Invasive procedures

in multifetal pregnancies

Luc De Catte

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Promotor : Prof. Dr. W. Foulon

To my parents, for their trust

To my wife, for her loving care

Dankwoord

Waarom ik in 1984 na enige aarzeling dan toch geselecteerd werd om de opleiding in Gynecologie, Verloskunde en Andrologie te mogen aanvangen, is me een raadsel gebleven. Vermoedelijk heeft de calligrafische kandidatuur hier toe bijgedragen, het moet haast zo zijn. Of heeft mijn wat traag zangerige wijze van spreken, eigen aan menigeen uit het “buitenland” (Limburg), de doorslag gegeven? Er was trouwens nog niemand uit die regio in opleiding, maar de moppen waren voorradig. Alvast ben ik Professor Amy dankbaar voor de opleiding, voor de geboden kansen en het vertrouwen om me te kunnen ontplooien in een snel evoluerend deelgebied van zijn Dienst Gynecologie-Verloskunde-Andrologie.

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Chapter 1

Introduction and Aims

The epidemiology of multiple pregnancies has changed dramatically in the last two decades. The introduction of ovulation induction, in vitro fertilization programmes including intracytoplasmic sperm injection, made the prevalence of multiple births increase to the levels comparable with those early in the 20th century.
The first aim is to shed some background information regarding multiple pregnancies. New, unpublished, data are incorporated on the outcome of triplet and twin pregnancies in Flanders.

The greater number of high order multiple pregnancies, and of aging women carrying a multiple pregnancy, and the introduction of new screening programs for fetal aneuploidy in the first trimester contribute to the higher need for prenatal diagnosis in multiple pregnancies.
The second aim is to elaborate on the specific considerations related to prenatal diagnosis in multiple pregnancies. What are the risks for chromosomal malformations? How correctly can chorionicity be identified by ultrasound in order to determine the pregnancy related risks for congenital malformations and the way to deal with them?

Amniocentesis has been the gold standard of prenatal diagnosis in the second trimester. In singleton pregnancies, chorionic villus sampling has been accepted as an equally valuable method for prenatal diagnosis from the first trimester onward.
The third aim is to demonstrate that first trimester chorionic villus sampling has become a valuable alternative for prenatal diagnosis in multiple pregnancies. Results of our published research together with new, unpublished data are presented.

The advent of new fertility treatment regimens introduced high numbers of higher order multiple pregnancies (≥3) and their associated pregnancy complications.

The fourth aim consists in demonstrating the advantageous outcome of reduced higher order multiple pregnancies. Do triplet pregnancies benefit from fetal reduction too? New, unpublished work is presented.

Congenital malformations in one of the fetuses jeopardize obstetric outcome. Uterus anomalies or cervical incompetence result in early preterm deliveries with high perinatal mortality and morbidity.
The fifth aim is to present an overview of existing data concerning (s)elective fetal reduction in multi- and monochorionic pregnancies. For this purpose our published research and new unpublished data are presented together with an overview of the literature.

In their search for normal outcome of their offspring after years of infertility and in the light of fetal reduction, parents are eager to submit to prenatal diagnosis.
The sixth aim will be to prove that prenatal diagnosis in association with fetal reduction is a safe and accurate procedure. In order to fully benefit from the results offered by prenatal diagnosis, we advocate the use of pre-reduction CVS over post-reduction amniocentesis. For this purpose our published research is incorporated in the whole of available information.

Chapter 2

The natural history of multifetal pregnancies

De Catte L, Martens Guy. Outcome of triplet pregnancies over 9 years in Flanders. In preparation.

De Catte Luc, Martens Guy. Outcome of twin pregnancies over 9 years in Flanders. In preparation.

De Catte L, Sleurs E, Goossens A, Foulon W. Favorable outcome of the pump-twin in association with an amorphous acardiac fetus. In preparation.

De Catte L, Camus M, Foulon W. Monochorionic high order multiple pregnancies and multifetal pregnancy reduction. Obstet Gynecol 2002;100:561-6.

1. Outcome of multifetal pregnancies.

Some general considerations about multiple pregnancies have to be discussed first. Several items are in the light of this work of lesser importance, and will therefore be mentioned only briefly. To correctly understand risks associated with multiple pregnancies in relation to invasive procedures, fetal and neonatal death and perinatal mortality will be discussed more extensively. Specific conditions related to invasive procedures in multiple pregnancies are clarified in more detail.

1.1 Embryonic and early fetal loss.

The incidence of fetal loss in multiple pregnancies is extensively higher than in singletons. The proportion of multiple pregnancies detected among spontaneous abortions ranges from 2-3%, two to threefold higher than the rate among pregnancies delivering after 20 weeks (*Boklage: In Keith, Papiernik, Keith, 1995*). Early first trimester loss of one or more fetuses in a multiple pregnancy is also referred to as a vanishing twin or vanishing multiplet. The majority of spontaneous embryo reductions occurs before the 7^{th} completed week, and are hardly ever observed after the 14^{th} week. Aetiologic factors for the vanishing twin phenomenon are chromosomal abnormalities (Callen, 1991; Reddy, 1991; Rudnicki, 1991), or the discordance for structural defects (Kapur, 1991).

The incidence of early fetal demise is inversely related to the gestational age at which the multiple pregnancy is diagnosed (Table 2.1). Of the 221 fetuses identified in 88 multiple pregnancies after ovulation induction at 5 to 6 weeks, including 54 twin, 26 triplet, 5 quadruplet and 3 quintuplet pregnancies, 107 vanished spontaneously (48%). The majority occurred at 12 weeks or less (Blumenfeld, 1992). Complete abortion rates in twin, triplet and quadruplet pregnancies were respectively 10.3, 7.7 and 20%. However, if the loss of at least one fetus in these multiple pregnancies is considered, the abortion rates would become respectively 22.2%, 26% and 20%.

Table 2.1: Spontaneous reductions in multifetal pregnancies in relation with gestational age.

Author	Fetal loss per gestational sac (%)	Fetal loss/embryos with heart activity (%)	Fetuses at delivery = gestational sacs seen (%)
Landy, 1986	26.8	-	48.1
Kelly, 1991	16.3	8.2	67.5
Blumenfeld, 1992	42.4	-	17.0
Kol, 1993	23.6	5.4	61.7
Manzur, 1995	25.4	14.3	47.4
Legro, 1995	28.0	5.7	41.9
Wisanto, 1995	-	-	71.7

A high starting number of fetuses and their abnormal chorionicity add to the risk of early fetal loss. Multiple pregnancies established after in vitro fertilization (IVF) will lead to fetal loss rates that differ according to the number of early first trimester viable fetuses (Seoud, 1992). Maternal age above 30 years increases significantly the chance of one or two sacs of a twin or a triplet pregnancy being reabsorbed. After documentation of viable twin and triplet pregnancies by ultrasound, women under 30 years of age have a 90% chance of giving birth to twins or triplets, where as in older women the chances are respectively 84 and 44% (Table 2.2)(Dickey, 1990).

Table 2.2: Percentage of twin and triplet pregnancies remaining in relation with maternal age and the gestational age at the time of investigation (After Dickey RP, 1990).

		Twin pregnancies	Triplet pregnancies
Maternal age < 30 y	Early first trimester (sacs)	63.1%	45%
	Late first trimester (fetuses)	90%	90%
Maternal age > 30 y	Early first trimester (sacs)	50.1%	17%
	Late first trimester (fetuses)	84%	44%

In IVF twin pregnancies about 27% of the gestational sacs spontaneously disintegrated at day 35 post-transfer. Complete abortion (<20 weeks) of a twin pregnancy with previously documented fetal heart motions occurred in 17/165 IVF twin pregnancies (10.3%), where as in an other 50 twin pregnancies one

fetus was lost (30.3%). So at least one fetus was lost in 67/165 twin pregnancies (40.6%); the total fetal loss rate was 84/330 or 24.5% (Seoud, 1992). However, no distinction in fetal loss rate according to the chorionicity was made. One might assume that the number of monochorionic twin pregnancies in IVF is rather low. Sampson (1992) described a higher fetal disappearance rate when viable twins were diagnosed before 6 to 7 weeks gestation compared with 7 to 9 weeks, irrespective of the way of conception. Benson (1994) documented a 4 fold increased fetal loss rate among dichorionic pregnancies between 6 to 8 weeks (16%) compared with those after 8-13 weeks (4%). In addition, they found that of 137 twin pregnancies on an early first trimester ultrasound scan, only 80.3% ended in the delivery of twins. The rates of complete pregnancy and fetal losses (< 24 weeks) in 456 twin pregnancies with live fetuses between 10 and 14 weeks were significantly lower in dichorionic than in monochorionic twin pregnancies (respectively 2.5% versus 12.7% ($p<0.001$) and 1.8% versus 12.2% ($p<0.001$)) (Sebire, 1997(a)). In addition, in dichorionic twin pregnancies with spontaneous death of one fetus at the 10 to 14 week scan there is a tenfold increase for subsequent miscarriage (Sebire, 1997(b)).

Bollen reported clinical triplet pregnancies, defined as the presence on ultrasound of 3 gestational sacs at 7 weeks of gestation, after transfer, GIFT or ZIFT of respectively 3 embryos, 3 oocytes or 3 zygotes in about 8.3% of cases. At 20 weeks 6.7 % of triplet pregnancies were ongoing, representing a loss rate of 19% (Bollen, 1991). Manzur examined 38 early first trimester triplet pregnancies starting from day 28 post ART. At least one embryo resorption was observed in 48.6%, resulting a triplet delivery rate of 51.4%. However, visualization of the fetal heartbeats reduced the chance of embryo wastage and increased the triplet delivery rate to 69.2% (Table 2.1) (Manzur, 1995). Analysis of the outcome of higher order multiple pregnancies after oocyte donation, showed that the probability of loss of a gestational sac after demonstration of fetal cardiac activity in that embryo, was 5.7% (Legro, 1995).

1.2 Late fetal, early neonatal and perinatal losses (Table 2.3)

Data on fetal and perinatal death rates in multiple pregnancies vary extensively because of various reasons (Lawson, 1994), but most commonly because of the gestational age at which late fetal death is registered. The "Studiecentrum voor Perinatale Epidemiologie" in Flanders considers fetal death from 22 weeks onward, or a fetal weight of more than 500 g, perinatal events are defined from 22 weeks onward including the first 7 days of life. This definition has been adopted for the purpose of our papers.

Table 2.3: Perinatal mortality in triplet pregnancies

Reference	Nr	Period/ Type of study	Spont pregn	Perinatal death	Type chorioni-city
Lipitz 1989	78	1975-1988 R/SC	15/78	93/1000 (>500g)	NA
Newman 1989	198	1985-1988 M-SC	110/198	39/594: 66:1000 (up to day 28)	NA
Gonen 1990	24	R/SC	NA	69.4/1000	NA
Weismann 1991	29	1978-1987 R/case study/	5/29	138/1000	NA
Boulot 1992	33	1985-1990	3/33	42/1000	NA
Friedler 1994	151	R/MC Fertility treatment!	13/151	72.8/1000 (≥ 26w)	NA
Albrecht 1996	57	1989-1994 R/SC	5/57	41/1000 (>20w)	NA
Fitzsimmons 1998	16	1985-1996 case control R/cohort/SC	0/16	6/96 (>20w) 62.5/1000	All trichorionic
Angel 1999	25	1993-1998 R/cohort/SC	-	107/1000	NA
Devine 2001	100	1992-1999	12/100	103/1000	NA
De Catte 2001	313	1991-1999 /population/		57/917 (62.2/1000)	NA

R: retrospective; Spont pregn: spontaneous pregnancies; Nr : number.

SC: single center; MC: multi center; M-SC: multicentric uptake, single center delivery

Pr: prospective

NA : not available

Population selection has contributed significantly in the differences in perinatal mortality statistics: ART related versus spontaneously conceived pregnancies, pregnancies managed in a tertiary care center versus community hospitals, multichorionic versus monochorionic or mixed populations, differences in maternal age at conception…. Perinatal mortality rates for twins from 1986 to 1995 range from 50 to 80 in most developed countries to as high as 400 per 1000 twins born in rural communities in developing regions (Petterson, 1998). Comparative data from Western Australia between singleton, twin and triplet pregnancies over the period 1980 to 1989, showed a perinatal mortality rate of respectively 122‰ (CI:117-126),65‰ (590-720) and 890‰ (570-1340) (Petterson, 1993).

Factors correlating with fetal, neonatal and perinatal death in multiple pregnancies are represented in table 2.4. For twin pregnancies, perinatal morbidity in monochorionic and dichorionic pregnancies were most significantly different (2.8 versus 1.5 %, respectively). However, the most important predictor of perinatal morbidity was the birth weight of the smaller twin. The strongest associations in triplet pregnancies are with gestational age at delivery (OR: 2.54; CI: 1.48-4.35), the way of delivery (OR: 4.13; CI: 1.55-11.05) and the way of conception (OR: 3.54; CI: 1.37-9.19) (De Catte, in preparation). Luke calculated the relative risk of fetal death among twin and triplet pregnancies in association with gestational age and birth weight (Luke, 1994,1996). In twin pregnancies, the lowest risk for fetal death was at 34-39 weeks, and at a birth weight of 2500 to 3100 g (RR of 1 at 36-37 weeks and 2500 g). In triplet pregnancies, the ranges were respectively 32-37 weeks and 1600 to 2200 g (RR of 1 at 34-35 weeks and 1900 g).

Fetal death rates and perinatal mortality in higher order multiple pregnancies (>3) have been addressed only in a handful of studies. The outcome of 65 quadruplet pregnancies over a 16 years period in France was conducted through questionnaires sent to 116 families with quadruplets (Pons, 1996). Fetal mortality rates were 39‰, and early neonatal mortality was 68‰ (perinatal mortality: 104‰). A significant bias may be introduced in these figures because of the low level of response (65/116: 56%), which might be related to bad obstetric outcome in the non-responding families (Pons,1996;). Wilson and co-workers investigated their experience with quadruplet pregnancies over a 12

years period (François, 2001, a-d). Of the 32 quadruplet pregnancies, 2 (7%) were lost before 20 weeks. They encountered no intrauterine fetal demises and only one neonatal death (perinatal mortality: 8.3‰).

Table 2.4: Factors determining fetal, early neonatal and perinatal mortality in multiple pregnancies

	Risk	
Factor	Twin pregnancy	Triplet pregnancy or >
Maternal age		-No difference (Elliot 1992) -perinatal mortality significantly lower at ≤ 30 years (De Catte, In preparation)
Mode of delivery		-no difference (Dommergues 1995) -higher perinatal mortality with C section (Wildschut 1995) -significantly higher with vaginal delivery (De Catte, In preparation)
Gestational age	-decreases up to 37 weeks, increases >37w (Luke, 2001) -lowest risk at 34-39 weeks (Luke, 1996)	-decrease with GA (Lipitz 1989) -decreases up to 34 weeks (Luke, 2001) -lowest risk at 32-37 weeks (Luke, 1996) -decreases significantly with GA (De Catte, In preparation)
Birth weight	-lowest risk between 2500-3100g (Luke, 1996)	-lowest risk between 1600-2200g (Luke B, 1996) -significantly lower for BW > 1500g (De Catte, In preparation)
Mode of conception	-Increased risk for ART (Daniel 2000) -No increased risk (Dhont 1999) -Lower risk for ART (Fitzsimmons 1998)	-No difference (Lipitz 1993) -No difference (Fitzsimmons 1998) -No difference between ART and ovulation induction (Friedler, 1994) -significantly higher fetal and perinatal death after natural conception (De Catte, In preparation)
Chorionicity	- increased in monochorionic twin (Sebire, 1997)	-increased in pregnancies with monochorionic component (Chasen, 2001) -increased in pregnancies with monochorionic component (De Catte, 2002)

Dommergues (1995), in a retrospective case-control study, did not find a significant difference in perinatal mortality after caesarean section. In a Dutch retrospective study comparing vaginal delivery in one center versus caesarean

section in another one, each of the centers delivering about 80% of their triplet pregnancies by one of both ways, Wildschut et coworker stated that planned caesarean section was associated with a higher perinatal mortality rate primarily because of respiratory distress syndrome (Wildschut, 1995).

