

Chromosome Abnormality Detection Rates of QF-PCR in Early Pregnancy Loss

GABRIELA POPESCU-HOBEANU^{1,2}, MIHAI-GABRIEL CUCU^{2,3},
ANCA-LELIA RIZA^{2,3}, IOANA STREATA^{2,3}, RAZVAN MIHAIL PLESEA^{2,3},
STEFANIA DOROBANTU^{2,3}, ADINA BARBU^{2,3}, LUCIAN GEORGE ZORILA^{4,5},
MARINA DINU^{4,5}, ANDA LORENA DIJMARESCU^{4,6}, STEFANIA TUDORACHE^{4,5},
DOMINIC ILIESCU^{4,5}, FLORIN BURADA^{2,3}

¹Doctoral School, University of Medicine and Pharmacy of Craiova, Romania

²Laboratory of Human Genomics, University of Medicine and Pharmacy of Craiova, Romania

³Regional Centre of Medical Genetics Dolj, Emergency Clinical County Hospital Craiova, Romania

⁴Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania

⁵Department of Obstetrics and Gynecology, Emergency Clinical County Hospital, 200642 Craiova, Romania

⁶Department of Obstetrics and Gynecology, "Filantropia" Clinical Municipal Hospital of Craiova, Romania

ABSTRACT: Early pregnancy loss (EPL) is the most common form of miscarriage and establishing its exact etiology is vital for the proper prognosis and management of possible future pregnancies. The aim of our study was to assess the incidence and types of chromosome abnormalities in product-of-conception (POC) samples analyzed by Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR). Two hundred fifty-seven POC samples meeting EPL criteria were referred to the Human Genomics Laboratory of the University of Medicine and Pharmacy of Craiova, Romania. DNA was extracted from both POC tissue and maternal blood samples, while PCR products were migrated on the ABI3730xl platform. A total of 124 samples were successfully analyzed, 46 cases (37.1%) showing various types of abnormalities, while no aneuploidies were found in the remaining 78 samples (62.9%). The most common abnormalities were monosomy X, followed by triploidy, trisomy 18, 16 and 15. The basic QF-PCR kit had an overall detection rate of 25.8%, but the detection rate rose to 37.1% when employing the extended kit. Our study proves that QF-PCR can be used as a first approach in the genetic analysis of POC, followed by conventional karyotyping (KT) or Chromosomal Microarray Analysis (CMA) as follow-up. QF-PCR is able to identify maternal cell contamination, as well as provide timely results.

KEYWORDS: Early pregnancy loss, product of conception, chromosome abnormality, aneuploidy, QF-PCR.

Introduction

Pregnancy loss is arguably a common traumatic experience in couples' lives. According to a review compiling data from nine large cohort studies in North America and Western Europe, the pooled risk of pregnancy loss was 15.3% of all recognized pregnancies, with approximately 23 million miscarriages occurring worldwide every year [1].

Early pregnancy loss (EPL) is the most common form of miscarriage, occurring in about 20% of pregnancies [2-4].

According to the American College of Obstetricians and Gynecologists (ACOG), EPL is defined as a nonviable, intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo or fetus without fetal heart activity within the first 12 6/7 weeks of gestation [5], while the European Society of Human Reproduction and Embryology (ESHRE) uses the term early pregnancy loss to

define any miscarriage occurring before 10 weeks of gestation [6].

EPL is a broad term which includes multiple clinical entities, such as: biochemical pregnancy loss, asymptomatic pregnancy loss, complete and incomplete pregnancy loss, as well as recurrent pregnancy loss (RPL) [7].

Establishing the exact etiology of EPL is vital for the proper prognosis and management of possible future pregnancies [8].

Chromosome abnormalities account for more than half of EPL cases between six and ten weeks' gestation [9,10], with aneuploidies mostly occurring *de novo* and being the product of random errors occurring mainly during oogenesis and early embryogenesis [11,12].

Endometrial decidualization defects are also involved in the etiology of EPL. The risk factors for endometrial breakdown include immune (insufficient local inflammatory response during implantation and placentation, intolerance to partner alloantigens, and defects in the decidual

immune surveillance of senescent cells) [13-15], endocrine (thyroid hormone levels) [16], and metabolic (obesity) [17] alterations.

Moreover, there is a strong association between female age at the moment of conception [18], as well as the number of previous miscarriages [19], and early pregnancy loss. Lifestyle factors also seem to play a role in the occurrence of EPL [20].

Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) is a fast, reliable, relatively easy and low-cost molecular technique that is high-throughput and does not require cultured cells. It can be used as either a stand-alone method for the quick detection of product-of-conception aneuploidy, or as a complementary technique to conventional karyotyping (KT), not only for the exclusion of maternal cell contamination (MCC), but also as a rescue method in case of cell culture failure [12,21,22].

QF-PCR is used for detecting chromosome copy numbers by amplifying chromosome-specific polymorphic DNA markers (short tandem repeats, STRs). Fluorochromes are incorporated into PCR amplification products by means of fluorescently-labeled primers specific to each STR [23].

The usage of the extended QF-PCR assay for POC analysis allows for the detection of aneuploidies of chromosomes 13, 14, 15, 16, 18, 21, 22, X, and Y, triploid and molar pregnancies, uniparental disomy (UPD), as well as the exclusion of MCC, while providing valuable information on the paternal and meiotic origin of aneuploidies [22,24,25].

The aim of our study was to assess the incidence and types of chromosome abnormalities in product-of-conception samples analyzed by QF-PCR.

Materials and Methods

All product-of-conception (POC) samples included in this study were referred to the Human Genomics Laboratory of the University of Medicine and Pharmacy of Craiova, Romania for QF-PCR analysis between January 2013 and September 2024, by the Departments of Obstetrics and Gynecology of the Emergency Clinical County Hospital and the "Filantropia" Clinical Municipal Hospital of Craiova.

Data regarding reproductive history, including maternal and gestational ages were recorded. In the present study, maternal ages ranged between 18 and 46 years old, with gestational ages varying between 6 and 12 6/7 weeks.

The study was conducted in accordance with the Declaration of Helsinki and approved by the

Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania (no. 44/24.03.2022).

DNA was extracted from POC tissue and maternal blood samples using PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) and Promega Wizard™ Genomic (Promega, Madison, WI, USA), respectively.

