Fırat Tıp Dergisi 2004;9(3): 72-74

***Clinical Research***

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# Cytogenetic and FISH Analyses of the Abortus Materials: A Useful Approach Towards Genetic Counseling for the Couples with Recurrent Spontaneous Abortions

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**ABSTRACT**

**Objective:** The cytogenetic analyses of the abortus material provide important information about the recurrence risks and possible management for the couples with recurrent spontanous abortions. The methods of cytogenetic analyses depend on primarily cell culture that has relatively higher (10-40%) failure. Since the most common chromosomal abnormalities in abortus materials are numerical chromosomal abnormalities; we evaluated the compatibility of the karyotypes and interphase-Fluoresan *in situ* Hybridization (FISH) results and the predictive value of interphase FISH technique in cases with tissue culture failure.

**Materials & Methods:** Interphase-FISH slides were prepared using touch preparation protocol from fresh tissue samples to eliminate the collagenase procedure. FISH procedure was carried out using specific probes for centromeric 13, 16, 18 and 21. At least 100 interphase cells for each slides were detected.

**Results and Conclusion:** Chromosomal abnormality incidence of our study (45.5%) seems to be compatible with literature. It is founded some numerical aberrations (chromosomal abnormalities) as 18.18%autosomal trisomies, 13.64% triploidies, 4.50% monosomies and 9% mosaicism in cases. Two out of twenty eight abortus materials with tissue culture failure were revealed trisomies by FISH. However, the tissue culture failure incidence of this study (56%) is higher than the reported incidences (10-40%). This shortcoming could be diminished by the improvement of the tissue procurement and transportation conditions for culture. With this approach, supportive data will be accumulated and utilized for future epidemiological studies by concurrent FISH preparations and culture set up. *©2004, Fırat Üniversitesi, Tıp Fakültesi*

Key words: *Cytogenetic and FISH, recurrent abortions, abortus material*

**ÖZET**

**Abortus Materyallerinin Sitogenetik ve FISH incelemeleri: Rekurren Spontan Abortuslu Çiftlerin Genetik Danışmanlığına Yönelik Yararlı Bir Yaklaşım**

**Amaç:** Abortus materyalinin sitogenetik analizleri, tekrarlayan abortuslu çiftler için tekrar riski ve çiftlere verilecek olan genetik danışmanlık hakkında önemli bilgiler sağlar. Primer hücre kültürüne dayalı olan sitogenetik analiz metodları nispeten yüksek sayılabilecek bir oranda başarısızlık gösterir (%10-40). Sayısal kromozom anormallikleri, abortus materyallerindeki en yaygın kromozomal anormallikler olduğu için karyotip ve interfaz-Floresan in situ Hibridizasyon (FISH) sonuçlarının uyumluluğu ve doku kültürü başarısızlığı gösteren vakalarda interfaz FISH tekniğinin kullanılabilirliği değerlendirildi.

**Gereç ve Yöntem:** İnterfaz FISH preparatları kollajenaz prosedürünü ortadan kaldırmak için taze doku örneklerinden dokundurma yöntemi kullanılarak hazırlandı. FISH çalışmaları 13, 16, 18 ve 21'in sentromerine spesifik problar kullanılarak yapıldı. Her bir preparat için en az 100 interfaz hücresi değerlendirildi.

**Bulgular ve Sonuç:** Bu çalışmadaki kromozomal anomali insidansı %45.5 olup literatür ile uyumludur. Vakaların %18.8'inde otozomal trizomiler, %13.64'ünde triploidiler, %4.50'sinde monozomiler ve %9'unda mozaik sayısal kromozomal anomaliler bulundu. Doku kültürü başarısızlığı olan 28 düşük materyalinin ikisinde FISH tekniği ile trizomi tanımlandı. Bununla birlikte doku kültür başarısızlığı insidansı (%56), rapor edilmiş insidanslardan (%10-40) daha yüksekti. Bu başarısızlık farkı, kültür için doku temini ve transport koşullarının iyileşmesi ile azaltılabilir. Bu yaklaşımla, destekleyici bilgiler toplanacak ve bunun sonucunda FISH preparasyonları ile kültür kurma arasındaki uyum, gelecekteki epidemiyolojik çalışmalarda faydalı olacaktır. *©2004, Fırat Üniversitesi, Tıp Fakültesi*

