

Research Article

Comparative Cytogenetic Analysis of Spontaneous Abortions in Recurrent and Sporadic Pregnancy Losses

Tatiana V. Nikitina^a Elena A. Sazhenova^a Ekaterina N. Tolmacheva^a
Natalia N. Sukhanova^a Anna A. Kashevarova^{a, b} Nikolay A. Skryabin^{a, b}
Stanislav A. Vasilyev^{a, b} Tatiana N. Nemtseva^c Sergey Y. Yuriev^{c, d}
Igor N. Lebedev^{a, b, e}

^aLaboratory of Cytogenetics, Institute of Medical Genetics, ^bLaboratory of Ontogenetics, Tomsk State University, ^cCenter of Perinatal Health, and Departments of ^dObstetrics and Gynecology and ^eMedical Genetics, Siberian State Medical University, Tomsk, Russia

What Is It about?

The majority of miscarriages are sporadic; however, 1–5% of couples experience recurrent pregnancy loss (RPL). Currently, there are conflicting reports regarding the rates of chromosomal abnormalities in RPL and sporadic abortions (SA). A comparative cytogenetic analysis of 442 RPL and 466 SA was performed. The frequency of embryos with abnormal karyotypes was significantly higher in SA compared to RPL, and abortions from young women were the main contributor to this difference. The incidence of recurrent abnormal karyotypes in subsequent miscarriages was significantly higher than random probability. Our findings highlight the variability in the risk of aneuploidy in recurrent abortion.

Key Words

Recurrent pregnancy loss · Sporadic abortion · Miscarriage · Cytogenetic analysis · Chromosomal abnormalities

Abstract

Background: The majority of miscarriages are sporadic; however, 1–5% of couples experience recurrent pregnancy loss (RPL). Approximately 50–60% of miscarriages result from chromosomal abnormalities. Currently, there are conflicting reports regarding the rates of chromosomal abnormalities between recurrent and sporadic pregnancy losses. **Methods:** A retrospective comparative cytogenetic analysis of 442 RPL and 466 sporadic abortions (SA) was performed. Maternal age and medical background were evaluated, and chromosomal abnormality rates were compared between groups. **Results:** The frequency of embryos with abnormal karyotypes was significantly higher in SA compared to RPL (56.7 and 46.6%, respectively),

Tatiana V. Nikitina
Laboratory of Cytogenetics, Institute of Medical Genetics
Naberezhnaya Ushaiki, 10
RU-634050 Tomsk (Russia)
E-Mail t.nikitina@medgenetics.ru

and abortions from women under 30 years of age were the main contributor to this difference. An age-dependent increase in the abnormal karyotype rate was observed in two groups of women – those with SA [53.0 and 70.1% for younger and older (≥ 35 -year-old) mothers, respectively] and those with idiopathic RPL without any concomitant reproductive pathology (46.5 and 78.4% for younger and older mothers) – but not in the group of women with RPL associated with concomitant reproductive pathology. The incidence of recurrent abnormal karyotypes in subsequent miscarriages was significantly higher than random probability (odds ratio = 22.75). **Conclusion:** Our findings highlight the variability in the risk of aneuploidy in recurrent abortion.

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Introduction

Humans have a high rate of embryo loss, which is caused by the high frequency of chromosomal abnormalities in oocytes and embryos in the early stages of development. Approximately 25% of unfertilised eggs and 29% of early human embryos have abnormal karyotypes according to cytogenetic analysis [1]. A recent study using array comparative genomic hybridisation found that 75% of oocytes, 83% of cleavage-stage embryos, and 58% of blastocysts were aneuploid [2]. Although these data were obtained after in vitro fertilisation and are likely higher than the rates for natural conceptions, it is obvious that human reproduction is characterised by an extremely high incidence of aneuploidy. More than 99% of chromosomally abnormal pregnancies result in miscarriage [3], and chromosomal abnormalities are found in 50–60% of dead embryos [4–7].

