Virchows Archiv A Pathol Anat (1990) 417:151–158

Virchows Archiv A
Pathological Anatomy and Histopathology

Surface and branching of placental villi in early abortion: relationship to karyotype

Scanning electron microscopic study

G. Röckelein[1], Renate Ulmer[2], and R. Schwille[1]

Institutes of [1] Pathology and [2] Human Genetics, University of Erlangen-Nürnberg, Krankenhausstraße 8–10, D-8520 Erlangen, Federal Republic of Germany

Summary. The placental villi of 61 early abortions with known karyotype and 7 legally induced abortions were investigated by scanning electron microscopy and documented in standardised enlargements. Five groups were established from the findings: uniformly branched villi with a velvety surface (group A) were found in 4 of the 7 induced abortions, abundant syncytial sprouts (group B) in 4 of the 6 cases with monosomy X; all 5 cases of triploidy were classified in the group bulbous or spherical villi (group C); 13 out of 25 cases of trisomy were found to have little branching and a surface densely covered with microvilli (group D), while 14 out of the 25 cases of euploidy belonged in the group with slender villi and surface with focal areas of denudation (group E). Forty of the 68 cases were properly assignable to the correct groups (58.8%). The non-uniformity of the villous morphology in the case of induced abortions shows that there is no uniform development of the (early) placenta. The variable morphology seen in abortions with euploidy reflects the various mechanisms of abortion applicable to this group.

Key words: Abortion – Placental morphology – Karyotype – Scanning electron microscopy – Cytogenetics

Introduction

Although magnifying glass and light-microscopic inspection of placental villi in early abortions frequently reveals differences in branching patterns and in the nature of the surface (Geisler and Kleinebrecht 1978; Göcke et al. 1982, 1985; Minguillon et al. 1988; Müntefering et al. 1982, 1987; Philippe 1973; Ornoy et al. 1981; Röckelein 1989; Röckelein et al. 1989) the three-dimensional structure of these placental villi has not been investigated.

Offprint requests to: G. Röckelein

The scanning electron microscope suggests itself for use in such an analysis, for its enormous depth of focus permits a good overview of three-dimensional structures.

Materials and methods

Portions of placenta, obtained by curettage from 61 spontaneous abortions, were studied in this investigation. Occasionally, spontaneously expelled material was also examined. Part of the tissue was placed in cell culture medium as quickly as possible for cytogenetic study; further portions of tissue were prepared for examination in the scanning electron microscope. The abortions occurred in the 6th–16th gestational week calculated. As a control group, we studied 7 placentas obtained from legal abortions performed in the first trimester for social indications. The karyotype of the induced abortions was not determined. All in all, 68 cases were employed in the study.

For the most part, chromosome analysis was carried out on the basis of direct preparations of spontaneous mitoses, without the need for prior tissue culture. As a rule, mitoses obtained from cell cultures were karyotyped as a control. In each case, at least five metaphases were analysed photographically. The cytogenetic findings represent the reference for our scanning electron microscopic investigations. Preparation of the tissue was carried out in accordance with the usual methods, and involved dehydration in acetone, critical point drying, and subsequent gold sputtering (for a description of the methodology see Reimer and Pfefferkorn 1973). All the cases were documented in standardised enlargements: about ×10 (overview), ×30, ×60, ×100 and ×300.

On the basis of estimated villous diameter, villous surface, branching pattern, number of syncytial sprouts and fibrin deposits, five "findings groups" were established, and the cases assigned to these groups (Table 1).

The villi were measured with the aid of the morphometry unit MOP (Kontron, Munich, FRG); the calibration of the morphometry device was done in accordance with the magnification scale to be seen in each of the photographs.

For determining the diameter and length of the unbranched portions of the villi. In each case, all of the villi to be seen in an enlargement were evaluated, the measurements being made at distances of 1 cm. Depending upon the size of the villi and the dimensions of the preparation, between 40 and 150 measurements were performed for each case. In the case of the measurement of the length of the unbranched portions of the villi, the number of measurements carried out was smaller (between 6 and 25). The mean figures for diameter and lengths, together with the standard

Table 1. Criteria for classification of placental villi of first trimester abortions by scanning electron microscopy

	Villus branching	Villus diameters	Surface	Syncytial sprouts	Fibrin deposits
Group A	**Regular**	Decreasing peripherally	Uniformly velvety	Many	Very little
Group B	Variable	Variable	Dense covering of microvilli	**Extremely abundant**	Very little
Group C	Irregular	**Very thick or bulbous**	Mostly velvety, frequent ridges	Many	Very little
Group D	Usually little branching	**Often cylindrical**	**Uniformly covered with microvilli**	Few	Very little
Group E	Sparse	Mostly very slender	**Focal areas of denudation**	Sparse	**Much**

deviations of the mean values, were computed for further analysis. The distribution of the parameters was evaluated in the form of a histogram with a logarithmic scale; the frequency in each class in the histograms was incorporated in further evaluation. Discrimination analyses to test diagnostic significance were obtained from the SPSS® (Statistical Package for Social Sciences, IBM PC version).

