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Original Research

Is Red Blood Cell Distribution Width (RDW) a Negative Predictor of Repeated Implantation Failure?

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Abstract

Background: Repeated implantation failure (RIF) after *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) can be a devastating reality for some patients with infertility. Our objective was to evaluate the potential role of the complete blood count (CBC) parameters, on treatment outcome in patients with repeated IVF implantation failure. **Methods**: This retrospective clinical study, involving a total of 173 patients, consisted of 64 patients with RIF who underwent a fresh IVF-ET cycle, underwent 3 or more IVF cycles, and 109 patients in the control group who became pregnant in the first IVF-ET cycle. **Results**: Duration of infertility, number of grade 2 embryos and red cell distribution width (RDW) were significantly higher in RIF patients ($p < 0.001$, $p < 0.001$, $p = 0.02$). The number of 2 pronucleus (PN) showed a significant positive correlation with the fertilization rate (FR) (r: 0.6; $p < 0.001$). To understand the effects of CBC parameters on FR, the model established with the number of RDW, number of grade 2 embryos and the number of 2 PN proved to be significant (ANOVA, $p < 0.001$). **Conclusions**: RIF patients have higher RDW, longer duration of infertility, and higher number of grade 2 embryos. Elevated RDW may negatively impact FR. The number of 2 PN increased FR.

Keywords: repeated implantation failure (RIF); fertilization rate; IVF- ICSI; red blood cell distribution width (RDW); CBC

1. Introduction

Repeated implantation failure (RIF) after IVF/ICSI-ET (*in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer) can be a devastating reality for some patients with infertility. It is a challenging topic for both clinicians and patients. The definition is inconsistent and differs from that of repeated IVF failure, which includes cases in which embryos cannot be transferred [1]. Reviewing the literature, one comes across different definitions [2]. They are as follows: (a) patients under 40 years of age with a negative pregnancy test after three consecutive embryo transfer (ETs) with good quality embryos, (b) patients under 40 years of age with at least 4 good-quality embryos in whom no clinical pregnancy (CP) has occurred despite three consecutive ETs, (c) patients without implantation despite two consecutive fresh or frozen transfers of at least 4 good quality cleavage stage embryos or at least 2 good quality blastocysts [3–5]. The definition includes both patients who do not show measurable signs of implantation, such as elevated human chorionic gonadotropin (hCG) levels and patients who later show positive hCG levels without showing a gestational sac on ultrasound [3].

In order to offer meaningful solutions to RIF patients, the etiology of RIF must first be determined. RIF may be due to embryologic, maternal, or both causes. Maternal factors include uterine anatomic problems, endometrial pathologies affecting endometrial receptivity, hypercoagulability conditions, and immunologic factors [6]. Various uterine pathologies, including uterine polyps, fibroids, uterine septum, and adhesions, can interfere with embryo implantation [7]. Another important maternal condition is endometriosis. In endometriosis, the quality, quantity, and implantation rate of oocytes and embryos are decreased; the spontaneous abortion rate increases [8]. Older maternal age, smoking in both parents, high body mass index (>25 kg/m^2) and stress also increase the risk of RIF. Embryo quality decreases with increasing maternal age [9]. This has a detrimental effect on implantation. Other factors determining embryo quality include oocyte and sperm quality and paternal chromosomal abnormalities [10].

In addition to the known factors underlying implantation, inflammatory, immunologic, and infectious causes as well as states of hypercoagulability are of interest and have been studied in detail. Natural killer (NK) cell and lymphocyte concentrations in the periphery are increased in RIF patients [11]. In addition, an increased Type 1 helper/Type 2 helper (Th1/Th2) ratio in peripheral blood has been associated with embryo rejection [12]. In the study of autoimmunity, there is no evidence that autoantibodies directly lead to implantation failure, although there is a strong association with RIF, particularly with antiphospholipid antibodies [9]. There are some data suggesting that hereditary thrombophilias may play a role in RIF, although this needs to be confirmed by further studies [13]. Some women with RIF

have been found to have chronic endometritis due to bacterial colonization without clinical signs of infection [14].

