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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Embryoscopy and karyotype findings of repeated miscarriages in recurrent pregnancy loss and spontaneous pregnancy loss

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Abstract

Purpose The aim of the study was to assess cytogenetic and embryoscopic characteristics in subsequent miscarriages of spontaneous pregnancy losses (SPL) and recurrent pregnancy losses (RPL).

Methods A retrospective cohort of 75 women was affected by repeated pregnancy loss. Of those, 34 had SPL, 24 primary RPL, and 17 secondary RPL. Ploidy status and morphology was analyzed by transcervical embryoscopic examination of the embryo and cytogenetic analysis of the chorionic villi in subsequent miscarriages.

Results Similar rates of recurrent ploidy status were observed between first and second miscarriage in SPL and RPL (82.4% recurrent ploidy status in SPL, $p > 0.999$; 73% recurrent ploidy status in RPL, $p = 0.227$). No difference was found regarding recurrent abnormal morphology between SPL and RPL ($p = 0.092$). However, secondary RPL resulted significantly more often in recurrent abnormal morphology compared to primary RPL ($p = 0.004$).

Conclusions High rates of recurrent normal/abnormal karyotypes were observed in all groups with a majority of embryos presenting with recurrent abnormal morphology. Secondary RPL presented significantly more often with recurrent abnormal morphology compared to primary RPL. These findings offer prognostic information for the affected patient and might impact treatment choice.

Keywords Abnormal embryonic development · Chromosome abnormalities · Missed abortion · Transcervical embryoscopy · Repeated pregnancy loss

Introduction

More than 50% of women experience one or more miscarriages in their lifetime. Most miscarriages occur until the 12th week of gestation and it is estimated that 15% of clinically recognized pregnancies are affected [1]. Not only in spontaneous pregnancy loss (SPL) but also in recurrent pregnancy loss (RPL)—defined as three or more consecutive pregnancy losses [2]—genetic causes lead to developmental arrest and miscarriage and a majority of embryos present with morphological defects [3].

However, in the cases of secondary RPL (live birth followed by RPL), abnormal karyotype rates were significantly higher than in primary RPL (RPL with no previous live birth) where maternal factors could play a more prominent role [3, 4]. Possible maternal factors affecting RPL are coagulation and immune disorders and anatomic abnormalities. Therefore, RPL checkup includes parental karyotype, maternal lupus anticoagulant, anticardiolipin antibodies, anti-ß2 glycoprotein 1, thyroid hormone, and prolactin serum levels as well as anatomic examination with hysteroscopy, hysterosalpingogram, or sonohysterogram [5].

To date, little is known about the recurrence rate of embryonic morphology and cytogenetic defects in spontaneous and recurrent pregnancy loss. In SPL, euploid karyotype of the first abortion was associated with recurrent euploidy in a consecutive miscarriage but there was no association of repeated trisomy [6].

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In RPL, more than 70% of embryos presented with recurrent abnormal karyotypes [4]. Other studies suggested low successive abnormal karyotype rates in RPL patients [7].

No study previously performed repeated transcervical embryoscopy analysis in subsequent miscarriages and analyzed the morphological and cytogenetic characteristics in RPL and SPL. Therefore, the objective of this study was to investigate differences in karyotype patterns and morphology between the first and second pregnancy losses in RPL and SPL.

Methods

Seventy-five women affected by first-trimester recurrent and non-recurrent pregnancy loss were included in the study. SPL was defined as less than three pregnancy losses irrespective of previous live births. Patients were included in the RPL group as soon as they presented with their third consecutive pregnancy loss. Only consecutive pregnancy losses were used for RPL diagnoses. Primary RPL was defined as three or more consecutive pregnancy losses with no previous successful pregnancy. Secondary RPL included women with three or more consecutive pregnancy losses after at least one live birth [2]. Only non-viable first-trimester pregnancies with ultrasonographic evidence of a negative fetal heartbeat between the 7th and 13th gestational weeks were included in this study. Patients have been referred for dilatation and curettage (D&C) and missed abortion diagnosis was confirmed by transvaginal ultrasound. All patients underwent transcervical embryoscopic and cytogenetic evaluation of the non-viable embryo in the Danube Hospital (Vienna, Austria).

