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☐ Original Article ☐

Studies on the Prenatal Chromosomal Analysis and the Changes of Maternal Serum Alpha-Fetoprotein Following Chorionic Villus Sampling

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Transcervical chorionic villus sampling (CVS) was performd in 174 patients between 7 & 12 menstrual weeks of pregnancy opting for prenatal diagnosis. Advanced maternal age was the most common indication for CVS (39.7%). The sampling success rate was 95.4% (166/174), representing 88.9% at 7 to 8 weeks, 98.9% at 9 to 10 weeks & 92.7% at 11 to 12 weeks gestation. In 139 of 174 patients (80%), successful sampling was acomplished in one or two catheter passages only. Four spontaneous fetal losses (2.3%) occured. The cytogenetic analysis routinely used was the direct overnight & long-term culture methods which revealed 4 abnormalities (2.4%). To date, 90 of the women have been delivered & all infants are doing well and the remaining 65 pregnancies are continuing uneventually.

Maternal serum alphafetoprotein (MSAFP) concentration was determined in 72 patients immediately before & after CVS. A significant increase of 20% or more, comparable to pre CVS levels, was noted immediately after sampling in 56 of 72 patients (77.8%). The increase in MSAFP concentration correlated with the amount of villi sampled ($r=0.498$, $p<0.001$) & with the number of sampling attempts ($p<0.05$). Estimated CVS related fetomaternal hemorrhage (FMH) ranged from 0.005 to 0.1552 ml and in 5 of 72 patients (6.90%) 0.06 ml or more of FMH was noted. Two of the 5 patients had FMH of 0.1 ml or more.

Key Words: Chorionic villus sampling (CVS), maternal serum α-fetoprotein (MSAFP), fetomaternal hemorrhage (FMH)

Chorionic villus sampling (CVS) can be performed in early pregnancy, usually at 9 to 11 weeks gestation and requires only a few days for obtaining results. This short waiting time would benefit the patient by lightening the physical and psychological burdens on the gravida should therapeutic termination according to the results be carried out. It is not surprising to see CVS gradually becoming an attractive alternative to amniocentesis as a method of prenatal genetic diagnosis.

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Admittedly, CVS has its pitfalls. Disputes on the safety of the procedure, reliability of the diagnosis based on karyotype obtained from CVS, discrepancy of maternal and fetal karyotypes, and complications arising after the procedure still need to be settled.

It is reported that aspiration of chorionic tissue is occasionally accompanied by fetomaternal hemorrhage (FMH), and that with FMH, the incidence of postsampling spontaneous abortion or maternal isoimmunization is high. Procedure-related FMH may also accompany amniocentesis, and even fetoscopy. Amniocentesis-related FMH has been studied using the maternal serum α-fetoprotein (MSAFP) method by many separate work groups Lele *et al.* 1982; Thomsen *et al.* 1983), but CVS-related FMH, despite its significance, has not been adequately studied. Measuring the difference in MSAFP before and after the procedure, however, is

considered a new method of diagnosing FMH (Lachman, 1977; Lele *et al.*, 1982; Scott & Warenski, 1982; Bowmann & Pollock, 1985). The method is superior to Kleihauer Betke test in several ways; with RIA, the sensitivity of the test is high enough to use the result of the method as the index of measuring FMH. CVS-caused FMH may raise the chance of postsampling spontaneous abortion and may trigger maternal Rh isoimmunization.

In this study, we have attempted to evaluate the success rate of CVS according to gestational weeks, subsequent fetal karyotypes, results of chromosomal analysis, and pregnancy outcome. Difference in MSAFP levels pre and post CVS was measured to detect CVS-related FMH. With this study, the authors hoped to determine the efficacy of transcervical CVS in relation to cytogenetic reliability of sampled chorionic tissue, and any correlation between the changes in MSAFP level in relation to the number of catheter passes for sampling and the amounts of obtained chorionic tissue.

MATERIALS AND METHODS

Material

Prenatal genetic counseling was given to 174 gravidas opting for prenatal genetic diagnosis. Informed consent was obtained from each CVS candidate. All candidates were Rh-positive, pregnant 7 to 12 weeks (menstrual age), and passed confirmatory ultrasonic fetal evaluation (realtime, linear, 3.5 MHz transducer). The information gained by ultrasound were fetal heart beats, placental location, the presence of multifetal pregnancy, and gestational age.