In our analysis of 313 consecutive triplet pregnancies, early neonatal and perinatal deaths were significantly higher after a vaginal delivery (respectively 5.9%, 17.8% and 25.0%) than after C section (respectively 1.8%, 2.2% and 3.7%)(p: 0.0001; RR: 3.3, 8.2 and 6.7). Conflicting results have been reported in relation with the mode of conception. In twin pregnancies, both natural conceptions as well as ART pregnancies have been associated with an increased perinatal mortality (Daniel, 2000; Dhont, 1999; Fitzsimmons, 1998). In triplet pregnancies, comparable data on perinatal losses have been found in naturally conceived, ART related and ovulation induced pregnancies. We found a significant increased number of fetal and perinatal deaths in naturally conceived pregnancies compared with ART pregnancies. The presence of a monochorionic component in the multifetal pregnancies is associated with an increased risk of adverse perinatal outcome (Sebire, 1997(a)). Chasen demonstrated that 30% of triplet pregnancies with a monochorionic twin had a twin-twin transfusion syndrome (Chasen, 2002). In addition, monochorionic high order multiple pregnancies have a high incidence of twin reversed arterial perfusion sequence (De Catte, 2002), associated with a 50% pregnancy loss rate.

1.3 Preterm delivery and intrauterine growth

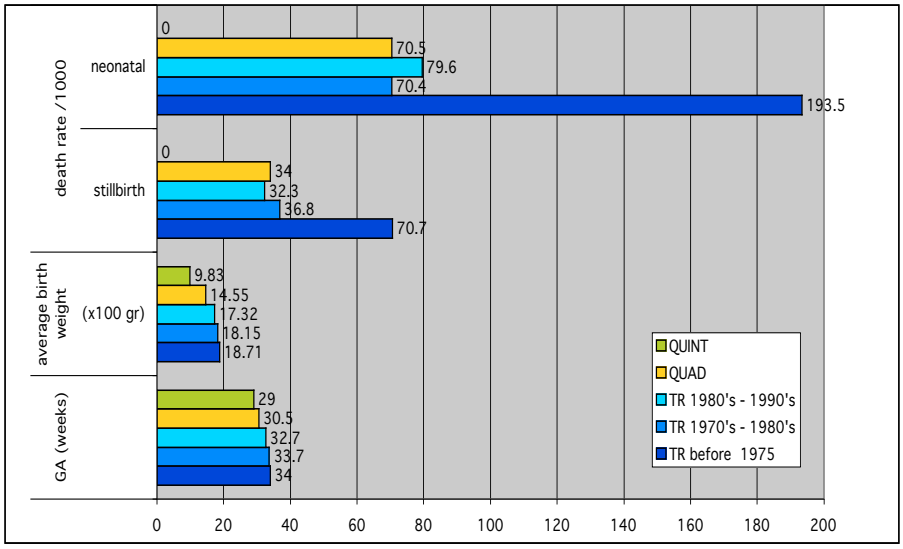
Historical trends in outcomes of multifetal pregnancies showed no significant improvement in gestational age at delivery since 1975 (Figure 2.1; adapted from Luke and Newman, 2000). As the number of fetuses increases, the duration of gestation decreases with a mean of three weeks per additional fetus (*Studiecentrum voor Perinatale Epidemiologie, Birth Register Flanders 1991-1999*). Approximately 50 % of twin pregnancies deliver at less than 37 weeks, and in nearly all high order multifetal pregnancies delivery occurs before 37 weeks (Petrikovsky, 1989). Increasing rates of preterm labour induction is responsible for the increase in preterm birth (Joseph, 2001). No single indicator could be isolated in this study, although maternal hypertension, fetal growth

retardation and placental abruption have been identified as primary causes for preterm delivery in twin pregnancies (Gardner, 1995).

The prevalence of preterm delivery before 30-32 weeks is at least twice as high in monochorionic than in dichorionic twin pregnancies (Sebire, 1997(a); Victoria, 2001).

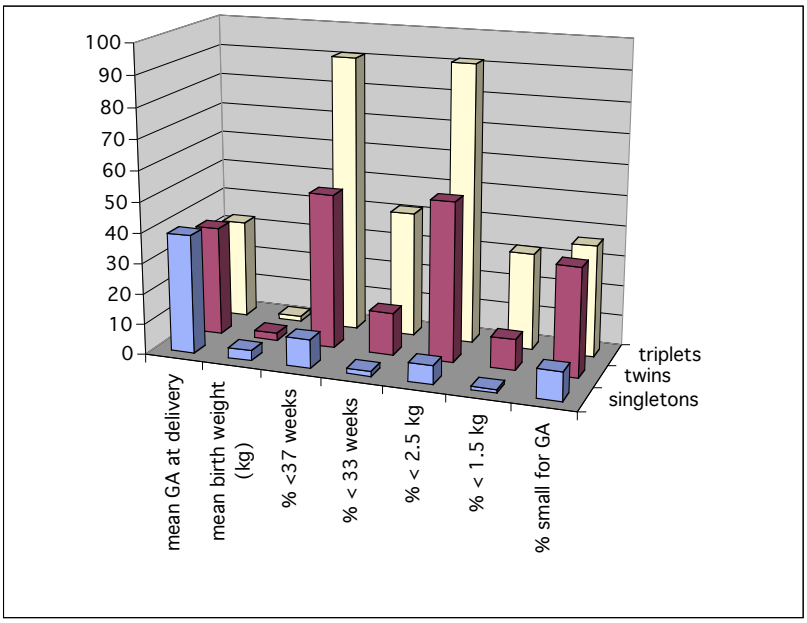
Preterm delivery occurs in more than 80 % of triplet and 100 % in quadruplet pregnancies with a mean gestational age at delivery of 33-34 weeks and 31-32 weeks respectively (Petrikovsky, 1989; Angel, 1999; Devine 2001).

Figure 2.1: Historical data on the outcome of triplet, quadruplet and quintuplet pregnancies (after Newman and Luke, 2000).



Intrauterine growth in high order multiple compared to singleton pregnancies decreases substantially in the third trimester. With increasing number of fetuses, the growth lag occurs earlier in pregnancy. For triplet infants born between 26 and 35 weeks, the median weight was at the 30th percentile for singletons. It decreases further to the 10th percentile after 37 weeks (Elster, 1991). For quadruplets, birth weight was at about the 25th percentile for singletons until 34 weeks, and below the 10th percentile beyond 34 weeks (Collins, 1990). Figure 2.2 shows the percentage of newborns with a birth weight of less than 2500 g and 1500 g for singleton, twin and triplet pregnancies.

Figure 2.2: Pregnancy outcomes related to the number of fetuses (Adapted from Alexander GR 1998).



1.4 Neonatal morbidity and long-term risks

Neonatal care admission (NICU), respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), grade 3- and 4 intraventricular haemorrhage (IVH) and retinopathy of the prematurity (ROP) increase with the order of the multiple pregnancy. Table 2.5 summarizes the ranges of neonatal morbidity from the literature for triplet and quadruplet pregnancies (*Chasen ST, In Blickstein I and Keith LG, 2001*).

Long term risks including mental and/or physical disability, have been reported more frequently among multiple pregnancies, including twins. Nearly 20% of triplet pregnancies and 50% of quadruplet pregnancies result in at least one child with a serious mental or physical disability. Most of these disabilities have been related to premature delivery, very low birth weight and intracranial haemorrhage.

Table 2.5: Neonatal morbidity in triplet and quadruplet pregnancies.

Type of pregnancy	NICU (%)	RDS (%)	Severe IVH (%)	NEC (%)	ROP (%)
Triplet infants	42.2-90.0	8.9-43.4	0-7.7	0-4.0	4.0-6.3

Quadruplet infants	75	34.8-75.0	0-8.7	5.0	0

NICU : Neonatal Intensive Care Unit admission; RDS : Respiratory Distress Syndrome; IVH : Intraventricular Haemorrhage; NEC : Necrotizing Enterocolitis; ROP : Retinopathy of Prematurity

In 1992, Luke calculated the rates of handicaps for those infants born in the USA in 1988 and who survived the neonatal period (Luke, 1992). Compared with singletons, the relative risk of moderate intellectual impairment was 1.3 fold higher for twins and 1.7 fold higher for triplets. The relative risk for severe impairment rose to 1.7 and 2.9 fold respectively. The global figures for handicap for singletons, twins and triplets was 9.0%, 12.6% and 17.9%. Yokoyama (Yokoyama, 1995(a)) found that in children from multifetal pregnancies born after 1977, at least one handicapped child was produced in 7.4% of the twin pregnancies, 21.6% of the triplet pregnancies and 50% of the quadruplet and quintuplet pregnancies. The prevalence of cerebral palsy after a singleton, twin and triplet pregnancy was respectively 1.6, 7.3 and 27.9/1000 survivors at 1 year of age. The prevalence was significantly higher considering infants of 1500 g or less. In association with fetal death of one co-fetus, the prevalence of cerebral palsy in the remaining fetus(es) was respectively 96.2 and 286/1000 survivors in twin and triplet pregnancies (Petterson, 1993).

Recently, Blickstein (1999) demonstrated that the estimated rate of cerebral palsy was significantly lower after spontaneous conception than after the transfer of three embryos (OR: 6.3; CI: 3.7-11.1), two embryos (OR: 3.3; 1.8-5.9), or after reduction of triplets to twins (OR: 3.8; CI: 2.2-6.8).

1.5 Maternal risks

1.5.1 Medical risks

Women carrying a multiple pregnancy are subject to a higher rate of operative deliveries, anaemia, hypertensive diseases, and maternal death (Table 2.6). Other non-life threatening maternal complications occur more frequently as pregnancy progresses: cholestasis, urinary tract infections, varicose veins, oedema, and shortness of breath (Albrecht, 1996; Malone, 1998; Devine, 2001).

Caesarean delivery in multiple pregnancies is performed because of malpresentation: in twin pregnancies with the presenting fetus in breech, and in nearly all triplet pregnancies after 28 weeks.

The incidence of anaemia in multiple pregnancies is mainly related to iron deficiency (Blickstein I, 1995).

Pre-eclampsia has been reported in about 19% of triplet and 38% of quadruplet pregnancies. The incidence rises further if the patient is nulliparous. (Hardardottir, 1996). There is still no valid explanation for this increased risk.

Maternal death rates are about 2.5 times higher in multiple pregnancies than in singleton. Senat (1998) reported 4.4 maternal mortalities per 100 000 live births among singleton pregnancies, and 10.2/100 000 in multiple pregnancies. This increased rate is partially related to the higher incidence of life threatening maternal complications as hypertensive disorders, haemorrhage and treatment with ß-mimetic agents for preterm labour. In addition, delivery by caesarean section increases maternal death rates two to four fold compared with vaginal deliveries.

Table 2.6: Maternal risks in triplet pregnancies.

	Albrecht JL, 1996	Malone HS, 1998
Number of triplets studied	20	55
Anaemia	58%	27%
Pre-eclampsia	33%	27%
Preterm PROM*	18%	20%
Postpartum haemorrhage	12%	9%
HELLP syndrome	11%	9%
Gestational diabetes	11%	7%

PROM : premature rupture of membranes

1.5.2 Psychological morbidity

The diagnosis of a high order multiple pregnancy is usually a shock to the parents. Although many of these couples have been treated for many months and even years, and often have been counseled about the risks of multiple pregnancies, their perception of the risks involved in a multiple pregnancy are weak (Gleicher, 1995). Frequently a multiple pregnancy is experienced as a compensation for childless years.

Maternal reactions to the birth of triplets were examined in 14 women at 4 months and one year (Robin, 1991). Depressive reactions were observed in 40%, and 50% of women developed a series of defense mechanisms to alleviate their suffering. The majority had no plans for the future, and only short-term plans are viewed as feasible. Forty percent of women felt abnormal about having triplets, and this enforces the need for contact with couples sharing the same experience. In 60% of the families, there was no enjoyment in exchanges with the infants at 4 months. The overload of tasks reduced interaction to predominantly utilitarian care. However at one year, as families got organized, there is increased pleasure in the interaction with the children and more mothers succeed in describing each triplet. Garel (1997) investigated 11 mothers of triplets for 4 years, and found emotional fatigue and stress in all of them. Four suffered from depression and used psychotropic medication. There was no improvement of their overall mental condition over the last 2 years. Four women spontaneously expressed their regrets about having triplets. In all instances, the children's psychomotor development was satisfactory. Women who were extensively helped and who received support from husband, relatives and friends appeared to have less problems.

1.6 Multifetal pregnancies and ART

The incidence of multiple births after assisted reproduction is at least 30% in most European countries. This group of pregnancies contributes almost exclusively to the increased incidence of multiple pregnancies in the general population. Several studies have shown that in-vitro-fertilization singleton pregnancies have higher complication rates than those in the general population (Doyle, 1992; Verlaenen, 1995; Tanbo, 1995; Koudstaal, 2000).

Third trimester complications, gestational age at delivery and preterm delivery rates were not significantly different in a small series of multiple pregnancies after IVF (Petersen, 1995). Particularly in twin pregnancies, perinatal outcome in IVF and normally conceived ones, differed only slightly : a higher rate of caesarean deliveries (Dhont, 1999), and a lower perinatal mortality rate in IVF pregnancies (Fitzsimmons, 1998). However, the incidence of pregnancy-induced hypertension (18.3%), vaginal bleeding (10.6%), premature contractions (36.5%),

intrauterine growth retardation (7.7%), fetal death (5.1%), and caesarean section (43.4%) were significantly higher in the ART established twin pregnancies than in naturally conceived ones (respectively 9.9%, 4.1%, 22%, 2.5%, 0.0%, 33.6%)(Daniel, 2000). It was remarkable to observe that the outcome of naturally conceived and ovulation induced twin pregnancies was similar. In a retrospective cohort study in Sweden, deliveries after IVF (1982-1995) showed a significantly higher preterm delivery rate, more low birth weight infants, a higher perinatal mortality, and more neural tube defects and oesophageal atresias. Most importantly, not the IVF technique itself, but the high frequency of multiple births and maternal characteristics like higher maternal age and lower parity in the IVF group were responsible for the adverse outcome (Berg, 1999). Correction for these maternal factors, as shown by Dhont made these differences in outcome disappear (Dhont, 1999). The mixed mono- and dizygotic twin population was one of the objections in the majority of these studies. Natural dizygotic twin pregnancies showed a significantly higher gestational age at delivery, a smaller early preterm delivery rate, a higher mean birth weight, a better mean APGAR score, and a lower number of perinatal deaths than assisted reproduction induced twins (Lambalk, 2001). Although these results were not confined by Koudstaal (2000), a significantly increased birth weight discordance rate was present in IVF twin pregnancies, compared to naturally conceived controls.

1.7. Outcome of 313 triplet and 8964 twin pregnancies in Flanders.
Data from the SPE, 1991-1999; Analysis by L De Catte (AZ VUB) and G Martens IR (UZ Gent). With permission of Dr A Bekaert, Chairman of the SPE.

