

Chromosomal study for Assessment of Recurrent Spontaneous Miscarriage by Fluorescence in situ Hybridization (FISH) technique in Erbil City Iraqi- Kurdistan Region

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Abstract:

Spontaneous miscarriage is the most frequent complication of pregnancy and, classically, defined as the occurrence of at least two miscarriages before 20 weeks of gestation. Over 50 % of first trimester pregnancy losses are attributed to chromosomal abnormalities. This study aimed to investigate aneuploidy in spontaneous miscarriage by fluorescence in situ hybridization (FISH) using probes for 13, 18, 21, X and Y chromosomes. A total of 100 tissue samples from concepts materials were collected and examined by FISH. The incidence and type of chromosomal abnormality and sex ratio were analyzed for each samples. Moreover, the relationship between the rate of aneuploidy and maternal age also the relationship between maternal age and type of aneuploidy and the difference in incidence of aneuploidy between samples from previous miscarriage and those with no previous miscarriage were investigated. Results obtained from this study revealed that, 52 of 100 cases were with aneuploidy. Trisomy 21, 18, and 13 was the major aneuploidy followed by monosomy X. Cases of miscarriages which contain chromosomal abnormalities were higher in females than males. Cases with advanced maternal age and history of previous miscarriage were significantly have higher aneuploidy rate compared with young age cases and those with no previous miscarriage. However, rates of trisomies 18, 13, and 21 of the advanced maternal age group were remarkably higher than those of the young maternal age group.

Keywords: Recurrent miscarriage, Aneuploidy, Florescence In Situ Hybridization, Maternal age, Sex Ratio.

Introduction:

Recurrent miscarriage is a recognized pregnancy that involuntarily ended before 20 weeks of gestation (1). At least 15–20% of clinically recognized pregnancies result in a spontaneous miscarriage (2). Despite the involvement of numerous etiologies in miscarriage including infection, maternal hormonal imbalances, abnormal uterine anatomy, hematological and immunological disorders, and fetal genetic defects, in most cases the etiology of repeated miscarriage remains obscure. Interestingly, fetal chromosomal abnormalities have been shown to be implicated in a majority of early pregnancy wastage (3). Over and above, numerical chromosomal abnormalities are the most common in over 90% of the chromosomal defects, with monosomy X, triploidy, and autosomal trisomy being frequently common (4).

Several authors have observed an increasing risk of spontaneous abortion with increasing maternal age (4,6 and 7).

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Previous spontaneous abortions and multi-gravidity are also well-established risk factors for spontaneous abortion in subsequent pregnancies (8 and 9) Although these factors are highly correlated, the interaction between them is complex and remains to be evaluated especially when considering a woman's reproductive history. Over decades, numerous alternative genetics tests have been used for diagnosis of products of conception, including conventional karyotype, comparative genomic hybridization (CGH) (10 and 11) array and fluorescent in situ hybridization (FISH) (12 and 13). Although conventional karyotyping is the gold standard of diagnosis, many difficulties can be faced while using this technology including; culture failure, infection of the sample, maternal cell contamination and poor chromosomal preparations. These limitations may influence the net result of the test and result in an overall failure rate of 21% (14).

FISH technology uses DNA sequences incorporated with fluorophore-coupled nucleotides as probes to detect the presence or absence of complementary sequences in fixed cells or tissues (15). The advantages of FISH technology including high sensitivity and specificity in recognizing tar-

geted DNA sequences, direct application to both metaphase chromosomes and interphase nuclei, and visualization of hybridization signals at the level of single-cell. The overall benefits of this technology is the increased analytic resolution to the gene level compared with that of Giemsa bands and enabled rapid detection of either numerical or structural chromosomal abnormalities (13). Consequently, these advantages make FISH technique the most reliable diagnostic method for chromosome aneuploidy (16). Of particular interest, determining the genetic cause of a miscarriage, the risk of frequency, and chances for a following successful pregnancy can impact family planning and management of future pregnancies thereby lessen suffering of couples with recurrent miscarriage (16). Therefore, the main aim of this study is to assess if there is correlation between fetal chromosomal abnormalities and missed abortion by FISH techniques. We also aimed to determine the sex ratio in the first trimester and compare the rate of aneuploidy in irregular/sporadic abortion with that of recurrent abortion. Finally, we attempted to determine the relationship between the rate of aneuploidy and maternal age.