QF-PCR testing involved the usage of the IVD Devyser Extend kit (Devyser AB, Stockholm, Sweden), which contains one mix allowing for the detection of chromosome 13, 18, 21, X, and Y aneuploidies, and an additional polymerase chain reaction (PCR) mix for the testing of chromosomes 15, 16, and 22. This allows for a cost-efficient step-by-step approach.

PCR products were migrated on the ABI3730xl platform (Applied Biosystems, Foster City, CA, USA), with GeneMarker v2.2 software (SoftGenetics, State College, PA, USA) being used for electropherogram interpretation. Upon analyzing amplicon length by an automated DNA sequencer, a normal heterozygous individual shows two equal peaks of fluorescent activity signifying the presence of two different alleles at one locus (1:1 peak ratio). In contrast, a trisomic individual may present either a 1:1:1 peak ratio (trisomic triallelic) or 2:1 (trisomic diallelic) [26].

All resulting data was subsequently analyzed by using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA: IBM Corp.

Results

Two hundred fifty-seven POC samples meeting EPL criteria were submitted for QF-PCR analysis, 133 (51.8%) being subsequently excluded due to MCC. A total of 124 samples were successfully analyzed and 78 samples (62.9%) showed no aneuploidies, while the remaining 46 cases (37.1%) showed various types of abnormalities (Table 1, Figure 1).

Table 1. Aneuploidy detected in POC samples via QF-PCR (rounded values).

Chromosome abnormality	<i>n</i>	% of cases with chromosome abnormalities	% of cases analyzed
Aneuploidy of chromosomes 13, 18, 21	11	23.9	8.9
Aneuploidy of chromosomes 15, 16, 22	14	30.45	11.3
Sex chromosome aneuploidy	13	28.25	10.5
Triploidy	8	17.4	6.4
No aneuploidy	78	-	62.9
Total	124	100	100

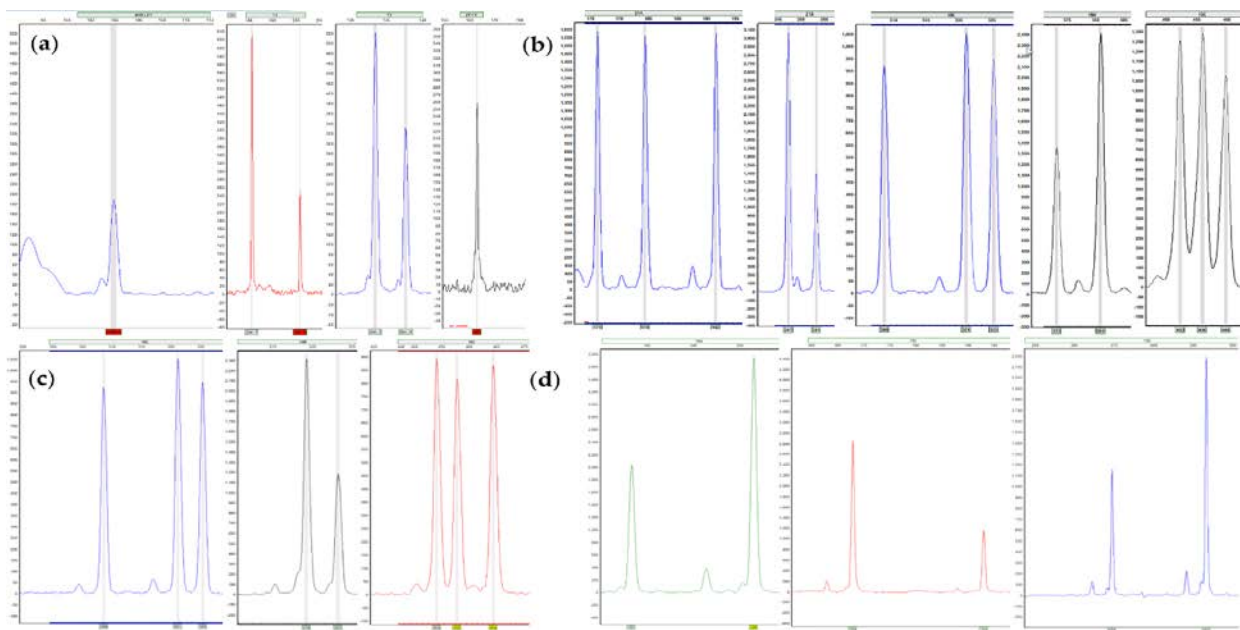


Figure 1. Partial electropherograms of chromosome abnormalities detected by QF-PCR (a) Monosomy X; (b) Triploidy; (c) Trisomy 18; (d) Trisomy 15.

The basic QF-PCR kit, accounting for aneuploidies of chromosomes 13, 18, 21, X, and Y, had an overall detection rate of 25.8%. When employing the extended kit, the detection rate rose to 37.1% out of all cases analyzed.

The most common autosomal aneuploidy detected was trisomy 18 (six cases, 13% of anomalies detected), followed by trisomy 21 (four cases, 8.7%). We found a single case of trisomy 13 in a case of pregnancy loss that occurred at 12 weeks' gestation. The male to female ratio for aneuploidies of chromosomes 13, 18, and 21 was 7:4.

The only sex chromosome aneuploidy found was monosomy X, accounting for 28.3% of all anomalies (13 cases).

We detected five cases each of both trisomy 15 and trisomy 16 (10.9% of anomalies detected), as well as four cases of trisomy 22 (8.7%). The gender of the products of conception in this category was overwhelmingly female, with a male to female ratio of 1:6.

Triploidies accounted for 17.4% of all anomalies detected (8 cases).

Out of the 46 chromosome abnormalities detected in the present study, we could establish the parental origin of the anomaly in 43 cases.

Autosomal aneuploidies were mostly triallelic (91.3%) and of maternal origin (87%), while most cases of monosomy X were of paternal origin. We also found that 57.1% of triploidies were digynic, while 42.9% were diandric (Table 2).

Table 2. The parental origin of aneuploidies detected by QF-PCR.

