BMC Pregnancy and Childbirth

RESEARCH

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Timing of diagnostic workups in Chinese population with recurrent pregnancy loss: a cross-sectional study

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Abstract

Background There are no specific guidelines regarding the definition, diagnostic workup and treatment of recurrent pregnancy loss (RPL) in China at present. Whether the diagnostic workup should occur after two or three or more pregnancy losses in the Chinese population is not clear.

Methods This cross-sectional study collected data from January 2017 to December 2022 from the RPL Clinic at Shengjing Hospital, affiliated with China Medical University. The results of diagnostic tests for evidence-based and possible risk factors of RPL,which is defined as two or more failed clinical pregnancies, were collected. The data collected include parental chromosomal karyotypes, immune factors (anticardiolipin antibody, anti-β2-glycoprotein I antibody, lupus anticoagulants, and antinuclear antibodies), endocrine factors (polycystic ovary syndrome, thyroid dysfunction, hyperprolactinemia, obesity, and glucose abnormalities), anatomical factors (uterine malformations, endometrial polyps, intrauterine adhesions, uterine fibroids or adenomyosis), coagulation factors (thrombelastogram, antithrombin III, and homocysteine levels) and other factors (vitamin D levels, MTHFR polymorphisms and ultrasound indices of endometrial receptivity). All these data were compared between patients with two or three or more pregnancy losses.

Results Among all 785 patients with RPL, the rates of abnormal anatomical factors (40.96% versus 32.94%, $P=0.021$, OR 1.41, (95% CI 1.05–1.89)), endometrial polyps (6.21% versus 3.06%, $P=0.034$, OR 2.10, (95% CI 1.04–4.23)) and obesity (13.76% versus 5.59%, $P<0.0001$, OR 2.69, (95% CI 1.62–4.49)) were significantly higher in people with three or more pregnancy losses than in people with two pregnancy losses. The rates of other diagnostic tests were not statistically significant between the two groups.

Conclusion Based on the high rate of abnormal test results in the Chinese RPL population, our findings may provide evidence for patients in our area begin routine etiological screening after two pregnancy losses.

Trial registration ClinicalTrials.gov Identifier: NCT03561766, 18/5/2018.

Keywords Recurrent pregnancy loss, Diagnostic workup, Obesity, Anatomical factors, Endometrial polyps

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Introduction

With a prevalence ranging from 1 to 2% worldwide, recurrent pregnancy loss (RPL) is devastating for women who desire to have children [1]. For patients with RPL, while the risk of many obstetric complications is increased, the negative emotions and mental harm caused by pregnancy loss should not be underestimated [2]. However, because of the differences in regional medical levels and populations, the definition of RPL varies among different institutions. The European Society of Human Reproduction and Embryology (ESHRE) defines RPL as pregnancy loss that occurs two or more times before 24 weeks of pregnancy [1]. The American Society for Reproductive Medicine (ASRM) defines RPL as the loss of pregnancy determined by ultrasound or histology after two or more pregnancies without the need for continuity. The Royal College of Obstetricians and Gynaecologists (RCOG) defines RPL as the loss of three or more consecutive pregnancies [3]. In China, there are no specific guidelines regarding the definition, diagnostic workup and treatment of RPL at present. A currently released expert consensus recommends the diagnostic workup should take place after two pregnancy losses based on a meta-analysis in which most studies only included non-Chinese patients [4].

In addition to the heterogeneity of definitions affecting people's research on RPL, the diversity and complexity of its risk factors have also increased the difficulty for scholars to conduct investigations. Well-established risk factors include parental chromosomal abnormalities, anatomical abnormalities, endocrine disorders (thyroid dysfunction, glucose abnormality and obesity), and acquired thrombophilia [5]. Possible risk factors include endocrine disorders (hyperprolactinemia, Polycystic ovary syndrome), inherited thrombophilia, psychological factors, and environmental and nutritional factors [6]. These factors have been investigated by many investigators, but most research has focused on one or several abnormalities, which often fail to explain the comprehensive cause of a patient's pregnancy loss. Given the heterogeneity of definitions, the lack of evidence in China and the incompleteness of previous studies, a comprehensive etiological study is required to investigate the proportions and roles of a variety of confirmed or possible risk factors of RPL. The aim of the present study was therefore to investigate the rates of abnormal diagnostic test results in the Chinese RPL population, describing and comparing the rates in the populations with 2 and 3 or more pregnancy losses, thus demonstrating the prevalence of various abnormalities in patients with RPL and providing evidence for determining the timing of screening.