Materials and Methods:

1. Study design

100 different samples including fetal tissues, chorionic vilus and placenta, were collected from patients who had spontaneous miscarriage submitted to cytogenetic laboratory at Genome Diagnosis Lab (GDL), Erbil, Iraq. All samples were collected from patients who had spontaneous abortion, at first trimester, in a natural pregnancy. Samples from patients with uterine abnormality or of genital organs defects as well as major diseases such as diabetes, Thalassemia, Thyroid problems and Hypertension were excluded. In order to assess the relationship between maternal age and aneuploidy, we divided the samples into 4 age groups (<25, 25–29, 30–40, and ≥40 years) according to maternal age. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethical Committees of Salahaddin University. Moreover, we got informed consent in writing from each participant before we collected and tested the samples.

2. Preparation of samples

Samples in this study, obtained either by curettage or from involuntary fetal loss, were examined under microscopy to avoid contamination. Samples were then washed in normal saline. Then cut into small pieces, digested in 37°C collagenase for 30 min, centrifuged (500 xg) and the supernatant was removed. The precipitate was incubated with a KCl hypotonic solution, centrifuged, and the supernatant was removed. The precipitate was fixed by methanol/acetic acid (3:1) for 15 min twice. Finally, the precipitate of each sample was smeared onto three slides for FISH (14).

3. Fluorescence in situ hybridization

FISH protocol

Two slides were prepared from each sample and incubated for 30 minutes in a 65°C oven. Then, slides were immersed in a 2× standard saline citrate (SSC) for 30 minutes at 37°C,

digested in a 0.9% NaCl pepsin working solution (160 mg pepsin/40 mL 0.9% NaCl) (pH 1.5) at 37°C for 10 minutes. The slides were then washed in phosphate buffered solution for 5 minutes at room temperature. Next, the slides were immersed in 1% formaldehyde and phosphate buffered solution at room temperature for 5 minutes each. Slides were dehydrated in 70%, 85%, and 100% ethanol for 2 minutes each at room temperature and air dried (14).

The AneuVysion kit, which consisted of centromere probes for chromosomes X, Y, and 18, and locus-specific probes for chromosomes 13 and 21 (Abbott Molecular, Abbott Park, IL) was used in this study. The probe sets, X, Y, 18 and 13, 21 were applied to two separate hybridization fields, cover-slipped, and sealed by rubber cement. Then, slides were denatured at 73°C for 5 minutes and hybridized overnight in a humidified chamber at 37°C. After ending of hybridization period, the slides were subjected to a post-hybridization wash in 0.4× SSC for 2 minutes at 73°C and then rinsed in 2× SSC/0.1% NP-40 for 1 minutes at room temperature. Finally, slides were counterstained with 4'-6,-diamidino-2-phenylindole (DAPI) and were cover-slipped (16).

4 Analytical criteria

Cytogenetic analysis was carried out in GDL, Erbil according to the International System of Human Cytogenetic Nomenclature. For each specimen, at least 100 non-overlapping nuclei were evaluated. The FISH slides were evaluated using an Olympus fluorescent microscope equipped with an appropriate filter wheel and cubes to visualize the Spectrum Orange, Spectrum Green, and Spectrum Aqua fluorophores. For each sample 100 consecutive non-overlapping nuclei were scored. After the analysis, the percentage abnormal nuclei were calculated. Normal cutoffs were then used to determine whether the sample was abnormal. The normal cutoffs for trisomy of chromosomes 13, 18, 21, X, and Y were 3%, 8.5%, 6%, 4.5%, 7%, 10%, and 10%, respectively. Monosomy X and triploidy had normal cutoffs of 20% and 5%, respectively (14).