In RIF of unexplained etiology, investigation of the above causes is both expensive and time-consuming. However, an ideal diagnostic marker for diagnosing many diseases should have high sensitivity and specificity, be rapidly accessible, inexpensive, and noninvasive [15]. Complete blood count (CBC) is also an ideal analysis for diagnosing many diseases. Some CBC parameters such as leukocytes, neutrophils, and neutrophil-to-lymphocyte ratio (NLR) are considered inflammatory markers [16,17]. In recent years, platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) have also been increasingly used as markers of chronic inflammation. There are some reports of NLR, MPV, and PLR in infertile women with polycystic ovary syndrome (PCOS) [18]. Red blood cell distribution width (RDW), also examined in blood counts, reflects the degree of heterogeneity of red blood cell volume (anisocytosis) and has traditionally been used for differential diagnosis of anemia [19]. More recently, RDW has been recognized as an inflammation-related marker and it has been suggested that it may play a role in predicting mortality in inflammation-related diseases [20].

In this study, we aimed to investigate the potential role of markers in a much simpler CBC analysis compared with many more complex tests and analyzes for RIF, in which there are still unexplained conditions in the etiology despite many of the causes and risk factors mentioned above.

2. Material and Methods

The retrospective clinical study presented was conducted in the Health Sciences University Hospital ART clinic. A computerized database reviewed one thousand five hundred thirty-five fresh IVF-ICSI cycles with good quality (grade 1 and 2) ET between September 2007 and June 2018. The study was approved by the institutional ethics committee.

2.1 Data Collection

Patients with 3 or more consecutive IVF cycles and a negative pregnancy test (presence of a detectable beta subunit of hCG in serum) were included in the RIF group. Some authors additionally used good ovarian reserve (follicle-stimulating hormone (FSH) $>$10 IU/L) as inclusion criteria and less than 4 follicles per hCG day, less than 4 oocytes in the previous cycle, endometriosis, hydrosalpinx, uterine abnormalities, and coagulopathy as exclusion criteria to define RIF [21,22]. Our study excluded all patients older than 40 years and patients younger than 40 years with endometriosis, hydrosalpinx, pelvic infections, and follicles with less than 4 hCG days. Also, cases with systemic diseases affecting CBC parameters (diabetes mellitus, hypertension, asthma, liver-kidney diseases, malignancies, hematologic diseases, all infectious diseases (including tuberculosis) and autoimmune diseases), endocrinologic abnormalities (thyroid diseases, hyperprolactinemia, etc.), use of anti-inflammatory drugs such as corticosteroids, a history of splenectomy, and cigarette and alcohol use were excluded from the study. Patients who became pregnant in the first IVF/ICSI-ET cycle formed the control group. Accordingly, 419 patients with a second cycle and pregnancy, 490 patients who did not become pregnant in the first cycle, 306 patients with CBC data at midcycle and/or during the cycle but without CBC data per cycle, and patients with thyroid disease (n: 104), tuberculosis (n: 4), celiac disease (n: 1), multiple sclerosis (n: 1), lymphoma (n: 1), asthma (n: 3), familial Mediterranean fever (n: 1), epilepsy (n: 1), patients with a history of heart disease (n: 1), endometrioma and surgery related to endometriosis (n: 12), chromosomal abnormalities such as translocations in parents (n: 7), and a number of follicles on hCG day below 4 (n: 11) were excluded from the study. According to the inclusion and exclusion criteria, a total of 173 patients, including 64 RIFs and 109 controls, were enrolled in the study (**Supplementary Fig. 1**).