Results

Twenty-five abortions were euploid, 14 with a male and 11 with a female karyotype (Fig. 1). Six cases had monosomy X, while in 25 cases autosomal trisomy presented (Fig. 1). One case showed trisomy 14 in connection with Robertsonian translocation 13/14; a further abortion had a partial trisomy 20 on the basis of translocation 14/20. In addition, 5 cases of triploidy were seen. In 2 cases, tissue culture revealed mosaicism with euploid female cells that were interpreted as maternal contamination.

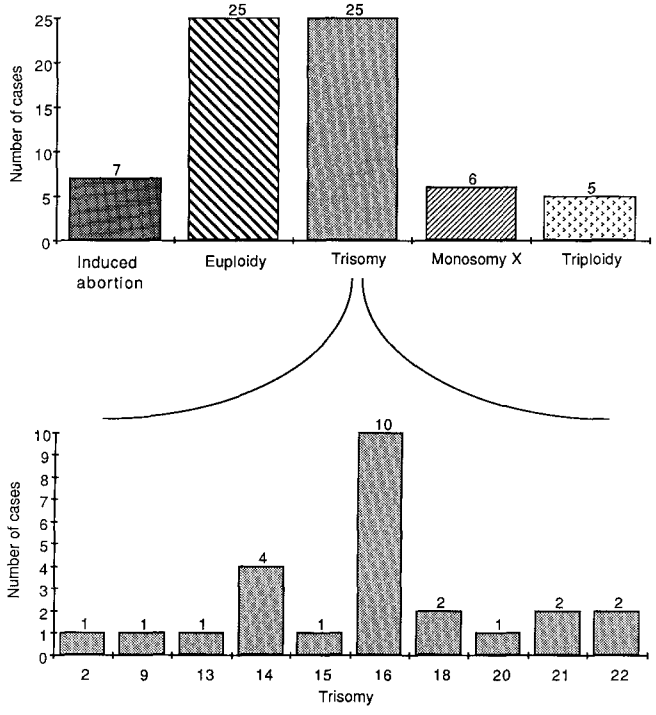

Fig. 1. Distribution of karyotypes in 61 early spontaneous abortions: the various autosomal trisomies are shown separately, the most common being trisomy 16

Table 2. Grouping by scanning electron microscopic criteria and karyotype

	Group				
	A	B	C	D	E
Induced abortions ($n=7$)	4			2	1
Monosomy X ($n=6$)		4	1	1	
Triploidy ($n=5$)			5		
Trisomy ($n=25$)		6	1	13	5
Euploidy ($n=25$)		2	5	4	14
Sum	4	12	12	20	20

Group A included 4 of the cases of induced abortions. Group B contained 12 cases (4 of the 6 monosomy X cases, 2 of the 22 euploid cases and 6 of the 25 trisomic abortions); group C contained 12 cases (1 each of trisomy and monosomy X, 5 each of euploid and triploid abortions). Group D contained 20 cases: 2 induced abortions, 4 euploid, 1 monosomy X, and 13 trisomic abortions. Group E also contained 20 cases: 1 induced abortion, 5 trisomic and 14 euploid abortions (Table 2). All cases of trisomy in group E had the karyotype 47,XX+16 or 47,XY+16. All 10 cases with trisomy 16 contained focal spherical villous thickening, even when the syncytial surface had degenerated.

All in all, 40 of the 68 cases were assignable to the various groups (58.8%). If groups B, C and D – in which branching is disordered – are pooled, they are found to contain 30 of the 36 aneuploid abortions, but only 11 of the euploid abortions.

Quantitative results

In the induced abortions, the measurement revealed a mean value for villous diameter of 141.7 μm, with clearly thicker villi in cases with trisomy (mean diameter 172.5 μm) and triploidy (303.8 μm). The villi of the euploidy group (161.6 μm) and the monosomy X group