3-Statistical analysis

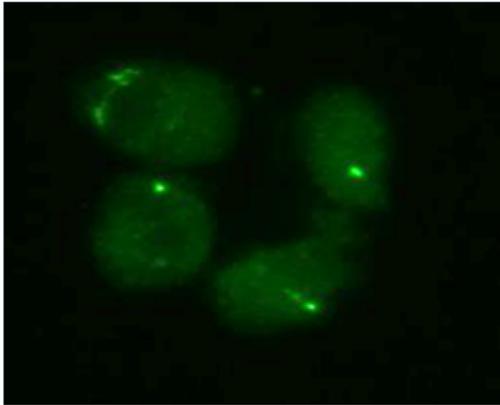
The means of the quantitative variables between the two groups were compared using the Student t-test for normally distributed data and the Mann-Whitney test for non-parametric data. ANOVA was used to compare the mean values between three or more groups. All tests were considered significant if the p-value was less than 0.05. Statistical Package for the GraphPad Prism, version 6.0, was used for the analyses.

Results:

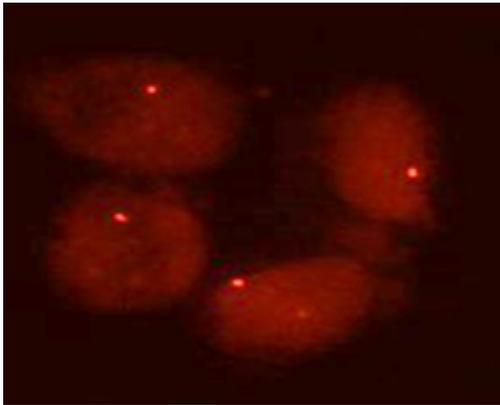
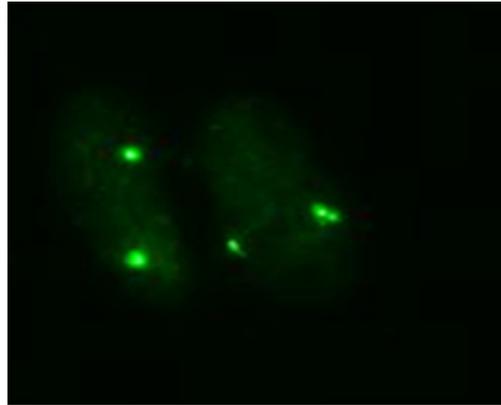
Frequency and type of chromosome aneuploidy

We obtained FISH results of 100 samples. Among these, there were 52 (52%) abnormal cases and the remaining were normal. Figure 1 showed results of normal XX and XY samples. Figure, 2, 3, 4, 6, 7, 8 and 9 showed the results of FISH studies of abnormal cases. Aneuploidies involved in the 13, 18, 21, and X chromosomes. The most prevalent trisomies identified by chromosome analysis were trisomy of chromosome 21 (18/52) followed by trisomy 18 (9/52), trisomy 13 (6/52). Sex chromosomes trisomies observed by FISH totaled

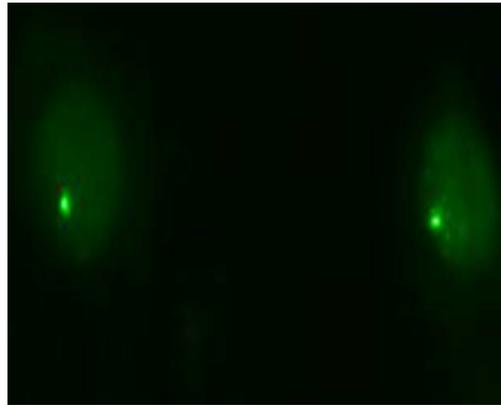
4 cases of XXX and 3 cases of XXY. The most frequent monosomy was X chromosome monosomy 12 cases (Table 1).