Oocyte fertilization was assessed by observation of 2 pronucleus (PN) 18–20 hours after ICSI. Fertilization rate (FR) was calculated as the ratio of 2 PN to mature oocytes. At 42–44 hours after ICSI, day 2 embryos were classified based on blastomere size, nucleation, and cytoplasmic morphology. At 61–65 hours after ICSI, day 3 embryos were classified based on cell number, size, symmetry, and degree of fragmentation using an embryo scoring system [23]. Grade 1 and 2 were classified as good-quality embryos, whereas grade 3 and 4 were classified as poor-quality embryos. Blastocysts with a day 5 (blastocyst) score $\geq$3 well-developed blastocysts (BB) were accepted as good-quality embryos [24]. Implantation was determined as a positive beta-hCG test 12 days after ET. Clinical pregnancy was adopted as identification of the intrauterine gestational sac by ultrasonographic examination. Biochemical pregnancy was defined as a pregnancy diagnosed only by the detection of beta-hCG in serum or urine and not resulting in CP. An abortion, on the other hand, was accepted as the loss of an initially ultrasonographically detected pregnancy in the first trimester.

The primary outcome of this study was a correlation analysis between IVF and CBC parameters and a comparison of CBC parameters between RIF patients and the control group. A regression analysis was performed for the influence of CBC parameters on FR, and the secondary outcome was whether the obtained model was significant.

2.2 Laboratory Evaluation

Blood tests, including CBC, are performed before all patients are enrolled in the IVF-ICSI treatment protocol. Venous blood samples were collected between 08:00 and 09:00 after a 12-hour fasting period in tubes containing ethylenediaminetetraacetic acid (EDTA). Complete blood count was performed using an automated blood analyzer

Table 1. The comparison of basal characteristics and IVF cycle results between RIF and control groups.

Parameter[a]	Group 1 (RIF)	Group 2 (control)	p^b value
Age (years)	29.84 ± 3.57	30.87 ± 2.55	0.081
BMI (kg/m^2)	26.87 ± 4.21	25.48 ± 5.16	**0.019**
FSH (mIU/mL)	6.78 ± 2.57	6.72 ± 2.42	0.768
LH (mIU/mL)	6.09 ± 3.74	5.63 ± 4.13	0.153
E_2 (pg/mL)	48.55 ± 29.94	48.56 ± 42.63	0.461
Duration of infertility (years)	91.08 ± 47.60	61.13 ± 37.59	**<0.001**
Number of antral follicles	16.08 ± 8.83	15.08 ± 8.30	0.504
Initial stimulation dose at day 3	227.54 ± 87.29	220.30 ± 70.44	0.661
Days of stimulation	10.02 ± 1.98	10.04 ± 1.65	0.606
Total gonadotrophin dose (IU)	2203.71 ± 950.08	2179.77 ± 907.43	0.733
Estradiol on hCG day (pg/mL)	2968.61 ± 1994.32	2864.04 ± 1432.03	0.790
Progesteron on hCG day (ng/mL)	1.07 ± 0.37	1.18 ± 0.65	0.854
On the hCG day ≥17 mm follicle number	3.72 ± 2.49	3.53 ± 2.38	0.657
Endometrial thickness on hCG day (mm)	10.30 ± 1.71	9.89 ± 1.98	0.226
Total number of retrieved oocytes	13.80 ± 8.12	12.69 ± 6.14	0.735
Number of mature oocytes	10.49 ± 6.66	9.85 ± 5.09	0.969
Oocyte quality index	5.32 ± 0.59	5.23 ± 0.71	0.647
Number of 2 pronuclei	5.47 ± 4.40	5.54 ± 3.65	0.499
Day 2 embryo scoring	4.04 ± 0.73	3.98 ± 0.79	0.849
Cleavage stage embryo scoring	4.07 ± 0.73	4.05 ± 0,65	0.698
Number of grade 1 embryos	0.88 ± 0.70	0.81 ± 0.48	0.638
Number of grade 2 embryos	0.78 ± 0.65	0.27 ± 0.46	**<0.001**

[a]Data are presented as mean ± standard deviation (SD), median (interquartile range) or number (percentage); [b]Student's *t*-test or Mann–Whitney U-Test for differences between normal and elevated progesterone groups; Bold, $p < 0.05$, statistically significant.

hCG, Human chorionic gonadotropin; E_2, estradiol; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; RIF, repeated implantation failure; IVF, *in vitro* fertilization.