Chromosome abnormality	n	Parental origin	
		Maternal n (%)	Paternal n (%)
Aneuploidy of chromosomes 13, 18, 21	9	8 (88.9)	1 (11.1)
Aneuploidy of chromosomes 15, 16, 22	14	12 (85.7)	2 (14.3)
Sex chromosome aneuploidy	13	3 (23.1)	10 (76.9)
Triploidy	7	4 (57.1)	3 (42.9)
Total	43	35 (81.4)	8 (18.6)

Discussion

Early pregnancy loss (EPL) is a burden on the mental health of women worldwide, the majority of patients reporting symptoms of depression, anxiety, and perinatal loss [27].

Establishing the exact cause of EPL not only can help reduce those symptoms [28], but also guide future pregnancies [29].

Around half of early pregnancy loss cases occur due to chromosome abnormalities, an overwhelming majority of those anomalies being aneuploidies [30].

Chromosome segregation during meiosis relies on both the integrity of the microtubule spindle apparatus and the checkpoint signaling pathway overseeing the attachment of spindle microtubules to kinetochores [31], therefore failures concerning these elements increase the

risk of missegregation and aneuploid daughter cells.

Non-disjunction seems to occur most frequently during the first meiotic division of oogenesis [32], with proven correlation between advanced maternal age and fetal trisomy [33].

Given the fact that maternal ages are currently on the rise [34], EPL will likely continue to be a major health issue.

Our study provides relevant information regarding the types and incidence of chromosomal abnormalities in early pregnancy loss patients, our results showing a chromosome

abnormality rate of 37.1%, as detected by the QF-PCR extended kit. We detected aneuploidies in 77.2% of all abnormal cases, in line with meta-analysis reports by van den Berg et al [12] and Smits and al. [35], the remaining cases being triploidies.

There is limited data regarding the usage of QF-PCR as a sole molecular technique for the genetic diagnosis of first-trimester pregnancy loss POCs.

However, we provide a limited literature comparison in Table 3 [22,36-39].

Table 3. QF-PCR abnormality rates in early pregnancy loss POC samples. Data adjusted to the terms of the present study (rounded values).

Study	Aneuploidy of chromosomes 13, 18, 21 (%) a)	Aneuploidy of chromosomes 15, 16, 22 (%) a)	Sex chromosome aneuploidy (%) a)	Triploidy (%)a)	Total abnormality rate (%)
Present study	23.9	30.45	28.25	17.4	37.1
Diego-Alvarez et al., 2005 [22]	45.8	16.7	12.5	12.5	36.4b)
Zou et al., 2008 [36]	no data	no data	no data	no data	36.7
Coelho et al., 2016 [37]	25.35	49.3	14.1	11.25	54.6
Pauta et al., 2021 [38]	28.3	39.1	15.2	8.7	42c)
Bozhinovski et al., 2023 [39]	14.8	34.3	10.4	15.3	56.25d)

a) % of cases with chromosome abnormalities detected

b) Includes one case of trisomy 2, one case of trisomy 7, and one unspecified double trisomy

c) Includes two cases of trisomy 7 and three unspecified double trisomies

d) Includes other trisomies, as well as partial and multiple chromosome abnormalities

In our present study, QF-PCR results showed abnormalities in 37.1% of cases (n=46) when using the extended kit, while a previously-published study conducted in our center showed that chromosome abnormalities were detected in

58.7% of cases when using conventional karyotyping [40].

In both our studies, the most common abnormalities detected were trisomies of chromosomes 15, 16 and 22, as shown in Table 4.

Table 4. Comparative view of chromosome abnormalities using KT and QF-PCR, relative to group values (rounded values).

Chromosome abnormality	% of cases analyzed		% of cases with chromosome abnormalities	
	KT	QF-PCR	KT	QF-PCR
Aneuploidy of chromosomes 13, 18, 21	10a)	8.9	17a)	23.9
Aneuploidy of chromosomes 15, 16, 22	17.8b)	11.3	30.4b)	30.5
Aneuploidies of other autosomes	6.1c)	-	10.4c)	-
Sex chromosome aneuploidy	10.4	10.5	17.8	28.5
Poliploidy	Triploidy	7.8d)	6.4	13.3d)
	Tetraploidy	2.2e)	-	3.7e)
Structural abnormality	3.1	-	5.2	-
Mosaic abnormality	1.3	-	2.2	-
Total	58.7	37.1	100	100

Note: a) Includes one double trisomy case (48,XXY,+13) and one 46,XY,der(13;21)(q10;q10),+21 case.

b) Includes one double trisomy case (48,XYY,+22), one 46,XX,der(14;15)(q10;q10),+15 case, and one 46,XY,der(15;15)(q10;q10),+15 case

c) Includes one double trisomy case, 48,XX,+6,+10, and one 46,XX,der(13;14)(q10;q10),+14 case

d) Includes two hypertriploidy cases, 71,XXY,+3,+20 and 71,XXY,+5,+9

e) Includes one hypertetraploidy case, 92,XXXX,+15

Out of the 230 cases previously analyzed by conventional karyotyping [40], 48.1% chromosome abnormality cases (28.3% of all cases analyzed, 65/135 cases) could have also

been detected by the QF-PCR kit focused on chromosomes 13, 18, 21, X, and Y. The extended kit would have increased the detection rate to 78.5% (106 cases out of 135 anomalies detected,

46.1% of all cases analyzed). The remaining 21.5% (29 cases) comprise of aneuploidies of other autosomes, tetraploidies, structural abnormalities, and low-level mosaicism cases which remain undetectable by QF-PCR alone.

Moreover, if we would have included STRs for chromosomes 8 and 9 (the next two common aneuploidies found by conventional karyotyping in our previous study), QF-PCR's detection rate would have reached 83% of all chromosome abnormalities (48.7% of all cases analyzed).

However, even with STRs for all chromosomes involved in undiagnosed aneuploidies (chromosomes 2, 7, 10, 12, and 14), the overall detection rate could not have been higher than 87.4% anomalies detected by QF-PCR.

On the other hand, QF-PCR would have identified four cases of unbalanced Robertsonian

translocation as trisomy 14, 15, and 21 respectively.

Robertsonian translocation trisomies hold great impact on couples' genetic counseling and future pregnancies, given the risk of recurrent pregnancy loss, potential Patau or Down syndrome live births, and the risk of UPD and imprinting disorders [41].

Therefore, parental karyotyping is recommended in all trisomy cases involving acrocentric chromosomes.