Method of villus sampling

After sterilization of the perineum, vulva, vagina and exocervix with Betadine solution, CVS was performed under real-time ultrasound vision (linear, 3.5 MHz transducer) with the cervix grasped with a tenaculum and by transcervically threading the tip of the plastic echogenic catheter by Holzgreve, Germany with accompanying stylet to the chorion frondosum. Upon reaching the chorion frondosum, the stylet was gently withdrawn and a medium-containing 20 ml plastic syringes was attached to the end of the catheter; then, 5 to 10 ml of negative pressure was applied and villi aspiration attempted. Under a dissecting microscope, the presence of chorionic villi was confirmed. The amount of sampled villi was determined according to the standard proposed by Simoni *et al.* in 1983. With less than 5 mg sampling, repeat sampling was allowed, but no more than 4 samplings per case were permitted.

Methods of cytogenetic analysis

Direct overnight incubation method: The aspirated samples were transported to the laboratory immediately. The carefully dissected villi from maternal decidual tissue under an inverted microscope were transferred to a 60 mm plastic petridish containing Chang's medium. Villi were incubated overnight at 37°C in a 5% CO_2 incubator. Colcemid was added to the medium at a final concentration of 0.04 mg/ml and the sample was left in a 5% CO_2 incubator. The medium was removed and replaced with 1% sodium citrate solution. The sodium citrate was removed, and 2 ml of methanol-acetic acid (3 : 1) fixative were added for 10 min and replaced with 0.5 to 1 ml of aqueous 60% acetic acid solution to induce cell dissociation. The suspension was then eventually distributed onto the surface of warm (40 ~45°C) slides.

Long-term culture method: For tissue culture the villi were placed in 60 mm plastic petridish containing 3 ml of Chang's media. Villi were incubated overnight at 37°C in a 5% CO_2 incubator and then placed in 3 ml of trypsin-EDTA solution for 1 hour (no. 610-5300AG, GIBCO). The villi were then transferred by forceps into a dish containing 0.1% of collagenase solution and the entire mixture was aspirated and centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and 3 ml of Chang's medium was added, well mixed and transferred into a culture flask. Cultures were monitored. During harvesting the cells were treated in the same way as for any other long-term culture. Slides were stained with 4% Giemsa and G-banding was carried out both in overnight and culture methods. In our study 25 metaphases were analysed from directly prepared specimens and cultured specimens.

Analysis of MSAFP

Peripheral blood of each CVS candidate was drawn immediately before and 15 minutes after CVS for determining the changes in MSAFP levels. The sampled blood was centrifuged and the centrifuged serum was subject to ELISA (ELISA, Behring Enzygnost AFP 1 IU/ml=1.5 ng/ml, sensitivity 0.5 IU/ml) immediately before and after CVS and were analysed in relation to gestational weeks, the

amount of sampled villi, and the number of cathter passes, respectively.

The values provided by Gitlin & Boesman (1966) for the early gestational period were used to calculate the volume of fetal blood that may have been transferred across the intervillous space to the maternal circulation for various delta MSAFP values. The calculation was based on the assumption that whole blood was transferred, that the fetal serum AFP concentration at 10 weeks gestation was approximately 2 mg/ml (Gitlin & Boesman, 1966), that the average fetal hematocrit at that age was 30% (Oski & Naiman, 1982), and that the average maternal plasma volume was 3000 ml (Lund& Donovan, 1967). Estimation of the volume (ml) of FMH for a given rise of MSAFP (△MSAFP) is by the following:

$$\frac{\text{Maternal plasma volume } (\mu l) \times \triangle\text{MSAFP (ng/ml)}}{\text{Fetal serum AFP (ng/ml)} \times 0.7 - \triangle\text{MSAFP (ng/ml)}}$$

Statistical method

Regression analysis (Spearman rank correlation) and Student t-test were used.

RESULTS

Chromosome analysis

Advanced maternal age was the single most common reason for undertaking CVS, accounting for 69 of the total of 174 cases (39.7%). It was followed by parental balanced translocation or previous chromosomally abnormal offspring (39/174; 22.4 %), previous birth of a child with congenital anomaly (35/174; 20.1%), prenatal exposure to radiation or chemotherapeutic agent (9/174; 5.2%), repeated spontaneous abortion (8/174; 4.6%), parental carriage of X-linked recessive gene (7/174; 4.0%), previous stillbirth or neonatal death of a child (5/174; 2.9%), and parental anxiety or other conditions (2/174; 1.1%) (Table 1).

Timing of CVS ranged from 7 to 12 weeks gestation. Success of sampling varied in relation to weeks of gestation. At 7 to 8 weeks gestation, the sampling was successful in 24 of 27 sampling cases. The highest success rate (91 in 92 cases) was seen in 9 to 10 weeks gestation. In 11 to 12 weeks gestation, success was seen in 51 fo 55 cases (Table 2).

The number of catheter-passes of successful sampling varied with 78, 61, 27, and 8 cases needing 1, 2, 3, and 4 passes, respectively; about 80% of the cases required only 1 or 2 passes for success (Table 3).

