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ORIGINAL ARTICLE

DNA ploidy of ectopic pregnancy and first trimester spontaneous abortion investigated by flow cytometry

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Background. To compare the success rate of DNA flow cytometry in determining the DNA ploidy status in ectopic pregnancy and first trimester spontaneous abortion.
Methods. Thirteen women with ectopic pregnancy (Group I) and 17 women with first trimester spontaneous abortion (Group II) were included into this study. DNA flow cytometric analysis was performed on all specimens. Aneuploidy was classified according to DNA index. The first trimester spontaneous abortions were also karyotyped after long-term culture of chorionic villi. Student-*t* test and Fisher's exact test were used in statistical comparisons.
Results. DNA aneuploidy was found in five women with ectopic pregnancy (38.5%) versus in 12 women with first trimester spontaneous abortion (70.6%), and it was comparable. A triploidy and a tetraploidy were detected in group I. Six tubal ectopic pregnancies were unruptured at laparatomy and four of them had aneuploid DNA content.
Conclusions. We believed that DNA flow cytometry was successful in determining the ploidy status of ectopic pregnancy and first trimester spontaneous abortion. In addition, it was interesting that ectopic pregnancies with aneuploid DNA content tended to be unruptured. However, this suggestion needs to be confirmed by further studies with larger numbers of cases.

Key words: chromosomal aberration; DNA flow cytometry; ectopic pregnancy; spontaneous abortion

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Chromosomal abnormalities, as high as 78%, have been claimed as one of the most important factors in the etiology of ectopic pregnancy (1). Although there have been conflicting reports regarding the actual incidence of chromosomal abnormalities in ectopic pregnancy, it remains unknown because of the difficulties in availability of karyotype analysis (1–4).

On the other hand, it has also been recognized that cytogenetic abnormalities are responsible in the etiology of spontaneous abortions and molar pregnancy like ectopic pregnancy (5–8). Recently there have been increasing numbers of flow cytometric DNA studies which have shown the chromosomal aberrations in spontaneous abortion, molar and ectopic pregnancy (9–13).

In this prospective study, we investigated the ploidy status in ectopic pregnancy and first trimester abortion and compared the success rate of DNA flow cytometry in determining the frequency of chromosomal aberrations in both groups.

Abbreviations:
DNA-FC: DNA flow cytometry, DI: DNA index, S: synthesis.

Materials and methods

A-Patients. Thirteen women with tubal ectopic pregnancy (Group I) and 17 women with first trimester spontaneous abortion (Group II) who applied to emergency services, were randomly included in this preliminary, comparative, prospective study. Women with habitual abortion were excluded from the study.

The women in group I underwent laparotomy and seven of them were found to be ruptured. All ectopic pregnancies were located in the ampullary portion of the fallopian tube and treated with salpingectomy or salpingostomy. The women in group II underwent dilatation and curettage. After obtaining the surgically removed materials of ectopic pregnancy and abortion, all were immediately fixed in 10% formalin solution and embedded in paraffin for histopathologic examination and DNA flow cytometry (DNA-FC). Unfortunately karyotype analysis could only be done in the specimens of group II, because we could not have enough chorionic villi from the women with ectopic pregnancy for karyotype analysis. Only the cases which had enough trophoblastic tissue for DNA-FC were included in this study. Therefore we excluded three women with ectopic pregnancies for which their histograms were technically insufficient.

B-Karyotype analysis. The materials from spontaneous abortions were put into the culture medium with RPMI 1640, immediately examined under an inverted microscope and chorionic villi were dissected from the direct preparation. At least 40–50 gram chorionic villi were collected and put into a dish which contained 4 ml trypsin EDTA (0.5% trypsin, 0.02% EDTA). After an hour, they were transferred to another medium which contained collagenase V (final concentration 1 mg/ml) and were mechanically divided into the pieces. They were left in a cabine at 37°C for 3–4 hours. After the cell suspension was centrifuged at 1000 rpm for 10 minutes, the pelete was taken out and 20% RPMI 1640 was added. The cells were cultured in the T 25 culture dish for long-term. In the cases with mosaicism 20 metaphases, but in non-mosaic cases 12 metaphases were analysed.

