Numerous erythroblasts in maternal blood are impervious to fluorescent *in situ* hybridization analysis, a feature related to a dense compact nucleus with apoptotic character

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Background and Objectives. The analysis by fluorescence *in situ* hybridization (FISH) of fetal erythroblasts enriched from maternal blood remains an attractive alternative for risk-free prenatal diagnosis of aneuploidies. However, current results are discouraging because of the low levels of sensitivity or the inability to detect fetal erythroblasts by FISH.

Design and Methods. Erythroblasts were enriched from 35 maternal blood samples by magnetic cell sorting (MACS), identified morphologically following May-Grünwald Giemsa staining and examined by FISH for chromosomes X, Y and 18.

Results. We observed that circulating erythroblasts comprised two distinct groups: one was clearly of maternal origin and could be reliably analyzed by FISH, whereas the other, which appeared to be of fetal origin, was largely impervious to FISH analysis. This latter feature seemed to be related to an abnormally dense nucleus with an apoptotic character. Since the oxygen tension in the maternal circulation is higher than that in the fetus, we cultured fetal cord blood erythroblasts in conditions mimicking this difference in oxygen concentrations and found that high oxygen concentrations rapidly induced shrinkage of the erythroblast nucleus, rendering it impervious to FISH analysis.

Interpretation and Conclusions. Our data show that circulating erythroblasts of presumed fetal origin cannot be reliably analyzed by FISH because of an abnormally dense nucleus. This nuclear phenotype appears to be induced by the higher oxygen tension present in the maternal circulation than in fetal blood.

Key words: non-invasive, prenatal diagnosis, fetal cells, FISH, oxygen concentration

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The number of women opting to have children at an age of greater than 35 years has increased considerably in the past decade. Because advanced maternal age is associated with an elevated risk of fetal aneuploidies, this demographic change has resulted in an increased need for prenatal diagnostic services.[1,2] Since these diagnostic procedures currently rely on invasive practices (amniocentesis, chorionic villus sampling or cordocentesis) which pose a risk to the mother and the unborn fetus, the need has been voiced for the development of risk-free methods for prenatal diagnosis.

One of the most promising strategies for the non-invasive, and hence, risk-free prenatal diagnosis of fetal aneuploidies is enrichment and analysis of rare fetal cells which have crossed the placental barrier and entered the maternal circulation.[1,2] The validity of this approach has been underscored by a number of proof-of-concept studies.[1,2] The fetal erythroblast has emerged as the target cell of choice in most approaches, because of its abundance in the fetal circulation, scarcity in normal adult blood, short lifespan, expression of potentially fetal-specific markers (embryonic and fetal hemoglobin molecules) and absence of genetic mosaicism.[1,2] Consequently the enrichment and analysis of this type of fetal cell have been the focus of several large scale studies, of which the most comprehensive was the multi-center, NIH-funded NIFTY study.[3,4] The results of this study, in which over 2700 maternal blood samples were examined, indicated that this approach is currently not suitable for clinical applications, as the sensitivity for detection of fetal erythroblasts was very low.[4] This ranged

from 13% in those cases in which erythroblasts were enriched by flow cytometry (FACS) to 44% in those cases employing magnetic cell sorting (MACS).[4]

Although disappointing, these results were still more promising than those of other studies in which either a preponderance of maternal erythroblasts or no fetal erythroblasts were detected. These latter findings suggested that fetal erythroblasts may not be present at all in the maternal circulation.[5] In addition, several studies have reported that fetal erythroblasts could only be detected in maternal blood samples drawn after an invasive procedure, such as chorionic villus sampling[6] or termination of pregnancy,[7,8] conditions which are known to lead to a large influx of fetal cells into the maternal peripheral circulation.[9]

We had previously shown, by the use of single cell polymerase chain reaction (PCR) analysis on single micro-manipulated erythroblasts identified by May-Grünwald Giemsa (MGG) staining, that almost half of these cells in maternal blood were of fetal origin,[10] and that this procedure could be used for the reliable determination of multiple fetal loci.[11] Therefore, a disparity appeared to exist between the analysis of fetal erythroblasts by PCR and FISH. To clarify this relationship we performed FISH analysis on erythroblasts similarly identified following MGG staining. In addition we examined the possible influence of different oxygen concentrations, akin to those in fetal and maternal blood, on the analysis of this type of cell by FISH.

