

(RESEARCH ARTICLE)



## Chromosomal analysis of pregnancy tissue from women with recurrent early pregnancy loss

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### Abstract

**Purpose:** This study aimed to determine the cytogenetical distribution of chromosomal disorders in couples after recurrent early pregnancy loss.

**Background:** One of the most important causes of pregnancy loss found in around half of the first trimester miscarriages is fetal chromosomal abnormalities, the role of fetal chromosomal disorders needs to be better evaluated.

**Method:** This study was conducted at two hospital and private clinics in Duhok, Iraqi Kurdistan from February 2017 to February 2022, and reviewed retrospectively. The study included 150 patients with history of Recurrent Early Pregnancy Loss were admitted for curettage because of miscarriage in early pregnancy. Patients were divided in two groups.

**Results:** The study population included a total of 150 fetal tissue specimens obtained during dilation and curettage after the diagnosis of spontaneous miscarriage. Patients were divided in two groups. Group 1 included 95 patients with an abnormal embryonic karyotype in the aborted material. Group 2 comprised 55 patients with a normal embryonic karyotype in the aborted products. Patients with a normal embryonic karyotype in the aborted products were significantly younger ( $p=0.0147$ ).

**Conclusion:** Young patients suffering from repeated miscarriages have a low probability to find chromosomal disorders in the embryonic tissue. Chromosomal analysis should be offered after previous miscarriages before further diagnostic methods are performed.

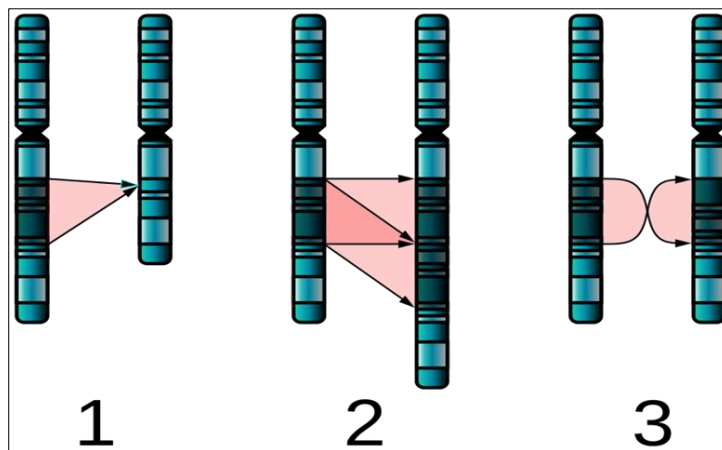
**Keywords:** Chromosomal Analysis; Recurrent Pregnancy Loss; Pregnancy rate; Pregnancy tissue

### 1. Introduction

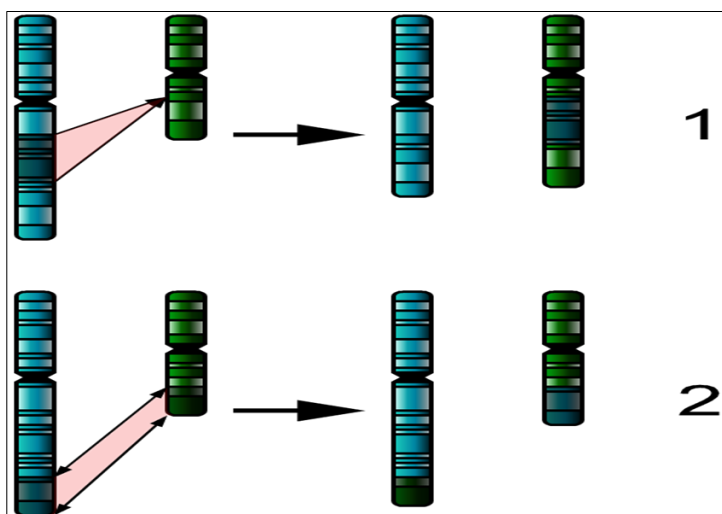
The normal diploid number of chromosomes in humans is 46. There are 23 pairs of chromosomes with 22 pairs of autosomes and two sex chromosomes, the X and the Y. Human females have two X chromosomes (46,XX), while males have one X and one Y chromosome (46,XY). The chromosomal aberration or mutation is the process of change in the chromosomes take place either due to the changes in the structure of the chromosomes or due to the abnormality in the chromosome number [1].