(Cell-Dyn 3700, Abbott®, Abbott Park, IL, USA). Leukocyte, lymphocyte, neutrophil, monocyte, platelet, MPV and RDW values were recorded; NLR, PLR and platelet mass index (PMI) were calculated and recorded.

2.3 Statistical Analysis

Statistical analysis was performed with the Statistical Program for the Social Sciences version 20.0 (IBM SPSS Inc., Chicago, IL, USA). The distribution of continuous variables was presented as mean and standard deviation (SD), whereas categorical variables were presented as ratios and percentages of the total. Comparison of continuous variables between groups was made with Student's *t*-test or Mann-Whitney U test, depending on the normality of the distribution, and comparison of categorical variables was made with Pearson's chi-square test or Fisher's exact test. Spearman correlation analysis was performed for the relationship between CBC parameters and FR, with correction for body mass index (BMI). Parameters with significant correlations were included in the model established by regression analysis and evaluated. The significance level was $p \leq 0.05$ for all statistical tests.

3. Results

A total of 173 patients, including 64 RIFs and 109 controls, were included in the study. Demographic characteristics and IVF cycle characteristics of the included patients (mean ± SD): age: 30.49 ± 2.99 yaers, BMI: 26.01 ± 4.85 (kg/m^2), duration of infertility: 72.27 ± 43.93 months and baseline FSH: 6.74 ± 2.47 (IU/mL), mean total gonadotropin dose: 2188.63 ± 920.75 (IU) and stimulation duration: 10.03 ± 1.77 days. On the day of hCG administration, the ovarian response parameters of the patients (mean ± SD) were as follows: hCG day E_2: 2904.57 ± 1665.78 pg/mL, the number of retrieved oocytes (>17 mm): 13.10 ± 6.94, endometrial thickness: 10.05 ± 1.88 mm, the number of 2 PN: 5.51 ± 3.93. There were no significant differences between the RIF group (group 1) and the control group (group 2) in age, basal FSH level, stimulation duration, total gonadotropin dose used in stimulation, number of mature oocytes, number of 2 PN, oocyte quality index, embryo score at day 2 and day 3, and number of grade 1 embryos. However, it was found that BMI, duration of infertility and number of grade 2 embryos were significantly higher in group 1 ($p = 0.019$, $p < 0.001$ and $p < 0.001$, respectively) (Table 1). No significant difference existed between groups in terms of ovarian stimulation pro-

Table 2. Comparison of IVF cycle parameters for groups 1 and 2.

