Hum. Genet. 37, 299—302 (1977)

Human Genetics

Rapid Determination of Polyploidy in Human Chorionic Tissue Sections

K. Riekki[1], J. E. Baillie[2], K. K. Nair[1], F. J. Dill[3], B. J. Poland[2], and D. L. Baillie[1]

[1] Department of Biological Sciences, Simon Fraser University, B. C. Canada V5A 1S6
[2] Department of Obstetrics and Gynaecology University of B.C. Vancouver, B.C. Canada
[3] Department of Medical Genetics, University of B.C. Vancouver, B.C. Canada

Summary. Chromosomal analysis from aborted tissue has become an important diagnostic aid. However, the necessary cultures are frequently unsuccessful due to the condition of the aborted tissue. Polyploidy, in particular triploidy, in the conceptus is a common cause of early pregnancy loss and unlike aneuploidy does not appear to be associated with an increased recurrence risk. The necessity to monitor a subsequent pregnancy with amniocentesis is therefore eliminated. Therefore, in cases where a chromosomal anomaly is probable, a fast simple method of identification of a polyploid karyotype would be valuable. In this presentation, we describe a method using a scanning light microscope and histologic tissue preparations. This method can accurately determine the ploidy of the aborted material in 5 days.

Introduction

Chromosomal analysis of tissue cultures from abortuses and still births has become an important aid in the diagnosis of the cause of a pregnancy loss. The identification of an associated chromosomal anomaly may affect the management, prognosis, and counselling in subsequent pregnancies. However, the tissue necessary for culture from these embryos and fetuses is often unsatisfactory (as a result of retention in utero) or impossible to culture if placed inadvertently in fixative.

Polyploidy, in particular triploidy, is a common finding in conceptuses from spontaneous abortion (Carr, 1971) and in humans is lethal either before or soon after birth (Niebuhr, 1974). Mittwoch (1968) has used nuclear DNA content and nuclear volume to assay for cell ploidy. Unlike the trisomic chromosomal anomalies, triploidy has not been shown to have an increased recurrence risk in a subsequent pregnancy and, therefore, monitoring of a subsequent pregnancy by amniocentesis would not be indicated. For this reason it is important to differentiate between aneuploid and triploid chromosome anomalies in the investigation of a pregnancy loss. Because spontaneously aborted tissue is difficult to

culture successfully, a fast simple method which could identify tissue with a polyploid chromosome complement in histologic preparations, would be of value. In this report we describe such a method using the scanning light microscope and histologic tissue preparations.

Material and Methods

The material used for this study consisted of histologic sections of placental tissue from spontaneous abortuses. Placental tissue was chosen because the cells remain in a better state of preservation, both in aborted tissue and in stillborn material, than other tissues of the embryo or fetus. A sample of amnion from each abortus was cultured for chromosomal analysis, using methods previously described (Poland et al., 1976) and karyotyped to determine chromosome complement. The placental specimens were fixed in 10% formaldehyde and processed through baths of alcohol and chloroform and finally embedded in paraffin wax. Sections 10 μm thick were cut from the paraffin blocks of tissue chosen from abortuses in which a diploid, triploid and tetraploid karyotype had been identified. In addition, tissue was processed in exactly the same way from a group of specimens in which culture had failed and the karyotype was unknown. Four sections consisting of the identified karyotypes and unknown karyotypes were arranged at random on a standard microscope slide. The three known samples provided an internal standard for comparison to the unknown and insured that all the tissue sections to be scanned were processed under exactly similar conditions. The slides containing the four sections were then dewaxed and prepared for histologic staining. They were stained in Schiff's reagent prepared according to Deitch (1966). Hydrolysis in 5 N HCl for 90 min and staining in Schiff's reagent (Basic Fuchsin, Harleco, Philadelphia) for 1 h was carried out in the dark at room temperature. After the bisulphite rinse and dehydration, the slides were mounted in oil of matching refractive index ($n_D = 1.56$, Cargille, Cedar Grove, N. J.). In some cases the aborted tissue was too macerated to take the Feulgen stain. A duplicate slide stained with hematoxylin and eosin (H & E) (Culling, 1974) gave an accurate indication of this type of unsatisfactory tissue. The measurements of the placental nuclei were made using the Scanning Microscope Photometer (SMP; Zeiss, Oberkochen, Federal Republic of Germany) interfaced to a PDP-12 computer (Digital Equipment Corporation, Maynard, Mass., USA). The scanning measurements were made with ultrafluar objective (X100, N.A., 1.25) and condenser (N.S., 0.8) at 0.5 μm steps across the nucleus. The diameter of the scanning aperture was 0.5 μm. Absorption measurements were made at 560 μm. Processing and integration of absorption measurements from individual nuclei used Apamos 2 version computer program (Zimmer, 1970). In order to insure that the nuclei chosen for measurement were fetal, only connective tissue nuclei contained centrally within intact placental villi were scanned. Nuclei from the syncytiotrophoblast and cytotrophoblast were avoided, since the nuclei in the trophoblast were too close together to get accurate scanning information. To eliminate bias, all measurements were made by operators who were not aware of the order of sections upon the slide.

