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ROUTINE CYTOGENETIC PRENATAL DIAGNOSIS USING DYNAMIC BANDING (RBG–GBG): A HIGHLY REPRODUCIBLE METHOD FOR AMNIOCYTES, FETAL CORD BLOOD, AND CHORIONIC VILLUS INVESTIGATIONS

J. B. SAVARY, F. VASSEUR, J. L. LAI, A. DAUDIGNON AND M. DEMINATTI

Service de Génétique Humaine et Pathologie Fœtale, Faculté de Médecine, 1 Place de Verdun, 59045 Lille CEDEX, France

SUMMARY

Dynamic banding (RBG–GBG) using pulse 5-bromodeoxyuridine (5-BrdU) incorporation during part of the last S-phase before harvesting has been used in prenatal investigations. This method has already been routinely applied in 1344 cytogenetic investigations. GBG and RBG bandings produced almost identical patterns to classical G- and R-banding methods except for heterochromatic portions and some euchromatic segments. Nevertheless, these discordances may be somewhat helpful for cytogenetic diagnosis (i.e., X numerical abnormalities). The results showed particularly good contrast and staining; 5-BrdU incorporation did not prevent additional staining. Likewise, previous RBG or GBG disclosure allowed further chromosomal identification with C-banding or nucleolar organizer staining. Simplicity and reproducibility were very helpful in cases with a low mitotic index. 5-BrdU had no significant effect on *in-vitro* damage because only 0·31 per cent of cells were affected; so, we believe that dynamic banding should be used more extensively in cytogenetic investigations. Moreover, the staining and contrast qualities were very suitable for automatic methods of analysis now in use: i.e., metaphase finding and computer-assisted karyogram creation.

KEY WORDS Dynamic banding 5-BrdU incorporation

INTRODUCTION

Classical Q, R, and G-bandings are global methods allowing the precise identification of each chromosome (Caspersson *et al.*, 1970; Dutrillaux and Lejeune, 1971; Seabright, 1971; Sumner *et al.*, 1971). Routine R- and G-bandings rely upon exogenous physical, chemical, and thermic (RHG banding) or enzymatic (GTG banding) treatments. Results with these methods reveal some variability from one tissue to another according to unstandardized factors such as ageing of preparations, hygrometry, and room temperature. Incorporation of 5-bromodeoxyuridine (5-BrdU) in chromosomal DNA is well known (Hsu and Somers, 1961; Zakharov and Egolina, 1972). Thus, to minimize exogenous factors we applied this endogenous event: 5-BrdU incorporation during part of the S-phase (Dutrillaux and Couturier, 1981).

This method was first applied to peripheral blood and then extended to fetal tissues routinely used in our hospital laboratory (Hospital Calmette Lille): amnion cells–

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cord blood–chorionic villus samples (CVS). High reproducibility and simplicity make this method suitable for routine use and analysis on automated systems.

MATERIAL AND METHODS

Chromosomal preparations were obtained with usual protocols: 24–48 h short-term culture (CVS), 72 h middle-term culture (fetal cord blood), and long-term culture (amnion cells—CVS).

R-banding with 5-BrdU incorporation (RBG)

Whatever the tissue may be, 5-BrdU was added to the medium at a final concentration of 10 μg/ml for the last 20 h (short-term culture), the last 6 h (middle-term culture), and the last 7 h (long-term culture). Fetal blood cell cultures were synchronized with 30 μg/ml thymidine according to Dutrillaux and Couturier (1981).

G-banding with 5-BrdU incorporation (GBG)

GBG banding was used for fetal blood cell culture only. After one night of synchronization with 5-BrdU (200 μg/ml), the blocking agent was removed and the lymphocytes were incubated with fresh medium containing thymidine (3 μg/ml) for a further 7 h before harvesting.

R- or G-banding disclosure

In any case to reveal R- or G-banding, we used an adaptation of the fluorochrome–photolysis–Giemsa (FPG) method (Perry and Wolff, 1974). Briefly, slides were stained for 20 min in an aqueous solution of Hoechst 33258 (10 μg/ml); rinsed in 2 × SSC (1 × SSC is 0·15 M NaCl and 0·015 M sodium citrate); mounted in 2 × SSC; and irradiated for 90 min at a distance of 10 cm from 360 nm UV black light (Duke F 20T9BLB). Preparations were then dipped for 5 min into pH 5·1 Earle's balanced salt solution at 87°C for RBG banding, or for 90 min in 2 × SSC at 60°C for GBG banding, and then washed in ice-cold distilled water and stained for 5 min in freshly prepared 1·5 per cent Giemsa (RAL), pH 6·8.