Compiling data reported in cytogenetic studies of early pregnancy loss POCs [40,42-48], we found that, if the authors would have chosen to employ the extended QF-PCR kit as a diagnostic method in their studies, they would have missed between 21.8 and 38.4% of chromosome abnormalities detected by KT (Table 5).

Table 5. Relative frequency of abnormality types detected by conventional karyotyping in early pregnancy loss POC samples. Data adjusted to the terms of the present study.

Study	Aneuploidy of chromosomes 13, 18, 21 (%) a)	Aneuploidy of chromosomes 15, 16, 22 (%) a)	Aneuploidies of other autosomes (%) a)	Sex chromosome aneuploidy (%) a)	Structural abnormalities (%)a)	Triploidy (%)a)	Tetraploidy (%)a)	Total abnormality rate (%)	Total abnormality rate detectable by the QF-PCR extended kit (%)
Our previous study [40]	17	30.4	10.4	17.8	5.2	13.3	3.7	58.7b)	78.5
Hassold et al., 1980 [42]	9.9	21.4	18	24.2	4.3	15.1	7.1	46.3	70.6
Ljunger et al., 2005 [43]	10.7	30.8	18.2	18.25	7.55	10.7	3.8	61.4	70.45
Menasha et al. (B), 2005 [44]	17.1	29.7c)	20.4c)	7.4	3.9	11.5c)	2.3d)	65.8	73.4
Zhou et al., 2016 [45]	21.1	29.5	13.2	13.3	4.9	14.3	3.7	48.75	78.2
Soler et al., 2017 [46]	19	35.4	16	10.1	5	13.1	1.4	70.3	77.6
Pylyp et al., 2018 [47]	20	26.2	16.6	8.2	7	17.8	4.2e)	50.1	72.2
Wu et al., 2021 [48]	10.9	30.1	21.1	11.8	5.7	8.2	3.1	53.2f)	61.6

Note: a) % of abnormality cases detected

b) Includes mosaic abnormalities

c) Only includes trisomies

d) Does not include unspecified mosaic cases, representing the remaining 0.3% of polyploidies

e) Includes tetraploidies and near-pentaploidies

f) Includes 3% mosaic cases (5.5% of abnormalities) and 1.6% (3% of abnormalities) consisting of other unspecified chromosome aneuploidies

Common techniques used for the genetic diagnosis of chromosome abnormalities in early pregnancy loss samples include conventional karyotyping, QF-PCR, Fluorescence in Situ Hybridization (FISH), and, more recently, microarray-based assays [49].

Conventional karyotyping of product-of-conception samples can detect balanced translocations and polyploidies [50,51], with major disadvantages lying in long wait times and a high risk of culture failure and maternal cell contamination [52,53].

FISH involves hybridizing a set of fluorescently-labeled probes to the interphase nuclei of uncultured cells [49].

Given the fact that the probes are chromosome-specific, the number of fluorescent signals is equal to chromosome copy numbers, FISH being able to detect aneuploidy, polyploidy, and the presence of male products of conception in samples with heavy MCC, but indistinguishable from fetal XX cells [2].

Therefore, interphase FISH is a fast, targeted approach limited to locus-specific probes, unable to detect structural rearrangements.

QF-PCR is a fast, reliable, cost-effective genetic technique to detect common or specific aneuploidies; however, it is unsuitable for the detection of structural chromosome

abnormalities, microdeletions, microduplications, and tetraploidy [22].

Structural chromosome abnormalities occur in ~5% of early pregnancy losses [44], while the reported incidence of microdeletions and microduplications (pathogenic copy number variants shorter than 10 Mb) is variable, generally ranging between 6.2 and 8.2% [54-56].

While QF-PCR can safely detect triploidy, tetraploidy, which can occur with an incidence as high as 7.1% [57] in EPL samples, is, in most cases, undetectable by this technique (e.g. when the mechanism is endoreduplication or cell fusion). In addition, QF-PCR can also detect mosaic cases, as long as the abnormal cell line is more than 15-20% [58]; however, this may prove difficult to do in practice and it also requires parental DNA [22].

However, a number of cases showing undetected chromosomal aberrations by QF-PCR would escape diagnosis, highlighting the need for comprehensive genetic (conventional karyotyping) or genomic analysis (Microarray-based Comparative Genomic Hybridization) in pregnancy loss [11,59].

Molecular karyotyping techniques, such as Chromosomal Microarray Analysis (CMA), have shown higher detection rates of genetic abnormalities compared to traditional karyotyping, identifying submicroscopic copy

number variations (CNVs) that are often missed by conventional methods. It may prove added benefits over karyotyping in EPL [60], as could SNP (single nucleotide polymorphism) microarray and shotgun sequencing (CNV-seq)/QF-PCR [61].

Microarray-based Comparative Genomic Hybridization (aCGH) scans the entire genome for chromosomal gains and losses at a higher resolution than conventional karyotyping [2].

However, it remains an expensive test that is not widely available in laboratories, and one that is not covered by many health insurance systems, especially for patients experiencing sporadic early pregnancy loss. Unlike aCGH, which can only detect CNVs, SNP microarray is also capable of detecting triploidy, as well as copy-neutral loss of heterozygosity (CN-LOH) regions across the genome in instances such as consanguinity and UPD, and some cases of MCC and placental mosaicism [62,63].

CN-LOH can be associated with an increased prevalence of autosomal recessive diseases, as well as imprinting disorders [64,65].

SNP microarray is also used in the diagnosis of molar pregnancy and its subtypes [66].

A comparative view of some of the genetic techniques used in the analysis of POC samples can be found in Table 6 [22,67-69].

Table 6. Advantages and limitations of genetic techniques used for POC analysis.

	KT	FISH	QF-PCR	aCGH	SNP microarray
MCC	male POCs	male POCs	+	- ^{a)}	+ ^{b)}
Aneuploidy	+	+	+	+	+
Triploidy	+	+	+	-	+
Tetraploidy	+	+	-	-	-
Balanced structural abnormalities	+	+	-	-	-
Large unbalanced structural abnormalities ^{c)}	+	+/- ^{d)}	+/- ^{d)}	+	+
Microdeletions/ microduplications	-	+/- ^{d)}	-	+	+
Mosaic anomalies	+	+	+/- ^{e)}	+/- ^{e)}	+/- ^{e)}
UPD	-	-	+	-	+
Parental and/or meiotic origin of anomaly	-	-	+	-	+

Note: a) can mimic POC mosaicism; high percentage of MCC might alter results

b) if MCC is over 5-10%

c) larger than 5 Mb

d) detectable with targeted probes or primers

e) not able to detect low-level mosaicism (<15-20%)

Shah et al. found substantial diagnosis overlap between KT, aCGH and SNP microarray ($\kappa=0,65$), following a comparative study performed on EPL POC samples.