Parameters		Group 1	Group 2	Total	*p* value
Ovarian stimulation protocol	Micro-dose-flare up	3 (4.7%)	7 (6.4%)	10 (5.8%)	$p^a = 0.382$
	Long luteal agonist	32 (50.0%)	44 (40.4%)	76 (43.9%)	
	Antagonist	24 (37.5%)	52 (47.7%)	76 (43.9%)	
	Hypogonadotropic hypogonadism	0 (0.0%)	2 (1.8%)	2 (1.2%)	
	Luteal E_2-Antagonist	4 (6.3%)	4 (3.7%)	8 (4.6%)	
	Femara-Antagonist	1 (1.6%)	0 (0.0%)	1 (0.6%)	
Total		64 (100.0%)	109 (100.0%)	173 (100.0%)	
Type of drug used during induction	RecFSH + HMG	28 (43.8%)	41 (37.6%)	69 (39.9%)	$p^a = 0.722$
	RecFSH	34 (53.1%)	63 (57.8%)	97 (56.1%)	
	HMG	2 (3.1%)	5 (4.6%)	7 (4.0%)	
Total		64 (100.0%)	109 (100.0%)	173 (100.0%)	
Assisted hatching	No	45 (70.3%)	69 (63.9%)	114 (66.3%)	$p^b = 0.389$
	Yes	19 (29.7%)	39 (36.1%)	58 (33.7%)	
Total		64 (100.0%)	108 (100.0%)	172 (100.0%)	
Embryo transfer day	2	2 (3.1%)	4 (3.7%)	6 (3.5%)	$p^a = 0.100$
	3	38 (59.4%)	44 (40.4%)	82 (47.4%)	
	4	0 (0.0%)	3 (2.8%)	3 (1.7%)	
	5	24 (37.5%)	56 (51.4%)	80 (46.2%)	
	6	0 (0.0%)	2 (1.8%)	2 (1.2%)	
Total		64 (100.0%)	109 (100.0%)	173 (100.0%)	
Cause of infertility	Male factor	26 (40.6%)	52 (47.7%)	78 (45.1%)	$p^a = 0.172$
	Poor ovarian reserve	4 (6.3%)	7 (6.4%)	11 (6.4%)	
	Unexplained infertility	27 (42.2%)	47 (43.1%)	74 (42.8%)	
	Tubal factor	7 (10.9%)	3 (2.8%)	10 (5.8%)	
Total		64 (100.0%)	109 (100.0%)	173 (100.0%)	

[a]Fisher's Exact Test; [b]Pearson Chi- Square test; Bold, $p < 0.05$, statistically significant.

RecFSH, recombinant follicle-stimulating hormone; HMG, human menopausal gonadotropin; IVF, *in vitro* fertilization.

Table 3. The comparison of inflammatory markers of CBC between RIF (group 1) and control (group 2) groups.

CBC parameters	Group 1	Group 2	p^a value
WBC (10^3/µL)	7.51 ± 2.99	7.14 ± 2.15	0.704
Lymphocyte (10^3/µL)	1.98 ± 0.68	1.97 ± 0.58	0.639
Neutrophil (10^3/µL)	4.87 ± 2.62	4.53 ± 1.77	0.704
RDW (%)	14.55 ± 1.45	14.14 ± 1.70	**0.02**
Monocytes	0.39 ± 0.16	0.38 ± 0.14	0.988
Platelets (10^3/µL)	282.39 ± 59.27	283.08 ± 71.58	0.895
MPV (fL)	8.17 ± 0.82	8.39 ± 1.01	0.156
PMI	2294.94 ± 471.11	2348 ± 549.93	0.688
NLR	2.44 ± 1.02	2.45 ± 1.09	0.853
PLR	153.04 ± 44.72	152.96 ± 50.06	0.612

WBC, White blood cell; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; MPV, Mean platelet volüme; CBC, complete blood count; PMI, Platelet mass index; RDW, Red blood cell distribution width.

[a]Mann–Whitney U-Test for differences between group 1 and 2; Bold, $p < 0.05$, statistically significant.

tocol, type of drug used in treatment (RecFSH/RecFSH + human menopausal gonadotropin (HMG)/HMG), assisted hatching, day of embryo transfer, and cause of infertility (p = 0.382, 0.722, 0.389, 0.100, 0.172, respectively) (Table 2). After adjusting for BMI, number of 2 PN was positively correlated with the FR (r: 0.527; $p < 0.001$). The RDW was significantly higher in group 1 (mean ± SD: 14.55 ± 1.45 (group 1); 14.14 ± 1.70 (group 2); p = 0.02) (Table 3). To understand the effects of CBC parameters on FR, the model established with the number of RDW, number of grade 2

Table 4. Predictive effect of number of 2 pronuclei on fertilization rate.