RESULTS AND DISCUSSION

Chromosome banding with 5-BrdU incorporation was first obtained from 1652 postnatal diagnoses (1638 RBG tests and 14 RBG+GBG tests). Because of the simplicity and high reproducibility allowing systematic R- and/or G-bandings, these methods were extended to 1344 prenatal diagnoses: 1104 amniocenteses, 166 cordocenteses, and 74 choriocenteses (Figure 1). Dynamic bandings were found to be especially useful for cultures of low mitotic index. Nevertheless, 5-BrdU incorporation did not interfere with subsequent standard staining, RHG banding, and GTG banding, or even after disclosure of the previous RBG or GBG banding, C-banding, or nucleolar organizer staining (Figure 2).

Banding patterns were in agreement with the international classification (ISCN, 1985). Both combined RBG and RHG haploid karyotypes (Figure 3) and GBG and

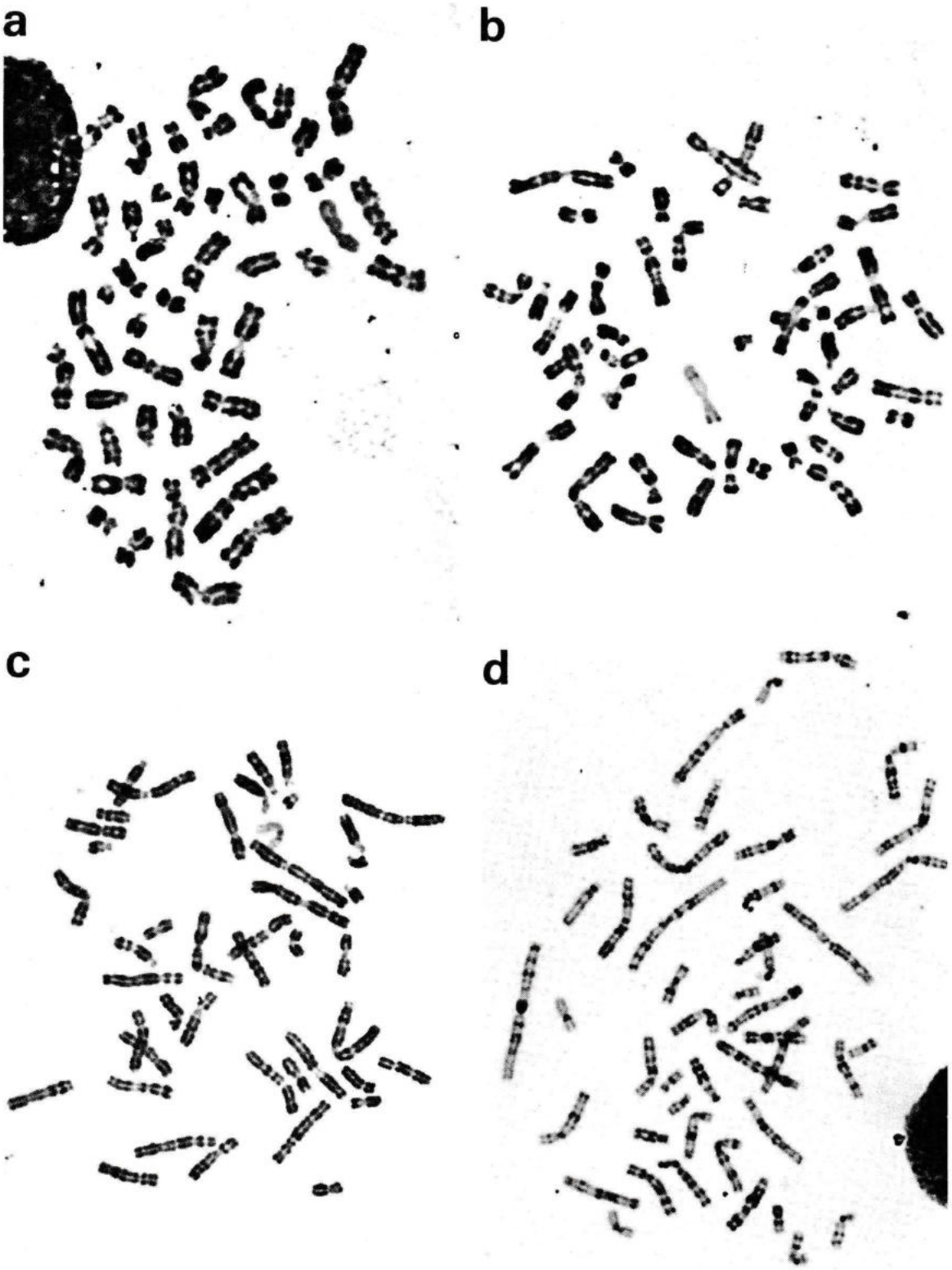

Figure 1. RBG dynamic banding: CVS 24 h short-term culture (a), fetal cord blood 72 h middle-term culture (b), and amnion cell long-term culture (c). GBG dynamic banding: middle-term lymphocyte culture (d)

GTG haploid karyotypes (Figure 4) exhibited great similarity between 5-BrdU incorporation banding (left) and classical methods (right) for global appearance, detail, and number of bands. However, obvious differences were noticed between classical and 5-BrdU dynamic bandings. These included late-replicating heterochromatic portions faintly stained with RBG banding and darkly stained with GBG