C-DNA flow cytometry (DNA-FC). The principles of DNA-FC have been addressed to the method of Hedley and associates (14). Fifty micrometer sections were cut from the embedded blocks. After controlling the sections under the light microscope, the cell suspension was prepared and stained with propidium iodine. DNA analysis was done using a Coulter Profile Flow Cytometry (EPICS) (Coulter Electronics, Hialeah, Florida) equipped with a 5 watt argon laser, wavelength of 488 nm. For each histogram, at least 10,000 cells were scanned. The mean coefficient of variations was 5.2 (2.8–12.1). Three histograms were disregarded since the coefficient of variations were beyond 15% and the number of scanned cells were below the limit of 10,000 cells.

DNA histograms were evaluated by using Multicycle Software Program (Phoenix Flow Systems Inc., SanDiego, California). This software program in the flow cytometry system determines cell cycle distributions of the presynthetic growth phase (G0/G1), synthetic phase or proliferative phase (S), postsynthetic and mitotic phases (G2/M). In this study, tubal cells were used as diploid control in the histograms. Aneuploidy was classified according to DNA index (DI) which is a ratio of channel number of aneuploid cell population to channel number of diploid cell population. DI 1.00 was considered as diploidy, DI ranging from 1.10 to 1.20 as near diploidy, from 1.40 to 1.60 as triploidy and above 1.90 as tetraploidy, respectively.

D-Statistical analysis. Student-*t* test and Fisher's exact test were used to comment on the significance of the results.

Results

The mean age of women in group I was 30.16 (22–37) and 29.81 (22–36) in group II. The mean gestational age in group I was 7.69 (s.d. 2.21) weeks (5–11) and 9.47 (s.d. 2.18) weeks (7–12) in group II.

DNA aneuploidy based on DI by flow cytometry was found in five tubal ectopic pregnancies (38.5%) versus in 12 first trimester spontaneous abortions (70.6%) (Tables I and II). DI of the aneuploid cells in the group I ranged from 1.2 to 1.91, whereas in group II it ranged from 0.85 to 1.51. When we com-

Table I. DNA-FC results of ectopic pregnancies

Cases	P	S (%)	DI	CVs	L
1	D	37.2	1.0	9.1	UR
2	D	7.4	1.0	5.6	R
3	D	25.4	1.0	2.8	UR
4	D	10.4	1.0	5.1	R
5	D	8.7	1.0	5.8	R
6	D	7.7	1.0	3.3	R
7	D	3.8	1.0	5.2	R
8	D	26.1	1.0	12.1	R
9	AN	25.1	1.2	2.3	UR
10	AN	40.1	1.2	4.6	UR
11	AN	0.0	1.47	3.5	R
12	AN	30.8	1.91	4.1	UR
13	AN	10.4	1.2	4.2	UR

P: Ploidy, S: Synthesis, DI: DNA Index, CVs: Coefficient of variations, L: Laparotomy, UR: Unruptured, R: Ruptured, D: Diploidy, AN: Aneuploidy.

Table II. DNA-FC results of first trimester spontaneous abortions

Cases	P	S (%)	DI	CVs	K
1	AN	8.5	1.20	8.1	47,XY,t(1;11),+16
2	AN	8.7	1.12	5.0	47,XX,+22
3	AN	12.0	1.47	5.9	69,XXY
4	AN	13.3	1.12	4.0	46,XX/47,XX,+16
5	AN	10.1	1.14	6.0	46,XY/47,XY,+3
6	AN	6.6	1.16	6.1	47,XY,+15
7	AN	10.9	0.85	7.3	45,X
8	AN	56.0	1.12	8.9	47,XY,+22
9	AN	66.3	1.51	6.7	69,XXY/71,XXY,+8,+17,t(11;5)
10	AN	24.4	1.24	8.8	47,XX,+16
11	AN	51.4	1.40	5.3	47,XX,+16/94,XXXX,+16,+16, t(12;13)
12	AN	7.1	1.10	4.1	47,XX,+18
13	D	14.8	1.00	7.8	46,XX
14	D	12.5	1.07	7.4	46,XX
15	D	45.0	1.10	6.2	46,XX
16	D	14.0	1.00	8.2	46,XX
17	D	8.2	1.00	7.7	46,XY

P: Ploidy, S: Synthesis, DI: DNA Index, CVs: Coefficient of variations, K: Karyotype, AN: Aneuploidy, D: Diploidy.