The study was approved by the ethics committee of the hospital, and informed consent was obtained from all patients. Transcervical embryoscopy was described in detail before [3, 8]. Briefly, transcervical embryoscopy and subsequent D&C were performed under intravenous general anesthesia. After careful dilatation of the cervix, the rigid hysteroscope (12-degree angle of view with both biopsy and irrigation working channel, Circon Ch 25–8 mm) was inserted transcervically into the uterine cavity and the pregnancy was visualized. To provide good operative visibility, continuous normal saline flow was used throughout the procedure (pressure ranging from 40 to 120 mmHg).

Embryoscopic findings were classified into three categories: [1] embryos showing normal development, [2] embryos with isolated or combined external defects, and [3] growth-disorganized (GD) embryos. All morphological defects detected in category 2 embryos were documented regarding location and severity. Category 3 embryos were subdivided according to their growth disorganization using the classification of Poland et al. [9]. Category 2 and 3 embryos were classified as "abnormal morphology" while category 1 embryos as "normal morphology." Cytogenetic results were categorized in euploid (normal karyotype), trisomies, monosomy X, polyploidies, structural anomalies, and others (double trisomies or a combination of cytogenetic defects). All non-euploid findings were classified as "abnormal karyotypes" and euploid samples as "normal karyotypes."

Karyotyping was attempted in all cases. Chorionic villi sampling was performed through direct chorion biopsy. The chorionic villi were placed in normal saline and carefully dissected. They were then placed in culture medium (Chang Medium C; Irvine Scientific, Santa Ana, CA) and immediately forwarded to the cytogenetic laboratory for further processing. Cytogenetic results were obtained using standard G-banding cytogenetic techniques or comparative genomic hybridization in combination with flow cytometry analysis (CGH/FCM) if standard karyotyping failed [10, 11].

Statistical analysis

Primary outcome measure was set as differences in karyotypes between the first and second pregnancy losses in SPL and RPL patients. Secondary outcome measures included differences in morphologic characteristics between the first and second pregnancy losses, recurrent karyotype aberrations, and recurrent euploidy. Subgroup analyses were performed in primary and secondary RPL patients.

To analyze differences in morphological and cytogenetic data between the first and second pregnancy losses, McNemar's test for paired nominal data was applied. Group-specific differences were assessed using Student's t test for continuous variables and chi-square test for nominal data. Two-sided statistical significance level was set to 0.05. Statistical analysis was performed with SPSS version 24 for Mac (IBM Corp., USA).

Results

Out of the 75 included patients, in 16 women one previous pregnancy loss had already been karyotyped before inclusion in the study but no embryoscopy had been performed, the remaining 59 women underwent repeated transcervical embryoscopy and karyotyping in subsequent pregnancy losses. Thirty-four patients had SPL and 41 RPL (24 primary RPL and 17 secondary RPL). Repeated transcervical embryoscopy was performed in 24 SPL patients (one patient with three pregnancy losses who did not fulfill RPL criteria due to a live birth in between the miscarriages) and 35 RPL patients (21 primary RPL (two patients with three pregnancy losses) and 14 secondary RPL (two patients with three pregnancy losses) patients) (Fig. 1).

The mean age was similar between RPL and SPL patients (34.72 vs. 33.05 years respectively, $p = 0.782$) and between primary and secondary RPL patients (34.53 vs. 35.00 years respectively, $p = 0.921$).