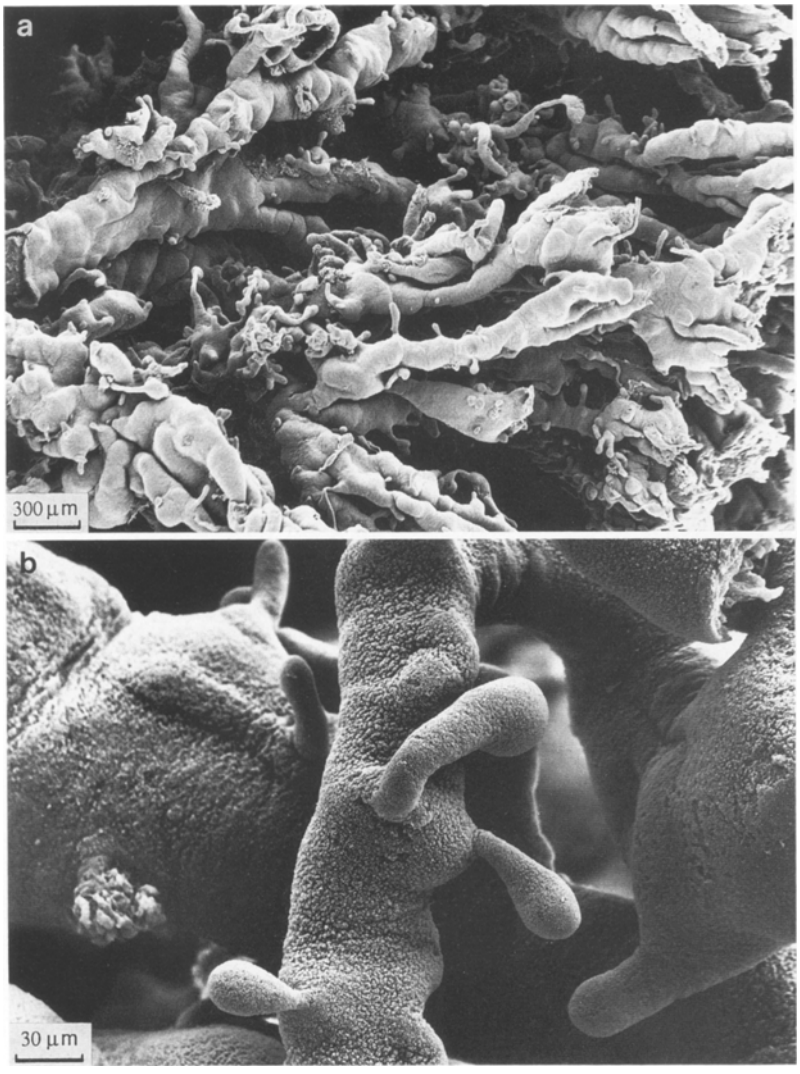

Fig. 2. Placenta – early pregnancy, with uniform branching (**a** induced abortion, ×30). The velvety surface comprises densely arranged microvilli of the syncytial trophoblasts, from which a number of club- or drop-shaped syncytial sprouts can be seen projecting (**b** induced abortion, ×300). The appearance corresponds to group A

(163.0 µm) were thinner. The F test revealed significant differences ($F=6.3$; 4 and 63 *df*, p 0.003, Fig. 7) which, for the most part, are due to the induced abortion group and triploidies.

With respect to the individual diameter classes, only in the 10th was there any statistically significant difference in the class frequency number (p 0.047). With respect to the mean figures of the unbranched villous portions, no significant differences were seen ($F=0.95$; 4 and 63 *df*, p 0.44, Fig. 7b); in contrast, the class frequency number of the 7th class of the length histograms revealed significant differences.

Discrimination analysis

Since descriptive statistics do not allow any statement to be made as to the diagnostic usefulness of variables, we submitted our results to a stepwise discrimination analysis that permits an analytical synopsis of a number of variables. At the same time, redundant information in this test procedure, resulting from correlations between the variables, is eliminated. If exclusively morphological variables are employed, 47 out of the 68 cases (69.1%) are correctly assigned (Table 3).

Table 3. Discriminant analysis including villous length and diameters

	Grouped as				
	Induced abortion	Monosomy X	Triploidy	Trisomy	Euploidy
Induced abortion ($n=7$)	2			3	2
Monosomy X ($n=6$)		3		1	2
Triploidy ($n=5$)			3	1	1
Trisomy ($n=25$)				21	4
Euploidy ($n=25$)	1	1		5	18

Correct classification: 47 of the 68 cases (69%)