The frequency of spontaneous abortion, which is approximately 15% of pregnancies [8], is similar in different human populations; however, some couples experience pregnancy loss more than once. If recurrent miscarriage is defined as two or more consecutive abortions, recurrent pregnancy loss (RPL) occurs in up to 2–5% of couples. Generally accepted causes of RPL include uterine anomalies, parental chromosomal abnormalities, antiphospholipid antibodies, polycystic ovary syndrome, diabetes mellitus, and hyperthyroidism [9]. However, in approximately half of all cases, the cause of RPL remains unexplained by conventional examinations [10, 11].

Are there any peculiarities regarding the frequency or distribution of chromosomal abnormalities in RPL versus sporadic abortions (SA)? The results of cytogenetic investigations of products of conception may be affected by maternal contamination and cell culture artefacts [12–14]; therefore, an accurate comparative analysis is possible only by comparing RPL and SA in the same study. Currently, only six published reports meet these conditions. Some authors did not find any statistically significant difference in the rates of cytogenetic abnormalities in abortions between couples with and those without RPL [15–17]. Others found that embryos with normal karyotypes were more common in RPL groups than in SA groups [18, 19], or, conversely, that cytogenetic abnormalities occurred more frequently in RPL groups than in SA groups [20]. Because of the limited sample sizes in most of these studies (ranging from 50 to 234 cases) and because of the inconsistency of the results obtained, our study aimed to compare the distribution of different types of chromosomal abnormalities in the largest study group to date (to the best of our knowledge) of miscarriages from couples with RPL and SA.

Subjects and Methods

Population

This retrospective cohort study analysed patients who were referred to the Laboratory of Cytogenetics of the Institute of Medical Genetics (Tomsk, Russia) from 1987 to 2014.

Material from 1st-trimester spontaneous abortions was obtained from gynaecologic and obstetric clinics in Tomsk and Seversk (Russia). Information was recorded about maternal and paternal age, gynaecological anamnesis of the women, the number and outcomes of previous pregnancies, and features of the present gestation.

The RPL group consisted of 442 abortions from women with a history of idiopathic recurrent miscarriage (≥ 2 consecutive spontaneous miscarriages). Subjects with established predisposing factors for RPL, such as antiphospholipid syndrome, hereditary thrombophilia, parental chromosomal abnormalities, structural uterine anomalies, hypothyroidism, or polycystic ovary syndrome, were excluded from the analysis. Immunological, endocrinological, and inflammatory diseases of the female reproductive tract (with the exception of aetiological factors for RPL) were recorded as concomitant reproductive pathologies. In total, 109 of the miscarriages were from mothers with such concomitant pathologies, and 239 abortions were from mothers with no concomitant reproductive pathology. The mothers of the other 94 embryos had incomplete medical information and were excluded from the analysis. In the RPL group, 166 patients had previous live births and 276 women were childless.

The data were also stratified by maternal age in two modes: (1) 5-year periods (≤ 24 , 25–29, 30–34, 35–39, and ≥ 40 years) and (2) younger (≤ 34 years) and older (≥ 35 years) women. The control SA group consisted of 466 miscarriages that were the first miscarriages experienced by women with prior normal pregnancies.

All products of conception were divided into two types: (1) missed abortions, with a developmentally arrested embryo in the gestational sac (no heartbeat or inconsistency between crown-rump length and current pregnancy term), and (2) anembryonic pregnancies (blighted ovum).

Sampling and Karyotyping

Tissue samples were obtained by curettage, collected, stored in sterile saline, and transferred to the cytogenetic laboratory. The products of conception were examined, and embryonic tissues were separated from decidua and blood clots. Metaphase chromosomes were obtained after long-term culture in DMEM/F12 (1:1) medium (Sigma, USA) supplemented with 20% foetal bovine serum (HyClone, USA). Colchicine (Sigma, USA) was added 4 h before chromosome harvesting, and the samples were processed using standard techniques. All specimens were G-banded using trypsin-Giemsa (Sigma, USA) to identify the chromosomes.