The amount of villi harvested ranged from 5 to 50 mg per case. The number of chromosomes observed per slide was 10.3, 16.7, and 18.1 with the villi amounts of 5 to 10, 10 to 20, and 20 to 30 mg, respectively. Higher metaphase counts were seen with larger amounts of villi. However, the number did not differ significantly with the sampled villi from 30 to 50 mg. When 5 mg or less amounts were used for diagnostic purpose, they were not included in the analysis of the success rate (Table 4).

Karyotyping was successful in 166 cases. Normal karyotypes were revealed in 162 of these 166 cases

Table 1. Indications of CVS

Indications	Patients	
	No	%
Advanced maternal age	69	39.7
Previous chromosomal anomaly	39	22.4
Previous congenital anomaly	35	20.1
Recurrent spontaneous abortion	8	4.6
Previous neonatal death or stillbirth	5	2.9
Carrier of X-linked recessive genetic disorder	7	4.0
X-ray irradiation or exposure to chemotherapeutic agent	9	5.2
Anxiety and others	2	1.1
Total	174	100.0

Table 2. Success rate of CVS

Gestation	No. of cases	No. of success	%
7- 8	27	24	88.9
9-10	92	91	98.9
11-12	55	51	92.7
Total	174	166	95.4

Table 3. Number of catheter passes

No. of passes	No. of cases	%
1	78	44.8
2	61	35.1
3	27	15.5
4	8	4.6
Total	174	100.0

Table 4. Amount of chorionic villi obtained and the mean number of suitable metaphase

Villi amount(mg)	No. of cases	No. of metaphases analyzed per slide[a]
No. Villi	3	NG
<5	5	NG
5-10	46	10.3
10-20	66	16.7
20-30	38	18.1
30-40	13	20.2
40-50	2	20.3
Total	174	

a: a minimum of 4 slides per case were analyzed.
NG: negligible

Table 5. Cytogenetic results of CVS

Karytotype	N	%
Normal	155	93.4
46, XX	82	49.4
46, XY	73	44.0
Normal polymorphism	7	4.2
46, XX, inv(9)(p11q13)	4	2.4
46, XY, inv(9)(p11q13)	1	0.6
46, XX, $15q^+$	1	0.6
46, XX, $21p^+$	1	0.6
Abnormal karyotype	4	2.4
47, XX, +21	1	0.6
45, X	1	0.6
47, XX, +7	1	0.6
46, XY, −14, +t(14q21q)	1	0.6
Total	166	100.0

(97.6%); of them, 88 were 46, XX, while 46 were 46, XY. Abnormal karyotypes, revealed in 4 (2.4%), were 47, XX, +21, 45, X, 47, XX, +7, 46, XY, −14, +t (14q21q), respectively (Table 5).

Reproductive outcome of the 166 cases where CVS was successful revealed ninety birthes to normal offsprings, and 65 in ongoing pregnancy. Four with abnormal karyotypes and 3 with normal karyotypes but with accompanying myoma had their pregnancies terminated. Spontaneous abortion occurred in 4; one of these 4 was with 46, XX, inv (9) (p11q13). The overall fetal loss rate was 2.3% (Table 6).

Table 6. Pregnancy outcome following CVS

No. of cases	N(174)	%
Failed CVS	8	4.6
Therapeutic termination	4[a]	2.3
Elective abortion	3[b]	1.7
Fetal loss	5[c]	2.3
Delivered at term	90	51.4
Ongoing	65	37.1

a: each case had an abnormal karyotype (47, XX, + 21; 45, X; 47, XX, +7; 46, XY, −14, +t (14q21q).
b: the cases were combined with uterine myoma.
c: included a case lost at 24 weeks gestation; fetal karyotype was revealed as 46, XX, inv (9).

Table 7. Concentration of MSAFP during pregnancy

Gestational weeks	Number	Median (IU/ml)	Range (IU/ml)
6	11	3.0	1.0- 4.7
7	4	3.0	3.0
8	6	3.0	1.0-10.2
9	8	3.0	1.0-16.0
10	8	3.7	1.3-29.0
11	8	5.9	1.8- 7.1
12	11	10.0	3.0-27.0

Change of MSAFP in relation to CVS

The level of MSAFP in normal pregnancy at 6 to 12 weeks: The level of MSAFP was measured at 6 to 8 weeks gestation. In determining MoM of MSAFP, such cases as twin pregnancy (including vanishing twin), missed abortion, and blighted ovum were excluded, as were the cases with abnormal fetal karyotype. MoM of MSAFP in uncomplicated singleton pregnancy at each gestational week from 6 to 12, obtained from 56 women, was 3.0, 3.0, 3. 0, 3.0, 3.7, 5.9, and 10.0 IU/ml, respectively. In our series, MSAFP was detectable from 6 weeks gestation onward and began to increase from 9 weeks gestation (Table 7, Fig. 1).