Design and Methods

Enrichment, identification and electronic position marking of erythroblasts

This study was approved by the Cantonal Review Board of Basel and written informed consent from participants was obtained in all instances. Fifteen milliliters of peripheral blood were drawn from 35 women with normal singleton pregnancies (18 cases with female fetuses, 17 cases with male fetuses). The mean gestational age was 12.4 weeks, the range was from 10 to 16 weeks. The blood samples were processed within 6 hours from the time of collection, using our established anti-CD71 MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) enrichment procedure, with the exception that a density gradient of 1083 g/L was used.[12] Following elution of the positively enriched cell fraction from the miniMacs® columns, the cells were transferred onto glass microscope slides by cyto-centrifugation (Shandon, Frankfurt, Germany) and differentially stained with MGG (Sigma, St. Louis, MO, USA). Mature erythroblasts were identified visually based on morphological criteria and their positions on the slides were electronically marked using an Axioplan 2 imaging microscope system (Carl Zeiss, Zürich, Switzerland) equipped with a digital automated location finder (Axiovision 3.1, Carl Zeiss, Zürich, Switzerland).

FISH analysis of electronically marked erythroblasts

FISH for X and Y chromosomes was performed using the directly labeled chromosome enumeration probe-cocktail X(α) spectrum green/Y(α) spectrum orange (Vysis/Abbott AG, Baar, Switzerland). FISH using two different Y-probes was performed with a combination of the X(α) fluorescein/Y(α) rhodamine-cocktail (Qbiogene, Basel, Switzerland) and the Yqh (classical satellite) rhodamine (Qbiogene)-probe. FISH for chromosomes 18 and X was accomplished using centromeric probes (18-fluorescein, X-rhodamine) (Qbiogene). The FISH procedures were all performed according to the manufacturer's instructions. All samples were scored in a blinded manner.

Microwave enhancement of FISH efficiency

Microwave treatment has been suggested to improve the efficiency of the FISH analysis.[13] To test this we used the protocol described by Durm and colleagues whereby the glass slides were mounted on a small glass filled with water and treated in the center of a microwave oven (700W for 40 sec).[14] In addition, we also tested whether treatment with dithiothreitol (DTT) improved the efficiency of the FISH analysis. This reducing agent has been reported to improve FISH analysis of very condensed nuclei, such as those of sperm, as it disrupts disulfide bonds thereby promoting decondensation.[15]

TUNEL assay

To detect nuclear fragmented DNA in enriched erythroblasts from maternal blood, these cells were examined by the TUNEL assay (Roche Molecular Biochemicals, Germany) according to the manufacturer's instructions and as described previously.[16] Positive and negative controls for TUNEL staining were incubated with DNase I and label solution, respectively.

Cell culture of cord blood erythroblasts with different oxygen concentrations

Six cord blood samples were collected post-partum, using EDTA as an anticoagulant, and cultured immediately in different oxygen concentrations as described previously by Kondo *et al.*[17] Briefly, 800 μL of each cord blood sample were incubated with 10 mL of PB-Max™ medium (Gibco Life Technologies, Basel, Switzerland) for 24h at 37°C with 3% or 20% oxygen. Cells were then transferred onto glass slides and stained with MGG.

Morphometric analysis

The diameter and circumference of erythroblasts, identified morphologically following MGG staining, were measured using the Zeiss Axiovision 3.1 measurement software (Carl Zeiss, Zürich, Switzerland).

Statistical analysis

To analyze differences in nuclear size between fetal cord blood erythroblasts following culture in either 3% or 20% oxygen concentrations, we used the Wilcoxon sign-rank test, with a p value <0.05 indicating statistical significance. To determine whether a significant difference existed between the size of nuclei amenable or impervious to FISH analysis, a T-test (2-tailed) assuming unequal variances was used.

Results

FISH analysis with centromeric probes

On average 13 (range 2-24) erythroblasts were recovered from each maternal blood sample analyzed following enrichment with our standard anti-CD71 MACS protocol.[12] Following positive identification, the position of each erythroblast on the glass slide was electronically marked using a Zeiss automated location finder. Each cell was then individually examined by FISH using Vysis centromeric probes for the X and Y chromosomes. This analysis indicated that in those samples obtained from pregnancies with male fetuses, on average only 5.3% of the erythroblasts were XY positive, while 50.9% were clearly of maternal origin as they contained XX signals (Table 1). The remaining erythroblasts (>40%) had aberrant or no FISH signals (Table 1; Figure 1).