Anahtar kelimeler: *Sitogenetik ve FISH, tekrarlayan abortus, abortus materyali*

More than 50% of spontaneous abortions have chromosomal abnormalities including primarily numerical abnormalities such as autosomal trisomies (29%), monosomy X (10%), poliploidy (10%) and mosaicism or structural chromosomal abnormalities (2%) (1, 2). The cytogenetic analyses of the abortus material provide valuable information about the recurrence risks and possible therapies for the couples with recurrent spontaneous abortions. The cytogenetic detections depend on providing viable tissue to perform primary culture and harvest metaphase chromosomes. The methods of the cell culture and chromosomal analyses are well described and accepted as a diagnostic method for last four decades (3). It is well known that these applications had some limitations such as relatively higher (10-40%) tissue culture failure and providing

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karyotypes not presenting concepts due to selective overproduction of maternal cells (4).

In the present study, we performed classical cytogenetic analyses of abortus materials and synchronously applied interphase-FISH technique to slides prepared by touch preparation method. The common probes were applied to touch preparation slides of the cases that could not be obtained karyotypes because of the tissue culture failure and the cases with abnormal karyotypes to compare and evaluate the predictive value of the interphase-FISH technique. Since the most common chromosomal abnormalities in abortus materials are numerical chromosomal abnormalities; we evaluated the compatibility of the karyotypes and interphase-FISH results and the predictive value of interphase FISH technique in cases with tissue culture failure.

## MATERIALS and METHODS

*Touch preparation protocol*

This procedure prepares fresh tissue samples for *in situ* hybridisation by eliminating the collagenase procedure. The small piece of fresh tissue was cut and gently touched onto a clean glass slide several times. The slide was immediately placed in cold methanol for 20 minutes and then immediately transferred to fresh fixative for 20 minutes. The slide was dehydrated in ethanol series, air dried overnight and stored at $-20^0$C until used.

Oncor's α satellite probes, D13Z1, D21Z1, D18Z1 and D16Z1, were blindly applied to all touch preparation slides.

At least 100 interphase cells for each slides were detected. The quality of the signals were adequate.

## RESULTS

Results obtained from chromosomal analyses of abortus materials and FISH in culture failure groups were shown in Table 1 and Table 2, respectively.

**Table 1.** The Results of The Chromosomal Analyses in Abortus Materials*.

| | |
|---|---|
| Normal | 12 (%54.68) |
| • 46,XX | 8 (%36.5) |
| • 46,XY | 4 (%18.18) |
| Anormal | 10 (%45.32) |
| Autosomal trisomies | 4 ( %18.18) |
| • 47,XY,+13 | |
| • 47,XY,+16 | |
| • 47,XX,+18 | |
| • 47,XX,+7 | |
| Triploidies | 3 (%13.64) |
| • 69,XXY | |
| • 69,XXY | |
| • 69,XXX | |
| Monosomy X | 1 (% 4.50) |
| Mosaic Numerical Chromosomal Abnormalities | 2 (% 9.00) |
| • 46,XX/92,XXXX (%80/%20) | |
| • 46,XX/ 47,XX,+11 (%67/%33) | |
| Toplam | 22 (% 100) |

*All cytogenetic results for cases with abnormal karyotype except the case with [46,XX/92,XXXX(%80/%20)] are compatible with interphase FISH method results.

**Table 2.** Results Obtained from FISH in Culture Failure Group*

| | |
|---|---|
| Tissue culture failure | 28 |
| Trisomy 16 | 1 |
| Trisomy 18 | 1 |
| Normal | 26 |

*Two cases with numerical chromosomal abnormality (Trisomy 16 and 18 ) were determined by FISH in tissue culture failure group

