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In Situ Hybridization for the Identification of Karyotype Anomalies in the Histological Analysis of Early Spontaneous Abortion

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An estimated 15% of known pregnancies terminate in spontaneous abortion, the majority (90% circa) within the XII° week of gestation: early spontaneous abortion (ESA). Spontaneous abortions are either occasional (first abortion in the patient's reproductive history), repeated (the second) or habitual (third or more). The possible causes are many, but about half are due to alterations in embryonic karyotype – 27% trisomy, 10% polipolidy, 9% monosomy and 2% structural rearrangements [1].
With histological examination a morphological diagnosis of chromosomal anomalies may be suspected, based on structural anomalies of the villous tree or alterations in vascularization, but a cytogenetic confirmation is required [2]. In cases where no fresh material is available for cytogenetic analysis, a different type of method utilising fixed and paraffin-embedded material is necessary to confirm morphological suspicion. This method is especially important and necessary during revision of material from occasional abortions which have, in the mean time, become repeated or habitual. The chosen technique must fulfil the following requirements: execution on fixed and paraffin embedded material is necessary, it must yield a high percentage of results, the signal must be stable in time and finally, the use of standard laboratory apparatus is mandatory [3].
The method which satisfies all these requirements is in situ hybridisation (ISH) with enzymatic staining on fixed and paraffin embedded tissue.
Chromosomal aneuplodies may be confirmed using a biotynilated centromeric probe (D7Z1, Quantum Appligene, Heidelberg, Germany) for chromosome 7, which is not subject to trisomy. Each chromosome 7 centromere is revealed by using GenPoint (DakoCytomation, Glostrup, Denmark), developed with DAB, counterstained with Evan's Hematoxilin and hence may be counted. Any difference in signal number (and hence in corresponding chromosome 7 number) from the normal double signal in diploid cells indicates aneuploidy.
Polyploidies represent 10% of causes of karyotype alterations and their identification is important in reproductive pathology counselling as they are usually occasional events which have a low frequency of repetition in future pregnancies [4].
The ISH technique has been amply tested and validated, as shown in the literature [5], however, with regards to fixed and paraffin embedded material, the phase using enzyme digestion was in need of improvement. Deparaffination and pre-treatment (pepsin) to widen the connective tissue mesh and partial enzymatic digestion (protease K) permit direct exposure of nuclei to the probes. Temperature, reagent concentrations and reaction times were calibrated to give the optimal balance between exposure of the nuclei to probes and

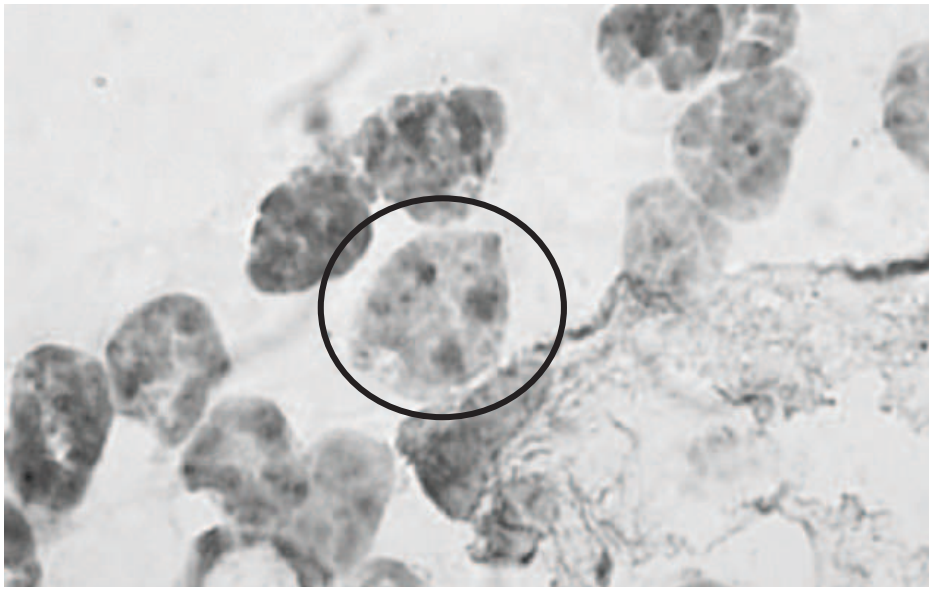

Fig. 1. Suspected chromosome anomaly confirmed - three separate signals confirm triploidy

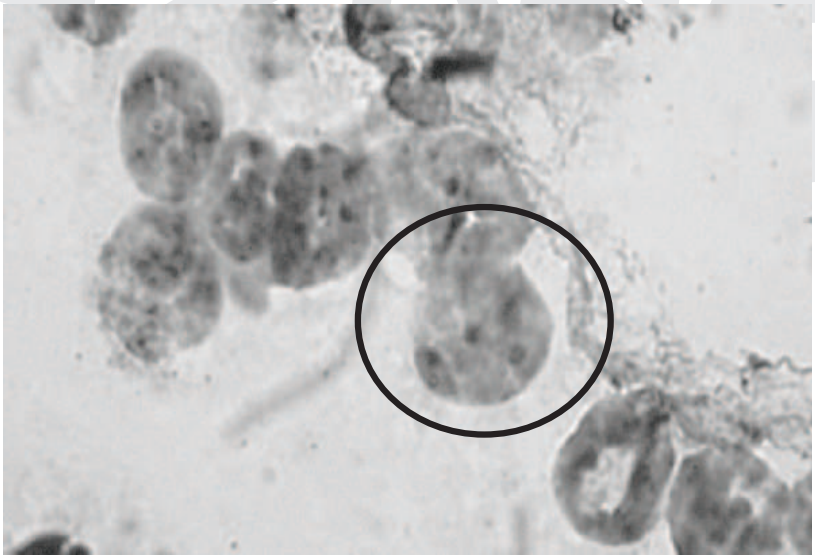

Fig. 2. Suspected chromosome anomaly confirmed - three separate signals confirm tetraploidy

preservation of cell morphology.
In conclusion it is thus possible to confirm cell aneupolidy by counting the mean number of coloured spots in cells from tissue from abortions which have been selected as having morphostructural alterations indicative of ploidy anomalies.

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