Statistical Analyses

The frequencies of embryonic aneuploidy and distributions of various types of cytogenetic abnormalities were compared between the groups using the χ^2 test and Student's t test. Correlations were analysed using Spearman's non-parametric rank test. Odds ratios (OR) were used to assess the risk of recurrent chromosomal abnormalities in families with more than one karyotyped abortion. A p value < 0.05 was considered statistically significant in all tests.

Ethics Approval

The collection and use of tissue samples from products of conception was approved by the local Ethics Committee of the Institute of Medical Genetics. Informed consent was obtained from all patients.

Table 1. Distribution of embryonic karyotype rates in RPL and SA

Karyotype	RPL (n = 442)	SA (n = 466)	p
Normal	236 (53.4%)	202 (43.3%)	0.0025
Abnormal	206 (46.6%)	264 (56.7%)	
Autosomal trisomies	98 (47.6%)	116 (43.9%)	n.s.
Including double trisomies	9 (4.4%)	2 (0.8%)	0.01^a
Numerical gonosomal abnormalities	22 (10.7%)	24 (9.1%)	n.s.
Triploidies	29 (14.1%)	43 (16.3%)	n.s.
Tetraploidies	37 (18.0%)	55 (20.8%)	n.s.
Structural aberrations	5 (2.4%)	5 (1.9%)	n.s.
Autosomal monosomies	2 (1.0%)	3 (1.1%)	n.s.
Other ^b	13 (6.3%)	18 (6.8%)	n.s.

Bold type marks statistically significant differences. n.s. = No significant difference.

^a Fisher's exact test. ^b Combination of different forms of abnormalities.

Results

Karyotypes were determined for 442 abortions from women with idiopathic RPL and 466 abortions from women with SA. In the RPL and SA groups, the average maternal age was 28.9 ± 6.1 and 28.5 ± 6.0 years, the average paternal age was 31.3 ± 6.9 and 31.3 ± 6.2 years, and the average gestational age was 9.6 ± 2.7 and 9.7 ± 2.8 weeks, respectively. The differences were not significant. The only significant difference was in the average number of pregnancies per woman, which was higher in the RPL group than in the SA group (406 and 362 pregnancies per 100 women, respectively; $p = 0.027$).

We compared the RPL and SA groups according to the severity of the embryogenesis disorder. All products of conception were divided into two types: missed abortions and blighted ova. No difference was found between the RPL and the SA group in terms of the frequencies of either type of embryogenesis disorder; the anembryonic rate was 77/442 (17.4%) for the RPL group and 85/466 (18.2%) for the SA group ($p = 0.74$).

Among the 442 recurrent miscarriages, 236 cases (53.4%) had a normal karyotype and 206 (46.6%) showed chromosomal abnormalities. Of the 466 SA, 202 (43.3%) had normal karyotypes and 264 (56.7%) had abnormal karyotypes. The frequency of abnormal karyotypes in the SA group was significantly higher than in the RPL group ($p = 0.0025$). Generally, the distribution of the specific types of chromosomal abnormalities in the recurrent miscarriages did not differ from that in the SA group. The most common chromosomal abnormality type in both groups was autosomal trisomies, followed by tetra- and triploidies, sex chromosome anomalies, and structural chromosomal aberrations (table 1).

Double trisomies were the only type of cytogenetic abnormality that differed between the RPL and SA groups (4.4 and 0.8% of abnormal karyotypes in RPL and SA, respectively; $p = 0.01$). Complete double trisomies (7 cases) were registered only among recurrent miscarriages, whereas mosaic variants were found in both groups (2 cases in each). All double trisomies were found in women older than 30 years. As the number of previous miscarriages increased from 2 to 7, the normal karyotype rate increased from 54.1 to 71.2%; however, this trend was not statistically significant ($R = 0.6$; $p = 0.21$).

Figure 1 shows the distribution of cytogenetically normal specimens in the RPL and SA groups, stratified for maternal age at the time of pregnancy loss into five age groups (≤ 24 , 25–29, 30–34, 35–39, and ≥ 40 years). A significant difference between RPL and SA embryos

Fig. 1. Rates of abortion karyotypes of women in different age groups (x-axis) with RPL and SA. Clear bars: normal karyotypes; dark bars: abnormal karyotypes.