1.7.1 Material & Methods.

The SPE (Studiecentrum voor Perinatale Epidemiologie) collects data on the maternal medical and obstetric history, and on perinatal events of each hospital birth in Flanders of more than 21 weeks gestational age or ≥ 500g at birth since 1987. Full cooperation of all departments of Obstetrics (n=80) has been established since 1991. The data are based on questionnaires completed by obstetricians and paediatricians in the early neonatal period. The data are sent to a data coordinator, who carries out a review for errors and omissions. Subsequently the files are stored anonymously onto a computer database. Each year, a complete analysis of the data is performed, resulting in a yearly global report and a report per obstetric unit.
For the purpose of this study, data collected on 313 triplet and 8964 twin pregnancies registered from 1991 to 1999 were analyzed. Pregnancy outcome was studied in terms of fetal, and early neonatal deaths. Obstetric outcome variables included gestational age at delivery, preterm and early preterm delivery rates, low birth weight (LBW) and very LBW rates, the method of delivery, and fetal and early neonatal death. Subclasses were established for each variable. Maternal age was broken up into 4 groups: ≤ 25 years, 26 to 30 years, 31 to 35 years and over 35 years. To assess the impact of previous births on pregnancy outcome, patients were classified as nulliparous or multiparous. Differences in pregnancy outcome were also related to the natural or artificial origin (artificial reproductive techniques including ovulation induction) of the conception, including a category where the mode of conception was unknown. Gestational age at delivery was divided into 4 subgroups – less than 28 weeks, 28 to 31 weeks, 32 to 36 weeks and beyond 36 weeks-, and birth weight classes into 3 classes: 500-1499 g, 1500-2499 g and more than 2500 g. The impact of vaginal delivery versus caesarean section on perinatal outcome was analyzed.

Statistical analysis included logistic regression analysis to determine the impact of maternal age, parity, the mode of conception, the gestational age at delivery, the mode of delivery, and birth weight on perinatal mortality rates. Furthermore, Pearson Chi-Square test and Fisher Exact test were used for comparison of differences in frequencies in the different subgroups of each individual factor. Differences in mean gestational ages at birth, mean maternal age, mean birth weights were analyzed with Student's T test. The level of significance was set at 0.05.

1.7.2 Results.

Incidence, maternal age, way of conception (Table 2.11).

Triplet pregnancies:

The incidence of triplet pregnancies over this period decreased from 0.8‰ in 1991 to 0.5‰ in 1999. Mean maternal age ranged from 28.6 to 30.1 years over the period of study. Overall, mean maternal age was 29.4 years (SD: 3.51; range: 20.4-40.0); it was respectively 28.5 years (SD: 3.33; range: 20.4-39.8) and 30.5 years (SD: 3.36; range: 22.5- 40) for primiparous and multiparous women. There were slightly more primiparous (166/313 or 53%) than multiparous women. The number of multiparous patients decreased from 52.9% to 36.3% over the time of the study. 251 pregnancies (80.2%) were established after the use of assisted reproductive techniques (ovulation induction and gamete/embryo transfer), 51 patients (16.3%)conceived spontaneously and in 11 cases (3.5%)the origin of the pregnancy was unknown. The frequency of pregnancies established after ART decreased over the years from 82.6% to 66.6%. Over the same time the total number of triplet deliveries/year has dropped from 57 to 30.

Twin pregnancies

The incidence of twin pregnancies increased from 1.6% in 1991 to 1.8% in 1999, representing a total yearly number of respectively 956 and 1097 twin pregnancies. Mean maternal age at delivery was 29.8 ±4.2 years (range: 16.7-47.7; CI: 29.74-29.86). The maternal age in 1992 was 28.9 ± 4.2 years, and it increased to 30.3 ± 4.2 in 1999. There were slightly more multiparous (4629;

51.6%) than primiparous women (4335; 48.4%). The percentage of primiparae increased from 44.6% to 52.7% in 1999.

Of the 8964 twin pregnancies, 5301 (59.1%) were conceived naturally, 961 (10.7%) after ovulation induction only, 1700 (19.0%) after IVF, 221 (2.5%) after ovarian stimulation and insemination, and 179 (2.0%) after ICSI. There was no information about the mode of conception available in 603 (6.7%) cases.

Method of delivery – birth weight (Table 2.11).

Triplet pregnancies:

Mean gestational age at delivery was 32.9 weeks (SD: 2.9 weeks; range: 22-38). Preterm delivery before 37, 33 and 28 weeks occurred in respectively 95.2%, 33.5%, and 7.31%. Mean gestational ages at delivery and the frequency of preterm and very preterm deliveries in prime- and multifarious pregnancies were comparable. Spontaneously conceived triplet pregnancies ended more frequently at ≤ 33 weeks (60.8%) than those established after ovarian stimulation (40.6%)(p: 0.0001) and IVF (49.7%) (p:0.023). Patients older than 30 years delivered significantly less frequently at ≤ 33 weeks (41.8% versus 51.6%; p: 0.007).

Only 10.8% (34/313) delivered vaginally, and 88.5% (277/313) by caesarean section. In 2 patients a caesarean section was performed after vaginal delivery of the first fetus. Of the vaginally delivered patients, 18/45 (40%) did so before 28 completed weeks, whereas 23/295 (7.8%) of the caesarean sections were performed at < 28 weeks. Only 27/299 patients delivered vaginally after 28 weeks (9%).

There was no difference in caesarean section rate between primi and multigravid patients (respectively 145/166, 87.3% and 134/147, 91.1%; p: 0.4).

Mean birth weight for baby A, B and C was respectively 1799±503 g, 1749±509 g and 1679±511 g. 279 (29.7%) of the 939 neonates had a birth weight of less than 1500 g, and 55 (5.9%) weighed more than 2500 g. The parity did not influence the risk of low and very low birth weight. The number of neonates with a birth weight of more then 2500 g was significantly higher after IVF (8.7%) than after ovulation induction (4.4%) and spontaneous conceptions (1.3%) (p: 0.03 and 0.003). In the younger patients the frequency of very low birth weight (>1500 g)

was significantly higher (32.1%) than in women over 30 years of age 24.5%) (p: 0.02).

Overall, of the 313 couples, 70 had one baby (22.4 %) less than 1500 g, and 146 (46.6 %) had at least one baby with a birth weight of less than 1500 g. In 57 couples (18.2 %), all three newborns were less than 1500 g; 19 couples (6.1 %) had 2 babies with very low birth weight.

Twin pregnancies

Mean gestational age at delivery was 35.9 ± 2.9 weeks (range: 20-41.0). Preterm delivery occurred in respectively 4398 (49.1%), 938 (10.5%) and 224 patients (2.5%) before 37, 33 and 28 weeks of gestation. The frequency of preterm delivery (<37w) was significantly higher in the ART group (51.0%; 1071/2200) than in the naturally conceived pregnancies (48.2%; 2553/5301) (p: 0.03). Of the twin pregnancies 9.2% (824/8964) delivered after 38 weeks.

A total of 3488 patients (38.9%) had a caesarean section for both fetuses, either as a planned or an emergency procedure. An additional 167 patients had a caesarean delivery for the second fetus (1.9%). The distribution of caesarean sections among primi and multiparae was significantly different (respectively 1877/4335: 43.3%, and 1611/4629: 34.8%; p: 0.0001). Significantly more caesarean deliveries were carried out for the second twin in multiparous than in nulliparous women (54/1931; 2.8% and 113/1724; 6.6%, p: 0.0001).

The caesarean section rate in preterm deliveries at less than 28 weeks was 73/224 or 32.6%.

The mean birth weights for babies A and B were respectively 2409±575 g and 2355±577g. 1451 fetuses (7.9%) weighed less than 1500 g, and 8053 fetuses weighed more than 2500 g (44.8%) The presenting fetus had less frequently a weight less than 1500 g (670 versus 74; p: 0.04) and more often one of more than 2500 g (4197 versus 3838; p: 0.0001). There were significantly more very low birth weight infants after ART (307/4198 or 7.3%) than after ovulation induction (158/1920 or 8.2%) or spontaneous conceptions (847/10601 or 8.0%)(p:0.0001). In addition, more newborns weighed over 2500 g after spontaneous conceptions (4843/10601 or 45.7%), than after ART (1799/4198 or 42.9%)(p:0.002).

Fetal loss (Table 2.11)

Triplet pregnancies

Of the 939 fetuses present at 22 weeks of gestation, 22 died in utero (2.34%) and 35 in the early neonatal period (3.81%). The perinatal mortality was 57/917 or 6.22%.

Stepwise logistic regression (Table 2.7), demonstrated that the gestational age at delivery, the mode of delivery and the way of conception were independent variables significantly determining fetal death. Maternal age, parity and birth weight had no significant bearing on fetal death in triplet pregnancies.

Table 2.7: Fetal loss in triplet pregnancies: logistic regression analysis.

	Level of significance	R	Odds	95% CI
GA at delivery	0.0007	-0.2135	2.54	1.48-4.35
Mode of delivery	0.0047	-0.1697	4.13	1.55-11.05
Mode of conception	0.0093	-0.1512	3.54	1.37-9.19

GA : gestational age

Fetal death was observed in 10.1% (7/69) before 28 weeks, of deliveries compared with 4.2% (7/165), 1.2% (8/660) and 0 (0/45) at respectively 28-31, 32-36 and more than 36 weeks (p respectively 0.15, 0.001, and 0.07). Fetal death was as much as 6.0% (14/234) in pregnancies ending before 32 weeks, compared with 1.1% (8/705) in those of 32 weeks or more (p: 0.0001). A vaginal delivery resulted in a fetal death rate of 8.7% (9/104) compared with 1.6% (13/835) (p: 0.0001) after a caesarean section. Triplet pregnancies naturally conceived had a significantly higher fetal death rate (8/153 or 5.2%) than those established after ovulation induction and artificial reproductive techniques (13/753 or 1.73%) (p: 0.02). There were 9 fetal deaths among the IVF/ART related pregnancies (9/366; 2.5%) and 4 in the pregnancies after ovulation induction (4/318: 1.3%).

There was no correlation between fetal death and advancing maternal age. Pregnancies at a maternal age of more than 30 years had a fetal loss rate (5/324 or 1.54%) not significantly different form that in younger women (17/587 or 2.89%; p:0.3). Even at maternal ages over 35 years, fetal death occurred with a comparable frequency (2/48 or 4.2%; p: NS). There was no significant difference

in fetal death among primi- or multigravidae (respectively 11.0 versus 11.0%, and 3.7 versus 3.9%). Although fetal death was unrelated to the birth weight (p: 0.07) there were significantly more fetal deaths in the newborns weighing less than 1500 g (16/279 or 5.7%) than in the group of 1500 to 2499 g (6/605 or 1.0% (p: 0.0001). In the newborns weighing 2500 g or more, there were no fetal deaths (0/55).

Twin pregnancies

From the 17928 fetuses in 8964 twin pregnancies evolving beyond 22 weeks, 292 fetuses died in utero (1.63%). In addition, 253 newborns died within the first week of life (1.44%). Perinatal mortality was 545/17636 or 3.09 %.
Logistic regression analysis showed that birth weight was the most determining factor (R: -0.2372; p: <0.0001) for fetal death (Table 2.8). Other independent variables determining fetal death are the mode of delivery (R: -0.0655) (p: 0.0001), the gestational age at delivery (R:- 0.0583) (p: 0.0005) and parity (R: -0.0342)(p: 0.02).

Table 2.8: Fetal loss in twin pregnancies: logistic regression analysis

Parity	Level of significance	R	Odds	95% CI
Parity	0.02	0.0342	0.75	0.6-1.0
GA at delivery	0.0005	-0.0583	1.37	1.2-1.6
Mode of delivery	0.0001	-0.0655	1.65	1.3-2.1
Birth weight	< 0.0001	0.2372	7.52	5.6-10.2

GA : gestational age

Fetal death occurred in 95/447 (21.10%) fetuses before 28 weeks, compared with 58/914 (6.3%) fetuses at 28-31 weeks, 76/7433 (1.02%) at 32-36 weeks and 63/9134 (0.69%) fetuses at >36 weeks (p respectively: 0.0001 (1-2, 1-3, 1-4, 2-3, 2-4), and 0.03 (3-4). Fetal death rates decreased significantly with increasing birth weights: 184/1415 (13%) in those < 1500 g, compared with 85/8197 (1.0%) at 1500-2499 g (p: 0.0001) and 23 /8316 (0.3%) >2500 g (p: 0.0001).

Early neonatal death (Table 2.11).

Triplet pregnancies

Overall early neonatal mortality was 35/917 (38/1000).

Stepwise logistic regression demonstrated that the gestational age at delivery and the mode of delivery were the only two independent variables significantly determining early neonatal death, whereas for perinatal death rates, the mode of conception and the maternal age at delivery were additional independent determining factors showing the levels of significance, the regression coefficient, and the odds with 95% lower and upper confidence intervals (table 2.9).

Table 2.9: Early neonatal mortality in triplet pregnancies: logistic regression analysis.

	Level of significance	R	Odds	95% CI
GA at delivery	0.0000	-0.4442	15.77	7.87-81.65
Mode of delivery	0.0163	-0.1127	2.99	1.22-7.30

GA : gestational age

Early neonatal mortality rates were significantly related to earlier gestational ages at birth. The mortality rates were 45.2% (28/62), 3.2% (5/158), 0.31% (2/652) and 0/45 at respectively less than 28(a) weeks, 28 - 31 weeks (b), 32-36 weeks(c) and more than 36 weeks (d) (p respectively 0.0001 (a-b), 0.003 (b-c), 0.3 (c-d)).

Neonatal death was significantly lower in the group delivered by caesarean section (18/822; 2.2%) than in the vaginal group (17/95; 17.9%)(p: 0.0001).

There was a trend (p: 0.06; R: 0.0695) towards higher neonatal death rates in younger women (11/117 or 9.4% in women less than 26 years old (a), 17/470 or 3.6% in those 26-30 years old (b), 5/278 or 1.8 % at 31-35 years (c) and 2/46 or 4.3% when aged more than 35 years (d); p <0.02 (a-b); 0.0013 (a-c)). Women 30 years or younger at the time of delivery had neonatal death rate of 4.7% compared with 2.2% in women older than 30 years (p:0.07).

The mode of conception was a factor significantly determining neonatal death: early neonatal deaths were not more frequent following pregnancies spontaneously conceived, than following those obtained after assisted reproductive techniques including ovulation induction (respectively 8/145 or 5.5%

and 23/740 or 3.1 %; p:0.23). However early neonatal death rates following spontaneously conceived pregnancies (8/145; 5.5%) and pregnancies after ovulation induction (19/314; 6.1%) were significantly higher than following pregnancies established after IVF treatment (4/426; 0.9%) (p respectively 0.0002 and 0.003).

Twin pregnancies

Neonatal death is predominantly determined by the gestational age at delivery, and to a lesser degree by birth weight and the mode of delivery (Table 2.10).

Table 2.10: Early neonatal mortality in twin pregnancies: logistic regression analysis.

	Level of significance	R	Odds	95% CI
Mode of delivery	0.04	-0.0300	1.37	1.02-1.83
Birth weight	0.0002	-0.0682	2.2	1.46-3.29
GA at delivery	<0.0001	-0.2827	6.80	5.25-8.80

GA : gestational age

Giving birth at less than 26 years, resulted in a neonatal death of 64/2964 (2.2%), compared with 114/8201 (1.4%) at 26-30 years (p: 0.005), 52/5394 (1.0%) at 30-35 years (p: 0.0001), and 23/1337 (1.7%) at more than 35 years (p: 0.4).
Neonatal death rates in babies born to primiparae were significantly higher than among those born to multiparous women (respectively 144/8669 or 1.7% and 109/9259 or 1.2%; p: 0.006).
Neonates born before 28 weeks showed a neonatal death rate of 166/447 (37.1%) compared with 38/914 (4.2%) if born at 28-31 weeks (p: 0.0001). The rates continue to drop significantly to 33/7433 (0.4%) if born between 32-36 weeks (p: 0.0001) and 16/9134 (0.2%) if born after 36 weeks (p: 0.0025). With increasing birth weights, neonatal deaths decreased significantly from 207/1415 (14.6%) at < 1500 g, to 31/8197 (0.4%) at 1500-2499 g and 15/8316 (0.2%) at > 2500 g (p respectively 0.0001(1-2) and 0.02 (2-3).