The differences in call rate arose from the individual limitations of each method, the authors recommending choosing a technique based upon availability and clinical context [70].

In another EPL comparative study, Cai et al. highlighted the difference in success rates between KT (78,5%) and SNP microarray (100%) owed to cell culture failure.

However, when analysis could be performed successfully, the chromosome abnormality detection rates were comparable (53,8% for KT and 53,3% for SNP microarray).

The authors suggest simultaneously testing POC samples by using both methods when possible; if not, KT should be performed, followed by SNP microarray in case of normal karyotype or cell culture failure [71].

A meta-analysis performed by Smits et al. found comparable chromosome abnormality rates between KT (47%) and aCGH (48%), with both lower than SNP microarray detection rates (60%), with the percentage of chromosome abnormalities not differing between spontaneous and recurrent pregnancy loss (46%) [35].

The detection of aneuploidy in the context of RPL can be reassuring for patients, giving the fact that aneuploidy seems to occur at the same rate as age-matched women not experiencing RPL [72].

Moreover, it can help guide further testing and discussions on prognosis [73].

In 2016, Chen et al. implemented a new molecular technique called High-throughput Ligation-dependent Probe Amplification (HPLA) [74], which aimed to detect aneuploidies of all 24 chromosomes by using 170 DNA probes in a single reaction. This method has already been reproduced successfully [75,76].

A dual QF-PCR/HPLA approach yielded an abnormality detection rate of 59.6% in EPL samples, similar to that of chromosomal microarray analysis (60.3%) [77], while also proving faster and most cost-efficient.

However, QF-PCR is also an excellent tool for excluding maternal cell contamination and determining the origin of the aneuploidy.

Since a larger number of samples are processed at the same time, it is also faster, less labor-intensive and less expensive than conventional karyotyping.

The genetic assessment of EPL product-of-conception samples relies on careful sampling, handling and dissection, since there is a relatively high chance that analysis could be hampered by MCC and/or bacterial or fungal contamination, and therefore providing no results [24].

The reported MCC rate ranges between 10.5 and 22% [53,78,79], but can go as high as 89.7% [80] in some cases, depending on sample inclusion criteria and the type of procedure used to obtain POC specimens [81].

In our study the MCC rate detected by QF-PCR was 51.8% ($n=133$), this molecular technique proving valuable not only in detecting MCC, but also in successfully analyzing samples where long-term cell cultures failed to be established.

Our higher MCC rate can be due to the inclusion of all samples meeting EPL criteria,

regardless of tissue quantity or quality. DNA analysis is independent of cell culture; DNA extraction can be performed on small tissue pieces, yielding adequate amounts of DNA for analysis [82].

Our study is limited by the small sample size, requiring further studies to develop a faster, cheaper, more reliable management protocol of product-of-conception samples in EPL cases, in order to provide more informed and detailed genetic counsel to patients experiencing EPL.

Conclusions

Our study shows that QF-PCR can be used as a first approach in the genetic analysis of POC, followed by conventional karyotyping or CMA as follow-up.

Genetic testing based on short tandem repeat analysis, including QF-PCR, remains the gold standard for MCC, as well as providing timely results.

At present, there is no single genetic diagnostic technique that is sufficient for the complete analysis of EPL products of conception; the integration of both targeted and broad-spectrum analysis techniques (e.g. conventional karyotyping, aCGH, SNP microarray, next-generation sequencing) can offer a more robust approach in EPL.

Acknowledgements

Genetic testing was funded through the National Health Program (“XIII Programul național de sănătate a femeii și copilului 2.3. Prevenirea bolilor genetice prin diagnostic pre-și postnatal”).

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, Brewin J, Ramhorst R, Lucas ES, McCoy RC, Anderson R, Daher S, Regan L, Al-Memar M, Bourne T, MacIntyre DA, Rai R, Christiansen OB, Sugiura-Ogasawara M, Odendaal J, Devall AJ, Bennett PR, Petrou S, Coomarasamy A. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. *Lancet*, 2021, 397(10285):1658-1667.
2. Hardy K, Hardy PJ. 1(st) trimester miscarriage: four decades of study. *Transl Pediatr*, 2015, 4(2):189-200.
3. Sonalkar S, McKean R. Changing the Landscape of Early Pregnancy Loss Care. *JAMA Netw Open*, 2024, 7(10):e2435861.
4. Varner C. Investing in streamlined care for patients experiencing early pregnancy loss in Canada would reduce substantial suffering. *Cmaj*, 2024, 196(34):E1171-e1172.

