Transcervical embryoscopic and cytogenetic findings reveal distinctive differences in primary and secondary recurrent pregnancy loss

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Objective: To assess the cytogenetic and embryoscopic characteristics of primary and secondary recurrent pregnancy loss.
Design: Clinical prospective descriptive study.
Setting: Tertiary care center.
Patient(s): Nine hundred and eighty-four women affected by first-trimester pregnancy loss; 145 patients with recurrent pregnancy loss (RPL) and 839 patients with nonrecurrent pregnancy loss as controls.
Intervention(s): Transcervical embryoscopic examination of the embryo before uterine evacuation, and cytogenetic analysis of the chorionic villi by standard G-banding cytogenetic techniques.
Main Outcome Measure(s): Aneuploidy frequency in the primary and secondary RPL group and the nonrecurrent pregnancy loss (non-RPL) control group.
Result(s): Patients with RPL showed statistically significantly fewer aneuploid pregnancy losses (odds ratio [OR] 0.596; 95% confidence interval [CI], 0.40–0.88). Primary RPL was associated with lower aneuploidy rates compared with the non-RPL group (OR 0.423; 95% CI, 0.27–0.66) while secondary RPL was not (OR 1.414; 95% CI, 0.67–2.99). Patients with primary RPL had statistically significantly more morphologically normal embryos compared with non-RPL and secondary RPL.
Conclusion(s): Patients' embryos after primary and secondary RPL show distinctive differences in aneuploidy and morphologic defect rates. These findings suggest different treatment approaches for the patients with primary and secondary RPL. (Fertil Steril® 2017;107: 144–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: Abnormal embryonic development, chromosome abnormalities, missed abortion, transcervical embryoscopy, repeated pregnancy loss

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It is estimated that 15% of naturally conceived pregnancies result in miscarriage, with the majority occurring in the first 12 weeks of gestation. Up to 50% of all women experience at least one sporadic miscarriage in their life (1). Recurrent pregnancy loss (RPL), however, is estimated to affect 1% of couples (2). The most common cause of miscarriage is aneuploidies, causing 50%–70% of all pregnancy losses (3, 4), but other factors such as coagulation or immune disorders and anatomic abnormalities have also been associated with recurrent miscarriage (5). Thus, the diagnostic workup after RPL typically includes an analysis of the parental karyotype, maternal lupus anticoagulant, anticardiolipin antibodies, anti-β_2 glycoprotein 1, evaluation of maternal uterine anatomy by hysteroscopy, hysterosalpingogram, or sonohysterogram, and evaluation of thyroid or prolactin anomalies as suggested by the corresponding American Society for Reproductive Medicine guidelines (6). If this workup does not reveal any pathologic results, the RPL is designated as unexplained, and expectant management is suggested (7). Several studies have failed to show any beneficial effects for treatment strategies such as

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low-molecular-weight heparin, progesterone, or preimplantation genetic screening (PGS) in this group of unexplained RPL (7–9).

However, due to the high prevalence of RPL, cytogenetic analyses of fetuses in recurrent miscarriage are of high interest to determine the causes of miscarriage and make conclusions for further treatment (10). It has been found that the fetuses in early miscarriage have a high degree of morphologic abnormalities, correlating with cytogenetic findings (11). This valuable information is often lost by conventional evacuation of the uterus, but it can be obtained by transcervical embryoscopy, which allows precise tissue sampling of the embryo for further genetic analysis, with minimal risk of maternal cell contamination (12). In the present study, for the first time we have assessed the morphologic and cytogenetic characteristics in primary and secondary RPL.

MATERIALS AND METHODS

The study population included 984 women who were affected by first-trimester recurrent and nonrecurrent missed abortion. Pregnancies included were both natural conceptions and in vitro fertilization/intracytoplasmic sperm injection (IVF–ICSI) conceptions. Only pregnancies with ultrasonographic evidence of a negative fetal heartbeat were included in this study. The patients had been referred for detailed transcervical embryoscopic and cytogenetic evaluation of the nonviable embryo to the Danube Hospital (Vienna, Austria).

The study was approved by the ethics committee of the hospital, and informed consent for transcervical embryoscopy was obtained from all patients. The transcervical embryoscopy has been described in detail elsewhere (12). Briefly, transcervical embryoscopy and subsequent curettage 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 implantation site of the pregnancy was visualized. Continuous normal saline flow was used throughout the procedure (pressure ranging from 40 to 120 mm Hg) to clean the operative field.