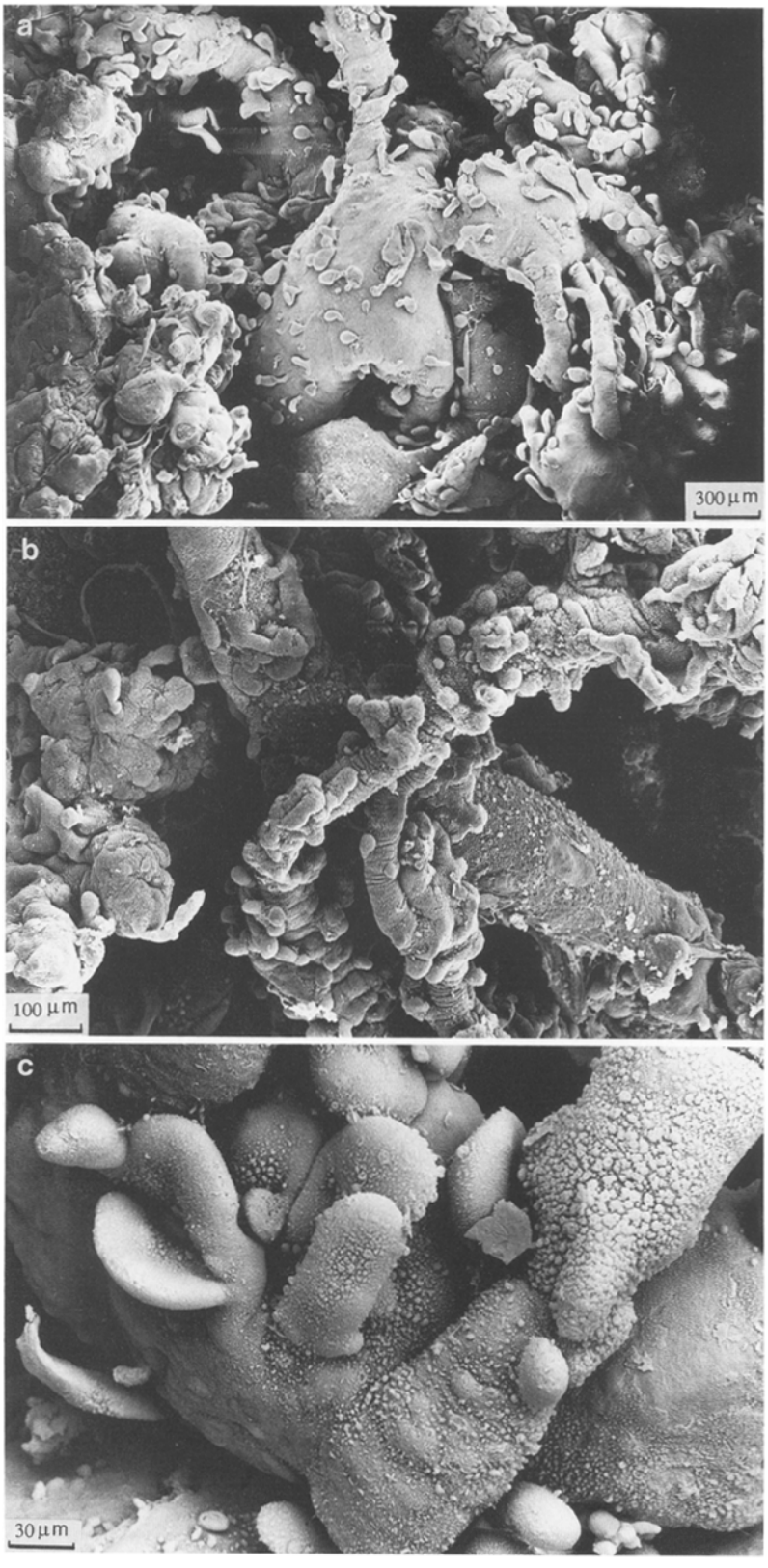

Fig. 3. Early abortion; long, little-branched villi with many syncytial sprouts (**a** trisomy 16, ×300, **b** monosomy X, ×100). The sprouts appear thick and finger-like, and even leaf-like (**c** trisomy 16, ×300). Group B

Discussion

Thanks to the direct preparation of trophoblast metaphases, contamination with maternal cells, which can falsify results, is largely excluded in our material. However, the quality of the mitotic figures is not as good as it is from cultured cells and the number of mitoses available for evaluation is also smaller (Eiben et al. 1987; Hansmann et al. 1986; Köhler 1988). For this reason, the possibility of diagnosing mosaicism is reduced

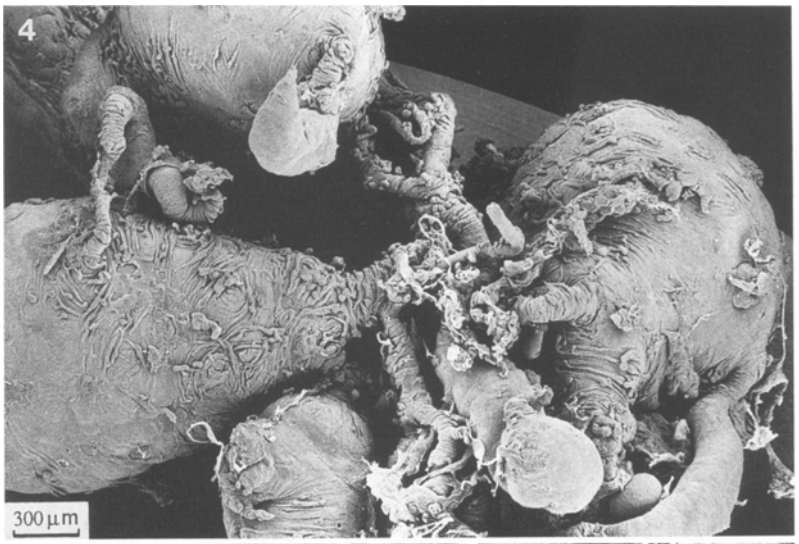

Fig. 4. Early spontaneous abortion with spherical villus, the surface of which shows many ridges (**a** euploidy 46, XY, ×30)

Fig. 5. Early spontaneous abortion with straight villi covered with an intact layer of syncytiotrophoblasts. Few club-shaped sprouts arranged in groups; no fibrin deposits (trisomy 9, ×100). Group D

(Maier 1987). The distribution of the karyotypes found in our material is in conformity with the reports of Boué et al. (1985), Warburton et al. (1980), Eiben et al. (1987) and Bernert et al. (1988).