5. ACOG Practice Bulletin No. 200: Early Pregnancy Loss. *Obstet Gynecol*, 2018, 132(5):e197-e207.
6. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, Goddijn M, Stephenson MD. Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. *Hum Reprod*, 2015, 30(3):495-498.
7. Cunningham F, Leveno KJ, Dashe JS, Hoffman BL, Spong CY, Casey BM. First and Second Trimester Pregnancy Loss. In: Cunningham F, Leveno KJ, Dashe JS, Hoffman BL, Spong CY, Casey BM (Eds): *Williams Obstetrics 26e*, McGraw-Hill, 2022, New York, 198-244.
8. Coomarasamy A, Gallos ID, Papadopoulou A, Dhillon-Smith RK, Al-Memar M, Brewin J, Christiansen OB, Stephenson MD, Oladapo OT, Wijeyaratne CN, Small R, Bennett PR, Regan L, Goddijn M, Devall AJ, Bourne T, Brosens JJ, Quenby S. Sporadic miscarriage: evidence to provide effective care. *Lancet*, 2021, 397(10285):1668-1674.
9. Tkach I, Huleyuk N, Zastavna D, Bezkorovaina G, Helner N, Benko O. Cytogenetic analysis of 2554 samples of the products of conception from early reproductive losses. *Faktori eksperimental'noi evolucii organizmiv*, 2024, 35:79-82.
10. Froeliger A, Deneux-Tharoux C, Sentilhes L. Early Pregnancy Loss. *Jama*, 2023, 330(9):878-879.
11. Essers R, Lebedev IN, Kurg A, Fonova EA, Stevens SJC, Koeck RM, von Rango U, Brandts L, Deligiannis SP, Nikitina TV, Sazhenova EA, Tolmacheva EN, Kashevarova AA, Fedotov DA, Demeneva VV, Zhigalina DI, Drozdov GV, Al-Nasiry S, Macville MVE, van den Wijngaard A, Dreesen J, Paulussen A, Hoischen A, Brunner HG, Salumets A, Zamani Esteki M. Prevalence of chromosomal alterations in first-trimester spontaneous pregnancy loss. *Nat Med*, 2023, 29(12):3233-3242.
12. van den Berg MM, van Maarle MC, van Wely M, Goddijn M. Genetics of early miscarriage. *Biochim Biophys Acta*, 2012, 1822(12):1951-1959.
13. Turco MY, Moffett A. Development of the human placenta. *Development*, 2019, 146(22):dev163428.
14. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol*, 2017, 17(8):469-482.
15. Lucas ES, Vrljicak P, Muter J, Diniz-da-Costa MM, Brighton PJ, Kong CS, Lipecki J, Fishwick KJ, Odendaal J, Ewington LJ, Quenby S, Ott S, Brosens JJ. Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. *Commun Biol*, 2020, 3(1):37.
16. Kakita-Kobayashi M, Murata H, Nishigaki A, Hashimoto Y, Komiya S, Tsubokura H, Kido T, Kida N, Tsuzuki-Nakao T, Matsuo Y, Bono H, Hirota K, Okaza H. Thyroid Hormone Facilitates in vitro Decidualization of Human Endometrial Stromal Cells via Thyroid Hormone Receptors. *Endocrinology*, 2020, 161(6):bqaa049.
17. Antoniotti GS, Coughlan M, Salamonsen LA, Evans J. Obesity associated advanced glycation end products within the human uterine cavity adversely impact endometrial function and embryo implantation competence. *Hum Reprod*, 2018, 33(4):654-665.
18. Bortoletto P, Lucas ES, Melo P, Gallos ID, Devall AJ, Bourne T, Quenby S, Bennett PR, Coomarasamy A, Brosens JJ. Miscarriage syndrome: Linking early pregnancy loss to obstetric and age-related disorders. *EBioMedicine*, 2022, 81:104134.
19. Coomarasamy A, Devall AJ, Brosens JJ, Quenby S, Stephenson MD, Sierra S, Christiansen OB, Small R, Brewin J, Roberts TE, Dhillon-Smith R, Harb H, Noordali H, Papadopoulou A, Eapen A, Prior M, Di Renzo GC, Hinshaw K, Mol BW, Lumsden MA, Khalaf Y, Shennan A, Goddijn M, van Wely M, Al-Memar M, Bennett P, Bourne T, Rai R, Regan L, Gallos ID. Micronized vaginal progesterone to prevent miscarriage: a critical evaluation of randomized evidence. *Am J Obstet Gynecol*, 2020, 223(2):167-176.
20. Yuan S, Liu J, Larsson SC. Smoking, alcohol and coffee consumption and pregnancy loss: a Mendelian randomization investigation. *Fertil Steril*, 2021, 116(4):1061-1067.
21. Mann K, Donaghue C, Fox SP, Docherty Z, Ogilvie CM. Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. *Eur J Hum Genet*, 2004, 12(11):907-915.
22. Diego-Alvarez D, Garcia-Hoyos M, Trujillo MJ, Gonzalez-Gonzalez C, Rodriguez de Alba M, Ayuso C, Ramos-Corrales C, Lorda-Sanchez I. Application of quantitative fluorescent PCR with short tandem repeat markers to the study of aneuploidies in spontaneous miscarriages. *Hum Reprod*, 2005, 20(5):1235-1243.
23. Muthuswamy S, Bhalla P, Agarwal S, Phadke SR. Performance of QF-PCR in targeted prenatal aneuploidy diagnosis: Indian scenario. *Gene*, 2015, 562(1):55-61.
24. Kato T, Miyai S, Suzuki H, Murase Y, Ota S, Yamauchi H, Ammae M, Nakano T, Nakaoka Y, Inoue T, Morimoto Y, Fukuda A, Utsunomiya T, Nishizawa H, Kurahashi H. Usefulness of combined NGS and QF-PCR analysis for product of conception karyotyping. *Reprod Med Biol*, 2022, 21(1):e12449.
25. Donaghue C, Davies N, Ahn JW, Thomas H, Ogilvie CM, Mann K. Efficient and cost-effective genetic analysis of products of conception and fetal tissues using a QF-PCR/array CGH strategy; five years of data. *Mol Cytogenet*, 2017, 10:12.
26. Adinolfi M, Pertl B, Sherlock J. Rapid detection of aneuploidies by microsatellite and the quantitative fluorescent polymerase chain reaction. *Prenat Diagn*, 1997, 17(13):1299-1311.
27. Cuenca D. Pregnancy loss: Consequences for mental health. *Front Glob Womens Health*, 2022, 3:1032212.
28. Nikcevic AV, Tunkel SA, Kuczmierczyk AR, Nicolaidis KH. Investigation of the cause of miscarriage and its influence on women's psychological distress. *Br J Obstet Gynaecol*, 1999, 106(8):808-813.
29. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertil Steril*, 2012, 98(5):1103-1111.
30. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol*, 2000, 14(5):855-865.
31. Compton DA. Mechanisms of aneuploidy. *Curr Opin Cell Biol*, 2011, 23(1):109-113.