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Aneuploidy, gain or loss of an individual chromosome, is more common, while Polyploidy is the gain of one or more complete set of haploid chromosomes such as (69,XXY). Abnormality of chromosomal structure as shown in figure 1 and 2, comprise those changes that are due to one or more breaks in a chromosome. Following a break, the separated fragments are likely to participate in chromosomal rearrangements. Structural chromosomal changes can result in a displacement of chromosomal regions without any loss or duplication of genetic material such as (balanced rearrangements) or they may be unbalanced. This can take several forms: Deletions: A portion of the chromosome is missing or has been deleted, Duplications: A portion of the chromosome has been duplicated, resulting in extra genetic material, Inversions: A portion of the chromosome has broken off, turned upside down, and reattached, therefore the genetic material is inverted, Insertions: A portion of one chromosome has been deleted from its normal place and inserted into another chromosome, Translocations: A portion of one chromosome has been transferred to another chromosome. There are two main types of translocations: Reciprocal translocation: Segments from two different chromosomes have been exchanged. Robertsonian translocation: An entire chromosome has attached to another at the centromere - in humans, these only occur with chromosomes 13, 14, 15, 21, and 22. Rings: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without the loss of genetic material. Isochromosome: Formed by the mirror image copy of a chromosome segment including the centromere [2].



**Figure 1** The three major single-chromosome mutations: deletion (1), duplication (2) and inversion (3)



**Figure 2** The two major two-chromosome mutations: insertion (1) and translocation (2)

Chromosomal mutations lead to abnormalities in the function of the cell and organism, as congenital anomalies, growth deficiency, and intellectual disability are findings often present in individuals with chromosome abnormalities, although some cytogenetic aberrations have little to no clinical effect.

Genetic abnormalities of the conceptus are a recognized cause of sporadic and recurrent pregnancy loss (RPL). Cytogenetic abnormalities are more common in spontaneous abortions (50 percent of fetal deaths <20 weeks)

It is possible to ascertain whether an early pregnancy loss is due to a genetically abnormal embryo or fetus (aneuploidy) by analyzing the pregnancy or fetal tissue [4]. Published studies have used a variety of genetic techniques (conventional karyotyping, fluorescence *in situ* hybridization [FISH], or array-based comparative genomic hybridization [array-CGH]). Analysis by conventional karyotyping is limited by the failure of tissue culture and the fact that it does not distinguish between maternal contamination and a normal (euploid) female fetus [5].

FISH is limited as it only uses probes for certain chromosomes, and therefore does not necessarily detect the chromosomal cause of the miscarriage. Array CGH is a better technique, and currently preferred technique, looking at all chromosomes and avoiding the limitations associated with karyotype and [4,6].

New techniques such as next generation sequencing (NGS) have not yet been extensively investigated in genetic analysis of pregnancy tissue but may be useful in the near future [7].

Several authors have suggested a strategy of karyotyping the pregnancy tissue of the second miscarriage and only proceeding to further maternal investigations (for thrombophilia, thyroid dysfunction, uterine malformations) for the cause of the recurrent pregnancy loss if the result is euploid [8,9,10].

Aneuploidy is a recognized cause of pregnancy loss, and the frequency of aneuploid early pregnancy losses increases with female age. Aneuploidies occur in comparable frequencies in both women with sporadic and recurrent pregnancy loss. Genetic analysis of pregnancy tissue has the benefit of providing the patient with a reason for the pregnancy loss and may help to determine whether further investigations or treatments are required, but it does not necessarily rule out other underlying conditions.

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## 2. Material and methods

### 2.1. Design and Setting

This study was conducted at two hospital and private clinics in Duhok, Iraqi Kurdistan from February 2017 to February 2022, and reviewed retrospectively. The protocols used in the study were approved by the Committee of Scientific research unit of Duhok Obstetrics and Gynecology Teaching Hospital. The written informed consent of all the participants was obtained. The study included 150 patients with history of Recurrent Pregnancy Loss were admitted for curettage because of miscarriage in early pregnancy. Patients were divided in two groups. Group 1 included those patients with an abnormal embryonic karyotype in the aborted material. Group 2 included those patients with a normal embryonic karyotype in the aborted products, both groups were compared.

The inclusion criteria included a sonographic presence of a gestational sac and the patient's consent to perform a chromosomal exam. Exclusion criteria were the couple who refuse the procedure

Documented parameters included parity, maternal age gestational age and cytogenetic results.

Chromosomal analysis was done by array-based comparative genomic hybridization [array-CGH] technique.

### 2.2. Statistical analysis

The data were collected and statistically analyzed using a software package, current versions IBM (SPSS) Statistic, descriptive statistics for nominal variables were interpreted as number and percentage(%), while quantitative variables were expressed as mean  $\pm$  standard deviation. Student's t-test was applied to difference of mean of quantitative variables. Chi-square test was applied to study the difference of frequency. For interpretation of results, p value < 0.05 was considered significant.