As a rule, only fetal membranes and/or pieces of the placenta are available for morphological studies on abortuses. The placenta is a relatively simple, robust and conservative organ that responds – even to chromosome anomalies that are fatal to the embryo – with only slight, and then quantitative, structural changes. Regular development of the placenta depends upon a viable embryo and intact blood circulation, both maternal and fetal. Early death of the embryo occurs frequently in pregnancies with chromosomal disorders, resulting in blighted ova or severely disorganised embryos (Baldwin et al. 1988; Boué et al. 1976; Bruyere et al. 1987; Canki et al. 1988; Kajii et al. 1980; Kalousek 1987; Kalousek and Poland 1984). Following the death of the embryo, the placenta can survive within the uterus for a long period, although no further development takes place, and regressive changes occur, which become increasingly significant the longer the placenta is retained. In the extreme case, a missed abortion occurs. Together with the arrest in placental development, branching disorders of the placental villi occur, which are particularly in evidence on inspecting the three-dimensional structure.

The normal development of the placenta in early pregnancy has been investigated electron microscopically by Wynn (1975), Ludwig et al. (1975) and Burton (1987). At these early stages, a regular dichotomous branching of the villi can be observed, while the surface is covered with a dense brush border of the syncytiotrophoblast (Bergström 1971). The findings described are in agreement with the criteria of our group A; at the same time, our examinations have revealed the variability of the normal placenta in early pregnancy: only 4 of the 7 placentas obtained from induced abortions showed the features of this orthologous placental type. This type of development was not found in any of the spontaneously aborted placentas.

Variably pronounced branching disorders form the basis of our findings groups B, C and D, to which monosomy X, triploidy and trisomies can be assigned as characteristic, but not absolutely specific, chromosome aber-

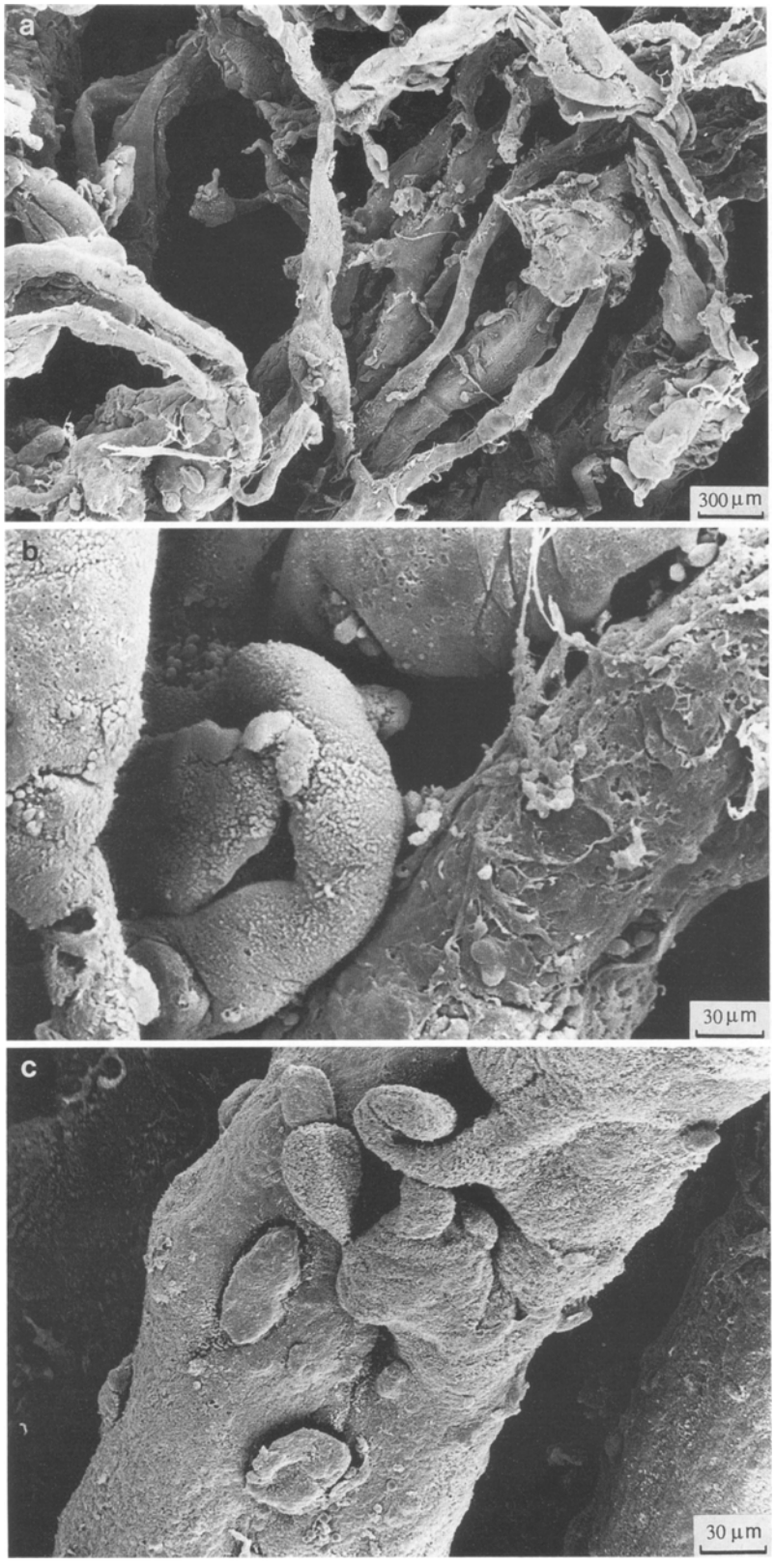

Fig. 6. Early spontaneous abortion with extremely thin placental villi with largely disrupted surface covered with abundant fibrin deposits (**a** trisomy 16, ×30). Nevertheless, trophoblast destruction is usually focal, and villi with intact syncytiotrophoblasts can also be found (**b** euploidy 46, XX, ×300). Sprouts may be flattened and already covered with fibrin deposits (**c** euploidy 46, XX, ×300)