The change of MSAFP level by CVS: Fifty six of the 72 CVS cases were observed with discrete increases in MSAFP levels after CVS; the percentage of increase ranged from 3 to as much as 1610%. More than 20% increase in MSAFP level was observed in 56 of 72 cases (77.8%). Of them, 50 to

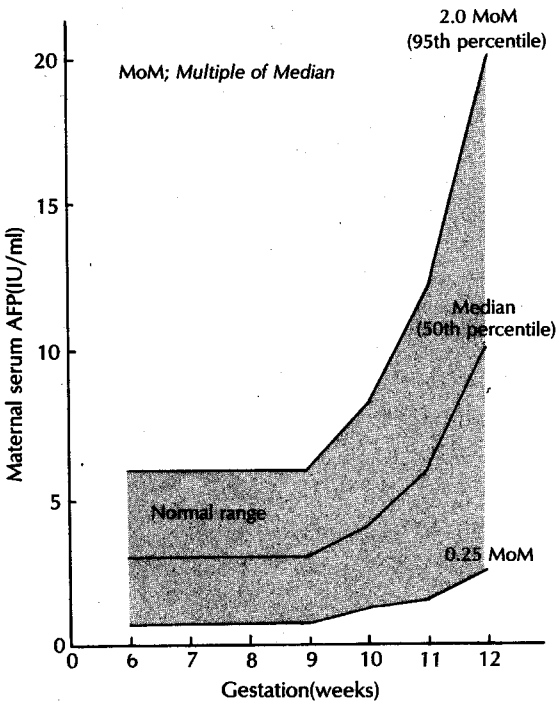

Fig. 1. *Normal range for maternal serum AFP (6-12 weeks).*

less than 100% increase was seen in 15 cases (20.8 %), and 100% or higher increase was seen in 30 cases (41.7%) (Table 8).

The MSAFP level and the number of catheter passes: There were 56 and 16 cases requiring 1 to 2 (group A) and 3 to 4 catheter passes (group B) for successful sampling. The cases needing 3 to 4 passes showed higher MSAFP level increase than those needing only 1 to 2 passes (Group A, $r=0.496$, $p<0.05$; Group B, $r=0.483$ $P<0.05$) (Fig. 2).

MSAFP and the amount of sampled villi: The sampled villi ranged from 5 to 50 mg, and a good positive correlation was present between the sam-

Table 8. Rise of concentration MSAFP after CVS

Percentage of rise	Patient number	%		
<20%	16	22.2		
20-49%	11	15.3	77.8	62.5
50-99%	15	20.8		
≥100%	30	41.7		
Total	72	100.0		

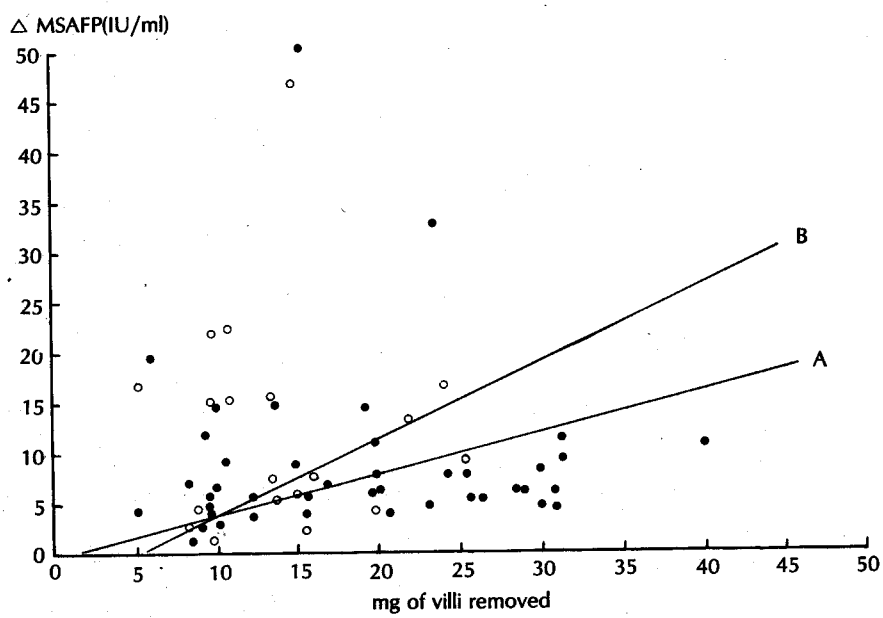

Fig. 2. *Relationship between delta maternal serum alpha-fetoprotein (△ MSAFP) value and amount of villi removed for one or two catheter pass (A) (closed circles, lower line, $n=56$, $r=0.496$ $p<0.05$) and for three and four passes (B) (open circles upper line $n=16$, $r=0.483$, $p<0.05$)*