Table 2.11: Obstetric outcome in twin and triplet pregnancies in Flanders between 1991-1999.

Population characteristics	Triplet pregnancies N=313	Twin pregnancies N=8964
Mean maternal age (±SD)	29.4 (3.51)	29.8 (4.2)
N > 35 years (%)	16 ((5.1)	1314 (7.3)
N primigravidae (%)	166 (53)	4334 (48.4)
Fertility treatment (%)	251 (80.2)	3061 (34.2)
Obstetric outcome		
Mean GA at delivery (SD)	32.9 (2.9)	35.9 (2.9)
< 37 w (%)	298 (95.2)	4398 (49.1)
< 33 w (%)	105 (33.5)	938 (10.5)
< 28 w (%)	23 (7.3)	224 (2.5)
N caesarean sections	277 (88.5)	3488 (38.9)
Mean birth weight BB A (g)	1799	3488
Mean birth weight BB B (g)	1749	2355
Mean birth weight BB C (g)	1679	-
N > 2500g (%)	55 (5.9)	8053 (44.8)
N :1500-2500g (%)	604 (65.4)	8424 (47.3)
N < 1500g (%)	279 (29.7)	1451 (7.9)
Fetal loss (%)	22 (2.34)	292 (1.63)
Early neonatal loss (%)	35 (3.81)	253 (1.44)

GA : gestational age

2. Specific conditions related to multiple pregnancies

A detailed description of these conditions is not within the scope of this work. However, some background knowledge is necessary to understand certain treatment modalities described later.

2.1 Fetal death in multiple pregnancies

Death of a single fetus complicates approximately 0.5-6.5 % of all twin pregnancies (Nicolini, 1998; Kilby,1994; Enbom, 1985). Survival of the second twin occurred in 82%, but only 54% of these survivors showed no morbidity (Enbom, 1985). Major differences in obstetric outcome of the co-twin have been found according to the type of chorionicity (Melnick, Lancet 1977, Saito, 1999).

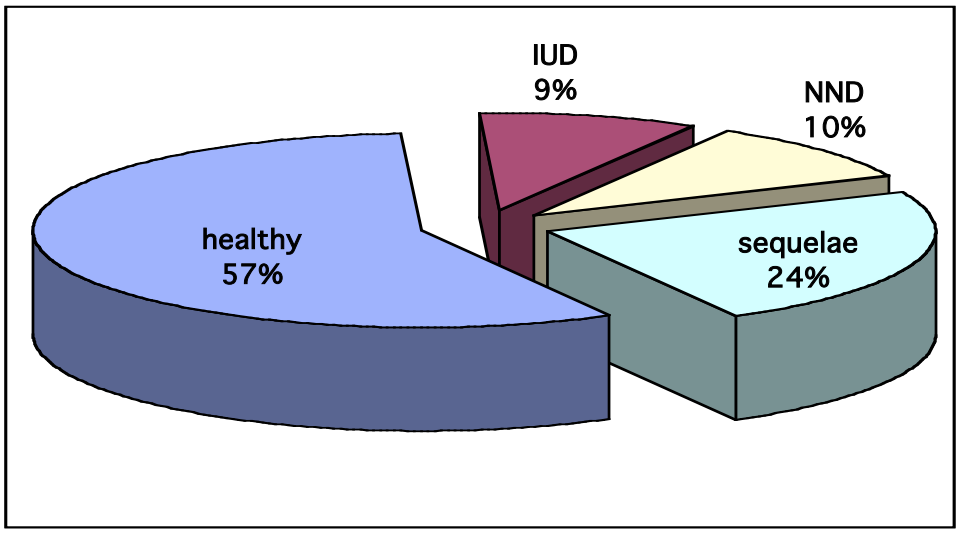
Demise of one fetus has more frequently been observed in monochorionic pregnancies (Kilby, 1994), resulting in the fetal death of the co-twin up to 38% (Fusi, 1990). In addition, the incidence of antenatal necrosis of the cerebral white matter was higher in monochorionic than in dichorionic infants, and significantly related to intra-uterine fetal death of the co-twin, polyhydramnios (as in twin-twin transfusion syndrome), and multiple placental vascular anastomoses (Bejar, 1990). However, older reports often do not state the type of placentation or do not match the control population for chorionicity (Santema 1995(b)), so that the outcome of assumed dichorionic pregnancies may look worse (Prompeler, 1994; Hagay, 1985; Enbom, 1985). Dichorionic pregnancies rarely show communicating placental vasculature. Nevertheless, there is a higher frequency of abnormal fetal heart rate monitoring, caesarean delivery, small for gestational age and hyperbilirubinemia (Carlson, 1989). Data from Geva (1998) suggest that multifetal pregnancy reduction after adjustment for IVF treatment, may be an additional factor for periventricular leukomalacia among premature infants, regardless of chorionicity (Geva, 1998). Exposure to fetal reduction in multifetal pregnancies results in an 18 fold increased risk for neurological damage (OR: 18.6; CI 1.8-140.3). Even after adjustment for twinning, periventricular leukomalacia was more frequently present in preterm delivered infants after fetal reduction (OR : 6.3 CI 1.3-30.3). No statistically significant differences were observed between the cases and the controls with regard to the gestational age at birth. The underlying mechanism may be related to an inflammation mediated release of cytokines caused by fetal death, comparable with the mechanism proposed in chorioamnionitis. However, the small number of infants with periventricular leukomalacia implies the need for larger studies.

Monochorionic fetuses however show a much higher complication rate in association with fetal death of one twin. If the co-twin does not succumb immediately, the fetus may experience multiple organ damage (central nervous system: 72%, lung: 8%, bowel: 19%, kidneys: 15%, limb, face and skin), and has a higher perinatal mortality rate (Nicolini, 1998; Kilby, 1994; Peterson, 1999; Szymonowicz, 1986). Reviewing 119 monochorionic pregnancies with one fetal death between 1985 and 1995, Nicolini found that nearly 40 of the initially surviving fetuses had died in utero or in the early neonatal period, or showed serious morbidity (Figure 2.3) (Nicolini, 1999). Only gestational age at the time of

the intrauterine death and the interval to the delivery predicted the outcome of the surviving twin. Fetal death occurring in the first trimester rather than in the second or third trimester, and a longer time interval to delivery had a significantly better outcome.

The presence of superficial A-A and V-V anastomoses rather than bi-directional A-V communications is held responsible for the high perinatal mortality rates (Bajoria, 1999). Recent data (Fusi, 1991; Okamura, 1994; Nicolini, 1998) support the mechanism of hypotension, blood loss and hypovolaemia of the surviving twin immediately following the death of the co-twin. Of the 4 fetuses sampled within 48 hours after the death of a monochorionic co-twin, all fetuses showed low haematocrits, and 2 were acidaemic. These fetuses were persistently immobile and subsequently had poor outcomes. Two of the evolving fetuses had postnatal evidence of porencephalic cysts.

Figure 2.3: Outcome of 119 monochorionic twin pregnancies with one fetal death between 1985-1995 (from Nicolini, 1999).



Management depends on chorionicity of the pregnancy, the gestational age at the time of diagnosis of the fetal death, and the time elapsed between the fetal death and the sonographic diagnosis. In 31% of the cases, preterm delivery is indicated because of deteriorating maternal or fetal condition. In more than 40%, a spontaneous preterm delivery occurred following the uterine death of one twin (Fusi, 1990). In dichorionic pregnancies, an expectant policy is adopted until

gestational age permits safe delivery of the co-fetus(es). Second trimester fetal demise usually necessitates a thorough sonographic study of the survivor(s) in search of damage, permitting interruption of pregnancy in affected cases. Sometimes immediate fetal administration of fluids or blood by intrauterine transfusion can correct fetal hypovolaemia and anaemia and thus prevent fetal damage and death. However, this technique may prevent fetal death, but not necessarily the development of brain injury (Tanawattanacharoen, 2001; Senat, 2002). Third trimester fetal death in a monochorionic pregnancy should only lead to expedite delivery when the time of diagnosis almost coincides with the fetal demise itself (Anderson, 1990).

2.2 Congenital malformations

Congenital malformations unique to multiple pregnancies are those related with monochorionic placentation: twin twin transfusion syndrome, twin reversed arterial perfusion (TRAP) sequence, and conjoined twins. Overall, congenital malformations are more frequent among multiple pregnancies. Their prevalence among fetuses of twin pregnancies is 1.2 to 2.0 times higher than among singletons, resulting in about 2.1% of major malformations, and about 4.1% of minor malformations. In referred populations, the incidence of congenital malformations may be as high as 10% (Allen, 1991). The most common of these are cardiac malformations, neural tube defects, facial clefting, urogenital abnormalities and abdominal wall defects. Moreover, this higher prevalence occurs primarily in like-sex twins and also in monochorionic pregnancies (Edwards, 1995). In 80 to 90% of twin sets, the fetuses are discordant for any structural defect. Among 245 consecutive twin pregnancies, Edwards (Edwards, 1995) found 24 infants (4.9%) with structural defects. There was only one pregnancy in which both infants were concordant for the presence of a malformation (4.2%).

Assisted reproductive technology, the largest contributor to the multiple pregnancy epidemic, has been associated with a significant increase in congenital malformations (Hansen, 2002). Infants conceived with ART were more than twice as likely to have major birth defects diagnosed during the first year of life than naturally conceived infants, and were also more likely to have multiple

major defects. No clear distinction between singleton and twin pregnancies was made in this study however. If matched for maternal age and parity, Dhont (1999) was unable to demonstrate a significantly higher number of congenital malformations in twin pregnancies after assisted reproduction than compared with naturally conceived twin pregnancies (3.5% and 2.9% respectively). Identical findings were reported by Lambalk (2001) in natural and assised reproduction related dizygotic twin pregnancies. In multifetal ICSI pregnancies, congenital malformations were present in 3.7% of the newborns (Bonduelle, 2002).

Factors associated with the increased risk may include the relatively advanced maternal age and the underlying cause of the infertility. Medication used to induce ovulation and to sustain pregnancy in the early stages, freezing and thawing of embryos, polyspermic fertilization and the delayed fertilization of the oocyte have to be considered as potential causal factors.

The presence of congenital malformations jeopardizes the outcome of the pregnancy. A higher incidence of pregnancy loss, preterm delivery, and neonatal death have been associated with the presence of a congenital malformation in one of the fetuses (Malone HS, 1996). Furthermore, there is an increased need for fetal surveillance. On some occasions the diagnosis of a malformation necessitates fetal treatment, in other cases a selective feticide of the abnormal twin.

2.3 Complications related to monochorionicity and monoamnionicity

Twin-to-twin transfusion syndrome (TTS), twin reversed arterial perfusion sequence (TRAP), and conjoined twins are rare conditions exclusively related to monochorionicity.

Twin-to-twin transfusion syndrome occurs in about 20 % of the monochorionic biamnionic twin pregnancies, and is the most frequent anomaly in this type of pregnancy. Although interfetal placental vascular anastomoses are found in nearly 100% of monochorionic placentas, the majority of A-A or V-V anastomoses are harmless. Yet A-V vascular unbalanced transplacental blood shunt from a donor fetus to its co-twin is usually a chronic process resulting in an oligo-polyhydramnios sequence, fetal anaemia and polycytaemia and finally heart failure because of hypovolaemia or vascular overload. In a small number of

cases, an acute feto-fetal transfusion leads to an instant hypovolemia and hypoxaemia resulting in immediate fetal death of the donor of both fetuses.
In higher order multiples pregnancies partially consisting of monochorionic fetuses, feto-fetal transfusion syndrome (FFTS) results in a dismal outcome comparable with monochorionic twins (Entezami, 1997). This spontaneous monozygotic triplet pregnancy complicated with FFTS resulted in one fetal death, one brain-damaged infant and one normally developing child.

Acardiac twinning or twin reversed arterial perfusion sequence is a rare condition occurring in 1/100 monozygotic twin pregnancies and in 1/35000 pregnancies overall. The incidence is even higher in higher-order multiple pregnancies (De Catte, 2002). Through umbilical artery-to-artery anastomoses, the normal twin pumps its deoxygenated blood directly into the co-twin. Hypoxaemia causes degeneration of the peripheral tissue resulting in a progressive distal to proximal limb hypoplasia/aplasia, a degeneration of the brain and head, and a destruction of the fetal heart. Typically, the acardiac fetus is perfused by a two vessel cord. Fetal death rates are at least 50 to 75%, depending on the development of polyhydramnios or hydrops fetalis in the pump twin, and the weight of the acardiac fetus. If the acardiac/normal twin weight ratio is 70% or more, almost all pregnancies deliver prematurally, 40% show a polyhydramnios and 30% of the pump twins develop hydrops (Moore, 1990). In 12 consecutive cases with early prenatal diagnosis, only 3 resulted in the live birth of the pump twin, all of which presented an amorphic acardiac fetus. In 3 other cases in which the acardiac fetus showed hydrops, the pump twin died (De Catte, in preparation). Recently, conservative management in 8 cases of acardiac twins resulted in a 7/8 pump twin survival rate, with a mean gestational age at delivery of 34.5 weeks (Sullivan, 2001).

Summary

Early fetal loss rates in multiple pregnancies are high. At least 26% of gestational sacs disintegrate spontaneously. After fetal heart activity is established, only 70% of triplet and 90% of twin pregnancies reach viability. After 10-12 weeks of gestation, the risk of fetal wastage is about 5-7% in twin pregnancies. With increasing maternal age and number of fetuses, the number of spontaneous pregnancy losses rises further.

Perinatal mortality rates in twin and triplet pregnancies are respectively about 5 and 8 times higher in population derived data. The presence of monochorionic fetuses increases perinatal mortality; however, chorionicity is rarely taken into account in most studies. The relation with fertility treatment regimes is unclear. Perinatal mortality rates among triplet and twin pregnancies in Flanders registered after 22 weeks are 61.5‰ and 30.7‰ respectively.

Early preterm delivery (<33weeks) is 8 times higher in twin and 24 times higher in triplet pregnancies. Very low birth weight infants are derived respectively 9 times and 29 times more often in twin and triplet pregnancies. The risk of growth restriction is 3 times higher in multiple pregnancies.

Neonatal morbidity is highly associated to the order of the multiple pregnancy and its chorionicity, and is reflected in the neonatal care admission rate, days of ventilatory support, the higher frequency of intraventricular haemorrhage and necrotizing enterocolitis.

Longterm morbity is reflected in mental and physical disabilities. At least one handicapped child is found in 7.4% of twin and 21.6% of triplet and 50% of quadruplet pregnancies. Cerebral palsy has been found respectively 5 times and 17 times more frequently in twin and triplet survivors than in singletons. There is clear correlation with monochorionicity. The presence of a very low birth weight infant or in utero death of one of the fetuses still increases this risk.

Maternal risks include anaemia, post-partum haemorrhage (10%), hypertension and pre-eclampsia (30%), gestational diabetes (10%), and maternal death (10/100 000). Psychological and psychiatric decompensation within 2 years after delivery of healthy triplets troubled 20% of the mothers.

Multiple pregnancies after fertility treatment, including ICSI seem to do worse than naturally conceived pregnancies. However maternal factors like age and parity may interfere.

Fetal death in multiple pregnancies is related with a higher morbidity and mortality for the remaining fetus(es). Particularly in monochorionic pregnancies there is a high rate of neonatal death and sequellae in the survivors. Expectant management is recommended until delivery of the surviving fetus(es) can be performed safely.