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. Additionally, the uterine cavity has been assessed regarding anatomical anomalies during embryoscopy.

Karyotyping was attempted in all cases. Chorionic villi were obtained by direct chorion biopsies. The chorionic villi were placed in normal saline and carefully dissected. They were then placed in culture medium (Chang Medium C; Irvine Scientific) and immediately forwarded to the cytogenetic laboratory for further processing. The tissue was subsequently cultured and analyzed cytogenetically, using standard G-banding cytogenetic techniques. Comparative genomic hybridization in combination with flow cytometry analysis (CGH/FCM) of paraffin-embedded or frozen placental tissue was performed in 51 cases in which traditional cytogenetic analysis had failed to provide results (13).

Primary RPL was defined as three or more consecutive pregnancy losses with no previous successful pregnancies. Secondary RPL included women with three or more consecutive pregnancy losses after a successful pregnancy (2). Patients were included in the recurrent miscarriage group as soon as they presented with their third consecutive pregnancy loss.

Statistical Analysis

As primary outcome measure, we chose aneuploidy frequency in the primary and secondary RPL group and the nonrecurrent pregnancy loss (non-RPL) control group. As secondary outcome measures, we chose frequency of morphologic defects in the primary and secondary RPL group and the non-RPL control group.

Categorical variables were analyzed using a chi-square test and multivariable regression analysis correcting for female age as a major confounder of aneuploidy. Continuous variables were analyzed using Mann-Whitney *U* test. All analysis were performed using SPSS version 23 (IBM) the statistical significance level was set to 0.05 two-sided.

RESULTS

Out of 984 investigated patients, 145 presented with recurrent miscarriage (95 primary RPL and 50 secondary RPL) and 839 controls with nonrecurrent pregnancy loss. Patients in the non-RPL control group were statistically significantly younger than the patients with RPL (Table 1). Out of 984 obtained samples, 961 could be used for further genetic analysis; 23 samples could not be analyzed due to growth failure. In multivariable regression analysis taking female age into account, patients with RPL showed statistically significantly lower odds of having an aneuploid embryo (odds ratio [OR] 0.596; 95% confidence interval [CI], 0.40–0.88; $P=.009$).

When we performed the subgroup analysis, the patients with primary RPL showed statistically significantly lower odds of aneuploid pregnancy compared with the non-RPL group (OR 0.423; 95% CI, 0.27–0.66; $P<.001$) and with patients with secondary RPL (OR 0.298; 95% CI, 0.13–0.70; $P=.006$). Patients with secondary RPL did not show any differences regarding aneuploid pregnancy compared with the non-RPL group (OR 1.414; 95% CI, 0.67–2.99; $P=.365$) (Fig. 1).

The distribution of the karyotype characteristics is visualized in Figure 2. Patients with RPL showed comparable numbers of previous abortions in the euploid and the aneuploid RPL groups (2.70 vs. 2.51 in the euploid vs. the aneuploid RPL groups, respectively; $P=.661$).

Patients with RPL did not show statistically significant differences regarding normally developed embryos compared with the non-RPL group ($P=.480$). In the subgroup analysis, patients with primary RPL had a statistically significantly higher number of normally developed embryos compared with the patients with secondary RPL ($P=.012$) and non-RPL ($P=.040$). The number of normally developed embryos was not statistically significantly different between the secondary RPL and the control group ($P=.080$) (see Table 1). Generally, aneuploidy was correlated with morphologic

TABLE 1

Characteristics of the recurrent pregnancy loss study population.

Characteristic	Total (n = 145)	Recurrent pregnancy loss		Control (n = 839)
		Primary (n = 95)	Secondary (n = 50)	
Age (y)	34.34 ± 5.76	33.94 ± 5.58[a,b]	35.10 ± 6.08[c]	31.48 ± 6.32[d]
IVF–ICSI, n (%)	17 (11.7%)	14 (14.7%)[a]	3 (6%)[e]	83 (9.9%)[f,g]
No. of previous abortions	2.59 ± 1.02	2.48 ± 0.90[a,b]	2.78 ± 1.22[c]	0.36 ± 0.59[d]
CRL	11.66 ± 10.21	12.65 ± 11.4[a]	9.78 ± 7.18[e]	12.68 ± 12.08[f,g]
Morphologic defects, n (%)	129 (89%)	80 (84.2%)	49 (98%)[e,h]	762 (90.8%)[f,i]
Amplified samples, n (%)	142 (97.9%)	94 (98.9%)	48 (96%)	819 (97.6%)
46, XX/46, XY karyotype, n (XX%)	26/24 (52%)	21/20 (51.2%)[a]	5/4 (55.6%)[e]	119/108 (52.4%)[f,g]
Aneuploidies, n (%)	92 (64.8%)	53 (56.4%)[b]	39 (81.3%)[e,h]	592 (72.3%)[j]