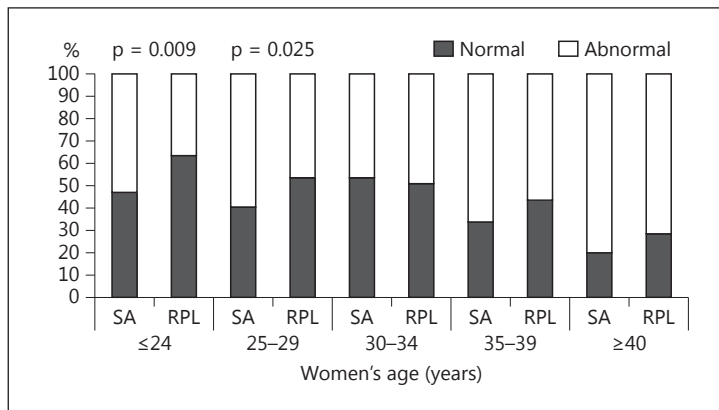
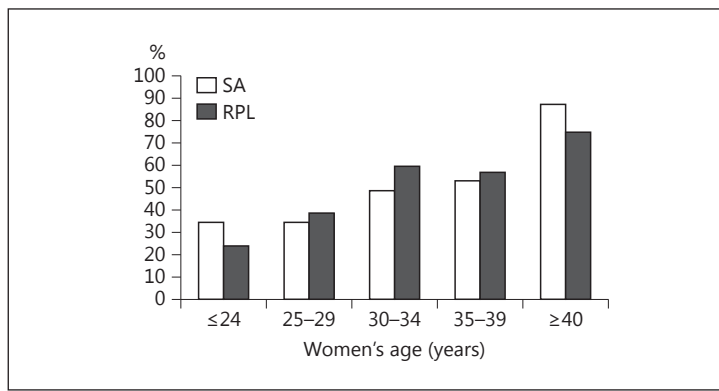


Fig. 2. Contribution of autosomal trisomies to general embryonic abnormality rates in relation to the mother's age.



was found only for women younger than 30 years [≤ 24 years: 63.2% (86/136) vs. 47.3% (62/131), $p = 0.009$; 25–29 years: 53.9% (76/141) vs. 40.4% (55/136), $p = 0.025$].

Autosomal trisomies were the main contributor to the increase in karyotypic abnormalities due to advanced maternal age. The proportions of autosomal trisomies were similar between the RPL and the SA group for women of various maternal ages ($p > 0.05$), and they increased from 12/50 (24.0%) and 24/69 (34.8%) for women aged ≤ 24 years to 15/20 (75.0%) and 14/16 (87.5%) for women aged ≥ 40 years, respectively (fig. 2).

There were 3 women with three and 30 women with two karyotyped miscarriages. In 42.2% of the latter cases, both abortions were abnormal. The probability of recurrence of normal or abnormal karyotypes in subsequent abortions is shown in table 2. The normal karyotype rate in subsequent abortions was 86.7% in women with a previous normal karyotype abortion and 22.2% in women with a previous abortion with an abnormal karyotype (OR = 22.75; 95% CI: 2.82–244.77; $p = 0.00022$). The average maternal age among women with normal previous abortions and abnormal subsequent abortions was significantly higher (39 years) compared to the other three groups (27.9–31.3 years). The abnormalities within any particular couple generally involved different types of alterations: trisomy and triploidy (4 families), trisomy and tetraploidy (2 families), trisomy and monosomy X (1 family), and autosomal monosomy and tetraploidy (1 family). Recurrence of trisomy 16 occurred in 1 family, and 5 women had trisomies of different chromosomes (heterotrissomy) in both abortions.

