the number of previous failed embryo transfer cycles, clinical management, endometrial thickness, the number of embryos transferred, the type of embryo transferred or the number of high-quality embryos transferred. The rate of miscarriage, ectopic pregnancy and the implantation were similar between 2 groups. But CPR (50.98% vs. 32.26%, P = 0.044) and LBR (41.18% vs. 22.58%, P = 0.033) were all significantly higher in the low D-dimer group than in the high D-dimer group.

Table 3
Clinical parameters and outcomes of lower D-dimer group (Group 1, the level of plasma D-dimer ≤ 0.22 mg/L) and higher D-dimer group (Group 2, the level of plasma D-dimer > 0.22 mg/L)

	Group1	Group2	Test value	P value
N	51	62		
maternal age (year)	33 (30,35)	31.5 (29,35)	z =-1.141	P = 0.254
BMI (kg/m^2)	23.44 (21.30,25.50)	23.20 (21.48,25.34)	z =-0.061	P = 0.952
primary infertility	72.54% (37/51)	77.42% (48/62)	χ^2 = 0.356	P = 0.551
infertility duration (year)	4 (3,7)	3 (2,5)	z =-1.367	P = 0.172
the number of previous failed embryo transfer cycles	3 (2,3)	3(2,3)	z =-0.572	P = 0.567
natural cycles	60.78% (31/51)	66.13% (41/62)	χ^2 = 0.346	P = 0.557
endometrial thickness (mm)	9.00 (8.00,11.00)	9.00(8.00,11.00)	z =-0.470	P = 0.638
the number of embryos transferred	1.82 ± 0.47	1.94 ± 0.47	t =-1.244	P = 0.216
the type of embryos transferred (cleavage stage embryos)	60.78% (31/51)	69.35% (43/62)	χ^2 = 0.909	P = 0.340
the number of high-quality embryos transferred	1.14 ± 0.63	1.05 ± 0.64	t = 0.739	P = 0.461
implantation rate (%)	31.18% (29/93)	21.67% (26/120)	χ^2 = 2.477	P = 0.116
miscarriage rate (%)	19.23% (5/26)	20% (4/20)		P = 1.000
ectopic pregnancy rate (%)	0.00% (0/26)	10.00% (2/20)		P = 0.184
clinical pregnancy rate (%)	50.98% (26/51)	32.26% (20/62)	χ^2 = 4.064	P = 0.044

BMI, body mass index. All data was recorded as mean ± standard, median (1st quartile-3rd quartile), or coefficient of variation (%). The significance of differences in proportions was tested using chi-square and Fisher's exact statistics. Differences in continuous variables were analyzed using Student's t-test and the Mann-Whitney U test.

	Group1	Group2	Test value	P value
live birth rate (%)	41.18% (21/51)	22.58% (14/62)	χ^2 = 4.526	P = 0.033

BMI, body mass index. All data was recorded as mean ± standard, median (1st quartile-3rd quartile), or coefficient of variation (%). The significance of differences in proportions was tested using chi-square and Fisher's exact statistics. Differences in continuous variables were analyzed using Student's t-test and the Mann-Whitney U test.

Discussion

Embryo implantation is a complex physiological process, which requires a number of complex events at the microvascular level, includes location, adhesion and invasion. Any failed steps could lead to implantation failure. In clinical work, many couples transfer good-quality embryos cycle by cycle but fail to achieve clinical pregnancy. The majority of these couples do not find any positive result in the routine examination, a condition called URIF. URIF not only causes physical and mental harm to patients but is also considered an important cause of infertility.

Interest in thrombophilia in RIF patients is largely influenced by the clinical association between alteration of hemostasis and a trend toward a hypercoagulable state and recurrent pregnancy loss. In recent years, several researchers have found that thrombophilic single nucleotide polymorphisms and acquired thrombophilia were more prevalent in the IVF failure group [12–13, 15]. A possible mechanism for URIF is thrombosis of the maternal vessels, especially the decidual or chorionic vessels, which can reduce perfusion of the intervillous space, preventing embryo implantation [16].

The prethrombotic state is a pathological state involving hemostatic, coagulation, anticoagulation or fibrinolytic dysfunction that could be caused by multiple factors. Although there are different mechanisms involved in the prethrombotic state, hypercoagulation and low fibrinolysis are the final stages. AT-III is an important physiological anticoagulant in plasma, which can directly bind to thrombin to achieve anticoagulant effects. The low level of AT-III may cause the tendency to form abnormal blood clots. As AT-III, the count of platelets is also supposed to have a role in the pathophysiology of thromboembolic disorders. Elevated level of platelets is prone to cause thrombosis. In our study, patients without live births had lower level of AT-III and higher count of platelets than patients with at least one infant in univariate analysis, which indicated patients without live births may have coagulation disorder. However, the statistic differences were not significant in multivariate analysis.