Materials and methods

Inclusion criteria

The China Medical University Birth Cohort is an ongoing prospective cohort study that includes a subcohort of patients with RPL specifically enrolled in the Recurrent Pregnancy Loss Clinic. This cross-sectional study collected data from January 2017 to December 2022 from the Recurrent Pregnancy Loss Clinic at Shengjing Hospital, affiliated with China Medical University. The inclusion criteria were as follows: 1) Patients diagnosed with RPL who completed a detailed history collection form for recurrent pregnancy loss; 2) Patients with complete and prepregnancy screenings for recurrent pregnancy loss conducted in this hospital; and 3) Patients who did not take medicines before screening that could have affected their test results (including traditional Chinese medicine). All enrolled patients were included in this study right after their first consultation in the RPL clinic. Patients were asked to undergo tests related to recurrent pregnancy loss at least three months after their last pregnancy loss. To avoid laboratory errors caused by the use of different test kits, the results of patient examinations (except for chromosome karyotype and hysteroscopy results) conducted in outside hospitals were not recorded. Patients who showed abnormalities at the time of the first test needed at least a second test to confirm the diagnosis. Sampling method used in the study was simple random sampling, which means every member of the population has the same probability of being randomly selected into the sample. This study was reviewed and approved by the ethics committee of China Medical University. All patients signed an informed consent to be screened for the etiology of RPL.

In this study, pregnancy was defined as urine/blood β-hCG positivity or ultrasound-confirmed pregnancy sacs. Pregnancy loss was defined as any spontaneous pregnancy loss or fetal weight $\leq$ 500 g before 20 weeks gestation. Patients with molar pregnancy, ectopic pregnancy and pregnancy terminations were excluded from the analysis. Implantation failure, which is defined as a lack of implantation after the embryo transfer, were also excluded.

Sample calculation

The sample size of this study was calculated using an online sample calculation site (http://riskcalc.org:3838/samplesize/). The collection of data from 100 patients in the preliminary stage allowed us to roughly determine the proportion of various abnormalities in the two populations (patients with two and three or more pregnancy

losses). The type I error rate set in this study was 0.05, the degree of certainty of the study was 0.9, and the approximate ratio of the sample size was 4:3 among people with two and three or more pregnancy losses. The least common abnormality was an abnormal AT-III level, which occurred in approximately 3% of the 100 patients. Because of the weak correlation in observational studies, the relative risk ratio was set at 3 [7]. After calculation, the minimum sample size for this study was 745, which suggested that at least 426 and 319 patients be included in the experimental and control groups, respectively.

Data collection

At the first visit to the clinics, all patients included in the study were asked to complete questionnaires about their basic personal information (including age, height, weight, cell phone number, etc.) and maternal history (including the number of pregnancy losses, the possible cause of the pregnancy losses, and the presence of ultrasound images confirming the occurrence of early intrauterine pregnancy). The information collected in the questionnaire was further verified by clinicians through consultation. The test results collected in this study can be divided into six sections: parental chromosomal karyotypes, immune factors, endocrine factors, anatomical factors, coagulation factors and other factors. If a patient had thyroid dysfunction, she was also identified as having endocrine dysfunction. The abnormal rates of parental chromosomal karyotypes and immune, endocrine, anatomical and coagulation factors were calculated according to this logic. All data were collected from the hospital's electronic medical records system.

Parental chromosomal karyotypes

We determined the chromosomal karyotypes for both parents, and all abnormal karyotypes were analyzed by clinical geneticists to determine whether they were associated with pregnancy loss. The results were considered "normal" if both parents had no abnormalities in their chromosomes or if one parent had a normal karyotype and the other had missing information. If at least one parent had a karyotype abnormality, the results were considered "abnormal."