## DISCUSSION

The cytogenetic analyses of the abortus material provide valuable information about the recurence risks and possible therapies for the couples with recurrent spontaneous abortions (3). It has been reported that there was no statistically significant difference in cytogenetic abnormality frequencies of five different racial groups among the populations including 1000 cases. In the present study, it has been concluded that the variation of the chromosomal abnormality frequencies in cytogenetic analyses of abortus material could be due to the differences of applied methods in selected tissues rather than the biological variations in the study groups (5). Although our study group is small, the chromosomal abnormality incidence of our study (45.5%) seems to be compatible with literature. However, the tissue culture failure incidence of this study (56%) is higher than the reported incidences (10-40%). This shortcoming has been gradually diminished by the improvement of the tissue procument and transportation conditions for culture. We developed a protocol with Obstetrics and Gynecology and Pathology departments to maintain rapid transportation of the viable abortus materials containing placenta without using any fixative agent in sterile tissue containers. After first evaluation and tissue culture procedure with synchronous touch preparation, it had been referred to Department of Pathology for pathologic examination. So, we could provide a satisfactory data about any abortus including natural history, cytogenetic and pathologic findings.

The recent studies on the recurrent spontaneous abortions have revealed that the frequencies of the normal embryonic karyotypes were associated with previous number of the spontanous abortions. They have implicated that the previous pregnancies with normal embryonic karyotype might be an indicator for an abortus and the frequency of the liveborn following an aneuploid abortion was higher than the liveborn following euploid one (6, 7). Although our data is not still available for this kind of analysis, this finding is important point for genetic counseling.

Cytogenetic analyses has revealed no significantly difference in chromosomal abnormality ratios of abortus materials between the women groups with and without spontaneous recurrent abortions (8). Studies involving couples with repeated abortus revealed that incidences of chromosomal anomalies, mainly balanced chromosomal rearrangements, were between 3.39-6.56% (9, 10, 11). Chromosomal anomalies in abortus materials were reported to be present in more than 50% of cases (1). Rates of spontan abortus due to reproductive problems were higher than congenital malformations. But, most of the aborted fetuses are composed of malformed and/or defective fetuses. Although sequences of spontan abortus and malformed babies are different, results including environmental factors obtained from spontan abortus cases have important place in evaluation of malformations (12). In

the light of these accumulated data, our studies on abortus materials are in progress as a comprehensive epidemiological study involving incidences of anomalies and contributing etiological factors.

Although FISH analysis of common aneuploidies with uncultured material has potential for diagnosis, confirmation of these findings with standart cytogenetic techniques is important for prevention of misdiagnosis of the structural chromosomal abnormalities (13). Aneuploidies observed about 50% of spontan abortus cases are one of the main reasons to cause miscarriage (14). In total of 10 cases with chromosomal abnormalities presented in this study, except one mosaic case, aneuploidies determined with interphase FISH method were confirmed with standart cytogenetic methods. Discordance observed in the mosaic case karyotyped as 46,XX/92,XXXX by cytogenetic method mighy be due to two main reasons. Either low level of mosaicism may not be detected with FISH method or aneuploidy might be a result of *in vitro* conditions. Second explanation might be more reasonable since mosaicism determined cytogenetically was about 20% in tetraploidic line and this kind of anomalies are frequently observed as artifacts in long term cultured cells. In our study, 8 of 12 cases with normal karyotype were determined to be female. This finding might be partially due to overproduction of maternal tissues.

When evaluating spontan abortus cases, results obtained from aborted materials are vitally important for couples with repeated spontan abortus. These results may help to direct risk of abortus in the future and possible treatment approachs as well as they provide important data in terms of epidemiology of congenital malformations (3, 6, 7, 12). Most commonly encountered failure reasons in cytogenetic analyses, which are the basic methodology used in studying aborted materials, are failure of tissue culture and overproduction of maternal originated female karyotypes which does not reflect fetuses (4). Rate of cases with abnormal karyotypes obtained from materials of aborted cases is somehow related with methodology rather than differences due to population. Therefore optimized standart methodology and protocol is necessary to obtain useful information from these studies. In the present study, we aimed at determining chromosomal anomalies constituting most of the aneuploidies under conditions where culture failure is observed by synchronized cultures with FISH preparations where related probes utilized to obtain supportive data.

In the light of accumulated data and findings of the present study, to minimize culture failure of aborted materials in cytogenetic analyses, there must be a tight colloboration between the departmens of Obstetrics and Gynecology and Pathology with the Department of Genetics. With this approach, supportive data will be accumulated and may be utilized for future epidemiological studies by concurrent FISH preparations and culture set up.

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*Kabul Tarihi:26.08.2004*