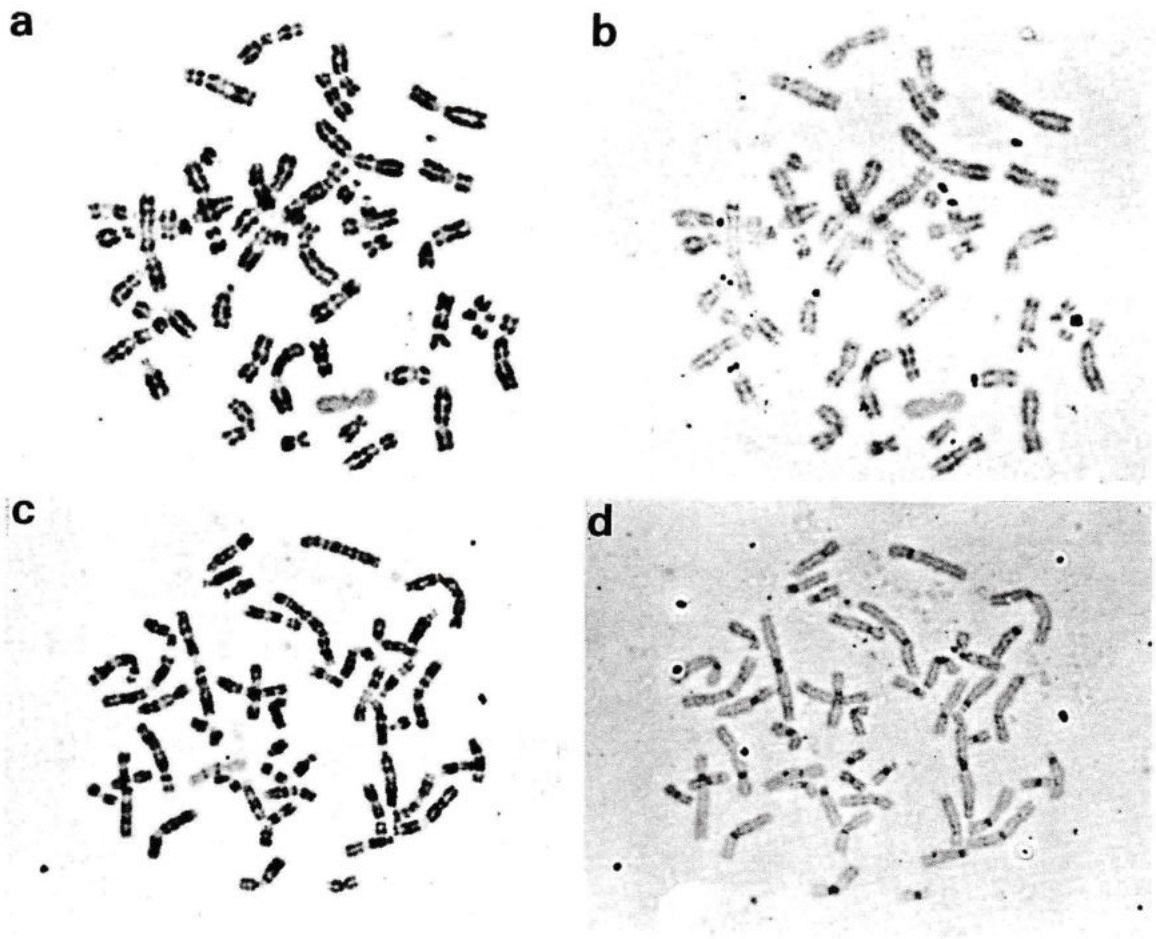

Figure 2. Metaphase cells with dynamic RBG banding (a,c) and subsequent nucleolar organizer staining (b) and C-banding (d)

banding (Figure 5). This property allowed the easy identification of secondary constrictions and their variants 1 qh+, 9 qh+, 16 qh+; acrocentric chromosome short-arms and their variants: Dp+, Gp+; Y heterochromatin and its variants: Yq+, the latter being sometimes asymmetrically stained. Owing to the fact that inactive X-chromosomes are GBG-positive and RBG-negative, the interpretation of X structural abnormalities may be somewhat more difficult especially with RBG banding; for example, fragile site X-chromosome identification when late-replicating X is affected. Nevertheless, this is a minor problem because in female heterozygotes expression of the fragile X site occurs randomly on either early- or late-replicating X-chromosome (Sutherland, 1983). X staining properties with dynamic bandings were of great interest, first to display X homogeneous or mosaic numerical abnormalities and second to screen *in-vitro* numerical X abnormalities when additional X-chromosomes did not appear late-replicating (RBG-negative) as they should do for *in-vivo* disturbance. Slight but significant differences were noticed for some euchromatic autosomal portions. So, our results showed 5p, 6p, and 10q RBG-negative telomeric segments, whereas these were RHG-positive. Higher resolution was achieved with RBG banding for 3q, 4q, and 15q segments and the whole 17 chromosome as compared with RHG banding. Very few contrast differences were noticed between GBG and GTG patterns, but RBG banding offered a better contrast than RHG. Discordance between GBG and GTG has been reported for euchromatic 5p, 8q, 11q, 13q, and 20q portions (Lemieux *et al.*, 1990); our results

