

## TRANSABDOMINAL CHORIONIC VILLUS SAMPLING: EXPERIENCE WITH 70 CASES

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### ABSTRACT

The first 70 cases of Transabdominal Chorionic Villus Sampling (TA-CVS) performed at the Serviço de Genética Humana da Associação Maternidade de São Paulo are reported. All the samples collected between November/87 and February/89 were adequate for analysis and the average number of needle insertions was 1.6. Eighty percent of the tests were done due to advanced maternal age ( $\geq 35$  years). Six cases (8.6 per cent) of chromosomal abnormalities were detected and the pregnancies terminated. The risk of pregnancy loss in the two week period after the procedure was 1.4 per cent. TA-CVS is an alternative method for prenatal diagnosis with no limitations of gestational age, but further investigation with respect to its safeness is needed.

### INTRODUCTION

Diagnostic techniques for examination of fetal health are being slowly but progressively incorporated into obstetrical propaedeutics. At present, the main methods for diagnosis available in Brazil are: ultrasonography, amniocentesis, transcervical chorionic villus sampling (CVS), transabdominal chorionic villus sampling (TA-CVS) and cordocentesis. Fetoscopy has never been used here, both because of the cost, and the high risk of pregnancy termination (5 per cent).

CVS was first used as a first trimester diagnostic procedure in China by Anguo *et al.* (1975) in 1970, and introduced into Italy by Brambati and Simoni (1983), into the United States by Jackson (1985) and into Brazil by Gollop *et al.* (1986). In

Brazil, Naccache *et al.* (1987) first reported the results of (250) CVS cytogenetic analyses.

Between 1984 and 1988 around 50,000 CVS were done all over the world, and data were collected by Dr. Jackson, in Philadelphia, PA. After this large sample of CVS was analysed the procedure was authorized at all the centres where prenatal genetic diagnosis is performed.

An alternative method for prenatal genetic diagnosis in the first trimester is early amniocentesis, done between the 9th and the 14th weeks of gestation and seems to result in similar pregnancy outcome of traditional amniocentesis (Elejalde *et al.*, 1990). The Canadian Collaborative CVS-Amniocentesis Clinical Trial Group (1989) published comparative results of CVS and early amniocentesis, and demonstrated similar maternal morbidity in both methods, a non significant difference in fetal losses between the procedures, though there was a shift to later losses in the CVS group. In CVS there were more problems with cytogenetic interpretation due to confined mosaicism and maternal cell contamination. The risk of maternal cell contamination can be reduced by careful laboratory management (Gollop *et al.*, 1988). Another of the most serious criticisms of CVS is the risk of chorioamnionitis. There is, undoubtedly, a minimum infection risk but we experienced no case of infection in 150 patients (Gollop *et al.*, 1988). It is clear that the low infection rate and minimum pregnancy loss rate (0.5 per cent) depends on the experience of those who collect the sample and on the introduction of the catheter no more than two times in the same patient.

The CVS is an outpatient procedure, needs no anesthesia and the patient does not have to stay for rest afterwards. It presents, however, an important limitation. It can be performed only between the 9th and the 11th week of gestation. Before the 9th week there is a risk of withdrawing an insufficient sample, and after the 11th week there is a higher risk of membrane perforation.

Up to 1987, when patients were referred to the Serviço de Genética Humana da Associação Maternidade de São Paulo after the 9th-11th week of gestation, we had no alternative for prenatal genetic diagnosis than to counsel an amniocentesis at the 17th week.

Amniocentesis continues to be useful, although it has two limitations which, together, interfere a great deal in its obstetric use here in Brazil: it has to be done in the second trimester and it takes four weeks, on average, to give results. For these reasons, Smidt-Jensen and Hahnemann (1984) suggested a transabdominal route of sampling trophoblastic tissue instead of the transcervical one, in order to prevent ascending microbial contamination from the external os and endocervical canal. Bovicelli *et al.* (1988) published their experience with 350 TA-CVS samples collected with a needle between the 9th and the 13th week of gestation. The authors reported 99.7 per cent success, an average of 1.8 insertions of the needle per patient, and 2.7

per cent fetal losses prior to 28 weeks of gestation and one perinatal death due to prematurity.

This paper reports our experience with our first 70 cases of TA-CVS.

## PATIENTS AND METHODS

The patients who will undergo any of the prenatal genetic diagnosis techniques at the Serviço de Genética Humana da Associação Maternidade de São Paulo are previously interviewed by one of its doctors (TRG or DH), to record data on the reproductive history of the patient and the family. The necessity of a test is evaluated and a detailed explanation is given on how the test is done, as well as its risks and limitations.