D-dimer is a degradation product of a cross-linked fibrin blood clot that increases during acute thrombotic processes due to secondary activation of the fibrinolytic system. It is an important reference marker for comprehending the hypercoagulable state. Normally, the level of D-dimer is low in human blood plasma. As the coagulation system activates, such as in the presence of thrombosis or disseminated intravascular coagulation, the level of D-dimer in plasma increases. A positive result for a D-dimer test

could contribute to the diagnosis of women with arterial and/or venous thrombosis. D-dimer levels may increase physiologically during pregnancy. The level of D-dimer in the first trimester of the pregnancy process (0.169–1.202 mg/L) was obviously lower than that in the second (0.393–3.258 mg/L) and third (0.551–3.333 mg/L) trimesters and in nonpregnant adults (< 0.5 mg/L) [17]. However, the level of plasma D-dimer in URIF patients is unknown. Our study found that the average D-dimer level in URIF patients was 0.23 (0.13, 0.45) mg/L in the day of embryo transferred.

In research on recurrent pregnancy loss, Wang T et al. found that D-dimer is a useful predictor of unexplained recurrent pregnancy loss [18]. Wang P et al. also found that the concentration of D-dimer can not only distinguish patients with recurrent pregnancy loss from those with normal fertility but also more accurately indicates recurrent spontaneous abortion prognosis [19]. However, only a few studies on female per-implantation blood coagulation status have elucidated the relationship between coagulation status and URIF [11–13, 15]. In this retrospective study, we explored the level of D-dimer before patients received FET cycles and compared the clinical data of patients with live birth and patients without live birth. We found that a higher level of plasma D-dimer existed in patients with worse pregnancy outcomes. The imbalance of hemostasis, coagulation, and anticoagulation processes comprise a pathological process that causes RIF. To determine whether the level of pre-embryo implantation plasma D-dimer could predict the pregnancy outcomes of women with RIF, we performed binary logistic regression analysis. After controlling for maternal age and other possible influential clinical variables, we found that the baseline D-dimer level before patients received embryo transfer was the most effective predictor of the CPR and LBR. With ROC analysis, we found the diagnostic threshold of basal D-dimer level to be 0.22 mg/L. In the prospective part of the present study, we verified this result. The implantation rate, CPR and LBR were higher in URIF patients with plasma D-dimer ≤ 0.22 mg/L than in those with plasma D-dimer > 0.22 mg/L when receiving prophylactic LMWH treatment during FET cycles. Plasma D-dimer levels assessed before FET could accurately predict pregnancy outcomes.

In recent years, researchers have not only focused on the cause of RIF but also sought to understand the potential therapeutic role of antithrombotic treatments in improving IVF-ET outcomes. A number of antithrombotic therapeutic interventions, such as LMWH, have been used to improve the success of IVF-ET [20]. Fertility clinics consider LMWH to be beneficial for placental circulation and alter uterine artery blood that may useful for obtaining a higher pregnancy rate [21–23]. However, the effect of LMWH as an adjunct to IVF treatment has been evaluated in several clinical trials that show conflicting results. Several studies have suggested that LMWH has beneficial effects in women with URIF, while other studies have shown no evidence of benefits [24–25]. Urman et al. performed an open-label randomized controlled trial (RCT) for women who had ≥ 2 implantation failures and were without coagulation disorders [26]. They empirically administered enoxaparin sodium 1 mg/kg/day SC to patients (n = 75) from the day after oocyte retrieval to the 12th gestational week to pregnant participants. The LBR, CPR and implantation rate of the LMWH group (34.7%, 45.3% and 24.5%) were higher than those of the control group (26.7%, 38.7% and 19.8%), in which patients were not treated. However, no statistically significant differences were observed between the two groups. The quasi-randomized study performed by Berker on women with URIF had similar results [27]. Compared with patients without enoxaparin treatment, patients who were

administered enoxaparin sodium 4000 IU (40 mg)/0.4 ml per day from the day of oocyte retrieval had increased CPRs, LBRs and implantation rates. However, there was also no statistically significant difference between them. The reason why previous studies did not demonstrate the therapeutic effect of LMWH in URIF patients may be due to the study design, small sample size, or heterogeneity among URIF patients. If there is no evidence of coagulation abnormalities or thrombosis, LMWH may serve no purpose.

Our data showed that the basal plasma D-dimer level was a strong predictor of pregnancy results in URIF patients receiving FET. The implantation rate, CPR and LBR were higher in URIF patients with plasma D-dimer ≤ 0.22mg/L than in those with plasma D-dimer > 0.22 mg/L when receiving empirical prophylactic LMWH treatment during FET cycles. The results indicated that URIF patients may have different coagulation statuses. D-dimer plasma levels could become helpful in monitoring the LMWH effects in those women who show a reduction in D-dimer plasma levels may be those who benefit the most from LMWH administration. Women with plasma D-dimer 0.22 mg/L have a low risk of implantation failure and can stop experimental anticoagulation treatment. The standard dose of LMWH to patients with elevated D-dimer may not be enough, and they may require individualized treatment, such as a higher dose of LMWH.

