ORIGINAL ARTICLE: EARLY PREGNANCY

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® 2016;■: ■–■. ©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