32. Angell R. First-meiotic-division nondisjunction in human oocytes. *Am J Hum Genet*, 1997, 61(1):23-32.
33. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum Genet*, 1985, 70(1):11-17.
34. Osterman MJK, Hamilton BE, Martin JA, Driscoll AK, Valenzuela CP. Births: Final Data for 2021. *Natl Vital Stat Rep*, 2023, 72(1):1-53.
35. Smits MAJ, van Maarle M, Hamer G, Mastenbroek S, Goddijn M, van Wely M. Cytogenetic testing of pregnancy loss tissue: a meta-analysis. *Reprod Biomed Online*, 2020, 40(6):867-879.
36. Zou G, Zhang J, Li XW, He L, He G, Duan T. Quantitative fluorescent polymerase chain reaction to detect chromosomal anomalies in spontaneous abortion. *Int J Gynaecol Obstet*, 2008, 103(3):237-240.
37. Coelho FF, Marques FK, Gonçalves MS, Almeida VC, Mateo EC, Ferreira AC. Detection of aneuploidies in spontaneous abortions by quantitative fluorescent PCR with short tandem repeat markers: a retrospective study. *Genet Mol Res*, 2016, 15(3):15038617
38. Pauta M, Badenas C, Rodriguez-Revenga L, Soler A, Grande M, Sabrià J, Illanes C, Borobio V, Borrell A. A New Stepwise Molecular Work-Up After Chorionic Villi Sampling in Women With an Early Pregnancy Loss. *Front Genet*, 2020, 11:561720.
39. Bozhinovski G, Terzikij M, Kubelka-Sabit K, Jasar D, Lazarevski S, Livrinova V, Plaseska-Karanfilska D. Chromosomal Abnormalities in Early Pregnancy Losses: A Study of 900 Samples. *Balkan J Med Genet*, 2023, 26(2):11-20.
40. Popescu-Hobeanu, G., Riza, A. L., Streata, I., Tudorache, S., Comanescu, A., Tanase, F., Dragusin, R. C., Pascu, C., Dijmarescu, A. L., Cara, M. L., Dorobantu, S., Petre-Mandache, B., Cucu, M., Sosoi, S. S., Ioana, M., Iliescu, D., Burada, F. Cytogenetic Analysis of Sporadic First-Trimester Miscarriage Specimens Using Karyotyping and QF-PCR: A Retrospective Romanian Cohort Study. *Genes (Basel)*, 2022, 13(12):2246.
41. Zhao WW, Wu M, Chen F, Jiang S, Su H, Liang J, Deng C, Hu C, Yu S. Robertsonian translocations: an overview of 872 Robertsonian translocations identified in a diagnostic laboratory in China. *PLoS One*, 2015, 10(5):e0122647.
42. Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA. A cytogenetic study of 1000 spontaneous abortions. *Ann Hum Genet*, 1980, 44(2):151-178.
43. Ljunger E, Cnattingius S, Lundin C, Annerén G. Chromosomal anomalies in first-trimester miscarriages. *Acta Obstet Gynecol Scand*, 2005, 84(11):1103-1107.
44. Menasha J, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med*, 2005, 7(4):251-263.
45. Zhou Q, Wu SY, Amato K, DiAdamo A, Li P. Spectrum of Cytogenomic Abnormalities Revealed by Array Comparative Genomic Hybridization on Products of Conception Culture Failure and Normal Karyotype Samples. *J Genet Genomics*, 2016, 43(3):121-131.
46. Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, Borobio V, Muñoz M, Sánchez A. Overview of Chromosome Abnormalities in First Trimester Miscarriages: A Series of 1,011 Consecutive Chorionic Villi Sample Karyotypes. *Cytogenet Genome Res*, 2017, 152(2):81-89.
47. Pylyp LY, Spynenko LO, Verhogyad NV, Mishenko AO, Mykytenko DO, Zukin VD. Chromosomal abnormalities in products of conception of first-trimester miscarriages detected by conventional cytogenetic analysis: a review of 1000 cases. *J Assist Reprod Genet*, 2018, 35(2):265-271.
48. Wu X, Su L, Xie X, He D, Chen X, Wang M, Wang L, Zheng L, Xu L. Comprehensive analysis of early pregnancy loss based on cytogenetic findings from a tertiary referral center. *Mol Cytogenet*, 2021, 14(1):56.
49. Massalska D, Zimowski JG, Bijok J, Pawelec M, Czubak-Barlik M, Jakiel G, Roszkowski T. First trimester pregnancy loss: Clinical implications of genetic testing. *J Obstet Gynaecol Res*, 2017, 43(1):23-29.
50. Christofolini DM, Bevilacqua LB, Mafra FA, Kulikowski LD, Bianco B, Barbosa CP. Genetic analysis of products of conception. Should we abandon classic karyotyping methodology? *Einstein (Sao Paulo)*, 2021, 19:eAO5945.
51. Zhou Y, Xu W, Jiang Y, Xia Z, Zhang H, Chen X, Wang Z, Ge Y, Guo Q. Clinical Utility of a High-Resolution Melting Test for Screening Numerical Chromosomal Abnormalities in Recurrent Pregnancy Loss. *J Mol Diagn*, 2020, 22(4):523-531.
52. Volozonoka L, Gailite L, Perminov D, Kornejeva L, Fodina V, Kempa I, Miskova A. Reducing misdiagnosis caused by maternal cell contamination in genetic testing for early pregnancy loss. *Syst Biol Reprod Med*, 2020, 66(6):410-420.
53. Ouchi N, Takeshita T, Kasano S, Yokote R, Yonezawa M, Kurashina R, Ichikawa T, Kawabata I, Kuwabara Y, Suzuki S. Maternal cell contamination in embryonic chromosome analysis of missed abortions. *J Obstet Gynaecol Res*, 2022, 48(7):1641-1647.
54. Dai YF, Wu XQ, Huang HL, He SQ, Guo DH, Li Y, Lin N, Xu LP. Experience of copy number variation sequencing applied in spontaneous abortion. *BMC Med Genomics*, 2024, 17(1):15.
55. Shao Y, Yang S, Cheng L, Duan J, Li J, Kang J, Wang F, Liu J, Zheng F, Ma J, Zhang Y. Identification of chromosomal abnormalities in miscarriages by CNV-Seq. *Mol Cytogenet*, 2024, 17(1):4.
56. Fan L, Wu J, Wu Y, Shi X, Xin X, Li S, Zeng W, Deng D, Feng L, Chen S, Xiao J. Analysis of Chromosomal Copy Number in First-Trimester Pregnancy Loss Using Next-Generation Sequencing. *Front Genet*, 2020, 11:545856.
57. Hassold TJ. A cytogenetic study of repeated spontaneous abortions. *Am J Hum Genet*, 1980, 32(5):723-730.
58. Donaghue C, Mann K, Docherty Z, Ogilvie CM. Detection of mosaicism for primary trisomies in prenatal samples by QF-PCR and karyotype analysis. *Prenat Diagn*, 2005, 25(1):65-72.