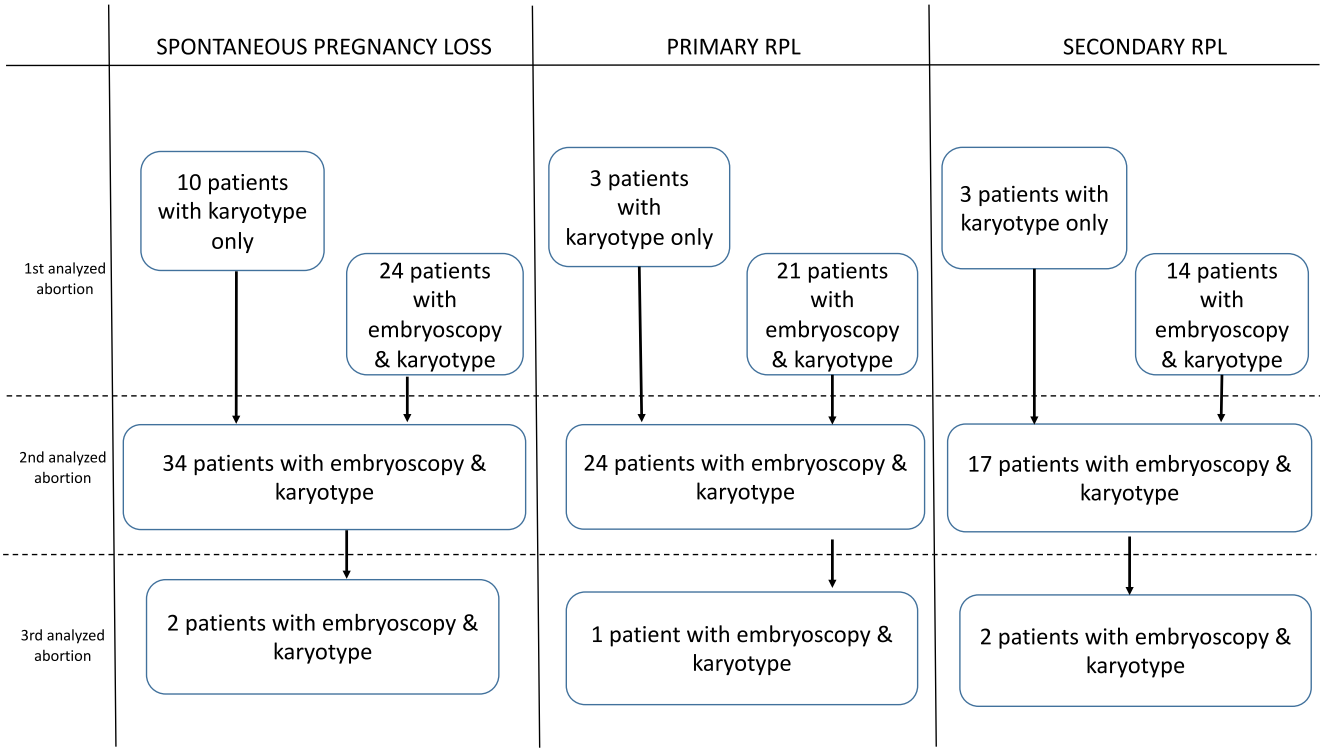

Fig. 1 Flow-chart showing patient recruitment in the spontaneous pregnancy loss and primary and secondary RPL groups in their first, second, and third analyzed miscarriage. Aberrations: RPL recurrent pregnancy loss

Morphology

Embryoscopy findings revealed that 81% of pregnancy losses had repeated abnormal morphology. In SPL, no significant difference could be found regarding morphology of the first and second miscarriage (92% recurrent abnormal morphology ($n = 22$); $p > 0.999$). In RPL, no significant difference was observed between the first and second pregnancy loss regarding normal or abnormal morphology (83% recurrent normal/abnormal morphology ($n = 29$); $p = 0.219$). No difference was observed when comparing SPL with RPL regarding frequency of repeated abnormal morphology ($p = 0.092$). However, statistically significantly higher numbers of recurrent abnormal morphology were observed in secondary RPL compared with primary RPL (100 vs. 57% of recurrent abnormal morphology in secondary and primary RPL, respectively; $p = 0.004$) (Table 1). Five patients underwent embryoscopy three times; of those, three patients (60%) had recurrent abnormal morphology and two patients a mixed pattern. Detailed morphology distribution in the different groups is presented in Table 2.

Cytogenetic results

In the cases of repeated karyotyping, 56% of samples showed recurrent abnormal karyotypes between the first and second pregnancy losses in the overall group ($p = 0.332$).

In SPL, no differences between the first and second pregnancy losses could be found (82.4% recurrent ploidy status ($n = 28$); $p > 0.999$). (Table 1).

Similarly, in patients with RPL, there was no statistically significant difference regarding abnormal or normal karyotypes between the first and second pregnancy losses (73% recurrent ploidy status ($n = 30$); $p = 0.227$). Comparable results were obtained when analyzing secondary RPL (76.5% recurrent ploidy status ($n = 13$); $p > 0.999$) and primary RPL (70.8% recurrent ploidy status ($n = 17$); $p = 0.125$). Recurrent abnormal karyotypes ($p = 0.350$) and recurrent euploidy ($p = 0.447$) were not statistically significantly different between primary and secondary RPL. Out of the six patients undergoing cytogenetic analysis three times, two patients (33.3%) showed recurrent karyotype aberrations, three patients (50%) recurrent euploidy, and one patient a mixed pattern. Detailed karyotype distribution in the different groups is presented in Table 3.