Our studies were limited by the small sample size and the retrospective nature of the first part of the study. In addition, our results did not take consider uterine receptivity [28] and other clinical parameters, such as thromboelastographic parameters, into account. A larger prospective study is needed to validate the results.

Conclusions

In conclusion, our results suggested that URIF patients with plasma D-dimer levels lower than 0.22 mg/L had a higher CPR and LBR in FET cycles. Plasma D-dimer levels may be used as predictors of clinical outcomes in URIF patients undergoing FET cycles and may guide appropriate anticoagulant treatment.

Abbreviations

URIF, unexplained recurrent implantation failure; FET, freeze-thaw embryo transfer; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; ET, embryo transfer; LB, live birth; LBR, live birth rate; CPR, clinical pregnancy rate; ROC, receiver operating characteristic; ORs, odds ratios; CIs, confidence intervals; AUC, area under the curve; LMWH, low molecular weight heparin; BMI, body mass index; APTT, thromboplastin time; PT, prothrombin time; TT, thrombin time; FIB, fibrinogen; AT-III, antithrombin III; ADP, platelet aggregation in response to adenosine diphosphate; Ara, platelet aggregation in response to arachidonic acid; PLT, platelet; APS, antiphospholipid syndrome.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking University First Hospital, and was carried out in accordance with the guidelines of ethical management. Due to the retrospective design, informed consent was not necessary, which is in accordance with the Ethics Committee of Peking University First Hospital.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

Methodology, Xin Li and Qing Xue; validation, Xin Li and Peili Wu; formal analysis, investigation and data curation, Xin Li, Peili Wu and Cheng Zeng; writing–original draft preparation, Xin Li, Jing Shang and Qing Xue; writing–review and editing, Xin Li and Qing Xue; supervision, Jing Shang and Qing Xue. All authors reviewed the manuscript. The authors read and approved the final manuscript.

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Not applicable.