Congenital malformations are more frequently observed in multiple pregnancies. In particular, their increased incidence after fertility treatment including ICSI remains controversial. Nevertheless, their presence may jeopardize pregnancy outcome.

Specific complications related to monochorionicity – TRAP sequence, Twin-twin transfusion syndrome, monamnionicity and cord entanglement, and conjoined twins - carry a tremendous high risk of pregnancy failure

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Chapter 3

Prenatal diagnosis in multiple pregnancies

De Catte L, Liebaers I, Foulon W, Bonduelle M, Van Assche E. First trimester chorionic villus sampling in twin gestations. Am J Perinatol 1996 ;13:413-7.

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1 Increased need for prenatal diagnosis

1.1. Introduction

The demand for prenatal diagnosis in multifetal pregnancies is mainly driven by 3 major issues.

-The highly effective artificial reproduction technology has led to a steep increase in the number of multifetal pregnancies.

-Women start their reproductive careers later in life, and therefore in addition to the higher risk for chromosomal abnormalities, are more likely to have multiple pregnancies.

-Screening for fetal aneuploidy for advanced maternal age is shifting towards non-invasive screening by first trimester nuchal translucency measurement. Invasive prenatal diagnosis may shift gears from second trimester amniocentesis to first trimester CVS.

Challenges for prenatal diagnosis are considerably greater when dealing with multiple pregnancies. Whereas prenatal invasive testing in singleton pregnancies addresses questions like what kind of procedure to perform according to gestational age, or which type of tissue to sample to establish a reliable diagnosis, one has never to question selective tissue sampling or selective pregnancy termination.

The issue of prenatal diagnosis in multiple pregnancies is in several ways related to the origin of the pregnancy. Although the zygosity of the pregnancy determines the risk of chromosomal malformations and structural defects, it is rarely revealed to the investigator. Chorionicity on the other hand helps predicting perinatal risks, determines management of discordant abnormality, and is relevant in the obstetric management of fetal compromise.

1.2 Multiple pregnancy boom

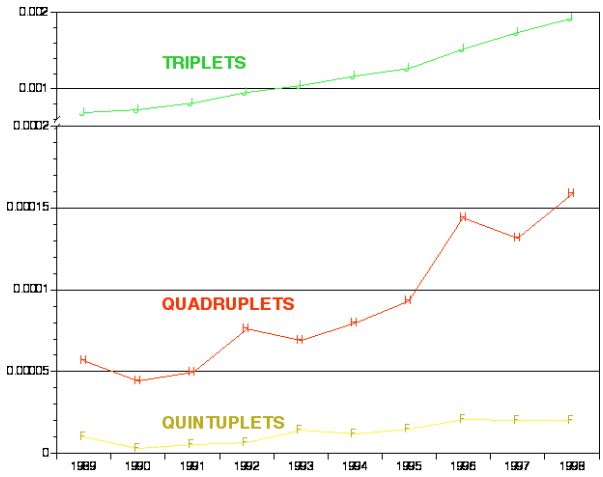
In Europe the prevalence of twin and triplet pregnancies has been fairly constant in the first half of the 20th century. The twinning rate was about 12/1000 maternities. In Sweden, where data are available from as early as 1756, twinning rates in the 18th and 19th century were as high as 18/1000 maternities. From 1960

onward, a steep decline in twin births occurred in Western European countries, reaching the lowest levels between 1970 and 1980. In most countries, the twinning rates dropped to 9 and 10/1000 and even lower in Luxemburg (1/1000) and Belgium (6/1000 or 1/167). Data from the Netherlands showed a coinciding decrease of the percentage of births in women at a maternal age of 30 and over from about 50% to 23 %. In other words, the decrease in multiple pregnancies coincided with a decrease in maternal age at conception. At this stage, fertility treatment regimes were not operational. In her analysis of changing patterns of multiple births in the United States, Barbara Luke found a parallel drop in singleton and twin births from 1960 to 1973. The number of twin births decreased from 86000 to 56000, resulting in a twinning birth rate of respectively 19.6/1000 and 17.7/1000. The triplet birth rate decreased from 33.8/100 000 to 29.0/100 000 live births. (*Derom in Keith, Papiernik and Keith, 1995*; Luke,1994; *Newman and Luke, chapter 1, 2000*). Other factors than an overall decrease in birth rates and younger maternal ages at conception might have contributed to some extent to the observed decline in twinning rates. Standardization for maternal age led to the discovery that increased urbanization, breakdown of isolation in a community, and deterioration in general physical fitness of the mother as a result of sedentary occupations are probably involved in the reduction of the twinning rate (Fellman,1990).

Since the mid-seventies, multiple birth rates have been rising again, and they reached levels as least as high as in the first half of the 20th century. Mainly two factors have contributed to this increase: the higher maternal age at procreation, and the use of medically assisted procreation techniques. Luke (1994) demonstrated that the expected twin to singleton births ratios calculated on figures of 1973, were actually much lower than those observed in 1990. The ratios were respectively 1/55 and 1/43. For triplet births, the discrepancies between the observed ratio (1/1341) and the expected one (1/3323) were even more dramatic. Today in Flanders, the incidence of twin deliveries is 1.8% (*SPE Yearbook 2000*) or 3.5% of all births. The rate of triplet pregnancies in Belgium increased from 9/100 000 (0.009%) in 1971 to nearly 50/100 000 (0.05%)in 1988. The frequency in Flanders rose to 0.09% in 1996, so that nearly for every 1100 pregnancies, there was one triplet pregnancy, a tenfold increase compared with 1971. Since then, the frequency of triplet deliveries has dropped, and is now

0.03%. Other European countries experienced identical changes in triplet pregnancy rates, although at a slower pace (*Derom, in Keith, Papiernik and Keith 1995*).

Figure 3.1: Frequency of live births by plurality in the USA, 1989-1998. Modified from Newman and Luke, chapter 1.



Few data exist on the incidence of high order multiple pregnancies. The US live birth tables differentiate twin, triplet, quadruplet and quintuplet pregnancies since 1989, and over a 10-year period (In Newman and Luke, 2000), as illustrated in figure 3.1. The increased rate is almost entirely related to artificial reproductive technology. Derom (1993) reported the increasing incidence of twin pregnancies after ovulation induction in the East Flanders Prospective Twin Survey. Between 1976 and 1992, the number of naturally conceived twin pregnancies did not change appreciably. The percentage of twin pregnancies obtained by ovulation induction rose from 4% to 35%. For triplet pregnancies, ovulation induction accounted for 90% of the incidence in 1992, compared with 18% in 1976. Following 3347 consecutive cycles of ovulation induction with human chorionic gonadotropin, 441 pregnancies occurred, of which 20.0% twins, 5.0% triplets and 3.9% higher order multiple pregnancies (Gleicher, 2000). Comparable data have been found after in vitro fertilization (Bassil,1997). Ovulation induction and medically assisted procreation were responsible for 69% of all higher multiple births (≥3) in Great Britain in 1989. Of the 156 multiple pregnancies, 47 triplet were conceived naturally (Levene, 1992). Comparison of

triplets and higher-order multiple births in the USA between 1972-1974 and 1985-1989 revealed a 113% increase in incidence among white mothers, but only a rise of 22 % among black mothers. In Caucasian mothers, the increase was not due to an upward shift in the maternal age distribution, but to the use of ovulation inducing drugs (Kiely, 1992). The proportion of multiple births attributable to ovulation induction in a register- based analysis in Italy over a one year period was 9.7% overall, 5.4% for twin births and 69.8% for triplet or higher births. The adjusted odds ratios for twins and higher order multiple pregnancies after ovulation induction were respectively 1.6 (CI: 0.8-3.1) and 66.0 (CI: 23.0-189.0) (Corchia C, 1996). Lynch analyzed the impact of assisted reproductive interventions on the rate of multiple births in 13 151 women delivering after 20 weeks. Treatment with any form of assisted procreation was significantly associated with multiple pregnancy (OR: 25; 95% CI: 18-35; $p < 0.001$) (Lynch, 2001). The attributable risk of a multiple birth in women exposed to ART was 48%.

Bollen and coworkers (Bollen, 1993) reported the incidence of multiple pregnancies after in vitro fertilization. In vitro fertilization-embryo transfers (IVF) with three embryos, gamete intrafallopian transfers (GIFT) with three oocytes, and zygote intrafallopian transfers (ZIFT) with three zygotes resulted each in a high multiple pregnancy rate. Of the ongoing pregnancies at 20 weeks, respectively 32.3%, 16.1 and 27.2% were multiple pregnancies after IVF, GIFT and ZIFT. In a Dutch study by Roest et al (1997) transfer of respectively 2, 3 and 4 embryos resulted in 22, 30 and 28% of multiple pregnancies in the early first trimester. Of the 72 clinical multiple pregnancies - 55 twin and 18 triplet pregnancies - 15 triplets and 54 twin pregnancies evolved beyond 20 weeks. “The Belgian Register for Assisted Procreation” (Belrap, 2001), covering the period 1990-1999, reported that of all pregnancies established after assisted procreation, the percentage of twin and triplet pregnancies had not changed and still remained respectively at approximately 30% and 3.6%. In more than 50 percent of the treated couples, 3 or more embryos had been replaced until 1997. In 1999, the percentage dropped to 42.6%, which was still unacceptably high. Similar frequencies of multiple pregnancies were described in 734 IVF pregnancies retrospectively analyzed: 24.7% twins, 5.8% triplets and 0.8% quadruplets. The number of multiple pregnancies rose with an increasing number

of embryos being transferred, but the numbers were lower at ≧ 37 years (Elsner, 1997). Svendsen found a significant increase in the frequency of triplet and twin pregnancies in women ≤ 34 years after IVF following the transfer of 4 versus 3 and 4 versus 2 embryos, without improving the chance of a singleton conception. The incidence of triplet pregnancies > 20 weeks was respectively 2.4 and 0.3%; the incidence of twin pregnancies respectively 7.4 and 1.3% (Svendsen, 1996). Data in Flanders from the Studiecemtrum voor Perinatale Epidemiologie from 1991-1999, show that the percentage of ART and ovulation induction related twin and triplet pregnancies changed over time. The percentage of singleton and multiple pregnancies after ovulation induction remained fairly constant (1.2% for singletons) or decreased (from 12.8 to 9.2% for multiple pregnancies). However there was a dramatic increase of multiple pregnancies after fertility treatment procedures: from 13.3% in 1992 to 28.5% in 1998.

Most multiple pregnancies after ovulation induction and ART are multichorionic. However, a rise in monozygotic twinning has been observed. There was a threefold higher frequency (1.2%) than expected in spontaneous pregnancies (0.45%) (Derom, 1987). Wenstrom found 3.2% monochorionic pregnancies after ART, eight times the background rate (Wenstrom, 1993). In a theoretical model, the calculated monozygotic twinning rate increased linearly with the increasing percentage of iatrogenic pregnancies in a population (In Blickstein and Keith, 2001). To understand this phenomenon, the comparison has been made with animal procreation and assisted hatching techniques. However, this would merely explain an increased incidence of dichorionic monozygotic pregnancies, and not that of monozygotic monochorionic ones. In human, ICSI procedures and zona pellucida manipulation by chemicals, drilling, opening and rubbing have also been related to monochorionicity in some centers. It is however unclear how one can relate these processes to monochorionicity.

Protocols to prevent multiple pregnancies therefore tend to reduce the number of embryos transferred and to intensify the monitoring of ovulation induction, and force cancellation of treatment in overstimulated cycles. (Vanthier-Brouzes, 1994; Matson, 1999; Coetsier, 1998; Staessens, 1993; Dean, 2000; Staessens, 1995; Strandell, 2000; Gerris, 2000). An increasing awareness of the risks and cost of the epidemic proportion of twin pregnancies resulted in some centers in replacement of no more than one top quality embryo with an ongoing

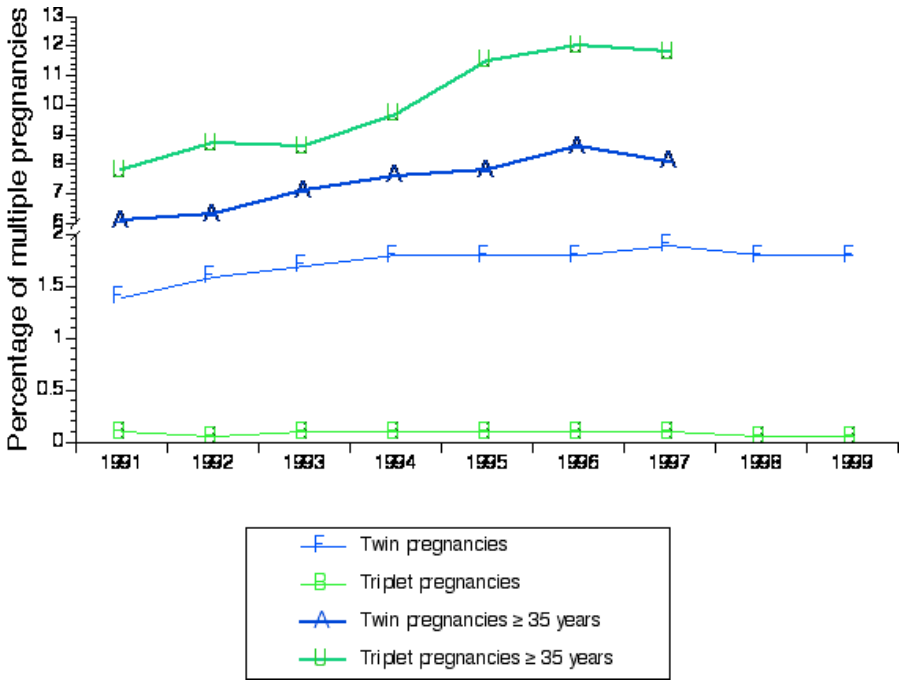
pregnancy success of 35% and more. Single embryo transfer in the first two IVF/ICSI cycles in mainly young patients may significantly reduce the amount of twin pregnancies (Gerris, 2000).

Several reports addressed the prevalence of major birth defects and chromosomal malformations after ICSI and in vitro fertilization. Recently it was shown that the prevalence of major birth defects after ICSI and IVF was respectively 8.6 and 9.0%, and twice that of natural conceptions (4.2%) (Hansen, 2002). Chromosomal malformations were present in 1.0% of ICSI and 0.7 % of IVF pregnancies, respectively (5 times and 3.5 times higher than in natural conceptions. Even higher rates of chromosomal malformations have been reported (in't Veld P, 1995; Bonduelle M, 2002), but mostly sex chromosomes are involved.

1.3 Advanced maternal age

In the early seventies, when the total yearly number of births had reached its lowest level, mean maternal age at delivery was 26.6 years. Since then, mean maternal age at the time of delivery has steadily increased from 27 years in 1984, to just over 28 years in 1992. The mean maternal age at delivery for primi- and multiparae was respectively 26.4 and 29.6 years in 1992 and respectively 27.5 and 30.7 years in 2000. There is a rapid evolution in birth rates at different maternal ages. In Flanders, the number of births per 100 women of 20 and 25 years of age dropped from respectively 8.4% and 16.4% in 1972 to 2.1% and 0.4% in 1998 (*Derom R in Keith,Papiernik, Keith 1995*). In contrast, there was a gradual increase in the number of births in women of 30 and 35 years of age from 10.1 and 2.5 % to 13.6 and 4.1%. In 2000, 26% of all primiparous and 55.9% of the multiparous women were 30 years or more at the time of delivery. In the Netherlands, the twinning rate increased steadily since 1975 in association with a parallel rise in the percentage of births in women older than 29 years of age. Since 1984 however, the twinning rate increased more rapidly than the expected rise in maternal age alone (*Derom R, in Keith, Papiernik, Keith, 1995*).