rations. Abnormally branched as well as abnormally configured villi are found (these latter villi have a perfectly velvety surface). Geisler and Kleinebrecht (1978) reported surprisingly good trophoblast in trisomies as seen under the light microscope, and are thus in disagreement with a report by Boué et al. (1976), who emphasised the presence of a flattening of the trophoblasts in the case of trisomy. Our own observations lend support to the report by Geisler and Kleinebrecht (see also Röckelein 1989; Röckelein et al. 1989). Fujikura et al. (1971)

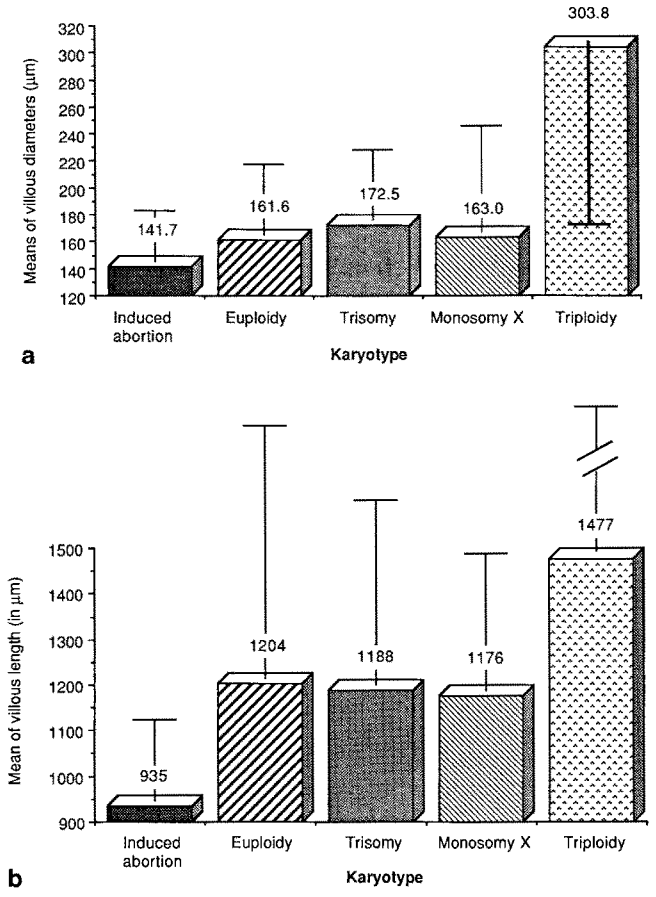

Fig. 7a. Mean values of villous diameter and associated standard deviations for the various abortion groups. The F test revealed significant differences: $F=7.2$; 6,62 *df*, p 0.000. **b** Mean values of lengths of unbranched sections of villi and associated standard deviations for the various abortion groups. The F test revealed no significant differences: $F=0.61$; 6,62 *df*, p 0.722

reported a reduction in syncytial sprouts in spontaneous abortions in comparison with induced abortions, but did not take account of the underlying karyotypes. Our own studies revealed that such findings are observed in only some of the spontaneous abortions and that there are even abortions that are associated with an increase in syncytial sprouts (group B). These observations are in agreement with our own light microscopic findings (Röckelein et al. 1989; Schröder 1989). Ockleford et al. (1989) investigated partial and complete hydatidiform moles in the scanning electron microscope and reported the occurrence of pathological microvilli. In partial hydatidiform moles, we often found trophoblast ridges that Ockleford et al. (1989) also reported to be characteristic, and which, in histological sections, represent the reported trophoblast proliferations (Ohama et al. 1986; Szulman et al. 1981; Szulman 1987a, b).