FISH analysis with satellite probes

Although our hybridization efficiencies on normal lymphoid cells were greater than 95%, we were concerned that these results may have been due to a less than optimal FISH procedure. Hence, we tested a different source of FISH probes (Qbiogene) in which two different Y probes (α- and classical-satellite) were used in combination with an α-satellite probe for the X chromosome. These examinations did not yield any significant improvements (Table 1), with on average only 6.4% of the erythroblasts being identified as male, while more than 30% of the cells had aberrant or no FISH signals. We also observed that in those cases in which no XY positive erythroblasts were detected, the number of erythroblasts with no FISH signals increased. In several instances we also observed loss of entire erythroblasts during the hybridization procedure.

Table 1. FISH analysis of morphologically assessed erythroblasts enriched from maternal blood samples.

Fetal Sex/FISH probe	*No. of cases/ Total no. of erythroblasts*	*XX+ Nuclei*	*XY+ Nuclei*	*00+ Nuclei*	*X0+ Nuclei*	*XXY+ Nuclei*	*Loss of erythroblast*
Male Vysis	7 113	50.9%	5.3%	21.0%	16.7%	2.6%	3.5%
Male Vysis + MW	6 111	50.4%	3.6%	17.1%	14.4%	5.4%	9.0%
Male Qbiogen	8 109	61.5%	6.4%	14.7%	8.3%	0.9%	8.1%
Male Qbiogen + MW	7 138	50.0%	4.4%	28.3%	5.0%	1.5%	10.8%
Female Vysis	7 56	41.1%	0.0%	26.8%	5.4%	0.0%	26.6%
Female Qbiogen	8 113	47.8%	0.0%	38.9%	8.8%	0.0%	4.4%

Vysis refers to the use of Vysis FISH probes for the X and Y chromosomes. Qbiogen refers to the use of X and 2 different Y FISH probes.
MW refers to microwave treatment.

FISH analysis in cases with female fetuses and with chromosome 18 probes

The inability to examine all erythroblasts by FISH did not appear to be restricted to pregnancies with male fetuses, since in pregnancies with female fetuses we also observed that on average only 44.5% of the erythroblasts were XX positive, while the remaining erythroblasts had aberrant or no FISH signals (Table 1). In addition, this phenomenon was not restricted to the sex chromosomes, as we observed similar results in an analysis of 6 cases for chromosome 18, since only 51% of the erythroblasts were correctly hybridized.

Microwave enhancement of FISH analysis

Since microwave treatment has been suggested to improve the efficiency of FISH analysis by enhancing the exposure of the chromosomes to FISH probes,[13,14] we examined this approach using both Vysis and Qbiogene FISH probes (Table 1). In our experiments, we did not observe that this approach produced a discernible improvement (Table 1). In this context, treatment with dithiothreitol (DTT) has also been reported to improve FISH analysis of very condensed nuclei, such as those of sperm, due to the disruption of disulfide bonds promoting nuclear decondensation.[15] In our hands, this strategy did not lead to any significant improvement in the FISH analysis of erythroblast nuclei (*data not shown*).