Figure(1): Normal female XX chromosome



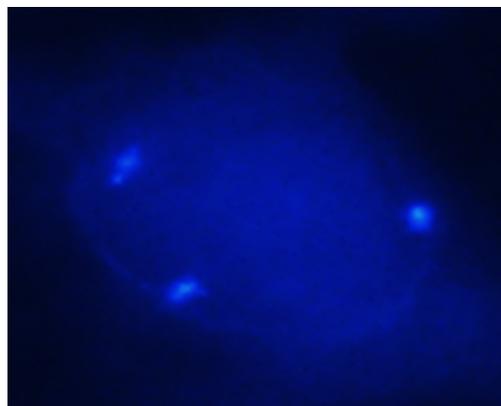
Figure(2): Normal male Y chromosome



Figure(3): Abnormal female X0 chromosome



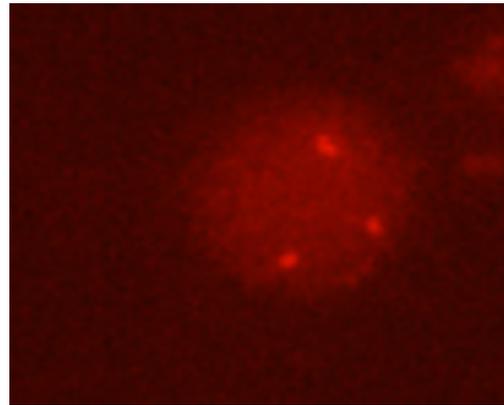
Figure(4): Normal chromosome 18



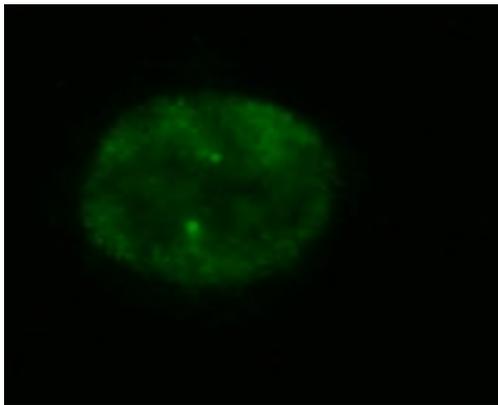
Figure(5): Abnormal chromosome 18 (trisomy 18)



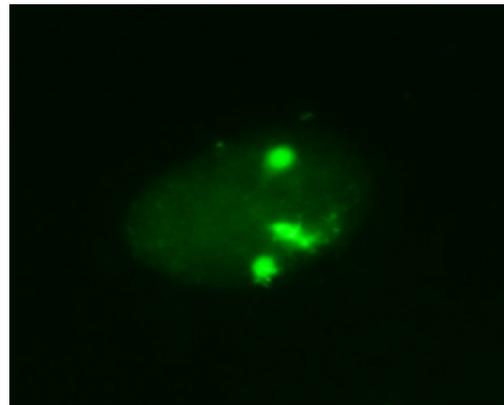
Figure(6): Normal chromosome 21



Figure(7): Abnormal chromosome 21 (trisomy 21)



Figure(8): Normal chromosome 13



Figure(9): Abnormal chromosome 13 (trisomy 13)

Table 1: The distribution of 52 cases with abnormal chromosomes

Chromosomal Abnormalities	Number of Patients	% Percent
Aneuploidy	52	
- Trisomy 21	18	34.61
- Trisomy 18	9	17.30
- Trisomy 13	6	11.54
- Trisomy X	4	7.69
- XXY	3	5.76
- X0	12	23.07

Sex ratio

To determine if there is a gender rate distortion in spontaneous abortions, fetal gender was determined for all abortuses using FISH technique. Results obtained from this investigation revealed that, of the 52 cases with chromosomal anomalies,

29 (55.77%) samples were females and 23 (44.23%) were males. The ratio of males to females was approximately 1:1.26. These results suggest the involvement of gender ratio distortion in recurrent miscarriages (Table 2).

Table 2: Sex ratio in 52 cases with abnormal chromosomes.

Sex	Number of Patients	% Percent
Males	23	44.23
Females	29	55.77

Relationship between aneuploidy and maternal age

In order to find out if there is association between maternal age and fetal chromosomal abnormalities, we studied the correlation between aneuploidy and maternal ages as shown in (Table 3). It was found that, the rate of aneuploidy of the advanced maternal age group (63.15%, as usually advanced maternal age is ≥ 35) was higher than that of the young ma-

ternal age group (45%). To further identify type of aneuploidy for each age group, we found that the rate of monosomy X was similar in the aged and young age groups. However, rates of trisomies 18, 13, and 21 of the advanced maternal age group were remarkably higher than those of the young maternal age group ($P < 0.01$) (Table 4),(Figure 10).