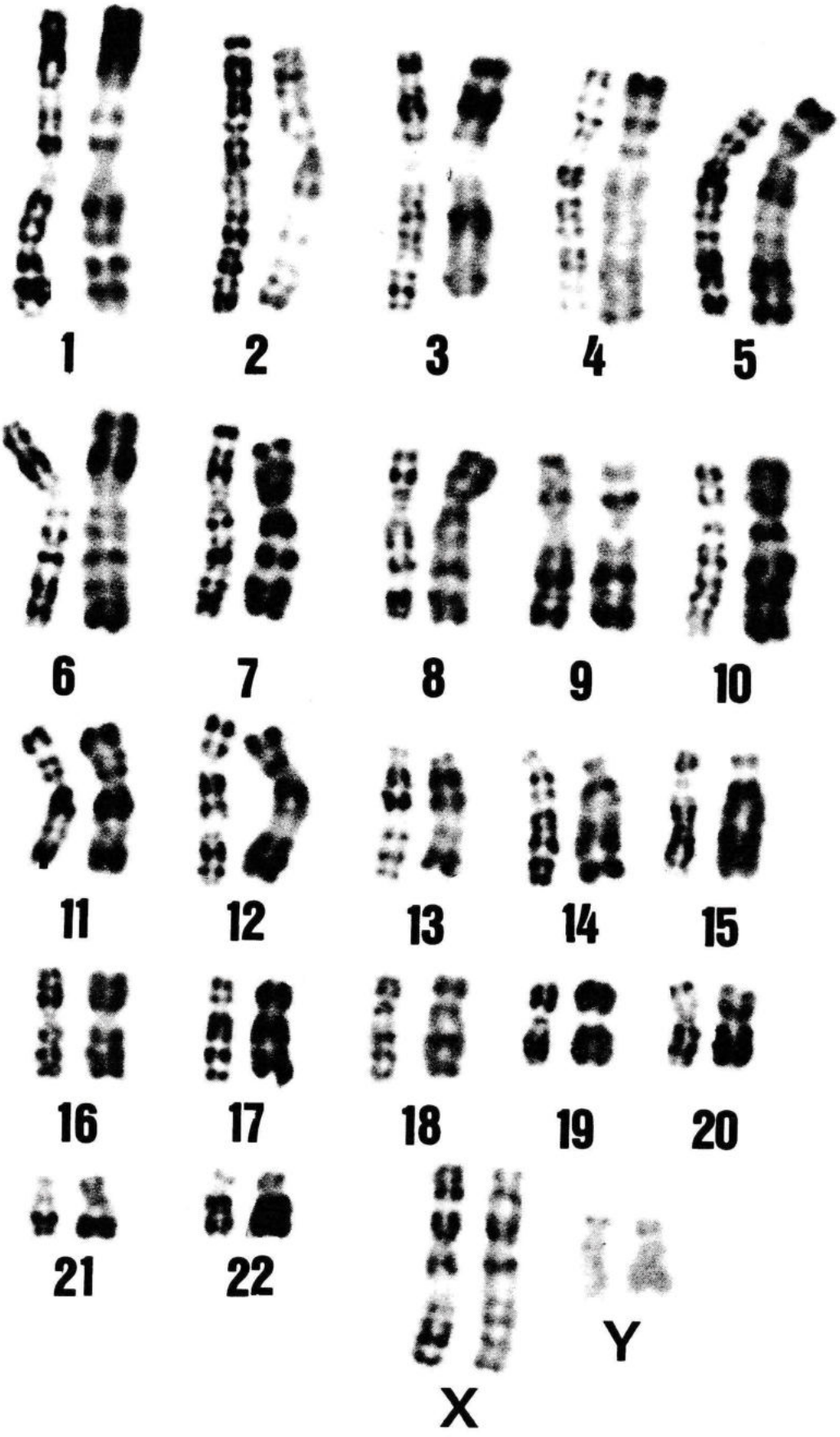

Figure 3. Combined haploid karyotypes: with RBG (left) and RHG (right) banding

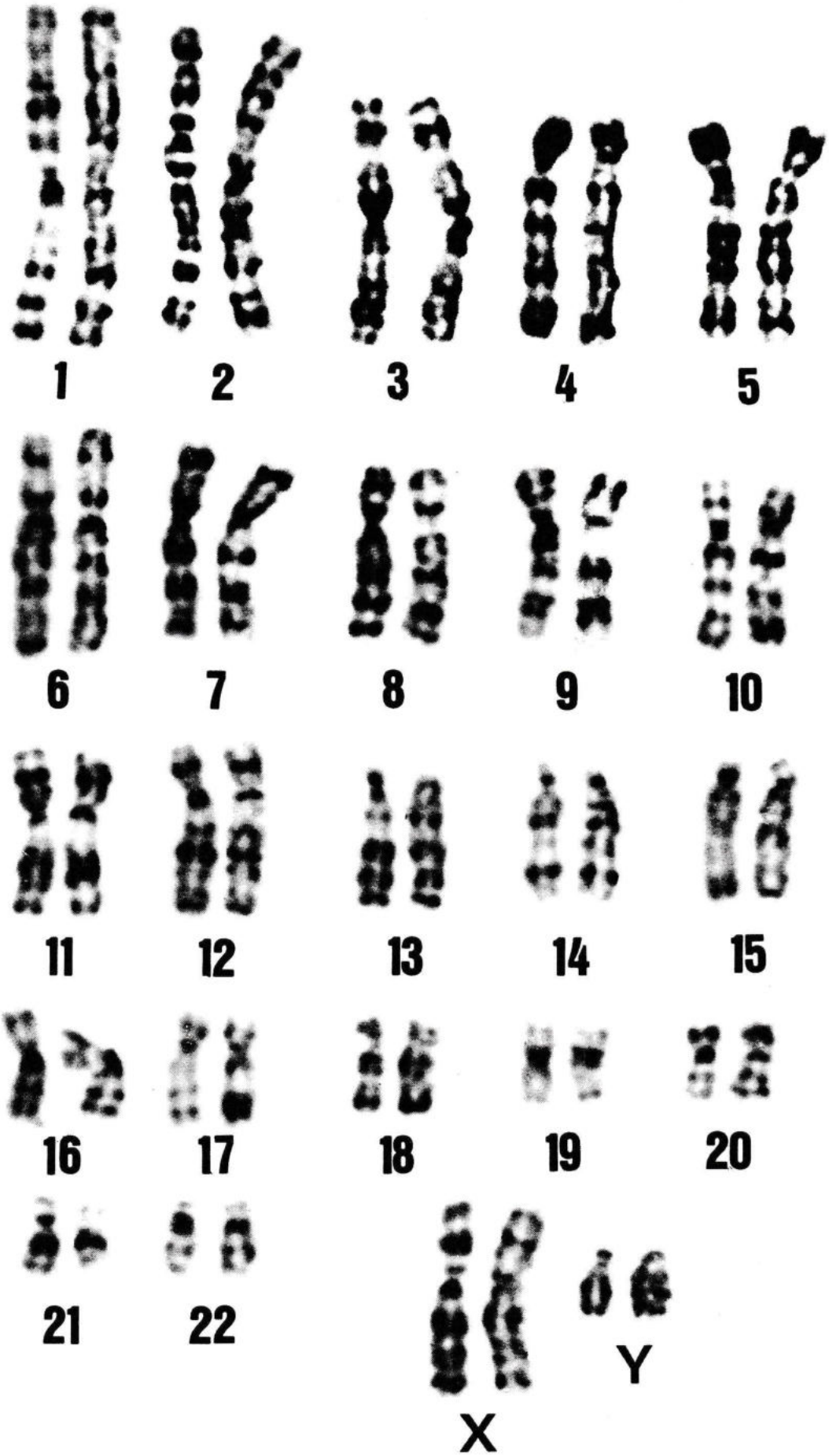

Figure 4. Combined haploid karyotypes: with GBG (left) and GTG (right) banding

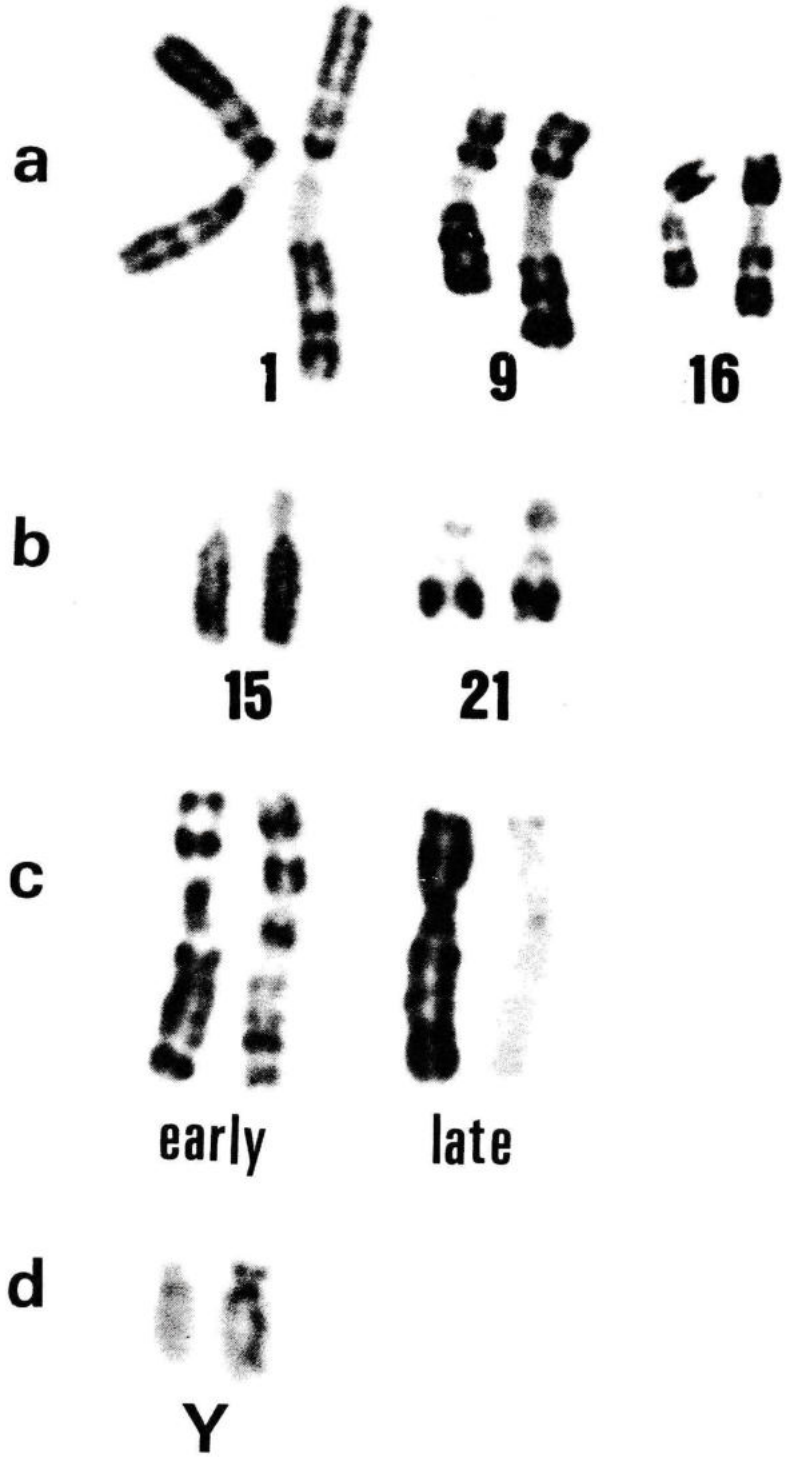

Figure 5. (a) 1, 9, and 16 normal chromosomes (left) and their qh+ variant (right) with RBG banding. (b) 15 and 21 normal chromosomes (left) and their p+ variants (right) with RBG banding. (c) Early- and late-replicating X-chromosomes with GBG (left) and RBG banding (right). (d) RBG-banded normal Y-chromosomes, with homogeneous (left) and asymmetrical staining (right)

exhibited only a 5pter difference (Figure 3). Dynamic bandings require the incorporation of a thymidine analogue in replicating DNA. These could be damage-prone methods. In our experience, isolated numerical or structural abnormalities involving a single cell have been assumed as *in-vitro* damage. Clonal abnormalities and 10q25 5-BrdU requiring fragile sites (Scheres and Hustinx, 1980) were thus left out. If 5-BrdU promotes clonal abnormalities, pulse incorporation during part of the last

Table 1. Proportions of cases and cells with *in-vitro* damage (IVD) after dynamic banding in prenatal diagnosis

Number of cases			Number of cells		
without IVD	with IVD	Total	without IVD	with IVD	Total
1286 (95·69%)	58 (4·31%)	1344	24 929 (99·69%)	79 (0·31%)	25 008