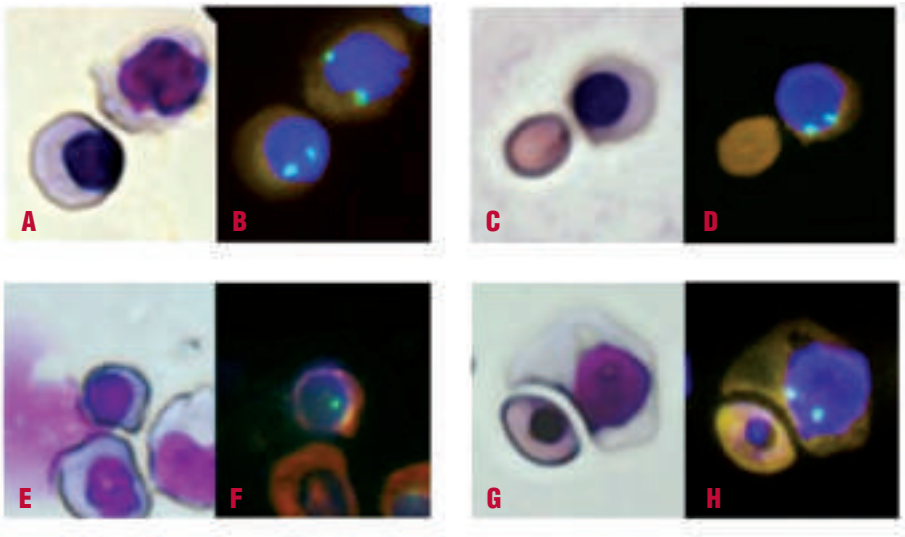

Figure 1. FISH analysis of erythroblasts enriched from maternal blood. A, B. XX positive erythroblast, characterized by a large nuclear diameter, enriched from a pregnancy with a male fetus. C, D. XX positive erythroblast, characterized by a large nuclear diameter, enriched from a pregnancy with a female fetus. E, F. XY positive fetal erythroblast, characterized by an intermediate nuclear diameter, enriched from a pregnancy with a male fetus. G, H. FISH negative erythroblast, characterized by very small nuclear diameter. All images were taken at 630× magnification.

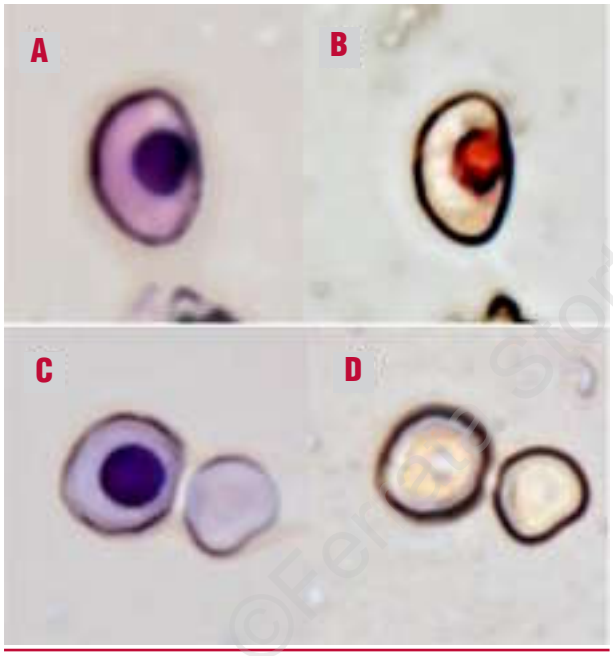

Figure 2. Examination of erythroblast nuclear size and presence of fragmented nuclear DNA in maternal blood. A, B. Erythroblast with a small dense nucleus which is TUNEL positive. C, D. Erythroblast with a large nucleus which is TUNEL negative. All images were taken at 630× magnification.

As we had previously observed, using single cell PCR on individual micro-manipulated erythroblasts,that almost 50% of these cells were fetal,[10] the combination of these two data sets suggests that those erythroblasts which are impervious to FISH analysis may be of fetal origin.

Morphometric analysis of erythroblast nuclei

In order to determine why only one fraction of circulating erythroblasts can be readily analyzed by FISH, we examined the morphologic characteristics of these cells in closer detail. This morphometric analysis indicated that those erythroblasts in maternal blood which had hybridized efficiently had a significantly larger nuclear diameter (average=6.5 μm for XX signals; average=6.2 μm for XY signals) and circumference (average=23.3 μm for XX signals; average=23.0 μm for XY signals) than those which had been impervious to the FISH procedure (average=4.7 μm; $p<0.01$; average=17.5 μm; $p<0.01$, respectively) (Figure 2). Since the differentiation of erythroblasts into enucleated erythrocytes involves fragmentation of the nuclear DNA, which can be detected by the TUNEL assay,[16] we next examined whether this aspect might be the reason for the low FISH efficiency. This analysis demonstrated that approximately 69% of those erythroblasts with a nuclear diameter larger than 5.6 μm were negative for the TUNEL assay, whereas almost 92% of those with a diameter smaller than this contained fragmented DNA (Figure 2). This latter phenomenon supports the notion that those erythroblasts with small nuclei containing fragmented DNA are of fetal origin, as it has previously been shown that these cells become TUNEL-positive upon entering the maternal circulation.[18]