TA-CVS was performed at an outpatient clinic in 70 pregnant patients. Previous to the procedure an ultrasound examination was done in order to verify signs of fetal vitality, the gestational age and to determine the insertion point of the trophoblast or placenta. Povidine-iodine solution was used for asepsis of the abdominal wall and xylocaine without adrenalin was used for local anesthesia of the needle insertion point.

The equipment used for ultrasonography was an ELCINT-ESI-1000 with a 3.5 MHz sectorial transducer. The samples were performed under direct ultrasound visualization with an 18 G needle, BD or Monoject type, manufactured in the United States. Once the needle was inserted in the desired place and the mandrel released, a disposable 20 ml syringe already containing 5 ml of culture 199 or F-10 medium was attached and negative pressure with the plunger removed about 20 mg to 50 mg of chorionic villi. Macroscopic confirmation of the sample taken is usually easy. The insertion point of the needle rarely bleeds and if so it can be covered with sterilized gauze and povidine. The patients with unsensitized Rh-negative blood type routinely received a dose of anti-Rh immunoglobulin.

Chromosomal analysis was done by a modification of the original method proposed by Bambati and Simoni (1985). Twenty-five cells were analysed for each patient and ten of them were G-banded.

The patients referred to our department attended it on their own (around 50 per cent of the total), or were sent through medical agreement, institutions (as e.g., the University of São Paulo, Associação de Pais e Amigos dos Excepcionais and Associação de Assistência à Criança Defeituosa - associations of parents and friends for handicapped children). Public Health Centres and private doctors. Patients pay a fee for the examination, but those with genetical indication and no financial resources were examined free of charge.

## RESULTS

TA-CVS was performed between the 10th and the 24th week of gestation (average: 14w 1d) and the needle was inserted a maximum of four times per patient (in the first examination). At present we are limiting this number to two, and when no satisfactory villus sample is obtained the test is postponed one week. In the 70 cases studied, the average number of needle insertions per patient was 1.6. There were two cases of chorioamniotic membrane perforation with removal of about 5 ml of amniotic fluid together with the chorionic villus. Both gestations went on and there was no fluid leakage via the vagina.

Ultrasonography was requested for all the patients at least 4 weeks after the TA-CVS in order to accompany the evolution of the pregnancy. However, only 21 patients (34 per cent) were monitored with ultrasound scan. None of them showed alterations of the placenta, retroplacental haematomas or oligoamnio.

Maternal age was the main indication for TA-CVS counting for 80 per cent of all cases. The mother's age range from 35 to 46 years, and 39 ys 7 ms was the average; 66.4 per cent of the patients referred to our department with gestational age less than 15 weeks came on their own, and 28.6 per cent of those with 15 or more weeks of gestation attended the department by their own decision.

Six (8.6 per cent) abnormal fetal karyotypes were found: two trisomies 21, two trisomies 18, one trisomy 22, and one trisomy 9 (Table I). Both trisomies 22 and 9 were found in all of the 50 cells analyzed, and an amnio or cordocentesis was suggested to check the TA-CVS results. One of the patients whose fetus showed trisomy 18 was referred to us in the 19th week of gestation because the ultrasound performed at the 18th week had shown multiple fetal anomalies. Besides the TA-CVS, a cordocentesis was performed and the lymphocyte culture also showed trisomy 18.

Table I - Chromosomal abnormalities observed in 70 cases of TA-CVS.

Trisomy 21 (Down's Syndrome)	2
Trisomy 18 (Edward's Syndrome)	2
Trisomy 22	1
Trisomy 9	1

In four (5.7 per cent) patients TA-CVS was performed because of a previous child with chromosomal abnormality. All of them were cytogenetically normal. In five (7.1 per cent) patients the test was performed due to parents' anxiety.

TA-CVS was performed during the 14th week of gestation in a patient who had already had a daughter with genital ambiguity due to congenital adrenal hyperplasia. The fetal karyotype was 46,XX and at the 17th week an amniocentesis was

done to 17 alpha-hydroxyprogesterone dosage; the level was normal, the gestation continued to term and a normal female was delivered.

All the patients whose tests showed abnormal karyotypes decided to terminate their pregnancies.