Discussion

In the present study, we provide morphological and cytogenetic characteristics in SPL, RPL, and RPL phenotypes in subsequent miscarriages. Recently, focus was set on RPL phenotypes, which were suggested to show distinctive differences in their etiology [3, 12]. Only few studies investigated cytogenetic findings in repeated miscarriages in RPL.

Table 1 Morphological and karyotype analysis of primary and secondary RPL as well as non-recurrent pregnancy losses in repeated embryoscopy

	Recurrently normal	Recurrently abnormal	Mixed
Karyotype overall ($n = 75$)	16 (21%)	42 (56%)	17 (23%)
Morphology overall ($n = 59$)	3 (5%)	48 (81%)	8 (14%)
Non-RPL karyotype ($n = 34$)	9 (27%)	19 (56%)	6 (28%)
Non-RPL morphology ($n = 24$)	0	22 (92%)	2 (8%)
RPL karyotype ($n = 41$)	7 (17%)	23 (56%)	11 (27%)
RPL morphology ($n = 35$)	3 (9%)	26 (74%)	6 (17%)
Prim RPL karyotype (n = 24)	5 (21%)	12 (50%)	7 (29%)
Prim RPL morphology ($n = 21$)	3 (14%)	12(57%)	6 (29%)
Sec RPL karyotype (n = 17)	2 (12%)	11 (65%)	4 (24%)
Sec RPL morphology ($n = 14$)	0	14 (100)	0

Previous studies suggest that, depending on maternal age, 30% up to more than 60% of RPL embryos present with chromosomal aberrations [13]. Nonetheless, when analyzing subsequent pregnancy losses, some studies failed to describe recurrent clinical karyotype patterns while others reported recurrent abnormal karyotype rates in > 70% in RPL; however, to our knowledge, no study analyzed recurrent embryo morphology pattern and no study distinguished RPL phenotypes [4, 7]. In the present study, 73% of patients with RPL had the same karyotype pattern (recurrent normal/recurrent abnormal karyotype) in their first and second recurrent pregnancy loss. Recurrent abnormal karyotype occurred in 56% of cases and no significant difference was found between the first and second miscarriage karyotype in patients of all groups. However, in primary RPL, there was a significantly higher number of recurrent normally developed embryos in the first and second analyzed pregnancy loss. These findings could suggest a more pronounced role of maternal factors in this group of patients. A large majority (> 90%) of both secondary RPL and spontaneous miscarriage embryos presented with recurrent abnormal morphology, while only 57% of primary RPL embryos were categorized as recurrently morphologically abnormal. A recent study could show significantly higher chromosomal aberration rates in secondary RPL compared to primary RPL. Hence, embryos of secondary RPL significantly more often presented with abnormal morphology compared to primary RPL [3].

Recurrent euploidy and high number of previous miscarriages were identified as negative prognostic factors for RPL patients [4, 14]. However, the etiology of abnormal embryonal morphology in case of euploid karyotype is not yet fully understood. Previous studies could detect submicroscopic genetic changes including copy number variants (CNV) in several euploid and morphologically abnormal embryos [11, 15, 16]. Another very recent study found compound heterozygous mutations associated with embryonic morphological abnormalities in half of the investigated families with idiopathic euploid recurrent pregnancy loss [17]. Coincidently, in those patients karyotyped three times in our study, a majority presented with recurrent euploidy but recurrent abnormal morphology.

These findings are of essential value for patients suffering from RPL. Primary karyotyping of the embryo in the second miscarriage was shown to be more cost-effective than performing routine maternal RPL screening [18, 19]. By adding morphological data through embryoscopy, additional insights in RPL etiology might be obtained and might affect future diagnostics and patients' treatment. Embryo morphology is closely correlated to karyotype abnormalities and can indicate undiagnosed genetic aberrations [20].