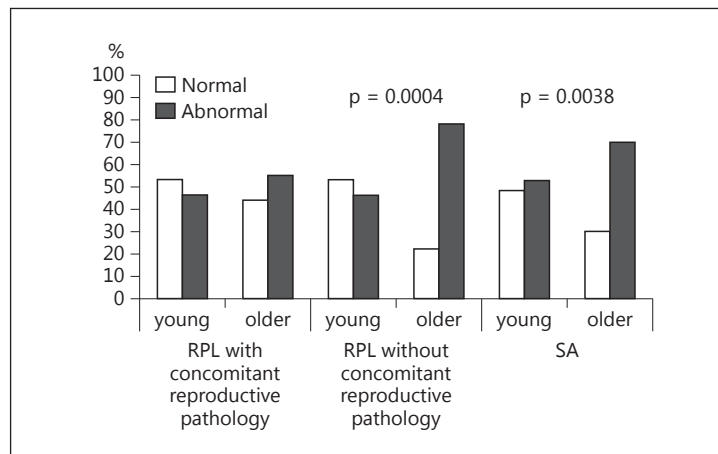
There were no significant differences in the chromosomal abnormality rate between abortions in RPL patients with previous live births [81/166 (48.8%)] and childless women [152/276 (55.1%), $p = 0.20$].

Table 2. Distribution of karyotypes in RPL families with two or more abortions studied

First abortion karyotype	Second abortion karyotype	Women, n (embryos)	Percentage	Average maternal age, years
Normal	normal	13 (26)	86.7	27.9
	abnormal	2 (5) ^a	13.3	39.0 (p = 0.002)
Abnormal	normal	4 (10) ^b	22.2	31.3
	abnormal	14 (28)	77.8	30.7

^a One woman had three karyotyped abortions (the first abortion had a normal karyotype, whereas the second and third abortions had abnormal karyotypes). ^b Two women had three karyotyped abortions (in both patients, the first abortion had an abnormal karyotype, whereas the second and third abortions had normal karyotypes).

Fig. 3. Rates of abortion karyotypes for women with SA and RPL according to the mother's age and the presence of concomitant reproductive pathology. Clear bars: normal karyotypes; dark bars: abnormal karyotypes. Young mothers: ≤ 34 years; older mothers: ≥ 35 years.



The chromosomal abnormality rates of abortions according to concomitant reproductive pathology and age of the RPL mothers are shown in figure 3. Among the 109 miscarriages in RPL women with concomitant reproductive pathology, abnormal karyotypes were found in 34/73 abortions (46.6%) from younger (≤ 34 -year-old) women and in 20/36 abortions (55.6%) from older (≥ 35 -year-old) women; the rates of abnormal karyotypes were similar between these groups (p = 0.38). For the 239 miscarriages from RPL women without concomitant reproductive pathology, the rate of abnormal karyotypes in the group of abortions from younger women was 46.5% (94/202), whereas this rate increased to 78.4% (29/37) for miscarriages among older women (p = 0.0004). Notably, the distribution of karyotype rates for SA was similar to that for RPL abortions in the group without concomitant reproductive pathology: abnormal abortions occurred significantly less frequently in younger than in older women [201/379 (53.0%) and 61/87 (70.1%), respectively; p = 0.0038] (fig. 3).

Discussion

To date, six articles have been published comparing sporadic and recurrent abortions in the same study, with conflicting results, as shown in table 3. We analysed more than 440 embryos in each group, and the frequency of abortions with normal karyotype was significantly higher in women with recurrent miscarriages compared with women with SA (53 vs.

Table 3. Rates of normal embryonic karyotypes in RPL and SA in different studies

Authors [Ref.], year	Population	RPL criterion ^a , n	Average maternal age, years	SA	RPL	p
Stern et al. [17], 1996	USA and Mexico	2	–	56/130 (43.1%)	40/94 (42.6%)	n.s.
Ogasawara et al. [18], 2000	Japan	2	31	27/114 (23.7%)	114/234 (48.7%)	0.000014
Sullivan et al. [19], 2004	USA	2	31	77/133 (57.9%)	91/122 (74.6%)	0.0051
Marquard et al. [16], 2010	USA	3	39	42/140 (30.0%)	11/50 (22.0%)	n.s.
Grande et al. [15], 2012	Spain	2	35	50/154 (32.5%)	73/199 (36.7%)	n.s.
Choi et al. [20], 2014	Korea	2	31	81/164 (49.4%)	31/86 (36.0%)	0.043
Present study	Russia	2	29	202/466 (43.3%)	234/441 (53.1%)	0.0034

n.s. = No significant difference. ^a RPL criterion: minimal amount of pregnancy losses as RPL indicator.