Table 3: Maternal age and chromosomal aneuploidy

Maternal age	Patients number	Patients with abnormalities	% Percent
35 <	38	24	63.15
35 – 30	26	14	53.84
30 – 25	20	9	45.00
25 >	16	5	31.25

Table 4: Association between maternal age and type of chromosomal abnormality.

Maternal age	Trisomy 21	Trisomy 18	Trisomy 13	X0	Trisomy X & XXY
35 <	12	4	3	3	2
35 – 30	1	2	2	4	5
30 – 25	3	2	1	3	0
25 >	2	1	0	2	0

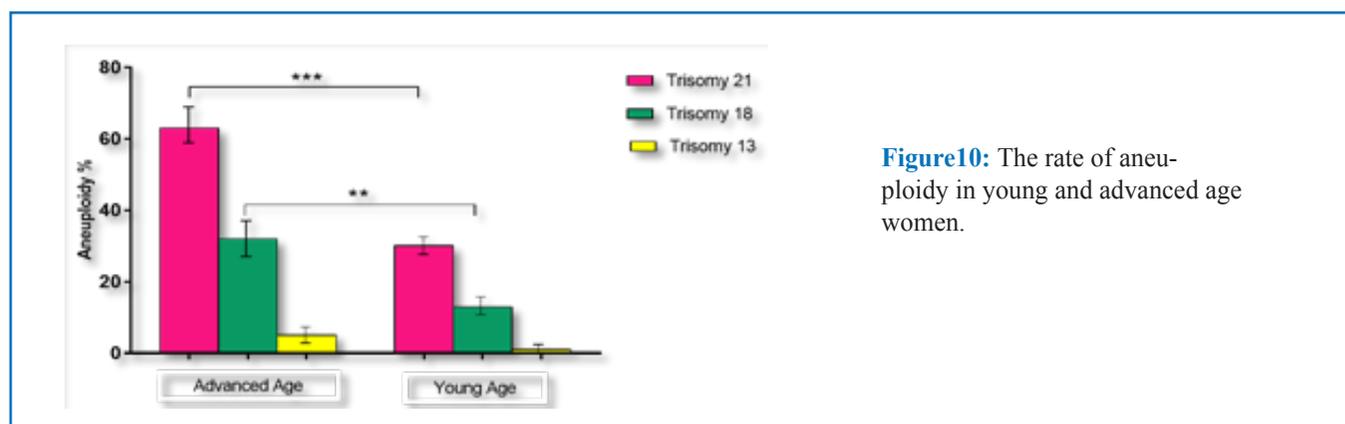


Figure10: The rate of aneuploidy in young and advanced age women.

Trisomy rates were determined in both groups and the difference was identified statistically using one way ANOVA. Trisomy 21 is significantly higher than young group ($***P < 0.001$). Trisomy 18 and 13 also were significantly higher in advanced group compared with young age group ($**P < 0.01$).

Difference between aneuploidy in consecutive and sporadic miscarriage

To examine whether common aneuploidy is one of the main causes of recurrent miscarriage, we divided the 52 cases into two groups. One group included cases with no or one previous miscarriage (19/52) and the other included those with two or more previous miscarriages (33/52). Of 33 cases having more than two miscarriages, 5 (15.62. %) had aneuploidy in each abortions. On the other hand of the 19 cases with sporadic abor-

tion, 2 (5.2%) had aneuploidy. There was a significant difference aneuploidy rate between the two groups ($P = 0.05$). These results indicates that recurrent aneuploidy may contributes to

recurrent miscarriage. Still, if it does so, it may influence a small number of patients.(Table 5).