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Figures

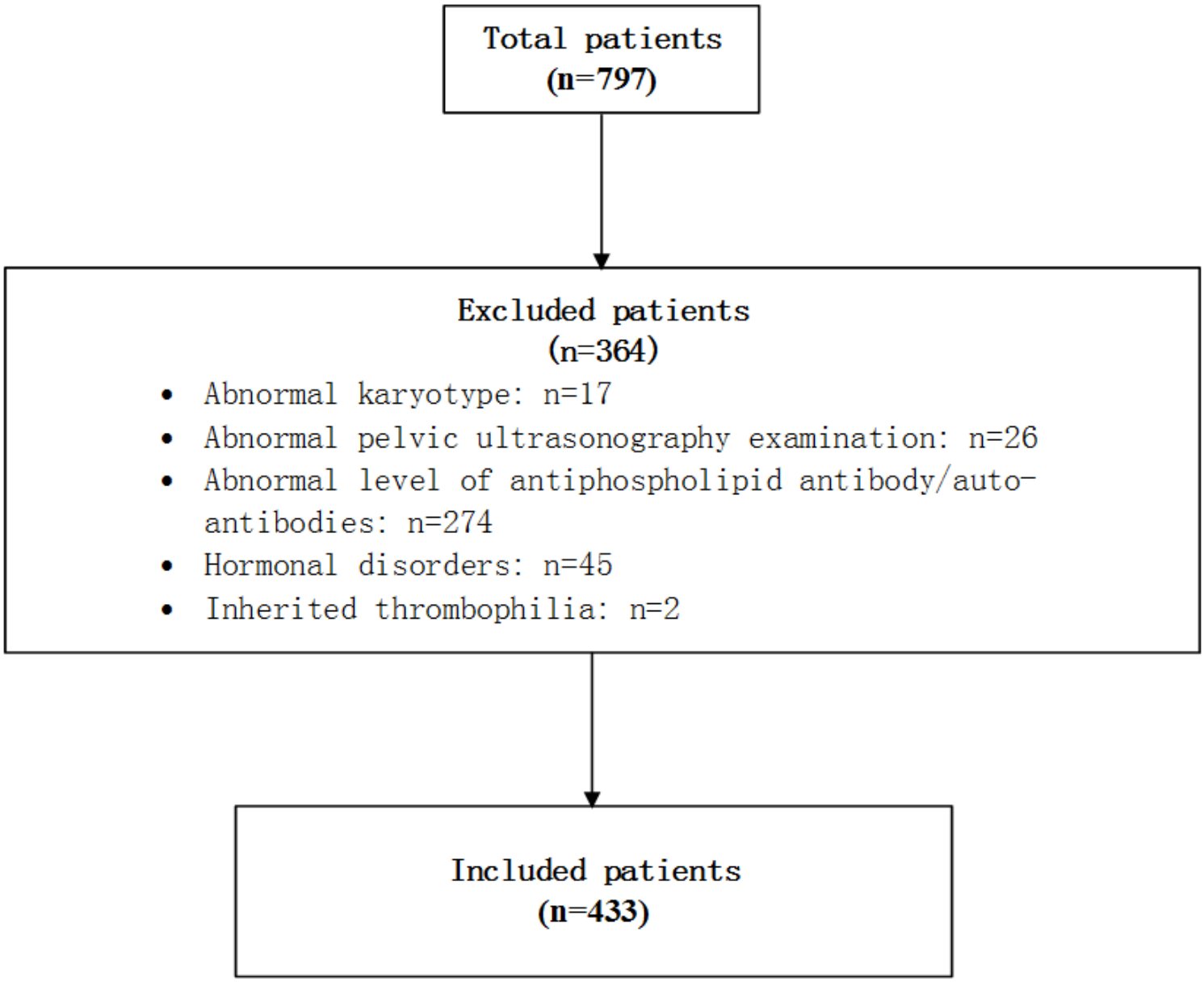

Figure 1

Overview of the inclusion and exclusion criteria in retrospective part.

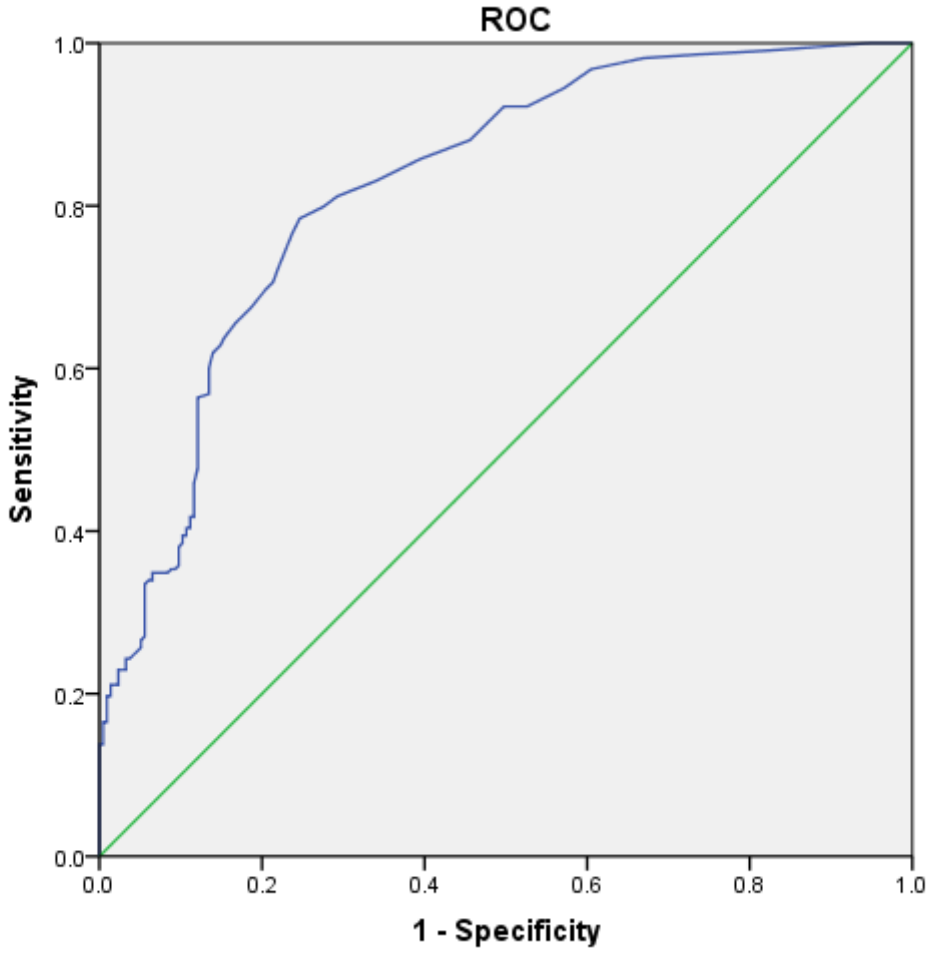

Figure 2

ROC curve analysis of D-dimer and clinical pregnancy rate in unexplained recurrent implantation failure (URIF) patients.

Receiver operating characteristic (ROC) curve evaluates the ability of plasma D-dimer to predict clinical pregnancy rate. The level of basial plasma D-dimer > 0.22 mg/L is associated with low clinical pregnancy rate, with a sensitivity of 71.3%, a specificity of 81.2%, and an area under the curve of 0.806.