43%; $p = 0.0034$), which agreed with the results of Ogasawara et al. [18] and Sullivan et al. [19]. To compare the results from different studies, it is necessary to consider the peculiarities of each sample, the most important of which are the criterion for RPL (2 or 3 miscarriages) and the patient age. Our investigation considered two or more pregnancy losses to be recurrent abortion, in agreement with most other studies. The average maternal age in the present study (slightly less than 29 years) was not advanced and was minimal in comparison with the other studies. Another difference between our study and the others is the control SA group, which included the first miscarriages that occurred only in women with a prior normal pregnancy, defined as a live birth or elective termination. Other authors considered SA as any first pregnancy loss.

The mother's age is the most important factor that directly affects the frequency of chromosomal abnormalities in the embryo [21]. This finding has been confirmed by most RPL studies. In agreement with Stephenson et al. [22], our study found a high rate of normal karyotype abortions among young women in the RPL group compared to the SA group, whereas no such pattern was found in older women. To ascertain the age limits of this phenomenon, we compared the frequencies of abortions with karyotypic abnormalities from women with sporadic and recurrent miscarriages by stratifying them into five age groups (≤ 24 , 25–29, 30–34, 35–39, and ≥ 40 years). We found that the rate of normal embryonic karyotypes was significantly higher for RPL versus SA only for women younger than 30 years. Perhaps this fact explains the minimal rate of karyotypically normal abortions and the absence of differences between RPL and SA groups in some studies with high average maternal ages [15, 16]. This finding suggests that in young RPL women, non-cytogenetic factors are more common, and they cause miscarriage more often.

In this study, we excluded patients with established predisposing factors for RPL (antiphospholipid syndrome, hereditary thrombophilia, parental chromosomal abnormalities, uterine structural anomalies, hypothyroidism, and polycystic ovary syndrome). When our idiopathic RPL data were analysed, taking into consideration the women's reproductive health (immunological, endocrinological, and inflammatory diseases of the female reproductive tract, recorded as concomitant reproductive pathologies), we found that the maternal age-related alterations in the rate of embryonic chromosomal abnormalities differed between RPL women with and those without concomitant reproductive pathologies. In the group with concomitant reproductive pathology, the frequency of chromosomal abnormalities in abortions did not change with increasing maternal age. In RPL patients without concomitant reproductive pathology, the proportion of embryos with abnormal karyotypes increased

substantially with maternal age (from 47 to 76%). A similar pattern was observed in women with sporadic miscarriages: abortions with abnormal karyotypes were significantly less common in younger than in older women (54 vs. 71%) (fig. 3). Therefore, the presence of concomitant reproductive pathologies is a continuous factor that increases the frequency of the loss of karyotypically normal embryos in older women.

How often is recurrent aneuploidy a cause of recurrent miscarriage? Because the mean chromosomal abnormality frequency in human abortions is approximately 50%, subsequent abortions should be cytogenetically abnormal in one half of cases, irrespective of the karyotype of the previous miscarriage. However, in our study, the abnormal karyotype rate among subsequent abortions was 77.8% after a previous abortion with an abnormal karyotype and 13.3% after a previous normal pregnancy loss (OR = 22.75). Our data agree with those of Hassold [23] and imply the possibility of a non-random distribution of abortion karyotypes in some women with RPL. Perhaps there are two different states: (1) RPL as the result of recurrent chromosomal abnormalities in embryos and (2) recurrent death of embryos with normal karyotypes.

We observed a high likelihood (42.4%) that both cases of pregnancy loss in families with two karyotyped abortions were caused by embryonic chromosomal abnormalities. In a similar study by Sullivan et al. [19], recurrent aneuploidy was detected in only 10% of 30 RPL families with two karyotyped abortions. Both groups of abortion represented idiopathic recurrent miscarriage, and the average maternal ages were similar (32.0 and 28.9 years in the Sullivan study and our study, respectively). The causes of this discrepancy may be related to the small number of families studied or to the influence of unidentified predisposing factors.