Independent variable	p^a value	Beta	95% CI	VIF
RDW	0.946	0.005	–0.021/0.023	1.020
Number of grade 2 embriyos	0.430	–0.077	–0.125/0.054	2.082
Number of 2 pronuclei	<**0.001**	0.547	0.028/0.046	1.047
Duration of infertility	0.94	–0.005	–1	1.111
BMI	0.439	–0.053	–2	1.034

[a]ANOVA: $p < 0.001$. Bold, $p < 0.05$, statistically significant.

Predictors: RDW, number of 2 pronuclei, number of grade 2 embryos, duration of infertility, BMI.

RDW, Red blood cell distribution width; VIF, Variance Inflation Factor; CI, confidence interval.

embriyos and the number of 2 PN proved to be significant (ANOVA, $p < 0.001$). While increasing the number of 2 PN increased FR (B = 0.547, $p < 0.001$) (Table 4).

4. Discussion

We could not find any study on RDW in the literature that included inflammation and blood count data on the etiology of RIF. In this regard, our study was the first in the literature to find that high RDW before IVF-ICSI treatment was associated with RIF.

Maternal and embryological factors play an important role in the etiology of RIF [6]. The most important maternal risk factors are advanced maternal age, high BMI, and smoking [9]. In addition, uterus abnormalities (uterine septum, etc.), uterine pathologies such as fibroids and polyps may interfere with implantation [7]. Endometriosis is known to decrease the implantation rate [8]. Therefore, in order not to compromise the results of our study, we excluded patients with uterine pathologies and patients with known endometriosis and endometrioma. Shapiro *et al*. [25] reported higher rates of embryo-endometrial mismatch, increased biochemical pregnancies, and lower live birth rates with increasing maternal age. We did not include patients older than 40 years in our study. It is also known that embryo quality is an essential factor affecting outcome, regardless of embryo developmental stage and number of embryos transferred [5]. Our study included only patients with good-quality (grade 1 and 2) embryo transfer.

There was no difference between the two groups regarding the cause of infertility (tubal factor, male factor, unexplained infertility, and low ovarian reserve). The low ovarian reserve was lower in group 1 (n: 4) than in group 2 (n: 7), and the difference was not significant. Patients with fewer than 4 follicles on hCG day were used as an exclusion criterion in the definition of RIF because the number of oocytes retrieved per cycle would decrease due to lower implantation [21]. We also excluded patients with fewer than 4 follicles on hCG day. The duration of infertility was significantly higher in RIF patients ($p < 0.001$). This suggests that patients here waited longer to take the baby home than in the other group. The mean BMI was >25 kg/m^2 in both groups; it was significant that it was higher in group 1 ($p = 0.019$). It is known that in obese patients, the response to ovarian stimulation is weaker and higher doses of gonadotropin are required to obtain more oocytes. Obesity is also thought to cause subclinical inflammation [26]. Consistent with this, we also observed a higher BMI in group 1. However, in the subsequent analysis, we corrected for BMI. There was no difference between the two groups in terms of oocyte and embryo quality index, number of grade 2 PN, and number of mature oocytes that would affect implantation (Table 1). However, the number of grade 2 embryos was significantly higher in RIF patients ($p < 0.001$).

Routine genetic evaluation is not performed, while embryo morphology is assessed for embryological factors. The prevalence of chromosomal aberrations, including translocations, mosaicism, inversions, and deletions in RIF patients is 2%, with the most common abnormality being a translocation [27]. Although the incidence is low, preimplantation genetic diagnosis, embryo coculture, and preferential blastocyst transfer are recommended in couples with RIF to minimize the embryonic component [4]. Assisted hatching seems to slightly increase the achievement of clinical pregnancy, but there is insufficient evidence for live birth rate [28]. In our study, there was no difference between the two groups in terms of ET day and use of hatching assistance. Coculture was used in only 3 patients. Because no information on preimplantation genetic diagnosis was available, patients with chromosomal abnormalities (such as translocation carriers) in the parents were excluded from the study. Our study found that RDW was significantly higher in the RIF group than in the control group ($p = 0.02$) (Table 3). The difference between the other CBC parameters was not significant ($p > 0.05$). The model established with the number of RDWs, the number of grade 2 embryos and the number of 2 PNs was significant (ANOVA, $p < 0.001$) (Table 4). In our literature review, we came across only one study on this topic. This study found a positive correlation between lymphocyte count and fertilization rate (FR) in IVF patients with unexplained infertility; however, inflammatory markers in blood were not associated with CP, biochemical abortion, and live birth [29]. Patel