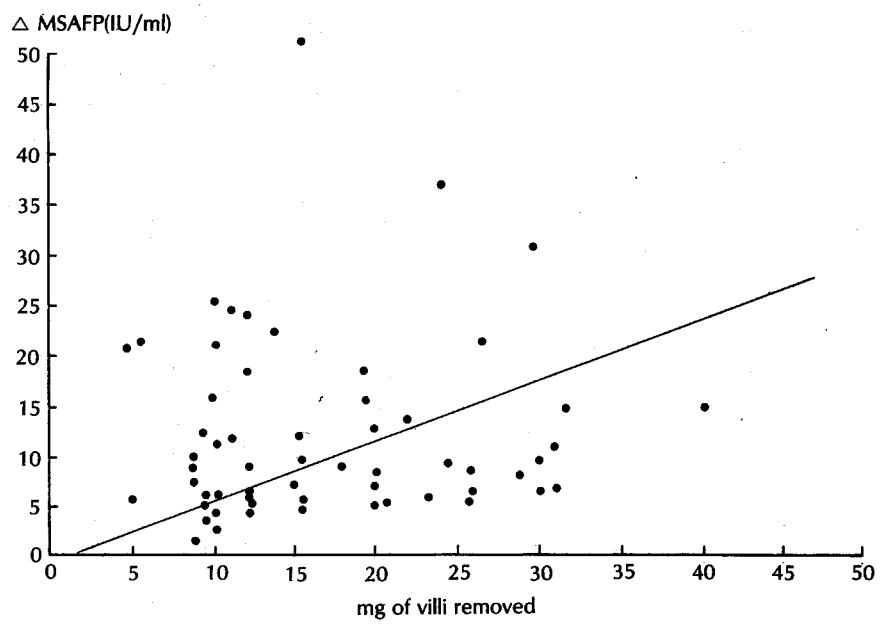

Fig. 3. *Relationship between delta maternal serum alpha-fetoprotein (△ MSAFP) value and amount of villi removed at CVS ($r=0.496$, $p<0.001$) in 72 women.*

pled villi amount and MSAFP level ($r=0.496$, $p<0.001$) (Fig. 3).

Post-CVS increase in MSAFP level and the amount of FMH: In 56 cases showing increased postsampling MSAFP levels, the lowest level of delta MSAFP before CVS was 0.15 and the highest after CVS was 48.30; the estimated CVS-related FMH thus ranged from 0.0005 to 0.1552 ml. Five cases (5/72, 6.9%) had 0.06 ml or more amounts of FMH (Case 3, 8, 18, 35, 59). In two of these (case 8, 35), the amount of hemorrhge were 0.1 ml or more (Table 9).

DISCUSSION

CVS requires a short time for results, usually from 48 hours to several days. Theoretically, CVS is based on the assumption that cells obtained from chorionic villi should have identical genetic composition and karyotype with the fetus (Watanabe *et al.* 1978; Pergament and Verlinsky 1982). However, it has been reported that cytogenetic reliability of the tissue obtained by CVS may be impaired by maternal cell contamination during CVS (Ridler and Grewal 1984), and mosaicism (Kalousek and Dill 1983).

Several different methods of CVS have been developed since the first introduction of CVS in 1968 by Mohr; thus, there are different data on the rates of sampling success. Szabo *et al.* (1984) reported the sampling success rate from their 82 cases of CVS to be 84%. Brambati *et al.* (1987), under ultrasound guidance, obtained sufficient amounts of villi in 95% of the time by a single catheter-pass and up to 98% by another pass. Others reported the success rate as high as 90 to 100% (Hogge and Sehonberg 1985; Simoni *et al.* 1984; Holzgreve and Miny 1987; Green *et al.* 1988).

The optimum time of CVS according to Simoni *et al.* (1983) was 7 to 9 weeks, since below 6 weeks, the chorion frondosum, the site of sampling, remains poorly demarcated under ultrasound, and over 10 weeks, the site of the placenta may not be ideal for transcervical CVS. Ward *et al.* (1984) on the other hand, obtained the highest success rate from 8 to 10 weeks, and suggested 9 to 10 weeks as optimum time for adequate sampling, and below 9 weeks, the period of completion of organogenesis, was unsuitable for sampling because of higher fetal risk. A Chinese group (1975) and Brambati *et al.* (1987) each proposed 7 to 12 weeks as opti-

Table 9. Expected volumes of fetal blood transferred for given rises in maternal serum AFP concentration