In group E, the damage observed was found in the trophoblast layer predominantly, which was focally destroyed and often replaced by fibrin deposits. For the most part, this group comprised euploid abortions. Vogel (1984) and Rüschoff et al. (1988) observed an accumulation of fibrin deposits in euploid spontaneous abortions. Mostly, fibrin and fibrotic villous stroma was considered to be associated with retention. Honoré et al. (1976) observed increased "focal villous infarction" in euploid abortions, together with trophoblast degeneration and villous fibrosis. These observations are in agreement with our criteria for euploid abortion. The findings seen in the scanning electron microscope are in agreement with our own light microscopic studies (Röckelein 1989; Röckelein et al. 1989). The difference in the nature of the surface suggests a variable pathogenesis of the abortions: in this group, the primary damage may lie in the trophoblasts. This group would then comprise abortions with a placental genesis, in which the death of the embryo is secondary. This assumption is supported by the vascular development detectable under the light microscope, and by the state of maturity of the erythrocytes which, in the case of the euploid abortions, are almost always more developed than in aneuploid abortions (Szulman 1988). It must, however, be admitted that euploid abortions represent a "melting pot" of abortions of highly variable genesis. Poor nutritional state of the trophoblast might also be due to an inadequate intervillous perfusion so that a discussion of the possibility of implantation damage (Vogel 1986) has been revived. The indistinct orthology of the (early) placenta and the heterogeneity of the material gives rise to diagnostic problems for the morphologist. The question of whether euploid abortions with variable predictive value for subsequent pregnancies can be identified via the morphological findings remains to be answered.

With the method employed morphometric evaluation of villous diameters provides true values, apart from possible shrinkage artefacts that might occur on critical point drying, but which, as systematic errors, affect all the cases investigated equally. In their trend, our results are compatible with those reported elsewhere on the basis of histological sections (Röckelein et al. 1989). The subjective impression that differences are to be found in the unbranched portions of the villi has not withstood a statistical examination.

All in all, in morphologically variable aborted placentas, the scanning electron microscope revealed characteristic findings criteria which, in a considerable number of the cases examined, permit conclusions to be drawn about the underlying karyotype. The observations made have provided further support for well-known light microscopic/histological findings in aborted placentas from the first trimester. Our results are thus in disagreement with those reported by Mingiullon et al. (1989), Novak et al. (1989) and Rehder et al. (1989) who, in studies of different design, were unable to demonstrate any correlation between the morphology of the placental villi and underlying karyotype. The additional consideration of the three-dimensional structure of the villi provides the pathomorphologist with greater confidence in his evaluation of early abortions.

References

Baldwin VJ, Kalousek DK, Dimmick JE, Applegarth DA, Hardwick DF (1988) Diagnostic pathologic investigation of the malformed conceptus. Pediatr Pathol 9:65–108

Bergström S (1971) Surface ultrastructure of human amnion and chorion in early pregnancy. Obstet Gynecol 38:513–524

Bernert J, Rauskolb R, Jovanovic V, Eiben B, Hansmann I, Schleiermacher E, Köhler A (1988) Zytogenetische Untersuchungen von Chorionzottengewebe aus Abortmaterial. Gynäkologe 21:107–109

Boué A, Gropp A, Boué J (1985) Cytogenetics of pregnancy wastage. In: Harris H, Hirschhorn K (eds) Advances in human genetics. Plenum, New York, pp 1–50

Boué J, Philippe E, Giroud A, Boué A (1976) Phenotypic expression of lethal chromosomal anomalies in human abortuses. Teratology 14:3–13

Bruyere JH, Ayra S, Kozel SJ, Gilbert EF, FitzGerald JM, Reynolds JF, Lewin SO, Opitz JM (1987) The value of examining spontaneously aborted human embryos and placentas. Birth Defects 23:169–178

Burton GJ (1987) The fine structure of the human placental villus as revealed by scanning electron microscopy. Scanning Microsc 1:1811–1828

Canki N, Warburton D, Byrne J (1988) Morphological characteristics of monosomy X in spontaneous abortions. Ann Genet 31:4–13

Eiben B, Borgmann S, Schübbe I, Hansmann I (1987) A cytogenetic study directly from chorionic villi of 140 spontaneous abortions. Hum Genet 77:137–141

Fujikura T, Ezaki K, Nishimura H (1971) Chorionic villi and syncytial sprouts in spontaneous and induced abortions. Am J Obstet Gynecol 110:547–555

Geisler M, Kleinebrecht J (1978) Cytogenetic and histologic analysis of spontaneous abortion. Hum Genet 45:239–251

Göcke H, Muradow I, Cremer H (1982) Morphologische und zytogenetische Befunde bei Frühaborten. Verh Dtsch Ges Pathol 66:141–146

Göcke H, Schwanitz G, Muradow I, Zerres K (1985) Pathomorphologie und Genetik in der Frühschwangerschaft. Pathologe 6:249–259

Hansmann I, Bartels I, Schübbe I (1986) Cytogenetic analysis of early human abortuses after preparation of chromosomes directly from chorionic villi. Hum Genet 72:189

Honoré LH, Dill FJ, Polland BJ (1976) Placental morphology in spontaneous human abortions with normal and abnormal karyotypes. Teratology 14:151–166

Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S (1980) Anatomic and chromosomal anomalies in 639 spontaneous abortuses. Hum Genet 55:87–98

Kalousek DK (1987) Anatomic and chromosome anomalies in specimens of early spontaneous abortion: seven-year experience. Birth Defects 23:153–168

Kalousek DK, Poland BJ (1984) Embryonic and fetal pathology of abortion. In: Perrin EVDK (ed) Pathology of the placenta. Contemporary issues in surgical pathology, vol 5. Churchill Livingstone, New York, pp 37–56