TUNEL analysis of erythroblast nuclei

In order to determine whether either the presence of fragmented DNA or a small nucleus hindered the FISH analysis of fetal erythroblasts in the maternal circulation, we first examined erythroblasts in cord blood, having previously observed that a significant proportion of these cells have TUNEL-positive nuclei.[16] Our analysis of such fetal cord blood erythroblasts indicated that although a high proportion of these cells were TUNEL-positive (57.3%), almost all could be reliably analyzed by FISH (82.3%), irrespectively of whether they were TUNEL-positive or negative. Interestingly,

our analysis of these post-partum cord blood erythroblasts indicated that these cells had relatively large nuclear diameters and circumferences (average=6.0 μm and 21.93), and were larger than those erythroblasts which could not be analyzed by FISH (average diameter=4.7 μm and average circumference=17.5 μm).

These results suggest that the presence of a dense nucleus rather than a nucleus containing fragmented DNA hinders the effective analysis of erythroblasts by FISH. We also observed that the DNA in these dense compact erythroblast nuclei hindered the reliable analysis of individual cells by single-cell PCR. Our analysis of pooled cells by PCR did, however, indicate that some of these cells were fetal.

Effect of oxygen tension

Taken together our results suggest that fetal erythroblasts undergo a fundamental change when they enter the maternal circulation, and that this change in phenotype involves an alteration in the size of the nucleus. We examined whether this change in nuclear size could be attributed to the different oxygen tensions in the fetal and maternal circulatory systems.[18] To test this hypothesis we cultured post-partum cord blood samples in different oxygen concentrations mimicking those in fetal and maternal blood (3 and 20%, respectively). This experiment clearly showed that the erythroblast nuclei did indeed shrink significantly (p=0.03) at the higher oxygen concentration (20%) when compared to the lower oxygen concentration (3%) (p=0.46) (Figure 3). We also determined that 24 hours of exposure to high oxygen concentrations hampered reliable analysis of these erythroblasts by FISH (XY cocktail probe from Vysis) (median=9%; range=25-77%), when compared to cells cultured in low oxygen conditions (median=88%; range=69-94%) and untreated control samples (median=91%; range=86-95%).

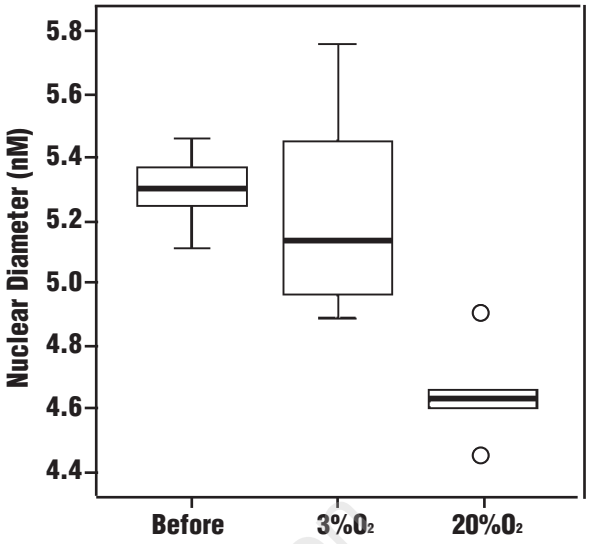

Figure 3. Influence of oxygen concentration on erythroblast nuclear size. Cord blood erythroblasts were briefly cultured in 3% and 20% oxygen concentrations, after which morphometric characteristics of their nuclei were examined. The data are presented by box plots, indicating the median value (line in the box), the 75th and 25th percentiles (limits of the box), and the 10th and 90th percentiles (upper and lower horizontal bars). Outliers are indicated by empty circles.