Immune factors

Information was collected on immune factors, including anticardiolipin antibodies (aCL antibodies), anti-β2-glycoprotein I antibodies (abeta2-GPI antibodies), lupus anticoagulants, and antinuclear antibodies (anti-ANA antibodies). All patients with a single positive test result were asked to undergo repeat tests at least 12 weeks apart. Enzyme-linked immunoassay (ELISA) was used for the detection of antibodies against aCL and abeta2-GPI antibodies. The 95th percentile and 99th percentile normal assay values were shown in Supplementary Table 1. Lupus anticoagulants were tested using the dilute Russell viper venom test and PTT-LA. Quantification of LAC activity using PTT-LA reagents was arbitrarily determined based on the 1:1 clotting time ratio between the tested sample and healthy combined plasma and the healthy combined plasma alone. Patients with a coagulation time ratio greater than 1.2 were considered to have an abnormality. Anti-ANA antibodies were detected by indirect immunofluorescence using the Fluro-Hepana assay (Aesku, Wendelsheim, Germany). The positive cutoff for ANA antibodies in this study was 1:80. Antinuclear antibody assays were performed using an immunoblotting assay with detectable antibodies.

Endocrine factors

Information on endocrine abnormalities, including polycystic ovary syndrome (PCOS), thyroid dysfunction, hyperprolactinemia, obesity, and glucose abnormalities, was collected. The diagnostic criteria for PCOS were based on the revised Rotterdam diagnosis. Thyroid dysfunction was categorized as abnormal thyroid autoimmune antibodies alone, elevated or reduced TSH levels alone, and abnormal TSH levels in combination with abnormal levels of autoimmune antibodies. Diagnostics of TSH abnormalities, hyperprolactinemia and glucose abnormalities were shown in Supplementary Table 1. Obesity was diagnosed based on the World Health Organization's (WHO) criteria for the diagnosis of obesity in China. The screening time for hormones was days 1–3 of menstruation.

Anatomical factors

Information on anatomical abnormalities, including uterine malformations, endometrial polyps, Asherman's syndrome, uterine fibroids or adenomyosis, was collected. Three-dimensional ultrasound of the uterus and hysteroscopy were used to establish diagnoses. In this study, patients with uterine fibroids were included only if they were diagnosed with submucosal or intramural myoma.

Coagulation factors

Information collected for coagulation factors included thromboelastogram (TEG), antithrombin III, and homocysteine levels. AT-III (normal: 83–128) levels were measured by ELISA using the Sysmex CA1500 automated coagulation analyzer (Sysmex Corporation, Japan). TEG parameters were obtained by using a TEG analyzer (Hemoscope, TEG 50000). The TEG results were ultimately summarized into three types of abnormalities: platelet aggregation, coagulation factor and fibrinolysis

abnormalities. Abnormal results were recorded if the fasting homocysteine level was greater than 15 mmol/L.

Other factors

Other factors collected in this study related to RPL included vitamin D levels, MTHFR polymorphisms and endometrial receptivity. Vitamin D was measured as 25-hydroxyvitamin D. Levels of 25-OH vitamin D in blood samples were measured by electrochemiluminescence. Diagnostics of vitamin D deficiency and insufficiency was shown in Supplementary Table 1. Abnormal results were recorded if the patients were heterozygous or homozygous for MTHFR C677T and/or A1298C polymorphisms. In this study, endometrial receptivity was examined using ultrasound. Specifically, we used Doppler ultrasound during the luteal phase (6–8 days after ovulation) and measured the thickness or type of endometrium and parameters related to uterine artery and endometrial blood flow.

Data not collected

In addition to the data we mentioned above, some information was not collected on risk factors associated with RPL, including protein C, protein S, Factor V Leiden and luteal dysfunction. Due to the low prevalence of protein C and protein S deficiency and Factor V Leiden thrombosis in the Chinese population and the limitations of cross-sectional studies in studying diseases with low prevalence, we did not collect information on protein C and protein S deficiency and Factor V Leiden thrombosis. In addition, luteal insufficiency was not included in this study due to the difficulty of diagnosis.

Bias

Bias in cross-sectional studies includes many aspects, and a variety of classifications of bias are summarized in the study by Wang et al. [8]. Because only baseline data collection involved question-based or questionnaire-based data collection (and these data were reviewed by clinicians), nonresponse bias, loss-to-follow-up bias, observer bias, interviewer bias, and recall bias are all relatively minor contributors to the total bias in this study. Furthermore, because the data collectors were not the originators of the study, they were unaware of the study objective. Therefore, the study would have generated less sampling bias, as well as less allocation bias. Prevalence bias is likely to be the largest source of bias in this study and is also referred to as Neyman bias, in which data from some patients with mild or severe diseases are missing in the data collection process. In this study, the situation that emerged was the lack of patients with mild disease. Because study data were collected from regional tertiary medical centers throughout the country, the patients had complex etiologies and relatively severe diseases. Furthermore, because inclusion in the study required that patients undergo at least 1 complete etiological screening, it also led to the loss of a proportion of patients with milder disease or low income. To address this bias, the study's conclusions should be similarly qualified. Tertiary care centers are more likely than local primary care centers to use the results or findings derived from this study.