Köhler A (1988) Zytogenetische Aspekte der Chorionzottenbiopsie. Gynäkologe 21:99–103

Ludwig H, Metzger H, Wolf H (1975) The placenta (man). In: Hafez ESE (ed) Scanning electron microscopic atlas of mammalian reproduction. Thieme, Stuttgart, pp 346–357

Maier DB (1987) Genetic and infectious causes of habitual abortion. In: Gondos B, Riddick DH (eds) Pathology of infertility. Thieme, Stuttgart, pp 201–218

Minguillon C, Vogel M, Eiben B, Hansmann I (1988) Histomorphologische Merkmale bei embryonalen Spontanaborten mit und ohne Chromosomenaberration. Verh Dtsch Ges Pathol 72:591

Minguillon C, Eiben B, Bähr-Porsch S, Vogel M, Hansmann I (1989) The predictive value of chorionic villus histology for identifying chromosomally normal and abnormal spontaneous abortions. Hum Genet 82:373–376

Müntefering H, Becker K, Schleiermacher E (1982) Korrelation zwischen morphologischen und zytogenetischen Befunden bei Spontanaborten. Verh Dtsch Ges Pathol 66:372–377

Müntefering H, Schmidt C, Geisler M (1987) Korrelation zwischen morphologischen und zytogenetischen Befunden an der Chorionzotte. Verh Dtsch Ges Pathol 71:454

Novak R, Agamonalis D, Dasu S, Igel H, Platt M, Robinson H, Shehata B (1989) Histological analysis of placental tissue in first trimester abortions. Pediatr Pathol 8:477–482

Ockleford C, Barker C, Griffiths J, McTurk G, Fisher R, Lawler S (1989) Hydatiform mole: an ultrastructural analysis of syncytiotrophoblast surface organization. Placenta 10:195–212

Ohama K, Ueda K, Okamoto E, Takenaka M, Fujiwara A (1986) Cytogenetic and clinicopathological studies of partial moles. Obstet Gynecol 68:259–262

Ornoy A, Salamon-Arnon J, Ben-Zur Z, Kohn G (1981) Placental findings in spontaneous abortions and stillbirths. Teratology 24:243–252

Philippe E (1973) Consequences des anomalies chromosomiques sur le developpement. In: Boué A, Thibault C (eds) Les accidents chromosomiques de la reproduction. Inserm Paris, pp 119–126

Rehder H, Coerdt W, Eggers R, Klink F, Schwinger E (1989) Is there a correlation between morphological and cytogenetic findings in placental tissue from early missed abortions? Hum Genet 82:377–385

Reimer L, Pfefferkorn G (1973) Rasterelektronenmikroskopie. Springer, Berlin Heidelberg New York

Röckelein G (1989) Abortursachen und Morphologie der Abortplazenta. In: Becker V, Röckelein G Pathologie der weiblichen Geschlechtsorgane. Pathologie der Plazenta und des Abortes. In: Doerr W, Seifert G (eds) Spezielle pathologische Anatomie, vol 20/1. Springer, Berlin Heidelberg New York, pp 155–254

Röckelein G, Schröder J, Ulmer R (1989) Korrelation von Karyotyp und Plazentamorphologie beim Frühabort. Pathologe 10:306–314

Rüschoff J, Chudoba I, Köhler A (1988) Zur Histopathologie von Chorionzotten im ersten Trimenon der Schwangerschaft. Gynäkologe 21:104–106

Schröder J (1989) Morphologische und morphometrische Befunde an Plazentazotten aus Frühaborten mit unterschiedlichem Karyotyp. Inaugural Dissertation, Erlangen

Szulman AE (1987a) Complete hydatidiform mole: clinicopathologic features. In: Szulman AE, Buchsbaum HJ (eds) Gestational trophoblastic disease. Springer, Berlin Heidelberg New York, pp 27–36

Szulman AE (1987b) Partial hydatiform mole. In: Szulman AE, Buchsbaum HJ (eds) Gestational trophoblastic disease. Springer, Berlin Heidelberg New York, pp 37–44

Szulman AE (1988) Timing of embryo/fetal death in spontaneous abortion (abstract). XVII International Congress of the International Academy of Pathology and 8th World Congress of Academic and Environmental Pathology. Dublin. JAP, p 282

Szulman A, Phillipe E, Boué JG, Boué A (1981) Human triploidy: association with partial hydatiform mole and nonmolar conceptuses. Hum Pathol 12:1016–1021

Vogel M (1984) Pathologie der Schwangerschaft, der Plazenta und des Neugeborenen. In: Remmele W (ed) Pathologie 3. Springer, Berlin Heidelberg New York, pp 509–574

Vogel M (1986) Placentabefunde beim Abort. Virchows Arch [A] 346:212–223

Warburton D, Stein Z, Kline J, Susser M (1980) Chromosome abnormalities in spontaneous abortion: data from the New York City study. In: Porter IH, Hook EB (eds) Human embryonic and fetal death. Academic Press, New York, pp 261–288

Wynn RM (1975) Development and ultrastructural adaptions of the human placenta. Eur J Obstet Gynecol Reprod Biol 5:3–21