Figure 3.2: Twin and triplet pregnancies rates and percentage of pregnancies in women ≥ 35 years in Flanders.



Although the incidence of twin and triplet pregnancies in Flanders has remained unchanged over the last five years, the percentage of twin and triplet pregnancies in women over 35 continues to increase (figure 3.2).

Assuming that the twin/singleton pregnancy ratio in 1975, before the era of medically assisted reproduction, was 1/110 (*Derom, in Keith, Papiernik, Keith, 1995*), a mean number of 562 (range: 537- 603) twin pregnancies would be expected yearly, depending on the yearly total number of deliveries between 1992 and 1999 (mean: 62 961; range: 61036-67477) (*SPE Yearly Reports*). The mean number of observed twin pregnancies in this period was 1124 (range: 1059-1163). Of these, at least 665 (range: 626-707) were conceived naturally. Subtracting the number of expected twin pregnancies from the spontaneously conceived ones leads to an extra mean number of 104 (range: 70-144) spontaneous twin pregnancies each year. This means that of all twin pregnancies in excess to the expected number based on the data of 1975, an estimated 18% (range: 13-26%) results from the rise in maternal age (Table 3.1).

A similar analysis for triplet pregnancies reveals the absence of any relationship between the rise in maternal age for procreation and the high incidence of triplet

pregnancies. At an estimated 1/10 000 triplet/singleton delivery ratio before the era of reproductive technology in 1975, the expected and observed numbers of spontaneously conceived triplet pregnancies between 1992 and 1999 are identical. The excess of triplet pregnancies in the nineties is entirely caused by ovulation induction and artificial reproductive technology (Table 3.1). A multivariate regression analysis showed that the adjusted odds ratio for maternal age was not associated with a significantly higher risk of multiple pregnancies (Lynch L, 2001).

Table 3.1: Percentage of twin and triplet pregnancies related to the increase in maternal age. Data derived from twin/singleton delivery ratio of 1/110 and triplet/singleton delivery rates of 1/10 000 in 1975 and the yearly perinatal reports of the "Studiecentrum voor Perinatale Epidemiologie" in Flanders.

	1992	1993	1994	1995	1996	1997	1998	1999
nr of deliveries	67477	65535	62637	62133	62304	62376	61038	60190
nr of singleton pregnancies	66378	64349	61472	60946	61106	61179	59870	59061
nr of observed twin deliveries	1059	1124	1121	1133	1143	1163	1149	1097
nr of expected twin deliveries	603	585	559	554	556	556	544	537
nr of naturally conceived twin pregnancies	673	707	703	655	651	638	670	626
extra nr of naturally conceived twin pregnancies	70	122	144	101	95	82	126	89
nr of non-expected twin pregnancies	456	539	562	579	587	607	605	560
% twin deliveries due to increase in maternal age	15	23	26	17	16	13	21	16
nr of deliveries	67477	65535	62637	62133	62304	62376	61038	60190
nr of singleton pregnancies	66378	64349	61472	60946	61106	61179	59870	59061
nr of observed triplet deliveries	37	60	43	54	54	33	19	31
nr of expected triplet deliveries	7	7	6	6	6	6	6	6
nr of naturally conceived triplet pregnancies	6	8	8	5	8	4	5	7
extra nr of naturally conceived triplet pregnancies	-1	1	2	-1	2	-2	-1	1
nr of non-expected triplet pregnancies	30	53	37	48	48	27	13	25
% triplet deliveries due to increase in maternal age	-2	3	5	-3	4	-8	-9	4

Number of expected twin deliveries: 1/11O x number of singleton deliveries (1/110 being the twin/singleton delivery ratio in 1975)

Number of expected triplet deliveries: 1/1000O x number of singleton deliveries (1/10000 being the triplet/singleton delivery ratio in 1975)

Extra number of naturally conceived pregnancies: number of naturally conceived pregnancies minus the expected number

Percentage deliveries due to maternal age increase: (extra number of naturally conceived pregnancies/number of unexpected pregnancies) x 100

However 69% of women having submitted to assisted reproductive technologies, 35% of hMG treated women and 23% of clomiphene citrate users were older than 35 years of age. It may just be that older women seek infertility treatment, rather than the traditional view of maternal age being a risk factor for multiple pregnancies.

Maternal age related risk for chromosomal malformations in dizygotic twin pregnancies is twice as high as in singletons. Rhodis (1990) and Prugmayer (1992) calculated that the risk of having a chromosomal malformation in at least one fetus of a dizygotic twin pregnancy would be 1.5 times higher than in a singleton gestation. However, the likelihood that both fetuses are concordant for a chromosomal malformation is extremely rare. Prenatal diagnosis in dizygotic pregnancies therefore should occur at an earlier maternal age. Maternal age related risks for chromosomal malformations in dizygotic twin pregnancies at 31 years of age are of the same magnitude as those of 35-year-old women with a singleton pregnancy (Meyers, 1997). Multiplying the maternal age related risk of a singleton pregnancy by 5/3 approximates the risk for fetal aneuploidy in a dizygotic twin pregnancy (*In Newman and Luke 2000*). Monozygotic twin pregnancies carry the same risk as singleton pregnancies: indeed since both fetuses originate from the same fertilized ovum, there is no increased risk associated with the number of fetuses. However, concordance for chromosomal abnormalities in these pregnancies is nearly total. Only a few exceptions have been reported (Nieuwint, 1999; Schmid, 2000).

If we assume a mean maternal age of 30 years, than the overall risk of carrying a fetus affected by Down's syndrome in a trichorionic triplet pregnancy in the first trimester would be 3/471 or 0.64% (*In Snijders and Nicolaides, 1996*). For a woman of 40 years of age, the risk for trisomy 21 at 10 weeks of gestation would be 3/51 (6%). These figures represent only rough estimates because no attention has been paid to the increased risk of pregnancy loss of high order multiple pregnancies, the small number of monochorionic components in these pregnancies, and the decrease in pregnancy loss following the fetal reduction procedures. However, these calculations illustrate that risks for fetal aneuploidy in triplet pregnancies at the age of 30 are higher than for singleton pregnancies at

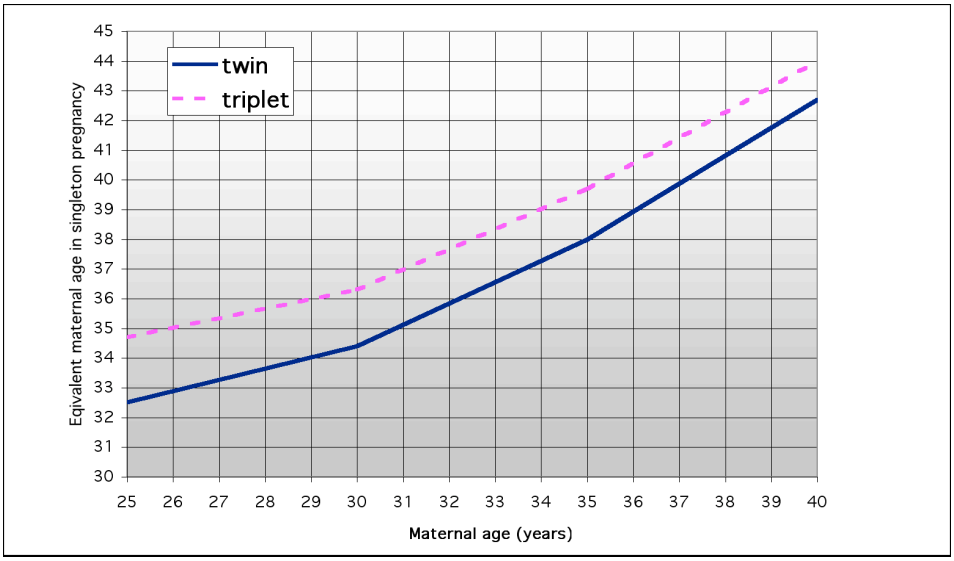
35 years of age, generally considered an indication for prenatal diagnosis (Table 3.2 and Figure 3.3).

It would take a large number of triplet pregnancies to calculate the risk/benefit balance of prenatal diagnosis in terms of fetal loss and birth of a chromosomally abnormal child. The largest compiled data set on multifetal reduction (Evans MI, 2001(a)) did not comment on chromosomal abnormalities in the remaining fetuses after fetal reduction as emphasized in anecdotic reports.

Table 3.2: Estimated risk of trisomy 21 (1/number given in the table) in trichorionic triplet pregnancies in relation to maternal age, gestation, and fetal reduction. (Modified from Snijders and Nicolaides)

Age (years)	10 weeks		16 weeks		Birth	
	Triplet	Reduced to twin	Triplet	Reduced to twin	Triplet	Reduced to twin
20	268	402	351	527	509	764
25	234	356	311	467	451	676
30	157	235	206	309	298	448
35	62	94	82	123	119	178
40	17	26	22	34	32	49

Figure 3.3: Equivalent maternal age risk in multiple pregnancies (Adapted from Cucle and Wald 1993 (chapter 32) and Evans 1993 (Chapter 41) in Brock, Rodeck & Ferguson-Smith, Prenatal Diagnosis and Screening, Churchill Livingstone, New York, 1992.



1.4 Screening for fetal aneuploidies by nuchal translucency thickness and nasal bone measurements

Nicolaides described the usefulness of fetal nuchal translucency (NT) measurement as first trimester marker for fetal chromosomal abnormalities a decade ago (Nicolaides, 1992). Since then, numerous reports have examined the impact of the NT measurement on the detection of Downs syndrome in routine practice. Because NT is gestational age dependent, the screening policy includes measurement of the CRL and NT in a standardized way. Subsequently the maternal age related risk for trisomy 21 in the first trimester is multiplied by a likelihood ratio depending on the deviation in NT from normal: the larger the measured NT, the higher the multiplying factor becomes and the higher the new risk. In singleton pregnancies, for a screen positive rate of 5%, the detection rate of trisomy 21 is 77% (Nicolaides, 1999). Invasive testing for those pregnant women with a calculated risk of ≥ 1/300 and subsequent selective termination of the affected fetuses would reduce the potential livebirth prevalence of trisomy 21 by at least 78% (Snijders, 1998).

In addition, in chromosomally normal fetuses, increased NT is associated with a wide range of fetal defects, among which fetal cardiac defects and skeletal malformations are of the most common ones. Moreover, the list of genetic syndromes associated with increased nuchal translucency is continuously growing (Souka, 1998).

Unlike biochemical markers for aneuploidy screening in the first trimester of pregnancy, nuchal translucency thickness measurements can be applied in screening for chromosomal abnormalities in multiple pregnancies (Table 3.3). Hence, the risk for aneuploidy can be calculated for each fetus individually. In dichorionic twin pregnancies, the sensitivity and the false-positive rate of NT screening are similar to those in singleton pregnancies (Sebire, 1996). However, in fetuses of monochorionic twin pregnancies the prevalence of increased nuchal translucency is higher (8.4%) compared with fetuses of dichorionic pregnancies (5.4%). Recently, Spencer (2001) detected only a 1.2% higher prevalence of NT in monochorionic (5.6%) compared with dichorionic twin pregnancies (4.4%), although the median NT MoMs in both groups were identical.

In monochorionic twin pregnancies this observation is unlikely to be associated with an increased for chromosomal abnormalities, but rather related to an early manifestation of cardiac failure due to twin-twin transfusion syndrome (Sebire, 1996).

The accuracy of nuchal translucency screening in multiple pregnancies may be related to the way of conception. Orandi (2002) found a 12% reduction in NT thickness in twin pregnancies resulting from IVF compared with spontaneously conceived ones. These differences were not present in singleton pregnancies. This might become of interest since the majority of multiple pregnancies results from assisted reproduction. However, unless more NT measurements for patients with different fertility treatment regimes are collected and analyzed, there should be no correction for the way of conception in the risk estimation calculations.

NT screening for fetal aneuploidy in multiple pregnancies may further be refined by adding additional markers. Although maternal serum screening in multiple pregnancies is of limited value, the addition of maternal serum PAPP-A and ß-HCG to the NT measurement in the first trimester increases the detection rate – for a 5% false positive rate - with 5% to about 80% (Spencer, 2000).

Identification and measurement of the nasal bone may further add to the reduction of the false positive rate or the increase in detection rate (Cicero, 2001).

Table 3.3: Fetal aneuploidy screening in multifetal pregnancies by means of nuchal translucency measurement.

Author	N	GA	Cut-off	Screen +	FPR	DR
Pandya 1995	8	11-13	≥2.5mm	10/16	1/6	9/10
Sebire 1996	344 $	10-14	>P95	5.4%	3.8%	11/12 *
Monni 2000	115	38-84mm	>P95	7.4%	6.7%	2/3
	100 TW			9.5%	9.1%	1/1
	70 TW$			8.6%	7.9%	1/1
Maymon 2001	174	38-84mm	≥P95	4.6%	3.2%	5/5

N: number; GA gestational age; FPR: false positive rate; DR: detection rate

$ only dichorionic pregnancies

* including 7/8 trisomy 21 and 4/4 other chromosomal abnormalities

2. Determination of chorionicity

2.1 Introduction

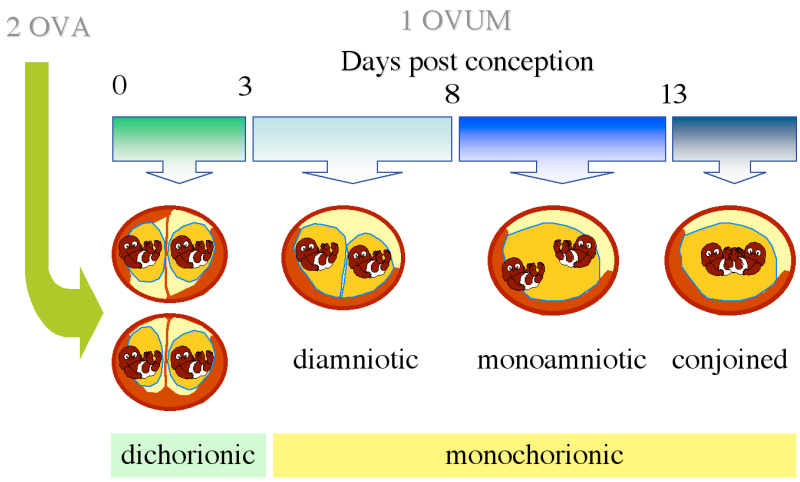
Zygosity is determined by the number of fertilized ova, whereas chorionicity relates to the number of chorionic masses implanting in the decidualized endometrium.

Dizygotic twins can only be dichorionic diamniotic. Both placentas may be on different sides of the uterine wall, making the diagnosis easy. When they align, they mimic a single placental mass. Monozygotic twins can present as dichorionic pregnancies if embryonic splitting occurred within the first 3 days after fertilization. Each embryo forms its trophoblastic mass, and implants as an individual pregnancy (*In Larson, 1998*) (Figure B2). About one third of monozygotic pregnancies are dichorionic, and hence cannot be distinguished from dizygotic pregnancies. The remaining monozygotic twins share one placental disk, and should be distinguished from dizygotic twins with fused placentas.

Determination of chorionicity should be performed routinely in the first trimester of pregnancy. The development of the gestational sac with its characteristic landmarks allows the most reliable determination of the chorionicity. The simplicity and accuracy of the determination of chorionicity and amnionicity in multiple pregnancies strongly depend on the gestational age at which the ultrasound scan is performed.