## DISCUSSION

TA-CVS has been counseled at our department as an equally quick and safe alternative to CVS or amniocentesis. From a strictly medical point of view we counsel the TA-CVS in those cases of very accentuated uterine rearversion with the chorionic plate inserted in the posterior wall; when the uterine cervix is stenosed and does not allow easy introduction of the catheter for CVS; in cases of cervix myoma or myoma in the catheter's normal route; in cases of suspicious ultrasound scans in order to obtain a rapid karyotype, and in cases of gestational age more than 11 weeks. There were, however, cases at an appropriate gestational age for CVS for which we counseled TA-CVS, e.g. acute herpes infection. We do not defend the idea that TA-CVS is a superior method and we do not intend to give up CVS.

CVS is an almost painless and elegant technique and its safeness depends on the experience and skill of who does it (as for any medical practice). From the patient's point of view, we noticed that there is a preference for CVS. Patients refer to it as "less traumatic", being a vaginal test, than "putting a needle near the baby". If on the one hand there is a small bleeding after CVS (mainly caused by the gripping of the uterine cervix) we often notice some complaints caused by an hypogastrium ache after TA-CVS. Among our CVS cases, the risk of gestational loss was 0.5 per cent (Gollop *et al.*, 1988). We will need to enlarge our TA-CVS study to make it comparable with the CVS group.

An undeniable advantage of TA-CVS over amniocentesis has to do with the results. Whereas the later takes four weeks on average to provide results, the former takes only four working days. This has a considerable role in decreasing the mother's anxiety, therefore we counsel TA-CVS instead of amniocentesis whenever that test is possible. We had no problems in placing the needle conveniently even in cases of rear chorionic plate or placenta, and there were also no accidents in those cases. At present, our average insertion number is 1.6 times per patient, and we are trying to bring this down to nearly one. There is no doubt about the need to avoid membrane perforation, but when this occurs it does not necessarily mean there will be a bad obstetrical evolution. Simoni (personal communication) stated that among more than 1,000 TA-CVS performed by his group there were membrane perforations in five cases and three of those pregnancies continued to term, while two were lost.

In all countries the main motive to undertake a genetical examination in the prenatal period has to do with the mother's age (Fraccaro *et al.*, 1985). It is not surprising, therefore that 80 per cent of the TA-CVS counseling within our department is

related to this factor. The same authors report that in Italy, about 50 per cent of the patients seek the tests on their own, without interference of their doctors. Here, the number of patients who do so is also quite high, representing an average of 47.5 per cent of all of them. This is evidence that the doctors charged with prenatal care of women with considerable genetical risk tend to avoid prenatal examination techniques due to the possibility of negative results and to the provisions of the law in our country.

The rather high frequency of tests showing chromosomal aberrations (8.6 per cent) can be explained by the fact that our sampling comes from patients with considerable genetical risk due to an advanced maternal age: 39 ys 7 ms, on average.

The cases of trisomy 9 and 22 need comment. Since both are very rare among newborns, cordocentesis or amniocentesis should ideally be performed in order to check these results. Both mothers even though they were thoroughly informed about the implications of these results, and despite our formal counsel to do a complementary examination, declined to do so.

CVS is formally indicated in cases of risk of congenital adrenal hyperplasia. The earlier the test is done, the sooner the fetal sex will be found out, making possible therapy with dexametazone. In the case we studied, the mother came to our department in the 14th week of gestation and so we could perform a TA-CVS.

The parents' anxiety is highly considered at our department, although we try at the first interview to dissuade the couple from taking the test, explaining about the risk of pregnancy loss and that it represents no guarantee of a perfect child. As a result, most of the couples who seek the procedures because of their anxiety desist and only seven per cent of our TA-CVS group is composed of patients who decided to undertake the test in spite of all the relevant information given.

Seventeen percent of the TA-CVS were performed free of charge for low income patients.

## ACKNOWLEDGMENTS

Publication supported by FAPESP.

## RESUMO

São analisados os 70 primeiros casos de Amostra de Vilo Corial por via Transabdominal (AVTA), realizados no Serviço de Genética Humana da Associação Maternidade de São Paulo, de Novembro de 1987 a Fevereiro de 1989. Em todos os casos obteve-se material adequado para análise e a média de inserções da agulha foi 1.6. Oitenta por cento dos casos foram realizados em função da idade materna avançada ( $> = 35$  anos). Seis casos (8.6%) revelaram anomalias cromossômicas sendo as respectivas gestações interrompidas. O risco de perdas gestacionais no período de 2 semanas que se seguiram a coleta foi de 1.4%. A AVTA é um método alternativo de diagnóstico genético no pré-natal, podendo

ser realizada sem limitações no que diz respeito a idade gestacional, embora investigações ainda sejam necessárias para que seja estabelecida a segurança do procedimento.

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(Received May 11, 1989)