Data analysis

Measurement data are presented as the mean plus or minus the standard deviation. Counting data are expressed as quartiles. In this study, We mainly conducted two parts of data analysis. 1) Continuous data such as age and vitamin D levels were compared using Students T-test. 2) we used a two-tailed Pearson chi-square test or Fisher's exact test to compare the incidence of various diagnostic abnormalities between people who had two pregnancy losses and those who had three or more pregnancy losses. All data analysis was performed in SPSS, Windows, Version 25.

Results

General features

The total number of RPL patients included in the study was 785, of whom 429 (54.65%) had two pregnancy losses and 356 (45.35%) had three or more pregnancy losses. The average age of the entire population was 32.01 ± 3.84 years, with a mean age of 31.60 ± 3.76 years in patients who had two pregnancy losses and 32.51 ± 3.88 years in patients who had three or more pregnancy losses. The difference in age between these two groups was significant ($p = 0.001$, −0.90, (95% Cl −1.44, −0.36)). The number of secondary RPL included in this study is relatively small due to China's previous implementation of the one-child policy. Further information of the enrolled patients are shown in Table 1.

Integrity of collected data

From the perspective of data integrity, we defined a full screening as the completion of all tests that we mentioned above in each part of the data collection process. Regarding parental chromosome karyotypes, 585 (74.52%) patients had complete screenings, and 142 (18.09%) patients were unilaterally screened for paternal or maternal chromosomes. Of the patients screened for immune factors, 763 (97.19%) completed the full screening. A total of 760 (96.82%) of the patients screened for coagulation factors completed the full screening. However, due to the incomplete collection of prolactin data, 682 individuals (86.88%) were completely screened for endocrine factors. A total of 779 (99.24%) patients

Table 1 Baseline characteristics and the proportion of maternal history

Characteristics	Percentage (proportion)
Age	
< 35	75.80 (595/785)
≥ 35	24.20 (190/785)
Parity	
0	97.07 (762/785)
≥ 1	2.93 (23/785)
Pregnancy loss	
2	54.65 (429/785)
3	33.89 (266/785)
4	8.54 (67/785)
≥ 5	2.93 (23/785)
Biochemical pregnancy*	
0	77.93 (611/784)
1	13.52 (106/784)
≥ 2	8.55 (67/784)
Pregnancy loss before 10 gestational week	
≤ 1	13.45 (96/714)
2	62.18 (444/714)
≥ 3	24.37 (174/714)
Pregnancy loss between 10 and 14 gestational week	
0	91.60 (654/714)
≥ 1	8.40 (60/714)
Pregnancy loss after 14 gestational week	
0	94.12 (672/714)
≥ 1	5.88 (42/714)

* Biochemical pregnancy is defined as detectible serum or urine beta-human chorionic gonadotropin and embryonic arrest prior to the development of a clinical pregnancy

completed the full screening for anatomical factors. Finally, 622 (79.23%) patients completed MTHFR screening; 673 (85.73%) patients underwent examinations for endometrial receptivity, and 767 (97.71%) patients completed serum vitamin D level tests.

Comparative results

Among the abnormal rate for each section, only anatomical factors differed significantly between the patients with 2 pregnancy losses and those with 3 or more pregnancy losses (40.96% versus 32.94%, $P=0.021$, OR 1.41, (95% Cl 1.05–1.89)). In the detailed screenings of each section, obesity was significantly more common in patients with three or more pregnancy losses than in those with two pregnancy losses (13.76% versus 5.59%, $P<0.0001$, OR 2.69, (95% Cl 1.62–4.49)). Similarly, polyps were found to be significantly more common in patients with 3 or more pregnancy losses than in those with 2 pregnancy losses (6.21% versus 3.06%, $P=0.034$, OR 2.10, (95% Cl 1.04–4.23)). The rates of other diagnostic tests were not statistically significant between the two groups. Tables 2, 3, and 4 show the rate of abnormalities in each of the tests in each component of the pathological screen and the *p* value compared with the population with different numbers of pregnancy losses.