In the case of euploidy and normal morphology, additional maternal evaluation should be performed. In aneuploid pregnancy loss with abnormal morphology, preimplantation genetic screening could be offered as a therapeutic approach. In euploid pregnancy loss with abnormal morphology, novel techniques like next-generation sequencing (NGS) should be applied to detect possible submicroscopic changes.

Euploid karyotype was associated with significantly more pathological findings in conventional maternal RPL screening than aneuploid karyotypes [21]. In secondary RPL however, due to the high recurrence of abnormal karyotypes, preimplantation

Table 2 Morphologic characteristics of embryos in spontaneous pregnancy loss (SPL) and recurrent pregnancy loss (RPL)

	Normal development	Isolated defects	Combined defects	Growth disorganized
SPL embryos ($n = 49$)	2 (4.1%)	2 (4.1%)	20 (40.8%)	25 (51%)
Prim RPL embryos ($n = 44$)	12 (27.3%)	1 (2.3%)	15 (34.1%)	16 (36.4%)
Sec RPL embryos ($n = 30$)	0 (0%)	3 (10%)	14 (46.7%)	13 (43.3%)

Table 3 Karyotype characteristics of embryos in spontaneous pregnancy loss (SPL) and recurrent pregnancy loss (RPL)

	Euploid	Trisomy	Monosomy	Polyploidy	Other
SPL embryos ($n = 69$)	26 (37.7%)	31 (44.9%)	5 (7.2%)	6 (8.7%)	1 (1.5%)
Prim RPL embryos ($n = 51$)	20 (39.2%)	20 (39.2%)	3 (5.9%)	1 (2.0%)	7 (13.7%)
Sec RPL embryos ($n = 37$)	9 (24.3%)	19 (51.4%)	3 (8.1%)	3 (8.1%)	3 (8.1%)

genetic screening (PGS) might be offered to patients as a therapeutic option. On the other hand, both secondary RPL and aneuploid RPL were associated with favorable cumulative live birth results compared to euploid RPL [4]. One recent study could show similar outcome in RPL patients when expectant management was applied compared to PGS [22]; however, this study did not distinguish between different RPL phenotypes and no information was obtained regarding cytogenetic pathologies in previous miscarriages. Future studies should focus on adapting treatment strategies according to RPL phenotypes. The application of PGS in non-infertile RPL patients should therefore be investigated in future studies, preferably focusing on secondary RPL patients or patients with a history of aneuploid recurrent pregnancy loss. Also, new genetic methods like next-generation sequencing (NGS) might provide additional insight to possible genetic factors related to RPL in the large group of supposedly euploid embryos with high rates of morphological defects [23].

To date no study described repeated embryoscopy in subsequent pregnancy losses. The strengths of our study include the analysis of morphological miscarriage characteristics in different RPL phenotypes with direct embryoscopy-guided sampling of the POC. Through standard dilatation and curettage (D&C), maternal contamination can occur resulting in false negative results [24]. In the present study, 44% of euploid samples were diagnosed as XY, which is in accordance with published literature ruling out maternal contamination [24]. As a main weakness of our study, sample sizes of subgroup analyses were small. Hence, in the subgroup analyses, p values were interpreted in an explorative manner. Another weakness is due to the retrospective character of our study.

To reach maximum statistical power, we also included karyotype samples of previous miscarriages where no embryoscopy was performed. Due to the small sample size, specific morphology and karyotype abnormalities could not be analyzed in detail. In all groups, a wide range of morphological defects and genetic aberrations was observed with different abnormalities occurring in subsequent pregnancies in a majority of patients.

Our data suggest that RPL (primary and secondary), as well as SPL, patients show high recurrence rates of abnormal karyotypes with a majority of embryos having recurrent abnormal morphology. However, in patients with primary RPL, there was a statistically significant lower number of recurrent morphologically abnormal embryos observed compared with secondary RPL. These findings might offer prognostic information for the affected patient and underline the potential different etiology of primary and secondary RPL. Future studies should further investigate the role of submicroscopic genetic changes in presumably euploid recurrent pregnancy loss and their impact on fetal wastage and morphologic anomalies.

Author contributions TP performed all embryoscopic investigations; AR performed all cytogenetic analysis; BH critically reviewed the manuscript and provided expert opinion. MF wrote the manuscript and performed the statistical analysis; all authors critically revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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