Sonographic characteristics of early singleton pregnancies apply. At $4^{3/7}$ weeks gestational age, the gestational sac can be observed by transvaginal sonography. One week later the yolk sac becomes visible, and a few days later the embryonic pole appears. Although present at 5 weeks, the amniotic cavity remains difficult to visualize in the 7^{th} week. Due to its rapid increase in size in the 8^{th} and 9^{th} week, the amniotic membrane looses its proximity to the embryo and is clearly distinguishable in the chorionic cavity. By the end of the first trimester, the chorionic cavity is obliterated by the expanding amniotic cavity, resulting in a fusion of amniotic and chorionic membranes, and the complete disappearance of the secondary yolk sac.

Figure 3.4: Schematic representation of the origin of monochorionic and dichorionic pregnancies.



2.2 Number of gestational sacs, chorionic and amniotic cavities

At an early gestational age (6-10 weeks), counting the number of gestational sacs provides an accurate estimate of chorionicity (Copperman, 1995; Hill, 1996). The number of the gestational sacs corresponds with the chorionicity of the pregnancy. Copperman correctly identified chorionicity in 47 sets of twins at 41 days post embryo-tranfer by vaginal ultrasound using the number of gestational sacs in addition to other markers. Hill reported correct identification of chorionicity in all of 179 examined twin pregnancies at 8 weeks or beyond. Before 6 weeks gestation, undercounting the number of sacs occurs in about 15% of dichorionic pregnancies (Doubilet, 1998) and in 16% of the high order multiple pregnancies. Determination of amnionicity and fetal poles at this stage is very unreliable, as 86% of monochorionic twins were mistaken for singleton pregnancies.

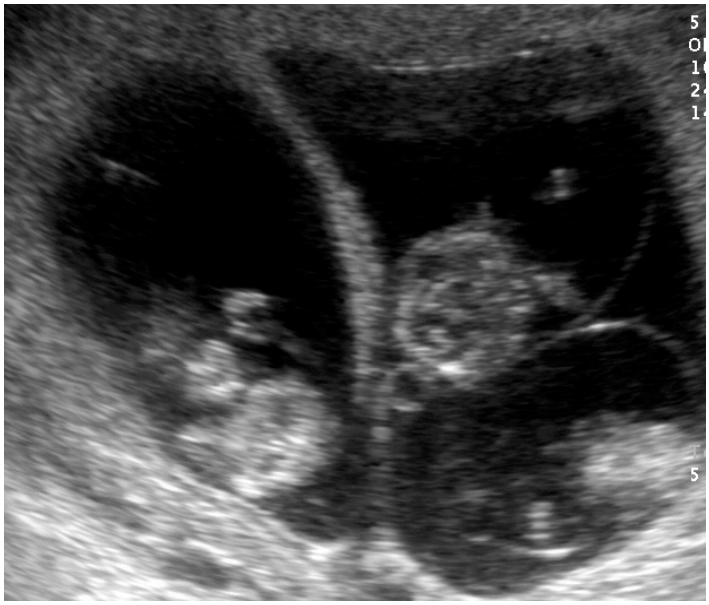
Counting the number of fetal hearts and comparing them with the number of gestational sacs, helps defining chorionicity and amnionicity (Copperman, 1995). The pregnancy is dichorionic (multichorionic) if the number of fetal heartbeats corresponds with the number of gestational sacs. However, if there

are more heartbeats than gestational sacs, the pregnancy should be monochorionic in case of twins, and at least partially monochorionic in high order multiple pregnancies.

Evaluation of the relationship between yolk sacs early in gestation in twin pregnancies can discriminate dichorionicity from monochorionicity (Malinowski, 1998). Yolk sacs separated by a septum belong to different gestational sacs, and to a dichorionic pregnancy, whereas non-separated or aligning yolk sacs indicate a monochorionic pregnancy. Additionally, the presence of the two yolk sacs are seen in a monochorionic twin pregnancies reliably identifies diamnionicity (Bromley, 1995). Bromley retrospectively examined 22 monochorionic twin pregnancies, and correctly identified all 20 monochorionic diamniotic pregnancies by the presence of two yolk sacs at 8 weeks of gestation. In the two remaining cases only one yolk sac was described in association with one gestational sac and two fetuses. These were monochorionic monoamniotic pregnancies. Visualizing the amniotic membrane was extremely difficult in half of the cases at 8 weeks or less. Counting the number of yolk sacs is feasible before proper identification of the amniotic membrane (Montaguodo, 1994). The identification of one yolk sac in the presence of two pulsating hearts almost certainly reveals a monoamniotic twin pregnancy.

Counting the number of amniotic cavities allows a more precise appreciation of amnionicity (Montaguodo, 1994)(Figure 3.5). From the 7^{th} week onward, the lining of the quickly expanding amniotic cavity can be visualized between the fetus and the chorion frondosum. The presence of two or more amniotic sacs in one chorionic cavity indicates a monochorionic di- or multiamniotic pregnancy. Two pulsating hearts in one amniotic cavity, in association with one yolk sac indicate a monochorionic monoamniotic twin pregnancy. Differentiation from a conjoined twin can only be made if both fetuses move independently. At the end of the first trimester of pregnancy, these easily sonographically detectable markers gradually disappear. From then on, one has to rely on other often subtler and more subjective parameters to identify chorionicity and amnionicity.

Figure 3.5: Counting two gestational sacs and three amniotic sacs: dichorionic triamniotic triplet pregnancy.



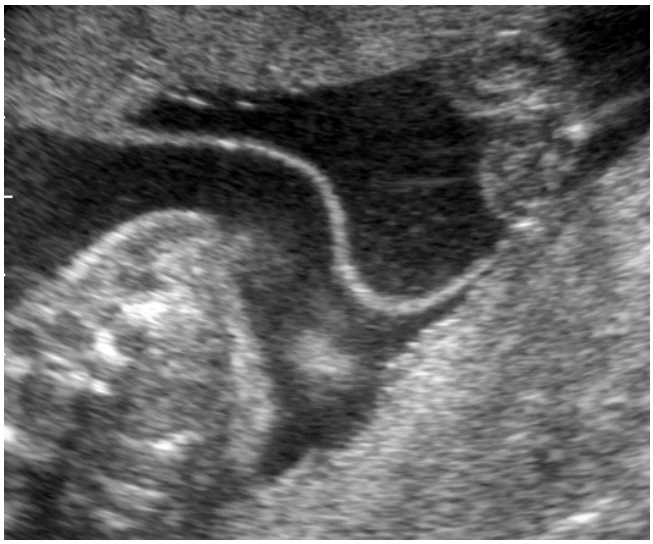
2.3 Intertwin membranes, lambda sign, T sign

The presence of a dividing membrane confirms at least diamnionicity. The rapid growth of the gestational sacs in the first trimester in dichorionic pregnancies results in the alignment of the membranes and the atrophy of the chorionic tissue in between. However a chorionic tissue wedge remains at the implantation site: this triangular structure is called the twin peak sign or lambda sign (Figure 3.6). Membranes in a monochorionic twin pregnancy on the contrary will show a perpendicular setting of the thinner fused amniotic membranes at the chorionic plate: the T-sign. In addition, the membrane is much thinner and wispy. Rarely it can be seen completely.

Several studies have examined the value of the lambda sign in the first, second and third trimester of pregnancy. The predictive value of this sign exponentially decreases with increasing gestational age and the presence of oligo-polyhydramnios sequence (stuck twin). Kurtz et al (1992) identified the lambda sign in 7% of dichorionic diamniotic (6/85) and 12% of monochorionic diamniotic twin pregnancies (2/16) evaluated by transabdominal ultrasound between 9 and 12 weeks gestational age. The sensitivity of the twin peak sign in

the detection of dichorionic pregnancies was only 42% in the second trimester; however it was 100% specific (Scardo, 1995). In 45 twin pregnancies between 12 and 40 weeks, Wood (1996) identified 34/36 dichorionic pregnancies using the lambda sign (sensitivity 94%, specificity 88%). Erroneous interpretation of chorionicity occurred almost exclusively in the third trimester; the corrected sensitivity for the first and second trimester was 100%. In a large prospective study, Sepulveda (1996) correctly assigned dichorionicity to the 100 twins of different sex using the lambda sign in the first trimester. Furthermore, in an additional 101 twin pregnancies initially examined between 10 and 14 weeks, he showed a decrease in sensitivity of the lambda sign after 16 weeks (Sepulveda, 1997). The absence of the sign at 16 weeks does not exclude dichorionicity.

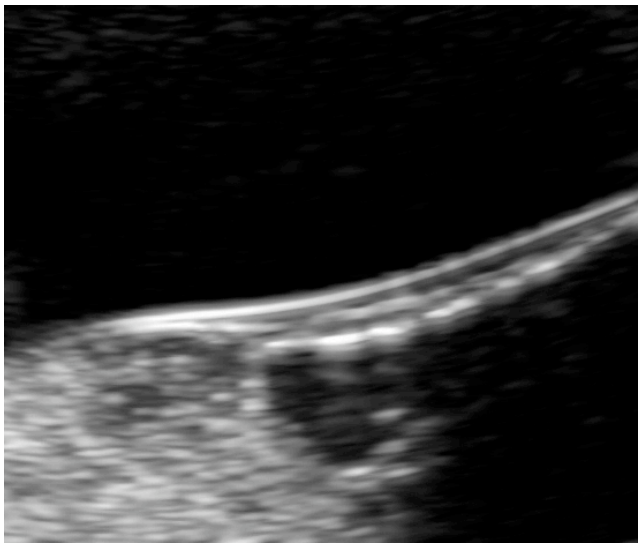
Figure 3.6: A thick intertwin membrane and a lambda sign indicate a dichorionic twin pregnancy.



The intertwin membrane is composed of four layers, with the two facing chorionic membranes enclosing the two inner amniotic membranes. Counting the number of membranes is an easy way to assess chorionicity between 8 and 12 weeks, with a 100% accuracy. Later on in the second trimester, the thickness of this dividing membrane predicts dichorionicity if it measures more than 2 mm, and if it is measured close to the chorionic plate. In a retrospective analysis of 32 twin pregnancies, Winn (1989) identified 91% of dichorionic pregnancies using a cut-off of 2 mm of more. A thick hyperechoic membrane of 2 mm or more was

found 78 out of 85 dichorionic pregnancies examined by Kurtz (1992). Four of the seven cases with a thin membrane or a membrane of intermediate thickness, showed separate placental disks, so that 3 out of 85 dichorionic pregnancies were mislabeled as monochorionic. The thickness of the intertwin membrane can be measured most reliably at a short distance from the chorionic plate. Stagiannis (1995) showed that differences in measurements in the second and third trimester were related to the subjects being measured, the type of pregnancy, the site of the measurement, and the observer. The interobserver variability is high and ranges from –60 to + 171% for dichorionic pregnancies. The intraobserver variability is acceptably low. Scardo found a thick dividing membrane in 55% of the dichorionic pregnancies in the second trimester with a false positive rate of 8.3%.

Figure 3.7: Layers of the intertwin membrane at the level of the placenta in dichorionic twin pregnancy.



Counting the number of layers in the dividing septum has been proven to be an accurate method (Figure 3.7). D'Alton found three or four layers in 51 out of 52 dichorionic twin pregnancies (sensitivity 98%) at 16 to 27 weeks, whereas in all 17 monochorionic pregnancies only two layers were seen (specificity 100%) (D'Alton, 1989). However, in 14% of the cases at least a second scan was necessary to establish the exact number of layers. A similar observation was done by Vayssiere (1996). Incorrect estimates of chorionicity were related to the

advanced gestational age and the presence of oligohydramnios. So, identification of the number of layers at this stage of pregnancy is highly operator and transducer dependent, and of little clinical use.

2.4 Combination of parameters

Combining several characteristics of multiple pregnancies allows for a faster and more precise identification of chorionicity and amnionicity. In early gestation, evaluation of the number of gestational sacs together with the number of yolk sacs, fetal poles, the presence of membranes and their thickness, and the lambda sign have been proven to correctly predict chorionicity and amnionicity in all cases in the first trimester from the eighth week onward (Copperman, 1995; Sepulveda, 1996, Sepulveda, 1997; Sebire, 1997). These data have recently been confirmed by Devlieger in 87 twin pregnancies. Once the operators were correctly instructed about the different ultrasound markers, previously acquired sonographic experience did not interfere significantly in the detection of chorionicity. Most importantly, no cases of monochorionic twin pregnancies were missed (Devlieger, 2001). However, in the second trimester, sensitivity of this approach is lower (Scardo, 1995). Although the sensitivity of the composite approach (the observation of separate placentas with fetuses of the opposite sex, a thick dividing membrane and the presence of a twin peak sign) scored nearly twice as high as each individual parameter, it failed to discriminate nearly 3% of dichorionic and 8% of monochorionic pregnancies.

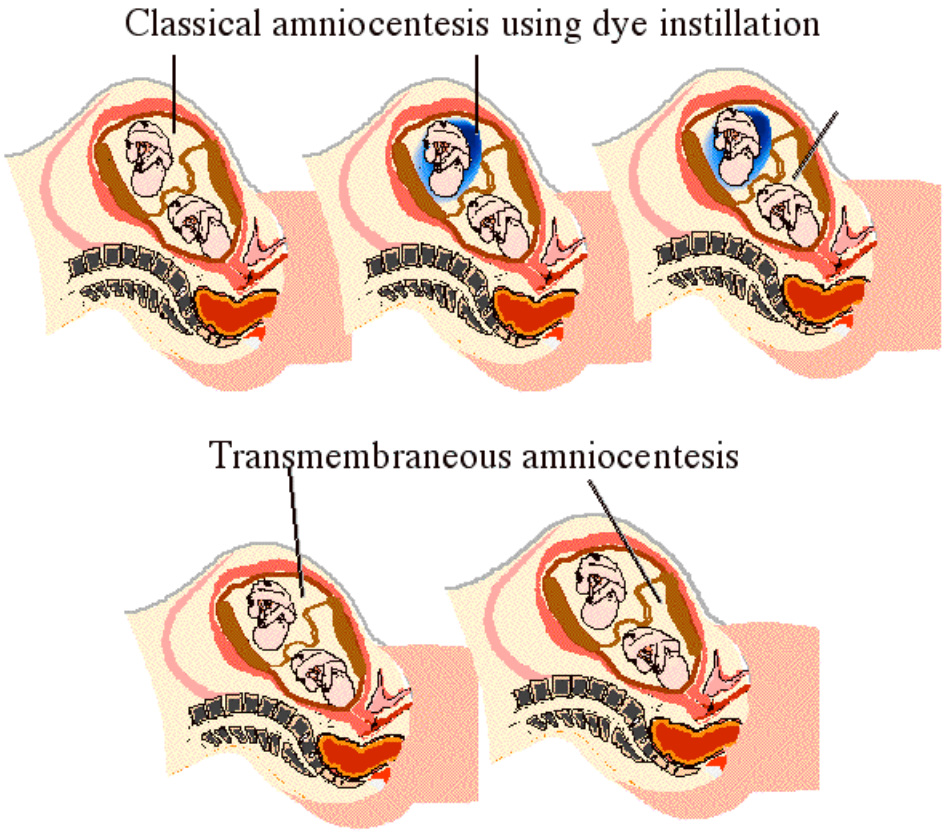
3. Invasive techniques

3.1 Amniocentesis

3.1.1 General considerations

Genetic amniocentesis in twin pregnancies was first described in 1980 (Elias S, 1980). Since then, it has been considered the golden standard: it is a safe and accurate technique when carried out between 15 and 20 weeks of gestation, under ultrasound guidance, and with the use of 20-22 gauge needles. Historically, the needle is sonographically guided into one of the gestational sacs, and amniotic fluid is withdrawn. Then, without moving the needle a contrast dye (indigo carmine) is injected to “mark” that amniotic compartment. A second needle is then introduced in the other sac. Obtaining dye free fluid ensures that the first sac has not been punctured again. This procedure can also be successfully performed in higher order multiple pregnancies (Figure 3.8).