Table 5: Difference between aneuploidy in consecutive and sporadic miscarriage

Cases	Number of cases	Cases with aneuploidy	% Percent
Consecutive	33	5	15.62
Sporadic	19	2	5.2

Discussion:

Chromosome aberrations are a clinically significant cause of pregnancy loss, congenital malformations, and cognitive impairment in humans. It has been demonstrated that numerical and structural chromosomal abnormalities are responsible for approximately half of miscarriages in the first trimester (17). Previous studies have reported that the rate of abnormality in chromosome number involved in spontaneous abortion was 23–61% (18). In present study, 100 abortion samples were analyzed using specific FISH probes targeting chromosomes X, Y, 21, 18 and 13, where 52% of cases were found to have an abnormal chromosomal complement. This finding is in accordance with the results of previous research using the same FISH technology (12,13,18). Current data showed that, of the 52 abnormal cases, the major kind of aneuploidy detected was trisomy, especially trisomy 21. X chromosome monosomy was the next common aneuploidy. It has been reported that, in second trimester, the most common aneuploidy is 21-trisomy (19). However, current study was conducted on samples of first trimester miscarriages. Therefore, results obtained here may indicates that most aneuploidies of fetal miscarriage spontaneously occur during the first trimester.

Of important note, the gender ratio of live births in the human species was found to be approximately 1.05 with males being slightly higher than females. This notion may suggest that there might also be a gender rate distortion in spontaneous abortions, where female fetuses may be more susceptible to abortion than male ones (20). However, conflicting results have been shown for the sex ratio of normal karyotype spontaneous abortions (21,20). Other reports have shown a greater number of females than males (22). According to current study, we found 29 females cases and 23 cases were males, with an approximate ratio of males to females of 1:1.26.

Previous reports have demonstrated that advanced maternal age is an important factor related to chromosomal aneuploidies. Our study showed a significant difference in the rate of aneuploidy between the advanced maternal age group (≥ 35 years) and the young maternal age groups (< 35 years). When the ratios of trisomies 21, 13, and 18 were compared between age groups, rates of trisomy were significantly higher in the advanced maternal age groups than in the young maternal age groups. For monosomy X, data presented here showed no differences between the two age groups. These findings indicate

that not all chromosome aneuploidies are related to advanced maternal age. Moreover, paternal age has been reported to contribute in fetal aneuploidy (23) (data was not available at the time of current study). Considering paternal age, it can be expected that fetal aneuploidy is a result of combination of maternal and paternal age.

Recurrent miscarriage is defined as the occurrence of at least two or more miscarriages [(24,25). In most couples, the rate of spontaneous fetal loss is 5% (1). It has been reported that, in women with recurrent pregnancy loss, the prevalence of chromosome aneuploidy was greatly varies (26,27). To examine whether aneuploidy is the main cause of recurrent spontaneous miscarriage, we compared the rate of aneuploidy of recurrent miscarriage with that of sporadic abortion. Results presented here demonstrated that women with recurrent miscarriage have a slightly higher rate of aneuploidy compared with those of sporadic abortion. These findings are in agree with previous studies by (21). This study investigated lymphocytes from couples with repeated miscarriage and proved that, there was an increased proportion of aneuploid cells in lymphocytes of recurrently aborting couples. Mitotic instability in the lymphocytes may indicate a predisposition to instability at meiosis leading to a chromosomal aberrations in the embryo and its subsequent abortion (21).

A limitation of this study was that we did not obtain all maternal and paternal karyotypes. However, the rate of abnormal karyotypes in parents with a history of an adverse outcome of pregnancy was approximately 2–3%. Therefore, we expected that chromosomal karyotypes of both parents may not have severely affected the results of this study.

It is concluded that chromosomal aneuploidy contribute to the underlying basis of reproductive failure in a varying proportion of cases and maternal age, previous miscarriage as well as sex ratios are risk factors. The identification of chromosomal abnormality as the etiology of spontaneous pregnancy loss will facilitate counseling and appropriate patient management. Furthermore, comprehensive characterization of chromosome aberrations could enable to calculate precise recurrent risk in the subsequent pregnancy. FISH is a reliable, sensitive and quick method to test aneuploidy in miscarriage compared with other conventional clinical methods.

Conclusions:

Aneuploidies involved in the 13, 18, 21, and X chromosomes. The most prevalent trisomies were trisomy of chromo-

some 21 followed by trisomy 18. Number of aborted case which contain chromosomal abnormalities were higher in females than males, It was found that, the rate of aneuploidy of the advanced maternal age group was higher than that of the young maternal age group, However, rates of trisomies 18, 13, and 21 of the advanced maternal age group were remarkably higher than those of the young maternal age group, . Cases with history of previous miscarriage were significantly have

higher aneuploidy rate compared with those with no previous miscarriage.