Case No.	MSAFP(IU/ml) pre CVS	MSAFP(IU/ml) post CVS	△ MSAFP (IU/ml)	Expected volumes of fetal blood transferred(ml)	Case No.	MSAFP(IU/ml) pre CVS	MSAFP(IU/ml) post CVS	△ MSAFP (IU/ml)	Expected volumes of fetal blood transferred(ml)
1.	3.00	20.86	17,87	0.0574	42.	4.24	14.78	10.54	0.0338
2.	11.44	12.16	0,72	0.0023	43.	21.20	27.31	6.11	0.0196
3.	3.69	27.02	23,33	0.0749	44.	5.32	19.08	13.76	0.0442
4.	4.64	7.03	2.39	0.0077	45.	3.26	9.50	6.24	0.0200
5.	3.00	18.05	15.05	0.0484	47.	7.10	22.09	14.99	0.0481
6.	7.10	22.00	14.90	0.0479	48.	6.20	10.17	3.97	0.0127
7.	3.70	10.00	6.30	0.0230	49.	5.20	12.73	7.53	0.0242
8.	3.00	51.30	48.30	0.1552	50.	4.30	11.78	7.48	0.0240
9.	1.30	10.60	9.30	0.0299	51.	3.40	11.24	7.84	0.0252
10.	5.80	20.70	14.90	0.0479	52.	10.20	18.73	8.53	0.0274
11.	2.60	7.90	5.30	0.0170	53.	7.80	14.53	6.73	0.0216
12.	1.00	16.60	15.60	0.0501	54.	11.2	21.33	10.13	0.0325
13.	5.30	17.70	12.40	0.0399	55.	20.10	24.58	4.48	0.0144
14.	2.40	5.50	3.10	0.0099	56.	15.70	27.12	11.42	0.0367
15.	10.24	15.73	5.49	0.0176	57.	6.20	10.70	4.50	0.0144
16.	21.82	26.53	4.71	0.0151	58.	7.20	11.42	4,20	0.0135
18.	3.00	22.60	19.60	0.0630	59.	21.20	43.30	22.10	0.0710
24.	5.67	5.82	0.15	0.0005	60.	10.20	15.62	5.42	0.0174
28.	2.14	8.06	5.92	0.0190	61.	15.70	20.15	4.45	0.0143
30.	2.40	3.55	1.15	0.0036	62.	5.20	10.17	4.97	0.0159
31.	3.20	4.30	1.10	0.0035	63.	11.00	15.02	4.02	0.0129
32.	11.20	14.10	2.90	0.0093	64.	7.30	15.74	8.44	0.0271
33.	6.21	9.01	2.80	0.0090	65.	7.10	22.00	14.90	0.0479
34.	7.21	14.90	7.69	0.0247	66.	13.40	20.38	6.98	0.0224
35.	22.10	55.37	33.27	0.1069	67.	12.10	17.30	5.20	0.0167
36.	10.10	16.22	6.12	0.0196	68.	5.30	11.24	5.94	0.0190
37.	6.30	11.97	5.67	0.0182	69.	10.10	16.22	6.12	0.0196
38.	5.70	11.24	5.54	0.0178	70.	2.4	10.35	7.95	0.0255
40.	2.70	13.95	11.25	0.0361	71.	15.2	19.67	4.47	0.0143
41.	3.21	17.54	14.33	0.0460	72.	3.7	10.70	7.00	0.0225

△ MSAFP: Delta maternal serum alpha-fetoprotein
Difference in maternal serum alphafetoprotein before and after CVS

mum. Simoni *et al.* (1984), Green *et al.* (1988) and Heim *et al.* (1985) each reported 8 to 12 weeks and 8 to 13 weeks gestation to be ideal. In our current series, in accordance with many authors, 9 to 10 weeks was associated with a higher success rate.

Chromosomal analysis was carried out in this series by direct and culture methods. The culture method produces many metaphase cells and high quality banding (Niazi *et al.* 1981; Blakemore *et al.* 1984), but increased maternal cell contamination and mosaicism, consequently raising the potential risk of diagnostic error (Miny *et al.* 1985). The direct method is cost-effective and is useful in precluding chromosomal aberration arising from maternal cell contamination or from long term culture as well as in obtaining a satisfactory number of high-quality metaphase cells in a short time, thereby reducing the time necessary for fetal karyotyping but may result in fewer metaphase cells and occasional suboptimal banding; some, however, observed accurate and satisfactory banding (Gregson and Seabright 1983; Sachs 1983; Simoni *et al.* 1984, 1986; Brambati *et al* 1985).