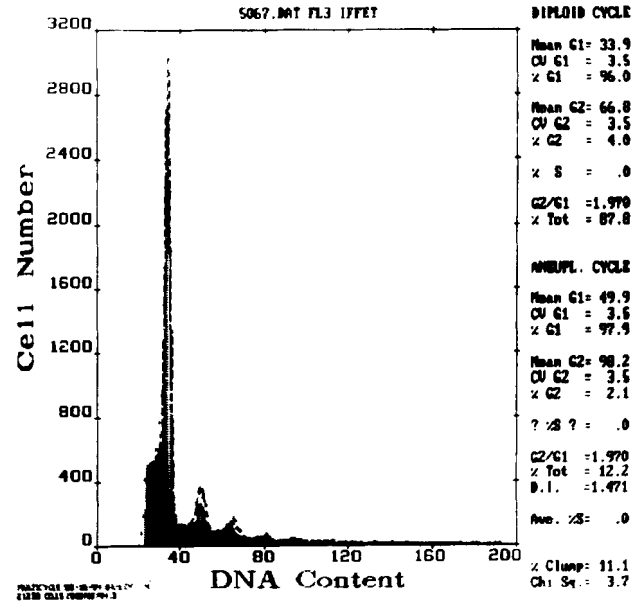

Fig. 1. Triploid DNA pattern on the histogram of a woman with tubal ectopic pregnancy.

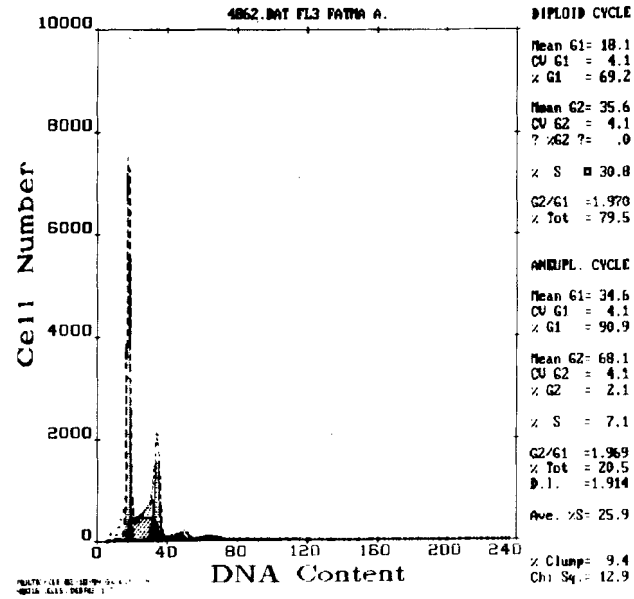

Fig. 2. Tetraploid DNA pattern on the histogram of a woman with tubal ectopic pregnancy.

pared the numbers of aneuploidy between the two groups, we could not find a statistical significant difference ($t=1.759$, $p>0.05$). In group II, ten cases which had DI above 1.1, were aneuploid. Five cases which had DI below 1.1 and four of them were diploid but one was aneuploid. Two cases which had DI equal to 1.1, showed one diploidy and one aneuploidy (Table II).

Triploidy was detected in a woman with tubal ectopic pregnancy. Her DNA-FC evaluation showed that a DNA aneuploid cell population consistent with triploidy (DI=1.47) (Fig. 1). In addition, tetraploidy was observed by DNA flow cytometry in another woman in group I (Fig. 2).

Six tubal ectopic pregnancies were found to be unruptured at laparotomy. Four of them had aneuploid DNA content (Table I). When we compared the frequencies of ruptured ectopic pregnancies with those with unruptured in terms of aneuploid DNA content by using Fisher's exact test, we could not find a significant difference ($p=0.086$, $p>0.05$).