Results

Scanning data were obtained from the tissues with known karyotypes as well as those which were unknown as a result of tissue culture failure. In all, 10 diploid, 10 triploid, and 3 tetraploid sections were scanned. Individual nuclei from within a section showed close agreement with one another (particularly if care was taken to measure only intact nuclei). The occasional nucleus was replicating and gave a high scanning value. Figure 1 shows the results of typical calibration curves for 8-min and 90-min hydrolysis times. For the 90-min hydrolysis curve

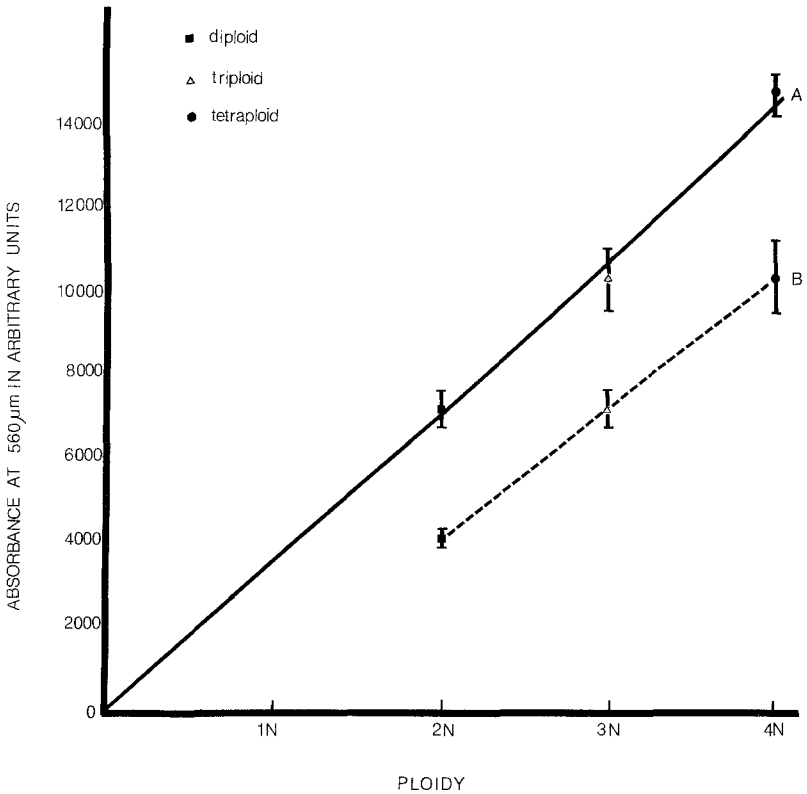

Fig. 1. Typical calibration curve of a karyotypically known diploid, triploid, and tetraploid. *A*: 90-min hydrolysis time, *B*: 8-min hydrolysis time

10 nuclei from each section were scanned and for the 8-min hydrolysis curve 25 nuclei were scanned. Note that even with the small sample size (10 nuclei) the 95% confidence limits (shown as bars around each of the points) were sufficiently small to assure accuracy in the assignment of ploidy to an unknown tissue section on the slide. In each case the determined ploidy was in agreement with the results of karyotyping from tissue culture. It should also be noted that even with a large variation in hydrolysis time (8 min, when optimum hydrolysis was achieved at 90 min) the results are sufficiently accurate to determine ploidy clearly.

Discussion

The important aspects of this method are the quality of the material and consistency of the staining methods. The tissue from fetal organs which has been retained in utero for up to 6 weeks may be unusable for tissue culture and although the placenta also may be poor, it is often in a better state of preservation than the organ tissue, while the karyotypes of both tissues are identical. The inclusion of known diploid, triploid, and tetraploid sections along with the

unknown makes ploidy determination possible even under suboptimal conditions (line B, Fig. 1).

To eliminate possible variations from one stain batch to another, all sections to be compared should be stained at the same time as a set of controls with known karyotypes.

It was found that any nuclei that stained with the H & E procedure also stained well with Feulgen. Nuclei that did not stain well with H & E did not take up the Feulgen stain. In order to screen 50 samples using a scanning light microscope, approximately 2 weeks of machine time are required. Since this comprises only a small percentage of total available machine time, the machine will thus be available to serve in other roles.

Conclusion

A method for the rapid determination of polyploidy in human chorionic tissue sections has been described. It would appear that in the majority of cases where the tissue is such that culture and exact karyotyping is impossible, an exact determination of the ploidy of the cell line is possible by this method within 5 days. This may be of considerable importance in the subsequent counselling and management of the patient.

Acknowledgement. Supported by Medical Research Council of Canada Grants MA 2740 and MA 5000, Simon Fraser University President's Fund and National Research Council Grant E-2465.

References

Carr, D. H.: Chromosome studies in selected spontaneous abortions. Polyploidy in man. J. med. Genet. **8**, 164—174 (1971)

Culling, C. F. A.: Handbook of histopathological and histochemical techniques (including museum techniques), 3rd ed. London: 1974

Deitch, A. D.: Introduction to quantitative cytochemistry, pp. 327—354. London-New York: 1966

Mittwoch, U.: Nuclear sizes in a human diploid/triploid cell culture. Nature **219**, 1074—1076 (1968)

Niebuhr, E.: Triploidy in man. Cytogenetical and clinical aspects. Humangenetik **21**, 103—125 (1974)

Poland, B. J., Dill, F. J., Styblo, C.: Embryonic development in ectopic pregnancy. Teratology (in press, 1976)

Zimmer, H.: Zeiss Information **74**, 126—132 (1970)

Received September 17, 1976 / March 22, 1977