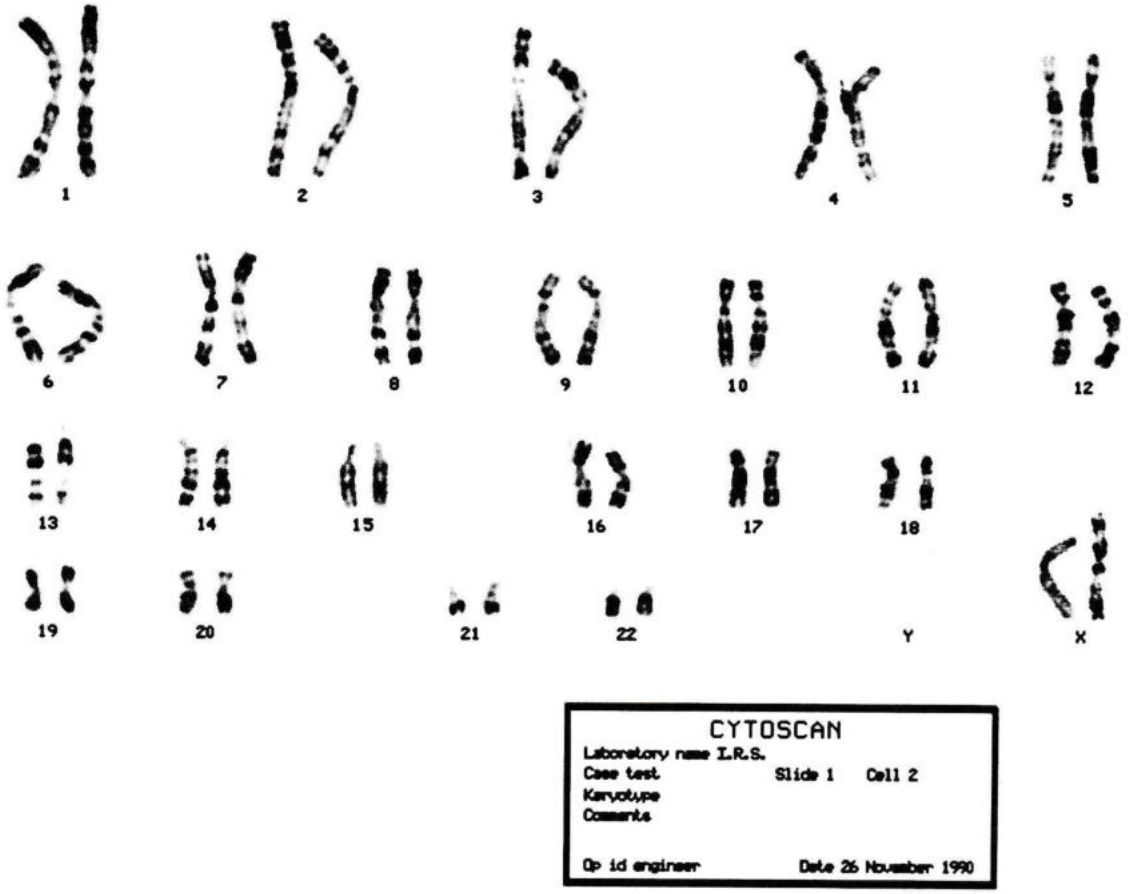

Figure 6. Automatic karyogram creation (RBG banding) with the Cytoscan RK 1 automated cytogenetic system

S-phase before harvesting does not allow enough time to involve more than one cell. Therefore every clonal aberration was finally assessed as *in-vitro* damage. As shown in Table 1, of 1344 prenatal diagnoses 58 analyses (4·31 per cent) presented *in-vitro* damage. Nevertheless, according to the total number of cells analysed (25 008), only 79 (0·31 per cent) were affected and the *in-vitro* damage was very uncommon.

Dynamic methods provide typical R- and G-banding patterns and the interpretation of numerical and structural abnormalities is not more difficult than with classical ones. An explanation for the high reproducibility could be the fact that the methods are based on the kinetics of incorporation into replicating DNA. The simplicity of the protocol, whatever the screened tissues may be, and the rapid

results make these methods suitable for routine prenatal cytogenetic analysis. Differences between classical RHG and RBG banding were noticed for heterochromatic portions and some euchromatic segments but these had no assessable outcome. Moreover, these may become additional 'bandings' useful for diagnostic purposes (i.e., X numerical abnormalities and qh+ chromosomes). One might expect the incorporation of a non-physiological nucleotide to be damage-prone. In fact, only 0·31 per cent of cells were affected by *in-vitro* damage; this is in agreement with too short a delay for damage effects and allowed routine safe use of dynamic bandings.

Technological development is moving toward automatic analysis of cytogenetic data. Two trends are in progress: metaphase finding and computer-assisted karyogram creation based on image analysis. Automatic finding is based on the discrimination of several parameters, e.g., area, shape, and optical density of metaphases. Computerized karyogram creation is achieved by use of classical parameters: the size of chromosomes, the centromeric index, the banding pattern, and the relative contrast between chromosomal bands. Dynamic banding with 5-BrdU has no effect on either shape or on area but offers reproducible and good quality staining minimizing artefact selection and so optimizing metaphase finding. Invariability in contrast, banding pattern, and grey level should offer reproducible material suitable for automatic karyogram systems as shown in Figure 6.

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