Discussion

Many human oocytes, perhaps as many as 30%, are genetically abnormal (15, 16). Spermatozoa have a lower incidence of genetic defects, approximately 8%, of which more than half are balanced translocations and deletions (17). Many embryos fertilized *in vivo* or *in vitro* inherit some form of chromosomal imbalance from the gamets, and others can arise during fertilization and cleavage. About 20% of human embryos growing *in vitro* are genetically abnormal, but this level increases by a factor of 2 or 3 if fertilization is delayed or if the embryos have fragmented blastomers during early growth (15). Many of these embryos are capable of implantation as are some triploid and tetraploid embryos (18). The majority of chromosomally abnormal embryos fail to implant or are lost as early abortions. Although the major etiologic factor in the tubal ectopic pregnancy has been regarded as due to anatomical changes in the fallopian tubes, chromosomal abnormalities have also been suggested as playing an important role (1–4, 12, 13).

In the present flow cytometric study, we found abnormal amounts of DNA content in 38.5% of the tubal ectopic pregnancies. In detecting the cytogenetic abnormalities in ectopic pregnancy, karyotype analysis or DNA flow cytometry can be used. By using karyotype analysis, it was reported that the frequency of chromosomal abnormalities ranged from 11.6% to as high as 78% in tubal ectopic pregnancies (1–4). The discrepancies between the results can be explained by the methodologic differences between the long-term culture for karyotype analysis and rapid karyotyping. On the other hand, in DNA flow cytometric studies, the frequency of DNA aberrations was quite constant (24% and 33%) and similar results were reported as in our study (12, 13).

The true rate of early pregnancy loss is unknown. Boue and associates claimed that at least 50% of early spontaneous abortions were cytogenetically abnormal based on karyotype analysis (5). In the present study, we observed that 70.6% of first trimester spontaneous abortions were cytogenetically abnormal when investigated by karyotype analysis. The same rate of aneuploidy was recognized by DNA-FC in women with early spontaneous abortion. Several recent studies have shown that aneuploid DNA content in spontaneous abortions and hydropic abortions was found to be much less (11%, 12%, 12.4% and 40%) than we found by DNA-FC (11, 19–21).

It has been reported that the subtle DNA changes and minor abnormalities concerning a single chromosome such as trisomies and monosomies could not be reliably detected by DNA-FC (13, 21). Contrary to these reports, in this study, when the DNA index limit of aneuploidy was accepted as 1.1, nine cases with aneuploid DNA content indicating eight trisomies and one monosomy were ascertained successfully by DNA-FC. Seven out of eight trisomic cases and a monosomic case were near-diploid, as shown in Table II. Therefore, present data has revealed a greater aneuploidy rate compared to other studies.

DNA-FC was found to be a reliable method in detecting the chromosomal aberrations in spontaneous abortion. Therefore we believed that its application to ectopic pregnancy was reasonable, although we were not able to perform karyotype analysis because of the scant materials.

We have in this article presented a triploid and a tetraploid pattern on the histograms in two women with ectopic pregnancies (Fig. 1 and 2). Montgomery and associates have recently reported a triploid case similar to ours (9). Triploidy may be observed in the histopathologic specimens of partial moles (8, 22). Since approximately 4% of patients with a partial mole develop non-metastatic gestational trophoblastic neoplasia (23), we have suggested that such a patient with triploid ectopic pregnancy should be followed up carefully.

Another interesting finding of the study was that the aneuploid ectopic pregnancies seemed to have a tendency to be unruptured as explored in laparotomy. It was shown that some forms of chromosomal abnormality in many embryos, such as mosaicism, haploidy, triploidy, trisomies, monosomies, are capable of implantation (15, 18, 24). On the other hand, transcription of both maternal and paternal genomes in the zygote are a prerequisite for normal embryonic development. Failure to express paternal and maternal genome would lead to defective trophoblastic development and embryonic growth failure respectively (25). Randall and associates noted that implantation and placentation within the tube are virtually identical to the equivalent process occurring in the uterus (26). Therefore it is reasonable to expect that the ectopic pregnancies with aneuploid DNA content would implant to the tube loosely without showing extensive trophoblastic destruction to the wall.

In conclusion, DNA-FC is a successful method in detecting the ploidy status in first trimester spontaneous abortion and ectopic pregnancy. We consider DNA-FC is a cost effective and time saving procedure. There may be a tendency indicating that the unruptured tubal ectopic pregnancies have aneuploid DNA content. Clearly a larger number of cases is required before any firm conclusions can be drawn from this data.

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