Its time-effectiveness which makes early therapeutic pregnancy termination possible by simple suction curettage should it be needed, minimizing maternal burden, both physical and psychological. For this reason, this method is valued by many researchers (Simoni *et al. 1983; Szabo et al.* 1984; Romero *et al.* 1984). Feasibility of cytogenetic eval-

uation of chorionic villi is often challenged by mosaicism (Per-gament and Verlinsky, 1982) thus the cytogenetic discrepancy caused by mosaicism, if arises, should be rectified by the result of amniocentesis of the respective patient; if the result is normal, pregnancy is carried to term. Our direct method has gradually produced sufficient number of metaphase cells and high banding quality as experience was gained, and results are now usually satisfactory. This indicates the potential of direct method as a means of providing fast and good quality results that are quite reliable when coupled with the results of the longterm culture method.

Advanced maternal age was the most common indication for CVS in this series. The incidence of chromosomal abnormality in this series was 2.4 percent (4/166). Trisomy was revealed in 2 cases (47, XX, +7; 47, XX, +21) with maternal age 35 or older. Translocation Down syndrome was noted in 1 case having balanced translocation on one parental side. The remaining 1 case was 45, X from a pregnancy following artificial insemination.

The incidence of first trimester chromosomal abnormality in apparently normal gravidas was 6.8% in one study (Yamamoto *et al.* 1975). Mikkelson (1985) in his 896 CVS cases observed 4.6% incidence of chromosomal abnormality with maternal age 35 to 39, and 9.2% with 40 or higher, thereby achieving the overall incidence of 6.0% with maternal age 35 or older; it thus was evident that increasing maternal age correlate with increased incidence of chromosomal abnormality. According to Green *et al.* (1988), the incidence was 2.9%, about 1.7 times that obtained by amniocenteiss (NICHD, 1976). The incidence, reported by Golbus from his CVS cases (1986), was 4.2%, also higher than 2.5% obtained by amniocentesis. However, a 7 U.S. center collaborative study recently reported by Rhoad *et al.* (1989) showed 1.4 and 1.8% incidence of aneuploidy from CVS and amniocentesis, respectively, and similar overall incidence of chromosomal abnormality by either CVS or amniocentesis.

Correlation between the amount of sampled villi and the success of chromosomal analysis was not always present. In a study by Simoni et al. (1984), in which overall of 20 to 50 mitoses were observed with the villi amount ranging from 5 to more than 50 mg; they noted sufficient numbers of mitosis with as little as 5 mg of villi, but only 9 mitoses with as much as 50 mg of partly misshapen villi. In this study, in 2 cases where the villi upon sampling was morphologically incomplete, 15 metaphases were observed with the villi amount exceeding 10 mg, while with as little as 5 mg of villi as many as 32 metaphases were noted. Nevertheless, the higher amount of sampled villi appeared to mean a greater number of obtained metaphases, and a minimum of 5 mg or more villi was necessary for achieving optimum number of metaphases. Heim *et al.* (1985) sampled from 2 to 150 mg villi in their series, and suggested a minimum of 5 mg of villi, provided they are at least moderately vascularized with prolific budding, would be enough for karyotyping.

The number of catheter-passes was from 1 to 3 in 95.4% of the cases in our series. There occurred 4 spontaneous abortions in the 174 CVS cases, giving the fetal loss rate of 2.3%; three of these four had normal fetal karyotype and the remaining one was 46, XX, inv (9)(p11q13). All these four cases had received two more catheter-passes. Whether there exists a correlation between the number of catheter-passes and the risk of fetal loss needs, however, to be examined further as more cases are accumulated.

Heim *et al.* (1985) reported 2 to 8% fetal loss rate and according to Hogge *et al.* (1985), it was 7.5%. Johoda *et al.* (1985) reported 3.3% abortion rate until 16 weeks pregnancy follwing CVS. Simoni *et al.* (1985) reported 5.2% in the Milano group study, and 10.2% in the Genova group study. Jackson (1986) reported 4.3%. Green *et al.* (1988) found the overall fetal loss rate of 2.4% of which only 0.6% was CVS-related. Brambati and Varotto (1985), Gilmore and McNay (1985), and Simpson (1984) each reported 2 to 4% abortion rates after sonographically confirming normal intrauterine pregnancy, and 2.4% was the rate of spontaneous abortion related to CVS. Jackson *et al.* (1989) in the 17th issue of "CVS Latest News", reported the experiences of 128 CVS centers according to which the average fetal loss rate was 3.49%. Rhoads *et al.* (1989) in a 7 U.S. center collaborative study found a slightly higher (by 0.8%) fetal loss rate following CVS than following amniocentesis when such factors as maternal age and gestational age were considered. A likely reason for the relatively higher rate of pregnancy loss from CVS than from amniocentesis is that the aneuploid fetuses, if ever conceived, usually do get aborted from 9 to 16 weeks, not reaching the weeks suitable for amniocentesis, thus becoming excluded from amniocentesis statistics. It thus seems that as also reviewed by Hogge *et al.* (1986), the fetal loss rate following CVS is not very different from 5 to 10% spontaneous abortion rates in early apparently normal pregnancy.