et al. [30] reported that increased RDW was significantly positively correlated with decreased red cell deformability. Therefore, venous thrombosis can be induced by large fluctuations in red cell volume, which increase blood viscosity and impair blood flow in the microcirculation [31]. Recently, RDW has also been recognized as a marker of inflammation [20]. Inflammation is also the main feature of endothelial dysfunction [16]. As a result of all these effects, an elevated RDW reflects a profound dysregulation of erythrocyte homeostasis, indicating both impaired erythropoiesis and abnormal red blood cell survival. This condition, shortening of telomere length, oxidative stress, inflammation, poor nutritional status, dyslipidemia, and hypertension can be attributed to underlying metabolic abnormalities such as red blood cell fragmentation and altered erythropoietin function [19]. Interestingly, a study of 3157 subjects examined telomere lengths of genomic DNA isolated from circulating white blood cells and found that shorter telomere lengths were associated with increased RDW [32]. In addition, the deterioration of iron metabolism during inflammation and the action of cytokines released during inflammation lead to a deterioration of the erythropoietin response, resulting in anisocytosis. Therefore, it is plausible that RDW is impaired during inflammation [33].

Considering all these mechanisms, high RDW may indicate insufficient trophoblast invasion due to increased inflammation, endothelial dysfunction, and thrombosis in spiral arterioles. In addition, shorter telomere length in the developing embryo could be a marker for short survival and biochemical abortion processes and predict RIF. Further studies are needed to understand whether there is a cause or effect here. Because the total number of patients with RIF per clinic in IVF clinics is small, multicenter and international research is needed to investigate the uncertainties surrounding this phenomenon in a good quality manner.

Our study was a retrospective study; no power analysis was performed and data from a single tertiary center were used. Despite evaluation over a period of more than 10 years, the number of patients was lower than expected because the group of RIF patients is relatively small and heterogeneous, and the cause was excluded. All patients included in the study did not have genetic analysis. This limits the ability of the results to reflect and generalize to the general population. Despite these limitations, this was the first study to examine the association between CBC parameters and RIF.

5. Conclusions

In our study, it was quite interesting that RDW stood out to be a significant negative predictive factor in RIF patients. This inexpensive and straightforward parameter provides valuable information for predicting subclinical and clinical diseases' general health status, presence, and prognosis. Therefore, closer monitoring of RIF patients with elevated RDW values and future therapeutic interventions to lower RDW may be beneficial for RIF patients in desperate situations.

Availability of Data and Materials

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

Author Contributions

Conceptualization: ÖKG; Data Curation: ÖKG, OA, RÖ; Formal Analysis: ÖKG; Investigation: ÖKG, OA, RÖ; Methodology: ÖKG, OA, EB; Project Administration: ÖKG; Resources: ÖKG, OA, RÖ, EB, SD, ÖMT; Software: ÖKG, OA, RÖ, SD, ÖMT; Supervision: ÖKG, SD, ÖMT; Validation: ÖKG, OA; Visualization: ÖKG, OA, RÖ, SD; Writing – Original Draft Preparation: ÖKG; Writing – Review & Editing: ÖKG, SD, ÖMT. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The research project and protocols were approved by the Etlik Zubeyde Hanim Research and Training Hospital Institutional Review Board (21/12/2018/ issue 90057706-799). Since it was a retrospective study, the consent form was waived by the ethics committee.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.ceog5010214.

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