Note: CRL = crown-rump length; RPL = Recurrent pregnancy loss; NS = not statistically significant.
[a] Primary versus secondary RPL (NS).
[b] Primary RPL versus control, $P<.001$
[c] Secondary RPL versus control, $P<.001$.
[d] Control versus RPL, $P<.001$.
[e] Secondary RPL versus control (NS).
[f] RPL versus control (NS).
[g] Primary RPL versus control (NS).
[h] Primary versus secondary RPL, $P<.05$.
[i] Primary RPL versus control, $P<.05$.
[j] RPL versus control, $P<.05$.

Feichtinger. Transcervical embryoscopy in RPL. Fertil Steril 2016.

defects ($P<.001$), but even in euploid embryos a majority of embryos appeared with morphologic defects. The distribution of the morphologic characteristics in euploid and aneuploid pregnancy losses is shown in Figure 3.

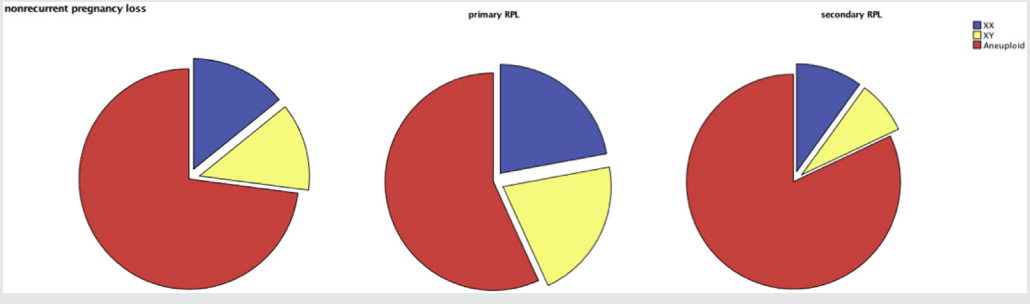
DISCUSSION

In the present study, women with RPL showed statistically significantly lower levels of aneuploidy compared with women with non-RPL. However, patients with secondary RPL showed no differences in aneuploidy rates compared with the non-RPL patients. Patients with primary RPL had a higher number of morphologically normal embryos compared with the non-RPL and secondary RPL group. Still, a majority of embryos presented with morphologic defects.

Patients with aneuploid and euploid RPL show distinctive differences in their prognosis. Euploid pregnancy loss raises the odds for a consecutive euploid pregnancy loss, with correlated detrimental prognosis compared with aneuploid pregnancy loss (3, 14, 15). On the other hand, embryos from patients undergoing preimplantation genetic diagnosis after viable and nonviable trisomic pregnancies were associated with high rates of aneuploidy, suggesting a high risk of repeated embryonic aneuploidy also in this group (16). The risk of suffering spontaneous pregnancy loss and RPL is associated with increased maternal age and fast-rising oocyte aneuploidy rates in this group (3). Consequently, maternal age and number of previous miscarriages are detrimental prognosis factors to achieve one healthy pregnancy (17).

It may be assumed that in the present study a large amount of patients after aneuploid secondary RPL already had aneuploid pregnancy loss before. Even though the probability to have a consecutive aneuploid conception is high (16), it may be emphasized that there is a chance for euploid conception after repeated attempts. However, if the RPL is caused by maternal factors, consecutive pregnancies will not lead to a live-birth, hence euploid RPL

FIGURE 1



Distribution of karyotypes in the control, primary recurrent pregnancy loss (RPL), and secondary RPL groups.