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## 3. Results

During the study period, from February 2017 to February 2022 there were women with history of recurrent pregnancy loss admitted for curettage because of miscarriage in early pregnancy 150 patient was offered a chromosomal examination with determination of the embryonic karyotype.

The study population included a total of 150 fetal tissue specimens obtained during dilation and curettage after the diagnosis of spontaneous miscarriage. Patients were divided in two groups. Group 1 included 95 patients with an abnormal embryonic karyotype in the aborted material. Group 2 comprised 55 patients with a normal embryonic karyotype in the aborted products.

### 3.1. Patients characteristics

The characteristics of the patients with history of recurrent pregnancy loss are summarized in Table 1. The mean age of patients from Group with abnormal karyotype were (32.24±5.13) years old and those from Group with normal karyotype were (30.2±4.41), respectively. Patients with a normal embryonic karyotype in the aborted products were significantly younger ( $p = 0.0147$ ).

Average parity in patients from Group with abnormal karyotype had  $0.75 \pm 0.84$  while those from Group with normal karyotype were  $0.6 \pm 1.0$  patients.

The number of miscarriages in previous pregnancies from group with abnormal karyotype were  $0.34 \pm 0.71$ , while in group with normal karyotype were  $0.46 \pm 0.82$ ,  $p = 0.3478$

The average gestational age in group with abnormal karyotype was 10.43 weeks ( $\pm 2.1$ ) comparable to the 10.06 weeks of gestation ( $\pm 2.2$ ) found in group with normal karyotype.

**Table 1** Characteristics of patients recurrent pregnancy loss

Characteristics of patients	Abnormal karyotype Group (95)	Normal karyo type Group (55)	P value
Maternal age (years)	32.24±5.13	30.2±4.41	0.0147
Parity	0.75 ± 0.84	0.6 ± 1.0	0.3278
Number of miscarriages	0.34 ± 0.71	0.46± 0.82	0.3478
Gestational age (weeks)	10.43 weeks ( $\pm 2.1$ )	10.06 weeks ( $\pm 2.2$ )	0.3085

Data are presented as mean  $\pm$  SDP < 0.05 = Significant, P < 0.001 highly significant

### 3.2. Cytogenetic findings of the fetal tissue

**Table 2** Cytogenetic findings of the fetal tissue

Abnormal karyo type Group	N = 95
Numeric aberrations	90(94.7%)
Trisomy 13	34(37.77%)
Trisomy 16	26(28.8%)
Trisomy 18	15(16.6%)
Trisomy 21	12(13.3%)
monosomy	3(3.3%)
Structural abettations	5(5.26%)
Deletion	2(40%)
Translocation	3(60%)
NORMAL KARYOTYPE GROUP	N = 55
Female	30(54.54%)
Male	25(45.45%)

Data are presented as number (percent)

The Cytogenetic findings of the fetal tissue in patients with history of recurrent pregnancy loss are summarized in Table 2.

Out of 150 aborted products, 55 showed normal karyotypes and 95 were abnormal. The most frequent abnormalities were numeric aberrations 90((94.7%)%)including.5(5.26%) cases showed structural aberrations only.

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#### 4. Discussion

Recurrent pregnancy loss are disaster condition for many couples. Embryonic chromosomal disorders are a frequent cause of early miscarriages.

Maternal age is a significant cause for chromosomal aberrations in aborted material. This has been clearly demonstrated in earlier literature as well [11, 12]. Increasing age is associated with a higher aneuploidy rate, especially trisomy risk. [13,14,15].Our finding accords the Scandinavian study of Roepke et al. [16]

There are other pathologies like thrombophilia, thyroid dysfunction, parental genetics or uterine malformations which are related to recurrent pregnancy loss and should be taken into account [17]. Since chromosomal aberrations are the most leading cause of miscarriage, the genetic analysis of aborted material is quite indispensable in case of recurrent pregnancy loss, despite the high costs.

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#### 5. Conclusion

The findings of this study revealed that young patients suffering from repeated miscarriages have a low probability to find chromosomal disorders in the embryonic tissue. the effect of maternal age seems to overcome the impact of the abort recurrence itself. Chromosomal analysis should be offered after previous miscarriages before further diagnostic methods are performed.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

There are no conflicts of interests to declare.

##### *Statement of ethical approval*

The protocols used in the study were approved by the Committee of Scientific research unit of Duhok Obstetrics and Gynecology Teaching Hospital.

##### *Statement of informed consent*

I would like to accept to participate in my research This research does not use any sedative medications, the participants has the right to ask questions about the contents and results of research before participation. The information that are taken from the participants does not include (name, address, email or phone number).

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