Chromosomal aneuploidy is a principal factor of miscarriage and maternal age, previous miscarriage are risk factor. Identification of these abnormalities helps to uncover to genetic etiology of miscarriage and estimate recurrence risks in future pregnancies.

References:

1. van den Berg MM., van Maarle MC., van Wely M. and Goddijn M. (2012) Genetics of early miscarriage. *Biochim Biophys Acta.*;1822:1951–9.
2. Petracchi F., Colaci DS., Igarzabal L. and Gadow E. (2009) Cytogenetic analysis of first trimester pregnancy loss. *Int J Gynaecol Obstet.*;104:243–4.
3. Maconochie N., Doyle P. and Prior S. (2007) Simmons R. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. *BJOG.*;114(2):170-86.
4. Mann K., Donaghue C., Fox SP., Docherty Z. and Ogilvie CM. (2004) Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. *Eur J Hum Genet.*;12:907–15.
5. Risch HA., Weiss NS., Clarke AE. and Miller AB. (1988) Risk factors for spontaneous abortion and its recurrence. *Am J Epidemiol.*;128:420–430.
6. Berkowitz GS., Skovron ML., Lapinski PH. and Berkowitz RL. (1990) Delayed childbearing and the outcome of pregnancy. *N Engl J Med.*;322:659–664.
7. Fretts RC., Schmittiel J., McLean FH., Usher RH. and Goldman MB. (1995) Increased maternal age and the risk of foetal death. *N Engl J Med.*;333:953–957.
8. Naylor AF. and Warburton D. (1979) Sequential analysis of spontaneous abortion. II: collaborative study data show that gravidity determines a very substantial rise in risk. *Fertil Steril.*;31:282–286.
9. Christiansen OB. (1997) Epidemiological, immunogenetic and immunotherapeutic aspects of unexplained recurrent miscarriage. *Dan Med Bull.*;44:396–424.
10. Lomax B, Tang S, Separovic E, Phillips D, Hillard E, Thomson T, et al. (2000) Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. *Am J Hum Genet.*; 66:1516–21.
11. Shimokawa O, Harada N, Miyake N, et al. (2006) Array comparative genomic hybridization analysis in first-trimester spontaneous abortions with 'normal' karyotypes. *Am J Med Genet A* ;140:1931–1935.
12. Lescoat D, Jouan H, Loeuillet-Olivo L, Le Calve M. (2005) Fluorescent in situ hybridization (FISH) on paraffin-embedded placental tissues as an adjunct for understanding the etiology of early spontaneous abortion. *Prenat Diagn.*;25:314–317.
13. Fejgin MD, Pomeranz M, Liberman M, Fishman A, Amiel A. (2005) Fluorescent in situ hybridization: an effective and less costly technique for genetic evaluation of products of conception in pregnancy losses. *Acta Obstet Gynecol Scand.*;84:860–863.
14. Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP. (2011) Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent in situ hybridization. *Genet Med.*;13(6):545-52.
15. Bryndorf T, Christensen B, Vad M, Parner J, Carelli MP, Ward BE, et al. 1996 Prenatal detection of chromosome aneuploidies in uncultured chorionic villus samples by FISH. *Am J Hum Genet.*;59:918–26.
16. Dória S, Carvalho F, Ramalho C, Lima V, Francisco T, Machado AP, et al. 2009 An efficient protocol for the detection of chromosomal abnormalities in spontaneous miscarriages or foetal deaths. *Eur J Obstet Gynecol Reprod Biol.*;147:144–50.
17. Carvalho B., Dória S., Ramalho C, Brandão O, Sousa M. and Matias A. (2010) Aneuploidies detection in miscarriages and fetal deaths using multiplex ligation-dependent probe amplification: An alternative for speeding up results?. *Eur J Obstet Gynecol Reprod Biol.*;153:151–5.
18. Menten B., Swerts K., Delle Chiaie B., Janssens S., Buysse K. and Philippé J., et al. (2009) Array comparative genomic hybridization and flow cytometry analysis of spontaneous abortions and mors in utero samples. *BMC Med Genet.*; 10:89.
19. Jia CW., Lan Y., Si YM., Ma YM., Lang Y. and Wang SY, et al. (2013) The analysis of the factors related to abnormal karyotypes of fetus. *Chin J Med Genet (Chin).*;30:635–7.
20. Del Fabro A., Driul L., Anis O., Londero AP., Bertozzi S., Bortotto L., and Marchesoni D. (2011) Fetal gender ratio in recurrent miscarriages. *Int J Womens Health.*; 3: 213–217.
21. Daniely M., Aviram A., Carp HJ., Shaki R. and Barkai G. (2001) The association between sporadic somatic parental aneuploidy and chromosomally abnormal placentae in habitual abortions. *Early Pregnancy.* Jul;5(3):153-63.
22. Jaslow CR., Carney JL., and Kutteh WH. 2010 Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril.*;93:1234–43.
23. Jacobs PA. and Hassold TJ. (1995) The origin of numerical chromosome abnormalities. *Adv Genet.*
24. Sullivan AE., Silver RM., LaCoursiere DY, Porter TF. and Branch DW. (2004) Recurrent fetal aneuploidy and recurrent miscarriage. *Obstet Gynecol.*;104:784–8. 1995;33:101–33.
25. van den Boogaard E., Kaandorp SP., Franssen MT., Mol BW., Leschot NJ. and Wouters CH., et al. (2010) Consecutive or non-consecutive recurrent miscarriage: Is there any difference in carrier status? *Hum Reprod.*;25:1411–4.
26. Ogasawara M., Aoki K., Okada S. and Suzumori K. (2000) Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril.*;73:300–4.
27. Suzumori N. and Sugiura-Ogasawara M. (2010) Genetic factors as a cause of miscarriage. *Curr Med Chem.*;17(29):3431-7.