Descriptive results

A total of 124 patients, or 15.80% of the total study population, were found to be antiphospholipid antibody-positive. There were 2 patients (2/785, 0.25%) with positivity for all 3 antibodies compared with 21 patients (21/785, 2.68%) with positivity for two antibodies. Thirteen of the 74 (17.57%) patients who were aCL antibody-positive were in the 99th percentile. Seven of the 43 (16.28%) patients who were abeta2-GPI antibody-positive were in the 99th percentile.

Among the 212 patients with abnormal thyroid function, 25 had abnormal TSH levels alone (25/212, 11.79%), 18 had abnormal TSH levels in combination with thyroid antibody positivity (18/212, 8.49%), and 169 had abnormal thyroid antibodies alone (169/212, 79.72%). Of the 169 patients with anti-thyroid antibody positivity alone,

Table 2 The rate of abnormal parental chromosomal karyotype, immune factors and endocrine factors among patients with 2 and 3 or more pregnancy losses

	Total	Times of pregnancy loss		*P* value
		2 (*n* = 429)	≧3 (*n* = 356)	
Chromosome karyotype	5.36 (39/728)	5.43 (22/405)	5.26 (17/323)	0.920
Immune disorder	30.67 (234/763)	29.98 (125/417)	31.50 (109/346)	0.649
Anticardiolipin antibody	9.68 (74/785)	10.49 (45/429)	8.15 (29/356)	0.263
Anti-β2-glycoprotein antibody	5.48 (43/785)	5.83 (25/429)	5.06 (18/356)	0.636
Lupus anticoagulant	3.96 (31/783)	3.96 (17/429)	3.95 (14/354)	0.995
Antinuclear antibody	14.71 (115/782)	14.95 (64/428)	14.41 (51/354)	0.830
Antinuclear antibodies	7.57 (58/766)	6.22 (26/418)	9.20 (32/348)	0.121
Endocrine disorder	50.15 (342/682)	50.66 (192/379)	49.50 (150/303)	0.764
PCOS	11.21 (88/780)	11.48 (49/427)	11.05 (39/353)	0.851
Thyroid Dysfunction	27.01 (212/785)	27.74 (116/429)	26.97 (96/356)	0.982
HPRL	6.22 (44/707)	6.91 (27/391)	5.35 (17/318)	0.392
Blood glucose abnormality				0.941
IGT	5.17 (39/755)	5.06 (21/415)	5.36 (18/336)	
DM	1.32 (10/755)	1.45 (6/415)	1.19 (4/336)	
Obesity	9.30 (73/785)	5.59 (24/429)	13.76 (49/356)	< 0.0001

Abbreviations: *PCOS* polycystic ovary syndrome, *HPRL* hyperprolactinemia, *IGT* impaired glucose tolerance, *DM* diabetes mellitus

Table 3 The rate of abnormal anatomical factors, coagulation factors and other factors among patients with 2 and 3 or more pregnancy losses

	Total	Times of pregnancy loss		*P* value
		2 (*n* = 429)	≧3 (*n* = 356)	
Anatomical disorder	36.59 (285/779)	32.94 (140/425)	40.96 (145/354)	0.021
Uterine malformation	6.42 (50/779)	5.88 (25/425)	7.06 (25/354)	0.503
Asherman's syndrome	24.65 (192/779)	23.29 (99/425)	26.27 (93/354)	0.337
Polyps	4.49 (35/779)	3.06 (13/425)	6.21 (22/354)	0.034
Fibroid/Adenomyosis	3.82 (30/779)	3.76 (16/425)	3.93 (14/354)	0.883
Coagulation disorder	42.63 (324/760)	43.94 (185/419)	41.00 (139/339)	0.415
Platelet aggregation				0.813
Increase	4.27 (33/773)	3.99 (17/426)	4.61 (16/347)	
Decrease	3.23 (25/773)	3.52 (15/426)	2.88 (10/347)	
Coagulation factor				0.755
Increase	26.32 (204/775)	25.93 (111/428)	26.80 (93/347)	
Decrease	1.94 (15/775)	1.64 (7/428)	2.31 (8/347)	
Fibrinolysis				0.770
Increase	1.29 (10/776)	1.40 (6/428)	1.54 (4/348)	
Decrease	0.90 (7/776)	0.70 (3/428)	1.54 (4/348)	
AT-III				0.969
Increase	0.65 (5/772)	0.71 (3/425)	0.58 (2/347)	
Decrease	0.91 (7/772)	0.94 (4/425)	0.86 (3/347)	
HCY	4.08 (32/782)	5.13 (22/429)	2.83 (10/353)	0.107
MTHFR	41.80 (260/622)	40.63 (141/347)	43.27 (119/275)	0.508
Vitamin D		15.38 ± 6.56	15.90 ± 6.45	0.270