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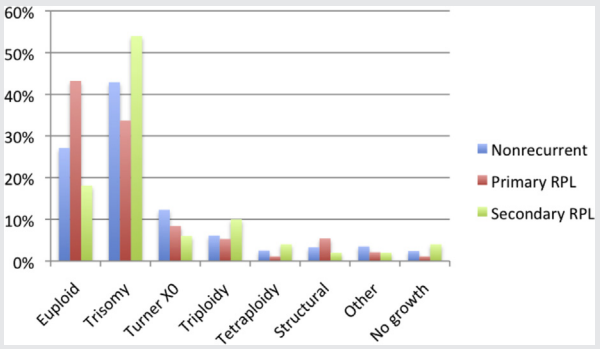
FIGURE 2



Visualization of the distribution of karyotype aberrations. RPL, recurrent pregnancy loss.

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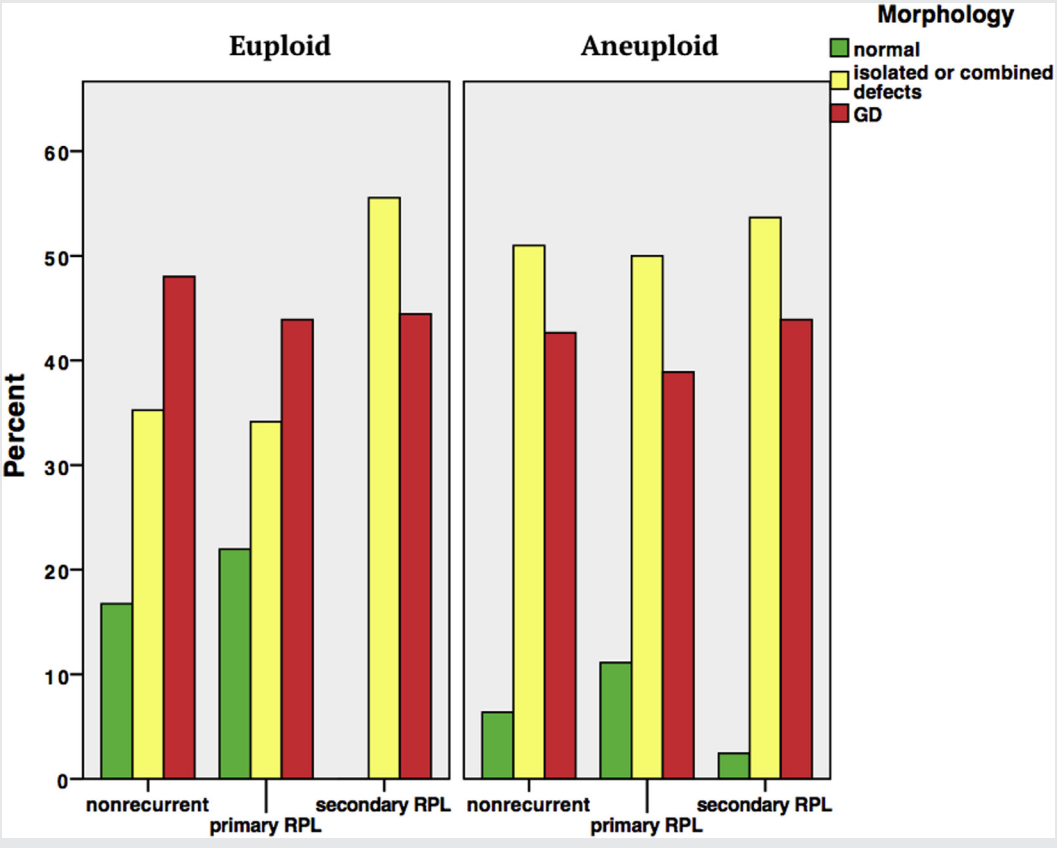
might be associated with an overall inferior prognosis (15). Therefore, routine checkup of RPL consisting of uterine anatomy evaluation, parental karyotypes, maternal lupus anticoagulant, anticardiolipin antibodies, anti-β_2 glycoprotein 1, and thyroid and prolactin levels is recommended (6). In cases with a family history of thromboembolic events, additional tests for coagulation disorders are encouraged (6).