دراسة كروموسومية لتقدير الاجهاض التلقائي المتكرر بواسطة تقنية التهجين في الموضع المفلور في مدينة اربيل / اقليم كردستان العراق

د. كetzال محمد سليمان

علوم الحياة/ كلية التربية/ جامعة صلاح الدين

الخلاصة:

ان الاجهاض التلقائي هي واحدة من التعقيدات الاكثر تكرارا اثناء الحمل وتعرف على انه حدوث على الاقل اجهاضين قبل الاسبوع العشرين من الحمل. فوق 50% من حالات الثلث الاول من الحمل تفقد والتي ترجع الى التشوهات الكروموسومية. ان هذه الدراسة تهدف الى البحث عن التضاعف الناقص في الاجهاض التلقائي بواسطة تقنية التهجين في الموضع المفلور بواسطة استخدام مسيرات لكروموسومات 13، 18، 21 و X و Y . تمت جمع وفحص 100 نموذج نسبي من بقايا الحمل بواسطة تقنية التهجين في الموضع المفلور، وقد تمت تحليل حدوث ونوع التشوهات الكروموسومية لكل نموذج. تمت دراسة العلاقة بين معدل التضاعف الناقص و عمر الام وايضا العلاقة بين عمر الام ونوع التضاعف الناقص والفرق بين حدوث التضاعف الناقص بين النماذج المؤخوذة من الحالات التي لها اجهاضات سابقة والآخرى التي ليست لها اجهاضات سابقة. تمت الحصول على النتائج من هذه الدراسة انه 52 من 100 حالة الاجهاض كانت لها التضاعف الناقص. ان الثلث الكروموسومات 13، 18، 21 كانت اكثر انواع التضاعف الناقص يليها تشوه احادى الكروموسومي. لقد لوحظت ان حالات الاجهاضات التي تحتوي على التشوهات الكروموسومية عالية في الاناث مقارنة بالذكور. ان حالات التي فيها تقدم في عمر الام وتاريخ عائلي لاجهاضات سابقة كانت معنويا لهل معدل تضاعف ناقص عالي مقارنة بحالات صغر عمر الام مع من هم ليست لديهم تاريخ باجهاضات سابقة. ان معدلات تثلث الكروموسومات 13، 18، 21 في المجموعة التي فيها تقدم عمر الام كانت ملحوظة بصورة عالية مقارنة بالمجموعة التي فيها اعمار الام صغيرة.