Discussion

In this study we found that, on average, only half of the circulating erythroblasts in maternal blood could be efficiently analyzed by FISH, and that these erythroblasts were largely of maternal origin, as they had a XX genotype. Only a small fraction of the erythroblasts in maternal blood could be reliably identified as being of fetal origin (<5%) on the basis of clear FISH signals for the X and Y chromosomes. The rest of this pool of circulating erythroblasts appeared to be refractory to FISH analysis. This feature was evident not only for the Y chromosome, but also for the analysis of chromosomes X and 18. Furthermore, this feature could not be overcome by decondensation strategies employing microwave or DTT treatment, which have previously been shown to be effective for the analysis of other compact nuclei, such as those of sperm.[14,15] Our data further indicated that this group of erythroblasts differs from those which are clearly of maternal origin, in that they have a small dense nucleus containing fragmented DNA. However, we determined that the inability to analyze these erythroblasts efficiently by FISH was probably not due to the presence of the fragmented nuclear DNA, but rather to the small and densely compact nucleus. This result was most evident in our analysis of cord blood erythroblasts, whose nuclei could all be efficiently analyzed by FISH despite being TUNEL positive. In previous studies using single-cell PCR on individual erythroblasts isolated by micro-manipulation, we had observed that almost 50% of the erythroblasts in the maternal circulation were of fetal origin.[10,19] Although the erythroblasts in this previous PCR study were not selected on the basis of having a small dense TUNEL-positive nucleus, it would appear that the erythroblasts in our current study, which are impervious to FISH analysis, are also of fetal origin. This hypothesis is supported by our ongoing studies using PCR which indicate that a number of these erythroblasts with a small dense nucleus are indeed of fetal origin. Our data also provide a possible reason for this difference between putative fetal and maternal erythroblasts in the maternal circulation, in that the change in the nuclear phenotype may be triggered by the difference in oxygen tensions existing between the mother and fetus. In this regard, it is interesting to note

that previous studies have shown that when fetal erythroblasts enter the maternal circulation their nuclear DNA becomes fragmented,[18] as detected by the TUNEL assay, and that this effect can be mimicked by exposing such cells to elevated oxygen concentrations.[17] Our investigations into this apoptotic phenotype suggested that this process of nuclear cleavage is associated with terminal differentiation and subsequent enucleation, rather than with apoptosis, as these cells do not express other characteristic apoptotic markers.[20] In our current analysis we demonstrate that 24-hour culture in elevated oxygen conditions leads to a significant reduction in the size of fetal erythroblast nuclei, and that this significantly reduces the efficacy of FISH analysis of these erythroblasts. As we only tested a single 24-hour time point, it is currently unclear whether shorter periods of exposure also lead to similar nuclear condensation. This aspect, which remains to be explored, may help to explain why under certain conditions, such as immediately post-termination, fetal erythroblasts can be reliably detected by FISH analysis.[7,8] Taken together, our data suggest that a large proportion of the erythroblasts in the maternal circulation cannot be reliably analyzed by FISH, and that it is probable that many of these cells are of fetal origin. It also appears that this change is induced by the higher oxygen tension present in the maternal circulation. Our data, therefore, provide an explanation for the apparent lack of fetal erythroblasts in the maternal circulation,[5] or the inability to detect them with high degrees of sensitivity when using FISH for their analysis.[4] This aspect will have to be considered in future potential diagnostic approaches. Alternative strategies may, therefore, focus on the use of primed *in situ* labeling (PRINS), as this method relies on smaller molecules than current FISH probes for the detection of fetal specific sequences, which may be able to enter the dense fetal erythroblast nuclei more successfully.

TB: performed the erythroblast enrichments, their analysis by FISH, as well as the culture and analysis of cord blood samples. TB prepared all the figures (1,2,3) and tables (1). SM: assisted and guided these experiments. SM assisted with the statistical analysis. GDN: performed the pilot study and made the initial observation. SH: performed the apoptosis studies. ST: recruited the samples for this study and assisted in the interpretation of the data. WH, SH: conception and design of the study, drafting the article, analysis and interpretation of data, generation of the final version to be published. All authors reviewed and approved the final version. *This study was supported by funds from the University Women's Hospital/Department of Research, Basel, Switzerland. The authors declare that they have no potential conflicts of interest.*