After routine checkup, a majority of patients will present with unexplained RPL (1). Several studies have investigated different therapeutic approaches in this group of patients. Neither progesterone supplementation nor low-molecular-weight heparin could provide positive effects in preventing unexplained RPL (8, 9). Other studies have suggested the treatment with intravenous immunoglobulins (IVIG) in cases of secondary unexplained RPL, but randomized studies failed to show any positive effect on pregnancy or live-birth rates (18–20). Taking our findings into account, this is not surprising because embryos of patients having RPL presented with high aneuploidy rates, with embryos of secondary RPL having the highest aneuploidy rates of all groups. Therefore, it seems unlikely that recently suggested treatment approaches such as tumor necrosis factor-α antagonists or granulocyte-colony stimulating factor in unexplained RPL will prove effective in larger prospective studies if not performed in a selected group of patients with immunologic aberrations (21, 22).

FIGURE 3



Visualization of the distribution of morphology characteristics in the different groups in euploid and aneuploid pregnancy loss. GD, growth disorganized; RPL, recurrent pregnancy loss.

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In RPL, PGS could serve as a possible tool to avoid recurrent aneuploidy and achieve a healthy live birth (23). However, the results with PGS in RPL patients have been contradictory, with some investigators reporting beneficial results and another recent study reporting no effect compared with expectant management (7, 24). Patient selection, not distinguishing between primary and secondary RPL, might explain these diverging results. Our data show high rates of aneuploidy in embryos of patients with secondary RPL and spontaneous abortions, a group who could benefit most from PGS, whereas patients with primary RPL could benefit from conventional RPL screening due to their higher number of euploid embryos.

Euploid RPL was associated with statistically significantly higher detection rates in conventional RPL screening compared with aneuploid RPL (25). Hence, considering that most embryos in primary and secondary RPL are aneuploid, primary cytogenetic analysis of the fetuses in RPL is less expensive and more cost effective than a first-line complete RPL checkup (26, 27). Our data support these findings, considering that even patients with primary RPL had more than 50% aneuploid embryos. Furthermore, cytogenetic testing of RPL is not only encouraged for therapeutic reasons but might also be valuable for psychological reasons (6).

As already observed by previous studies, embryos show morphologic defects in most cases, even when a euploid karyotype is present (12). In patients with primary RPL we found statistically significantly more normally developed embryos compared with secondary RPL and non-RPL patients. However, even in this group, fewer than 20% of embryos showed normal development. The reason why euploid embryos sometimes display severe morphologic defects is not fully understood yet. Some investigators have reported DNA-copy number variation (CNV) aberrations in more than 30% of euploid embryos with morphologic defects (28, 29). So genetic factors contributing to morphologic embryonal defects seem to be underreported when classic karyotyping is applied. More modern microarray techniques, compared with standard karyotyping, offer the advantages of higher resolution, fewer tissue culture failures, and less maternal contamination (13, 30).

Due to the endoscopic-guided sampling of chorionic villi in our study we could support specific sampling of sufficient material for further analysis. Previous studies reported much lower aneuploidy rates in RPL (25%–30%) compared with our present analysis, which showed aneuploidy in more than 50% of cases (31, 32). This effect might be attributed to higher rates of contamination with maternal cells when standard uterine evacuation is performed, with more than 50% of sampled XX embryos misdiagnosed (33). In case of euploid XX results after karyotyping, some investigators therefore suggest microsatellite analysis (MSA), short-tandem-repeat (STR), or single-nucleotide-polymorphism (SNP) techniques to rule out maternal contamination (34, 35). In our present study the XX/XY rate was around 1, much lower that usually found in studies performing classic uterine evacuation, correlating with studies using sensitive SNP or microsatellite analysis to rule out maternal contamination (33, 36). Furthermore, due to the embryoscopic sampling in our study, 97.7% of samples could be genetically analyzed, a much higher rate compared with previously published data reporting around 50% to 75% of usable material (15, 31).

These advantages of the technique used in our study and the fact that age was correlated for as a cofactor in the statistical analysis might explain the higher rates of aneuploidy observed in our data. One previous study showed no differences in aneuploidy rates in primary and secondary RPL, but that study did not take the patients' age into account, a major factor contributing to aneuploidy (31).

Ours is the first study to evaluate the morphologic and cytogenetic characteristic of embryos in primary and secondary RPL. We found distinctive differences in patients affected by primary RPL compared with secondary RPL. Because high levels of aneuploidy have been observed in the secondary RPL group, these patients might benefit from PGS. All three groups showed high rates of morphologic defects, even in euploid embryos, with the primary RPL group showing statistically significantly higher numbers of morphologically normal embryos. Future studies should aim at evaluating the effect of PGS in secondary RPL patients and at further investigating the possible causes for morphology defects in euploid embryos.

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