Abbreviations: *AT-III* antithrombin-III, *HCY* homecysteine

Table 4 The ultrasound indices of endometrial receptivity among patients with 2 and 3 or more pregnancy losses

	Total	Times of pregnancy loss		*P* value
		2 (*n* = 371)	≧3 (*n* = 302)	
Thickness of endometrium		0.83 ± 0.24	0.83 ± 0.23	0.844
Volume of endometrium		2.87 ± 1.66	2.87 ± 1.66	0.973
Type of endometrium				0.395
A	17.29 (115/665)	19.02 (70/368)	15.15(45/297)	
B	67.82 (451/665)	66.85 (246/368)	69.02 (205/297)	
C	14.89 (99/665)	14.13 (52/368)	15.82 (47/297)	
Right uterine artery				
PI		2.41 ± 0.63	2.49 ± 1.00	0.215
RI		0.87 ± 0.45	0.83 ± 0.06	0.166
S/D		6.67 ± 4.22	6.57 ± 2.88	0.771
AEDV or REDV	7.73 (52/673)	7.82 (29/371)	7.62 (23/302)	0.923
Left uterine artery				
PI		2.44 ± 0.60	2.57 ± 1.18	0.085
RI		0.83 ± 0.05	0.84 ± 0.11	0.256
S/D		6.43 ± 2.05	6.42 ± 2.25	0.943
AEDV or REDV	6.54 (44/673)	6.74 (25/371)	6.29 (19/302)	0.815
Endometrium artery				
PI		0.76 ± 0.82	0.72 ± 0.32	0.413
RI		0.46 ± 0.14	0.47 ± 0.15	0.619
S/D		2.11 ± 1.27	2.08 ± 0.99	0.786
AEDV or REDV	7.13 (48/673)	7.82 (29/371)	6.29 (19/302)	0.444

Abbreviations: *PI* pulsatility index, *RI* rigidity index, *S/D* ratio of systolic and diastolic blood pressure, *AEDV* absent end diastolic velocity, *REDV* reversed end diastolic velocity

73 had anti-Tg and anti-Tpo antibody positivity, 73 had anti-Tg antibody positivity alone, and 23 had anti-Tpo antibody positivity alone.

Finally, it is worth mentioning that the rate of vitamin D insufficiency and deficiency in our study was 97.00% (744/767). Serum vitamin D levels were satisfactory in only 23 patients (3.00%). Next, we performed a correlation analysis of each section and did not find any correlation of the abnormalities between any of these two sections.

Finally, we conducted a simple analysis of the number of abnormalities in the sections (apart of other factors). Of the 785 patients, 290 had abnormalities in only 1 section, and 260 patients had abnormalities in two sections. A total of 115 patients had abnormalities associated with 3 sections, and 21 patients had abnormalities associated with 4 sections. Only 1 patient had a combination of abnormalities in five sections.

Unexplained recurrent pregnancy loss

As recommended by the ASRM, screening for recurrent pregnancy loss should include the evaluation of the chromosome karyotype, antiphospholipid antibody positivity, TSH, HbA1C, and prolactin levels, and anatomical factors. These tests can only explain the well-established or possible risk factors of RPL in 42.42% (333/785) of patients. Compared to the examination recommended by the ASRM, 87.52% (687/785) of patients have abnormal screening results of the risk factors included in this study (excluding abnormalities in MTHFR polymorphisms, vitamin D levels, and endometrial receptivity).

Discussion

The incidence of parental chromosomal abnormalities in the population with RPL has been reported to be 2–8% and is greater than that in the normal population, where the incidence is nearly 0.7% [6]. Although a recent meta-analysis comparing the incidence of parental chromosomal abnormalities in individuals with two or more pregnancy losses did find that the incidence of anomalies was slightly higher in those with three or more pregnancy losses (6.3% vs. 5.5%), the difference was not statistically significant [4]. Consistent with these findings, our study found the rates of parental chromosomal abnormalities to be 5.43% and 5.26% in the two groups, again with no significant differences.