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References

1. Bianchi DW. Fetal cells in the maternal circulation: feasibility for prenatal diagnosis. Br J Haematol 1999; 105:574-583.
2. Hahn S, Holzgreve W. Prenatal diagnosis using fetal cells and cell-free fetal DNA in maternal blood: what is currently feasible? Clin Obstet Gynecol 2002; 453:649-56.
3. Bianchi DW, Simpson JL, Jackson LG, Evans MI, Elias S, Holzgreve W, et al. Fetal cells in maternal blood: NIFTY clinical trial interim analysis. DM-STAT. NICHD fetal cell study (NIFTY) group. Prenat Diagn 1999;19:994-5.
4. Bianchi DW, Simpson JL, Jackson LG, Elias S, Holzgreve W, Evans MI, et al. Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. Prenat Diagn 2002;22: 609-15.
5. Slunga-Tallberg A, el Rifai W, Keinanen M, Ylinen K, Kurki T, Klinger K, et al. Maternal origin of nucleated erythrocytes in peripheral venous blood of pregnant women. Hum Genet 1995;96: 53-7.
6. Oosterwijk JC, Knepfle CF, Mesker WE, Vrolijk H, Sloos WC, Pattenier H et al. Strategies for rare-event detection: an approach for automated fetal cell detection in maternal blood. Am J Hum Genet 1998; 63:1783-92.
7. Choolani M, O'Donnell H, Campagnoli C, Kumar S, Roberts I, Bennett PR, et al. Simultaneous fetal cell identification and diagnosis by ε-globin chain immunophenotyping and chromosomal fluorescence in situ hybridization. Blood 2001; 98:554-7.
8. Choolani M, O'Donoghue K, Talbert D, Kumar S, Roberts I, Letsky E, et al. Characterization of first trimester fetal erythroblasts for non-invasive prenatal diagnosis. Mol Hum Reprod 2003;9:227-35.
9. Bianchi DW, Farina A, Weber W, Delli-Bovi LC, Deriso M, Williams JM, et al. Significant fetal-maternal hemorrhage after termination of pregnancy: implications for development of fetal cell microchimerism. Am J Obstet Gynecol 2001; 184:703-6.
10. Troeger C, Zhong XY, Burgemeister R, Minderer S, Tercanli S, Holzgreve W, et al. Approximately half of the erythroblasts in maternal blood are of fetal origin. Mol Hum Reprod 1999; 5:1162-5.
11. Di Naro E, Ghezzi F, Vitucci A, Tannoia N, Campanale D, D'Addario V, et al. Prenatal diagnosis of β-thalassaemia using fetal erythroblasts enriched from maternal blood by a novel gradient. Mol Hum Reprod 2000;6:571-4.
12. Troeger C, Holzgreve W, Hahn S. A comparison of different density gradients and antibodies for enrichment of fetal erythroblasts by MACS. Prenat Diagn 1999; 19:521-6.
13. Yurov YB, Saias MJ, Vorsanova SG, Erny R, Soloviev IV, Sharonin VO, et al. Rapid chromosomal analysis of germ-line cells by FISH: an investigation of an infertile male with large-headed spermatozoa. Mol Hum Reprod 1996;2:665-8.
14. Durm M, Haar FM, Hausmann M, Ludwig H, Cremer C. Optimized fast-FISH with α-satellite probes: acceleration by microwave activation. Braz J Med Biol Res 1997;30:15-23.
15. Miharu N, Best RG, Young SR. Numerical chromosome abnormalities in spermatozoa of fertile and infertile men detected by fluorescence in situ hybridization. Hum Genet 1994;93:502-6.
16. Hristoskova S, Holzgreve W, Hahn S. More than one-half of the erythroblasts in the fetal circulation and cord blood are TUNEL positive. Clin Chem 2001;47: 1870-1.
17. Kondo T, Sekizawa A, Saito H, Jimbo M, Sugito Y, Okai T. Fate of fetal nucleated erythrocytes circulating in maternal blood: apoptosis is induced by maternal oxygen concentration. Clin Chem 2002; 48:1618-20.
18. Sekizawa A, Samura O, Zhen DK, Falco V, Farina A, Bianchi DW. Apoptosis in fetal nucleated erythrocytes circulating in maternal blood. Prenat Diagn 2000;20: 886-9.
19. Zhong XY, Holzgreve W, Li JC, Aydinli K, Hahn S. High levels of fetal erythroblasts and fetal extracellular DNA in the peripheral blood of a pregnant woman with idiopathic polyhydramnios: case report. Prenat Diagn 2000; 20:838-41.
20. Hristoskova S, Holzgreve W, Hahn S. Fetal nucleated erythrocytes in maternal circulation do not display a classic membrane-associated apoptotic characteristic (phosphatidylserine exposure) despite being positive by terminal dUTP nuclear end labeling. Clin Chem 2003;49:1934-7.