59. Szewczyk K, Bik-Multanowski M. Molecular karyotyping in early miscarriages: potential for the routine use of cytogenetic microarrays. *J Obstet Gynaecol*, 2018, 38(4):585-586.
60. Pauta M, Grande M, Rodriguez-Revenga L, Kolomietz E, Borrell A. Added value of chromosomal microarray analysis over karyotyping in early pregnancy loss: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*, 2018, 51(4):453-462.
61. Xue H, Guo Q, Yu A, Lin M, Chen X, Xu L. Genetic analysis of chorionic villus tissues in early missed abortions. *Sci Rep*, 2023, 13(1):21719.
62. McQueen DB, Lathi RB. Miscarriage chromosome testing: Indications, benefits and methodologies. *Semin Perinatol*, 2019, 43(2):101-104.
63. Dugoff L, Norton ME, Kuller JA. The use of chromosomal microarray for prenatal diagnosis. *Am J Obstet Gynecol*, 2016, 215(4):B2-9.
64. Kearney HM, Kearney JB, Conlin LK. Diagnostic implications of excessive homozygosity detected by SNP-based microarrays: consanguinity, uniparental disomy, and recessive single-gene mutations. *Clin Lab Med*, 2011, 31(4):595-ix.
65. Liu J, He Z, Lin S, Wang Y, Huang L, Huang X, Luo Y. Absence of heterozygosity detected by single-nucleotide polymorphism array in prenatal diagnosis. *Ultrasound Obstet Gynecol*, 2021, 57(2):314-323.
66. Maisenbacher MK, Merrion K, Kutteh WH. Single-nucleotide polymorphism microarray detects molar pregnancies in 3% of miscarriages. *Fertil Steril*, 2019, 112(4):700-706.
67. Lamb AN, Rosenfeld JA, Coppinger J, Dodge ET, Dabell MP, Torchia BS, Ravnan JB, Shaffer LG, Ballif BC. Defining the impact of maternal cell contamination on the interpretation of prenatal microarray analysis. *Genet Med*, 2012, 14(11):914-921.
68. Sahoo T, Dzidic N, Strecker MN, Commander S, Travis MK, Doherty C, Tyson RW, Mendoza AE, Stephenson M, Dise CA, Benito CW, Ziadie MS, Hovanec K. Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. *Genet Med*, 2017, 19(1):83-89.
69. Rana B, Lambrese K, Mendola R, Xu J, Garrisi J, Miller K, Marin D, Treff NR. Identifying parental and cell-division origins of aneuploidy in the human blastocyst. *Am J Hum Genet*, 2023, 110(4):565-574.
70. Shah MS, Cinnioglu C, Maisenbacher M, Comstock I, Kort J, Lathi RB. Comparison of cytogenetics and molecular karyotyping for chromosome testing of miscarriage specimens. *Fertil Steril*, 2017, 107(4):1028-1033.
71. Cai M, Lin N, Xu L, Huang H. Comparative clinical genetic testing in spontaneous miscarriage: Insights from a study in Southern Chinese women. *J Cell Mol Med*, 2021, 25(12):5721-5728.
72. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Hum Reprod*, 2002, 17(2):446-451.
73. Hyde KJ, Schust DJ. Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med*, 2015, 5(3):a023119.
74. Chen S, Liu D, Zhang J, Li S, Zhang L, Fan J, Luo Y, Qian Y, Huang H, Liu C, Zhu H, Jiang Z, Xu C. A copy number variation genotyping method for aneuploidy detection in spontaneous abortion specimens. *Prenat Diagn*, 2017, 37(2):176-183.
75. Yang L, Tao T, Zhao X, Tao H, Su J, Shen Y, Tang Y, Qian F, Xiao J. Association between fetal chromosomal abnormalities and the frequency of spontaneous abortions. *Exp Ther Med*, 2020, 19(4):2505-2510.
76. Mao J, Wang H, Li H, Song X, Wang T, Xiang J, Li H. Genetic analysis of products of conception using a HPLA/SNP-array strategy. *Mol Cytogenet*, 2019, 12:40.
77. Wang Y, Zhou R, Jiang L, Meng L, Tan J, Qiao F, Wang Y, Zhang C, Cheng Q, Jiang Z, Hu P, Xu Z. Identification of Chromosomal Abnormalities in Early Pregnancy Loss Using a High-Throughput Ligation-Dependent Probe Amplification-Based Assay. *J Mol Diagn*, 2021, 23(1):38-45.
78. Lathi RB, Gustin SL, Keller J, Maisenbacher MK, Sigurjonsson S, Tao R, Demko Z. Reliability of 46,XX results on miscarriage specimens: a review of 1,222 first-trimester miscarriage specimens. *Fertil Steril*, 2014, 101(1):178-182.
79. Popescu F, Jaslow CR, Kutteh WH. Recurrent pregnancy loss evaluation combined with 24-chromosome microarray of miscarriage tissue provides a probable or definite cause of pregnancy loss in over 90% of patients. *Hum Reprod*, 2018, 33(4):579-587.
80. Jarrett KL, Michaelis RC, Phelan MC, Vincent VA, Best RG. Microsatellite analysis reveals a high incidence of maternal cell contamination in 46,XX products of conception consisting of villi or a combination of villi and membranous material. *Am J Obstet Gynecol*, 2001, 185(1):198-203.
81. Cholkeri-Singh A, Zamfirova I, Miller CE. Increased Fetal Chromosome Detection with the Use of Operative Hysteroscopy During Evacuation of Products of Conception for Diagnosed Miscarriage. *J Minim Invasive Gynecol*, 2020, 27(1):160-165.
82. Khorami Sarvestani S, Rafati M, Soltanghorae H, Hoseini A, Soltani A, Jalilian K, Ghaffari SR. Detection of Aneuploidies in Products of Conception and Neonatal Deaths in Iranian Patients Using the Multiplex Ligation-Dependent Probe Amplification (MLPA). *Avicenna J Med Biotechnol*, 2021, 13(3):143-148.

**Corresponding Author: Florin Burada, Laboratory of Human Genomics,
University of Medicine and Pharmacy of Craiova;
Regional Centre of Medical Genetics Dolj, Emergency Clinical County Hospital Craiova, Romania,
e-mail: florin.burada@umfcv.ro**