Thrombophilia can be divided into inherited and acquired thrombophilia. Common inherited thrombophilia includes

Factor V Leiden mutation, thrombin G20210A mutation, protein C or protein S deficiency and antithrombin deficiency. It is still controversial whether inherited thrombosis can lead to poor pregnancy outcomes and whether it is associated with RPL. Due to the low prevalence of inherited thrombophilia in the Chinese population, we chose to use TEG in an attempt to detect the coagulation status in patients with RPL. In the TEG results, the most common abnormality was increased levels of coagulation factors, with a mean abnormal rate of 26.32%, and we found no difference in rates between the two groups in the TEG analysis. Similarly, we did not observe differences in the incidence of AT-III deficiency, hyperhomocysteinemia or MTHFR polymorphisms between the two groups.

Acquired thrombosis was more clearly associated with RPL than inherited thrombosis. In this study, 15.8% of the patients were aPL antibody-positive, which is similar to the rate of 14% found by Clifford et al. [9] and 16.8% found by Jaslow et al. [5] aPL antibodies were three times more prevalent in women with RPL than in women without RPL [10, 11]. As the number of positive aPL antibodies in patients increases, the live birth rate also decreases. Only 30% of patients who show positivity for all three antibodies had a live birth [12]. Only 2 of the 785 patients in our study showed positivity for all 3 aPL antibodies, and 21 patients showed positivity for 2 aPL antibodies. We also found that the 99th percentile for both aCL antibody and abeta2-GPI antibody positivity in RPL accounted for approximately 17% of the antibody-positive population, suggesting a high proportion of non-standard obstetrical APS among all patients with RPL. The proportion of ANA antibody-positive individuals in the normal population was reported as 4 to 13% [13], and the reported rate in the RPL population ranged from 2 to 45.7% [14]. In this study, the positivity rate for ANA antibodies was 14.71%, and the positivity rate for ANA antibody assays was 7.57%, while the combined positivity rate for both antibodies was 2.29%. The relationship between ANA antibodies and poor pregnancy outcomes is still not clear. One study suggests that the presence of ANA antibodies in patients with RPL combined with autoimmune disease may indicate a poorer prognosis [15]. One possible mechanism is that the presence of ANA antibodies enhances complement activation, which in turn affects embryo quality and development.

Our study used Rotterdam diagnostics and found the rate of PCOS to be 11.21% in patients with RPL. A recent meta-analysis of the prevalence of PCOS in the RPL population concluded that the mean incidence was 14.3% [16]. Of note, this study found that approximately 35% of PCOS patients had comorbid obesity, which is believed to be associated with pregnancy loss [17, 18]. In the present study, we found that obese patients were more common among women with a greater number of pregnancy losses. In particular, the ESHRE guidelines recommend that patients be informed that obesity is associated with lower rates of live births and poorer health outcomes during counselling, and our findings suggest that more attention needs to be given to obesity in patients with higher rates of pregnancy loss [1]. As with obesity, abnormal blood glucose levels are also associated with pregnancy loss. In this study, the rate of abnormal glucose tolerance was 5.17%, and the rate of diabetes was 1.32%. In women of all ages in China, the prevalence of diabetes is 11% [19]. The lower rate in the present study may be because diabetes is more common among older adults and in economically developed regions.

Thyroid dysfunction is currently a hot topic in the research field of RPL. Recent research suggests that abnormal levels of TSH or the presence of thyroid antibodies alone are associated with pregnancy loss [20]. The incidence of thyroid antibodies is 14 to 18% in women of childbearing age, and their occurrence is usually a predictor of abnormal thyroid function [21]. Thyroid autoantibodies were more common in the RPL population than in the normal population, and the rate of thyroid antibody positivity in the present study was 23.82%. In the present study, hyperprolactinemia occurred in 6.22% of patients. A randomized trial found that high prolactin levels increased the risk of spontaneous pregnancy loss in women with RPL [22]. The role of hyperprolactinemia in RPL remains poorly defined, but given the risk of infertility and desire for a child, preconception hyperprolactinemia also requires active treatment.