A high rate of recurrent aneuploidy in abortions was found in an analysis of 2,856 karyotypes that were obtained in prenatal diagnoses of women with previous trisomic pregnancies or spontaneous abortions with trisomy [24]. The authors found an increased risk of heterotrismy (trisomy of another chromosome) in a subsequent pregnancy if the previous pregnancy had been trisomic. Data from preimplantation embryos suggest that the risk of trisomy varies among younger women of the same age and that a history of trisomic conception is associated with an increased risk of another aneuploid conception [25]. Delhanty et al. [26] found that some women produce 'chaotic' embryos more often than do other women. These findings support the hypothesis that some women have an increased risk of chromosomal non-disjunction in oogenesis or later in early embryogenesis compared with other women of the same age.

A special study of families with multiple aneuploid abortions found no increased frequency of the same anomaly in the family; therefore, gonadal mosaicism is quite rare and does not make a significant contribution to the aetiology of recurrent miscarriage [27]. Data from Munné et al. [25] also do not support gonadal mosaicism as a common cause of an increased rate of aneuploidy. Possible causes of an elevated frequency of meiotic non-disjunction may include genetic variability in the meiotic recombination rate, variation in genes involved in oocyte maintenance or division, mutations in the genes that control the process of meiosis, changes in meiotic spindle formation, and the biological process of ovarian ageing, which may vary between women of similar chronological age [28–30]. Although a recent study of the associations between embryonic aneuploidy and maternal genome variants did not find any relationship between the mother's genotype and the rate of meiotic errors, the authors found a strong genetic association between the mother's genotype and the rate of observed mitotic errors in early embryogenesis [31]. Perhaps there exist exogenous factors that influence the fidelity of the meiotic process [32].

It was previously reported that a normal chromosomal status of a dead embryo significantly increased the likelihood that subsequent abortions would have a normal karyotype

[33, 34]. In our work, if the first abortion had a normal karyotype, the likelihood was 86.7% that a subsequent pregnancy loss would be euploid; the only exception was embryos from older women (table 2).

A normal karyotype of previous abortions in RPL women was associated with poor prognosis in a subsequent pregnancy. Previously, it was reported that 62% of following pregnancies would end with miscarriage in patients with karyotypically normal abortions, whereas only 38% of women with karyotypically abnormal abortions subsequently aborted ($p = 0.001$) [18]. In our study, although the mean ages of the women in the RPL and SA groups did not differ, the number of pregnancies was significantly greater in the RPL women than in the SA women. Thus, RPL women of the same age had more pregnancies and more losses of embryos with normal karyotypes than had SA women.

The contributions of various causes to the RPL aetiology differ for women of different ages: non-cytogenetic factors prevail among the causes of recurrent miscarriage in young patients, whereas chromosomal non-disjunction is a greater contributor among older women. Three types of idiopathic recurrent miscarriage can be distinguished: (1) pregnancy losses that occur accidentally in women who have no actual permanent pathological factors [35]; (2) abortions that occur in women with an increased frequency of chromosomal non-disjunction as a result of embryonic karyotypic abnormalities, and (3) repeated loss of embryos with normal karyotypes due to pathologies unidentified by conventional clinical studies. The first category has a favourable prognosis for live birth in a subsequent pregnancy (i.e. the mean population level at this age). Patients in the second group can increase the likelihood of a successful pregnancy through preimplantation genetic screening [36, 37]. The prognosis for women in the latter group of idiopathic RPL is less favourable. This group may be the most valuable for studying possible reasons for idiopathic recurrent embryonic death, including epigenetic abnormalities [38], copy number variations [6, 39, 40], failure of endometrial selective function [41, 42], sperm DNA fragmentation [43], telomere DNA deficiency [44], environmental exposure, and other circumstances [32].

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Disclosure Statement

The authors declare that no conflicts of interest exist.

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