Various methods of sampling chorionic villi have

been developed since the initial use of the endoscope-assisted suction guillotine biopsy. Among the various methods of CVS, the transplacental aspiration technique using a flexible echogenic plastic catheter by Holzgreve under ultrasound guidance is chosen here in order to compare its results with those of other methods.

In this study, 95.7% sampling success by transcervical CVS, higher than 93% in our earlier series (Yang *et al.*, 1989) indicates improved sampling technique.

FMH is considered as a significant hazard in amniocentesis and may result in Rh isoimmunization in Rh-negative mothers, abortion, and, even trophoblastic embolization. Likewise, FMH caused by CVS may influence the abortion rate and fetal growth (Symposium, 1984; Williams *et al.* 1987). Therefore, knowing the estimate of the volume of FMH is important. Whether a small placental hemorrhage caused by CVS may generate maternal isoimmunization is not clear. The issue, however, has significant impact since it involves the decision of administering prophylactic anti-D IgG to Rh-negative women after CVS. To date, there exists a number of articles addressing the issue of amniocentesis-related FMH. It appears, CVS, with its invasive nature, like amniocentesis, is associated with certain risks of procedure-related FMH. FMH is thought to occur by the disruption of barrier between fetal and maternal blood compartments following mechanical damage onto the villi portion of placental tissue. In fact, the incidence of FMH is known to be higher when placental location is anterior, a situation where the chance of disrupting placental tissue by amniocentesis is greater, than otherwise. The Kleihauer-Betke acid elution test has been the gold standard for identifying the presence of fetal red cells in the maternal circulation. The method is based on the observation that the concentration of fetal AFP approximately 30,000 times that of MSAFP at the same gestational age is high enough to cause a change in MSAFP with only a slight FMH.

FMH associated with CVS is also caused by mechanical damage onto placental tissue, and is considered to be best calibrated with MSAFP measurement. Brambati *et al.* (1985) estabilished cutoff values of 20% and 40% increase, respectively, in post-procedure MSAFP level from the immediate pre-procedure level for FMH, above which level diagnosis of FMH can be reached. In this series, the hemorrhage occured in 6 of 27 cases (59.3%). Positive correlation between the degree of MSAFP level increase and the amount of sampled villi was found by Blackmore (1986) and Brambati *et al.* (1986). However, such relationship was not evident according to Warren *et al.* (1985). Our results ($r = 0.496$, $P<0.001$) are supportive of the former. As similarly reported by Warren *et al.* (1985) and Blakemore *et al.* (1986), a positive correlation ($P< 0.05$) between the number of catheter-passes and the degree of MSAFP level increase was present in this series when the group given 1 to 2 catheter-passes was compared with the group given 3 to 4 passes.

Rh-isoimmunization may result as a consequence of FMH. The amount of FMH that could invoke maternal sensitization is considered, according to Scott *et al.* (1982) to be at mininum about 0.1 ml. Experimentally, Ziprusky (1967) noted maternal sensitization with several challenge doses of 0.1 ml of fetal blood. It is now considered a routine practice to administer a dose of 300 μg of immunoglobulin to D negative gravida following CVS irrespective of the presence and amount of the procedure-related FMH. There are conflicting reports on the effect of D immunoglobulin on the fetus, but at the present time no adverse fetal effect of maternally administered D immunoglobulin is believed to exist.

It has been reported that with the level of MSAFP significantly rising after amniocentesis or CVS, that in other words is in the presence of FMH, the incidence of spontaneous abortion and low birthweight infants increase. Thomsen *et al.* (1983) noted the increased incidence of low birth weight infants in the group that showed increased MSAFP immediately following amniocentesis. However, Simpson (1986) and Brambati *et al* (1986) noted no such difference. Data on such vital statistics are lacking with CVS, although Brambati *et al.* (1986) reported no significant difference in the incidence of spontaneous abortion, low birth weight infants, perinatal mortality, and intrauterine growth retardation in relation to CVS-associated MSAFP increase.

In this pilot study in Korea, these results suggest that CVS in early pregnancy is a relatively safe and a reliable method of prenatal genetic diagnosis capable of replacing genetic amniocentesis in many clinical circumstances and should be done preferably by experienced hands. And also during sampling, as small a sample of villi as necessary for diagnosis should be taken with as few catheter passes as possible, In particular, the direct overnight incubation method used for chromosomal preparation has the advantage of being fast as well as accurate in diagnosis, and coupling with the longterm culture

method can complement each other's potential pitfalls.

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