In RPL, the incidence of anatomical abnormalities ranged from 15 to 42% [23], and the mean rate of abnormalities in this study was 36.59%. Abnormalities in anatomy can be further subdivided into congenital and acquired abnormalities. Congenital malformations typically include unicornuate uterus, septate uterus, bipolar uterus, and so on, while acquired anomalies include Asherman's syndrome, polyps of the endometrium, and adenomyosis or myoma of the uterus. Polyps of the endometrium were reported in 1.6% to 6% of the RPL population [23]. In the present study, the overall incidence of endometrial polyps was 4.49% compared with 3.06% and 6.21%, respectively, in the two groups. It is worth noting that while endometrial polyps have long been linked to infertility, the impact on RPL is still inconclusive. However, due to uncertainty, clinicians also primarily choose to remove polyps when they find them. Based on the results of this study, clinicians need to be more aware of the possibility of endometrial polyps in individuals with a greater number of pregnancy losses. In our study, we did not find any significant differences between other anatomical abnormalities other than polyps of endometrium.

However, the incidence of each anatomical abnormality was higher in patients with three or more pregnancy losses, which finally resulted in a significant difference between the general rate of abnormalities in the anatomical section. Besides detecting anatomical abnormalities, endometrial receptivity parameters were also collected by ultrasound. However, we did not detect significant differences in the results.

Vitamin D may regulate the process of metamorphosis and the implantation of embryos at the maternal–fetal interface by appropriately promoting inflammatory responses, and many retrospective studies have shown that vitamin D supplementation can increase live birth rates [24]. Vitamin D deficiency, however, is common among women of childbearing age in China. Li et al. found that up to 70% of pregnant women had vitamin D deficiency, with only 1.6% achieving normal vitamin D levels [25]. Our study suggests that vitamin D concentrations are normal in only 3% of patients. Although there are still no guidelines or recommendations regarding vitamin D supplementation for all women of reproductive age, vitamin D supplementation is required for the vast majority of the population based on the prevalence of vitamin D deficiency.

Fifty to 60% of URSA cases have been reported in the literature. In this study, 57.58% of patients were diagnosed with URSA based on the ASMR's recommended evaluation for RPL. While it has not yet been demonstrated that many of the tests included in this study are associated with RPL, the rate of URPL diagnosis was 12.48% in this study. Propescu et al. found that 24 chromosome microarray analysis of pregnancy loss tissue combined with ASRM assessment of pregnancy loss accounts for almost 95% of cases of recurrent pregnancy loss [26]. Both our findings suggest that URPL patients may represent a much smaller proportion of the overall population with RPL than the incidence of more than 50% reported in prior studies.

Overall, this study comprehensively demonstrated and compared the rate of a variety of abnormal diagnostic workup results associated with RPL among individuals with two and three or more pregnancy losses in Northeast China. We found that the rate of abnormal results in the Chinese RPL population is high. Meanwhile, among all the diagnostic tests, only the rate of abnormal anatomical factors, endometrial polyps and obesity were significantly higher in people with three or more pregnancy losses than in people with two pregnancy losses. Due to the similarity in the majority of etiological screening results between the two groups, it may be more reasonable to start etiological screening after two pregnancy losses instead of waiting for the patient to experience a third pregnancy loss. Our results provide new evidence for the timing of screening, and further research is required to confirm the need for some controversial tests regarding the screening of RPL.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07330-8.

Supplementary Material 1: Table 1. Details regarding diagnostics.

Supplementary Material 2.

Acknowledgements
We thank all women who participate in the China medical university birth cohort.

Authors' contributions
LZ and YD designed the study and conducted data analysis. JZ and CL were involved in the data collection. JL advised on the conduct of the study. CQ had the conception for the study. All authors listed made important intellectual contribution to the work and approved the final version of the manuscript for publication.

Funding
This work was supported by National Key R&D Program of China(2016YFC1000404); The National Natural Science Foundation of China (81370735); General Program of National Natural Science Foundation of China (81771610); Science and Technology Project of Shenyang (20–205-4–004); Livelihood Science and Technology Joint Project of Liaoning Province(No: 2021JH2/10300123).

Data availability
The data of this study is available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the ethics committee of China Medical University. All patients signed an informed consent to be screened for the etiology of RPL.

Competing interests
The authors declare no competing interests.

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Received: 25 January 2024 Accepted: 14 February 2025
Published online: 29 March 2025

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