Figure 3.8: Schematic representation of amniocentesis in twin pregnancies.



More recently, two other dye free approaches have been used with success. Most operators will identify and map the amniotic cavities, and direct the sampling needles towards clearly demarcated zones to ensure separate sampling paths. However, this approach cannot confirm selective sampling in all cases. Inadvertent re-sampling was noticed in experienced centres in 1-3.5% of the pregnancies (van der Pol, 1992; Deslisle, 2001). A second approach consists in a transmembraneous puncture of the second sac (Jeanty, 1990; van Vugt, 1995; Buscaglia, 1995) (Figure 3.8). Once the amniotic fluid has been retrieved for the first amniotic cavity, the needle is advanced through the interfetal membrane into the other amniotic cavity. After aspiration and discarding the first few milliliters, the second sac is sampled. Withdrawing the needle through the membrane once again confirms the selective way of sampling.

Several studies report on the outcome of twin pregnancies after amniocentesis (3-18) (Table 3.4 and 3.5). Papers from the early 80's do not reflect the significant contribution of ultrasound in the guidance of the needle and the reduction of "dry taps". Therefore, these papers have lost their impact in modern amniocentesis, and will not be discussed.

Three major changes have occurred over the years.

1: The number of needle insertions and dry taps have dropped considerably. The mean number of insertions per pregnancy is close to two, one for each fetus. In cases of monochorionic pregnancies, commonly one amniotic sac was aspirated merely for two reasons: (a) discrepancies in cytogenetic results in monochorionic twins is exceptionally rare, and has been described in case reports (Costa, 1998; Nieuwint, 1999; Schmid, 2000), (b) in the presence of a discordant cyctogenetic result, only the entire pregnancy could be terminated. However, as recently newly developed techniques for fetal reduction in monochorionic pregnancies have emerged, the sampling of each amniotic cavity has been recommended (Wapner, 1993; Dommergues, 2002). Finally, using this approach, one might obtain a more complete overview of the true incidence and different types of discrepant results in monochorionic pregnancies.

More recently, some studies advocated the transmembraneous approach for the second amniotic cavity to reduce the number of needle insertions (Jeanty, 1990; van Vugt, 1995; Buscaglia, 1995). Yukobowich reported on 476 twin pregnancies in which amniocentesis was carried out through separate needle insertions for

each fetus; the number of needle insertions solely and significantly determined the risk of pregnancy loss (Yukobowich, 2001). Small series of single transabdominal needle insertions for twin amniocentesis have been reported.

Table 3.4: Double needle insertion studies

Reference	Number	Indication: mat age (%)	Fetal loss to 20 w (%)	Total fetal loss (%)	Sampling/ Lab/ Contamination failure
Tabsh 1985	48 – IC	84%	0	7	/1/NA
Pijpers 1988	83 - IC	47%	2	8	6/0/0
Anderson 1991	330 twins 9 triplets	91%		31/633 (4.9%)	2/NA/NA
Antsaklis 1991	60		0		
Pruggmayer 1991 (MC)	98 IC:54 MB:39	100% (>33y)	6.1(n:12)	21	NA
Pruggmayer 1992 (MC)	529 IC:351 MB:123		24 (2.3)	93 (8.8)	NA/NA/NA
Beekhuis 1992	63 Hb	NA	NA	5(4)	9/0/9
Wapner 1993	72	74.1%	1.4	13 (9.3)	0/1/0
Ghidini 1993	101 - IC	72%	0	7 (3.5)	NA
Kidd 1997	227	83.8%	NA	24/454 (5.3%)	Cave: exclusion fetal death < 20 w (1.5%)
Ko 1997	136	79% (≥34y)	0	5(1.8%)	0/ /0
Yukobowich 2001	476	47% ≥35y	2.7*	NA	NA/NA/NA
De Catte 2000	108	46%	1.9	3.3	0/2/0

MC: multicentre study

IC: indigo carmine

MB: methylene blue

fetal loss: excluding TOP and fetal death due to severe congenital malformations

*fetal loss considered up to 4 weeks after amniocentesis

** excluding balanced translocations and inversions

Jeanty (1990) described this new technique and its advantages and potential pitfalls in 18 patients. The advantages of a short sampling time, the reduced discomfort of a single needle insertion, and the high degree of reliability of selective sampling without injections of dyes are clearly greater than the unlikely

events of trapping of fetal body parts in an enlarged puncture opening (Gilbert, 1991) or the likelihood of chromosomal mosaicism from the second sampled sac. However, advantages of this technique are operator and sonographer dependent, and probably increase with experience. Sampling failures of the second amniotic cavity were by 5 to 15% reported in the learning phase (Buscaglia, 1995; Sebire, 1996b). In addition, the importance of the number of needle insertions on the outcome of the twin pregnancy has been challenged by other investigators (Pijper, 1988; Pruggmayer, 1992).

Table 3.5: Single needle insertion studies

Reference	Number	Sampling failure	Additional samplings	Total fetal loss
Jeanty 1990	18	1	1	0 4 pregnancies ongoing/ 1 no information
Van Vugt 1995	27	0	0	1/53
Buscaglia 1995	55	7	9	0 no information on 10 pregnancies (1 lost, 9 ongoing)
Sebire 1996	176	0	0	4/346 (4.0%) 6 fetuses TOP 2 karyotype culture failures
De Catte 2002	16	0	0	2/32 2 fetuses TOP no karyotype failures

2: The use of dye as a marker for the punctured amniotic cavity has progressively become obsolete. Fetal death and bowel atresia have been associated with the use of methylene blue (van der Pol, 1992; Nicolini, 1990; Kidd, 1996). The use of indigo carmine did not result in a significant increase in congenital malformations (Pruggmayer, 1992; Cragan, 1993), however it has vasoconstrictive properties, and therefore should be avoided. Specially prepared maternal haemoglobin has been injected by Beekhuis et al, but its preparation is time consuming and does not prevent inadvertent sampling of the same amniotic cavity in cases of brownish discoloured amniotic fluid (Beekhuis, 1992). More recently, sonographic mapping of the pregnancy and careful identification of each amniotic cavity have reduced intertwin sampling failures due to resampling the same sac (Ko, 1998). However, one should be aware that in cases of concordant chromosomal

malformations in both twins of a dichorionic twin pair, the likelihood of non-selective sampling is very high (van den Berg, 1999).

3: Finally, it must be stressed that the most important improvement over time is the significant decrease in fetal loss rate. In the 80's and early 90's, total fetal loss rates ranged between 7 and 10%. More recent data revealed risks of fetal loss of less than 5%, and probably, less than 3%. In a case control study, Ghidini (1993) found a fetal loss rate after amniocentesis of 3.5 % compared to 3.2% in the control group. Both groups were comparable except for the significantly higher maternal age in the amniocentesis group. In a retrospective cohort study, Yukobowich examined the early fetal loss within 4 weeks after amniocentesis in 476 dichorionic twin pregnancies, and compared it with an untested twin population and singletons undergoing amniocentesis (Yukobowich, 2001). The fetal loss rate in twins undergoing amniocentesis was 2.73%, and was four times higher than in non-tested twins (0.63%) or tested singletons (0.6%). None of the fetal losses were found to have an abnormal karyotype. However, the number of needle punctures and a cloudy aspect of the amniotic fluid, correlatet significantly with the risk of pregnancy loss ($p<0.03$; OR: 0.24; CI: 0.068-0.8 and $p=0.035$; OR: 0.29; CI: 0.63-1.38, respectively).
Increased fetal loss rates have been attributed to the higher spontaneous loss rates in multiples (Grobman WA, 1998), the advanced maternal age, the number of needle insertions, the use of methylene blue or the presence of structural defects in one of the fetuses. The impact of these different factors separately on the fetal loss rate after amniocentesis is hard to determine.

When dealing with prenatal diagnosis in multiple pregnancies, selectivity must be ensured at different levels. Clearly, the position of the fetuses, their placental sites and cord insertions must be schematically drawn. Only in this meticulous way selective termination of pregnancy can be performed if discordant results are obtained. What is more, conventional cytogenetic, metabolic or DNA analysis after prenatal diagnosis by amniocentesis in multiple pregnancies is time consuming. Often results are not available before a gestational age of 17 to 18 weeks. Discordant results subsequently lead to difficult and lengthy counseling about selective feticide. If proper mapping of the fetuses is lacking, or genetic diseases without structural fetal defects or growth restriction are present,

identification of the affected fetus may be arduous. Proper fetal identification and making a record of the different landmarks displayed by each fetus are mandatory at the time of the diagnostic intervention. However, selective fetal reduction at this stage in pregnancy is associated with more psychological discomfort and a worse outcome than earlier in pregnancy (Evans, 1999; Berkowitz, 1997; De Catte, 2002). Diagnosis of numerical aneuploidy by FISH on uncultured amniocytes for chromosomes 21, 18, 13, X and Y has attracted much attention, since it takes only a few days (Eiben, 1999; Weremowicz, 2001; Fiddler, 2001).

General recommendations for amniocentesis include the use of small gauge needles (22G), extensive sonographic expertise in screening multiple pregnancies, selectivity in handling and processing the individual fluid samples, rapid sample analysis by FISH, and vigilance towards concordant chromosomal aberrations in dichorionic pregnancies.

3.1.2 Pregnancy outcome of 124 mid-second trimester amniocenteses in twin pregnancies at the AZ VUB

Over a period of 12 years (between 01-1989 and 03-2001), 124 twin pregnancies underwent an amniocenteses under ultrasound guidance were carried out at a mean gestational age of 16.1 ± 1.9 weeks in 124 twin pregnancies (range (13-23 weeks). In 16 patients both amniotic cavities were sampled by a single transmembraneous puncture (13%); in 104 patients the two amniotic cavities were punctured separately (84%), and in 4 patients only one of the two amniotic cavities was punctured (3%). Indications for amniocentesis included maternal age (≥ 35years at the time of delivery) in 65 patients (52.4%)(62 patients maternal age only, 3 patients maternal age + ICSI), cytogenetic evaluation of ICSI pregnancies in 24 patients (19.4%), karyotyping for structural fetal anomalies in 11 patients (8.8%), and psycho-social reasons in 12 patients (9.7%) (Table 3.6). Ten other patients had an amniocentesis for an abnormal triple test (n=7), a history of chromosomal abnormality (n=3), Rhesus alloimmunisation (n=1), and seroconversion for toxoplasmosis (n=1). Cytogenetic analysis was available for all 244 examined amniotic fluid samples. Thirteen fetuses (5.3%) had a chromosomal abnormality: 6 inversions, 2 mosaicisms, 3 translocations, 1 marker

chromosome and 1 trisomy 21. An additional fetal blood sampling was performed in two cases. The obstetric outcome is summarized in Table 3.7. Three patients (4 fetuses) elected to have a termination of pregnancy because of structural defects in one or both fetuses (one conjoined twin; one twin pregnancy discordant for neural tube defect). Another 9 fetuses were lost spontaneously (3 complete pregnancies and 3 intra-uterine deaths) (3.7%). There was one neonatal death (0.5%). Perinatal mortality rate was 10/233 or 4.3%. No significant difference (p:0.3) in fetal loss rate was observed between the classical (7/210; 3.3%) and transmembraneous approach (2/32; 6.3%). Mean gestational age at delivery in 118 twin pregnancies evolving beyond 22 weeks was 36.2 ± 2.4 weeks. (range: 28-40 weeks). Sixteen patients delivered at 33 weeks or less (13.6%). Mean birth weights in twin A and B were respectively 2445 ± 537 g and 2354 ± 494 g.

The observed differences in fetal loss rates fit well with the natural losses observed in twin pregnancies between first trimester and the early second trimester. Grobman noticed a fetal loss rate of 11% in twin pregnancies sonographically identified at a mean gestational age of 10.1 weeks, whereas Ghidini et al (1993) observed a 3.2% fetal loss rate in twin pregnancies after a normal ultrasound examination between 14 and 20 weeks of gestation. Comparable data were observed in the Danish randomized trial, which examined the difference of spontaneous fetal loss between CVS and amniocentesis in singleton pregnancies. From the time of the first trimester intake into the study to the moment of the procedure was carried out, an excess of 2 % fetal loss was found in the group in which amniocentesis was to be performed (Smidt-Jensen, 1992).

Table 3.6: Differences in indication for prenatal diagnosis by CVS in the first trimester (De Catte, 2000) and by amniocentesis in the second trimester (unpublished data).

	CVS N = 262	Amniocentesis N = 124	p
Maternal age	82 (31.3%)	62 (50%)	0.0005
ICSI	114 (43.5%)	24 (19.4%)	<0.0001
MA + ICSI	33 (12.6%)	3 (0.8%)	0.0007
Other indications	33 (12.6%)	35 (28.2%)	0.0003

Pregnancies evolving beyond 22 weeks have very comparable outcomes in terms of mean gestational age at delivery, early preterm delivery rates (≤33 weeks), and mean birth weights for twin A. Comparison of these data with the obstetric outcome after CVS in 262 twin pregnancies (Table 3.7) (De Catte, 2000) is subject to several biases among which the different gestational age at the time of inclusion and the differences in indication are the most obvious ones. There were 6 pregnancy losses observed in the CVS group between the time of the sampling and 16 weeks of gestation, which might be attributed to the CVS procedure.

Table 3.7: Obstetric outcome after prenatal diagnosis in twin gestations by CVS (De Catte, 2000) or by amniocentesis (unpublished data).

	CVS N = 262	*Amniocentesis N = 124*	*p*
Total fetal loss (%)	28/509 (5.5)	9/242(3.7)	NS
Perinatal loss (%)	34/493 (6.9)	10/233 (4.3)	NS
Mean GA at delivery ± SD (weeks)	35.9±2.9	36.2 ± 2.4	NS
Preterm delivery (≤33 weeks) (%)	35 (13.8)	16 (13.6)	NS
Mean BW Twin A ± SD (g)	2429±589	2445 ± 537	NS
Mean BW Twin B ± SD (g)	2378±589	2354 ± 494	NS

However, there is no way to find out retrospectively the number of twin pregnancies scheduled for amniocentesis that aborted spontaneously before the procedure could be performed. In addition, the proportion of women having CVS for ICSI was considerably higher than in the amniocentesis group.

3.2. Chorionic villus sampling

3.2.1 Introduction

Chorionic villus sampling (CVS) became popular for first trimester diagnosis in the mid-80's. Only two approaches have remained: transcervical aspiration by a flexible catheter and transabdominal double or single needle aspiration technique.

Mainly three points of discussion emerged with the introduction of CVS for first trimester prenatal diagnosis:

-the safety of the procedures in terms of fetal loss and infections
-fetal malformations caused by CVS
-the cytogenetic mosaicism associated with rapid and long term culture of chorionic tissue.

3.2.2 Safety of first trimester prenatal diagnosis by CVS.

"Considerable data support the procedure-related pregnancy loss rate being comparable in CVS and traditional amniocentesis in experienced hands" (Kuliev, 1999). The fetal loss rate and risk for infection are not significantly influenced by a transcervical or transabdominal approach, although initial reports favoured transabdominal aspiration. Although the Danish collaborative study on CVS and amniocentesis showed a significant higher fetal loss rate after transcervical CVS compared with transabdominal CVS or amniocentesis (Smidt-Jensen, 1992), the USNICHD collaborative CVS study group failed to do so (Jackson, 1992). However, the introduction of the transabdominal technnique in a frist trimester protocol diagnosis based on transcervical CVS only, may significantly reduce fetal loss rates (Chuch, 1995). It certainly deserves strong recommendation to be skilled in both sampling routes when performing CVS in multiple pregnancies.

Boyd described an upper limb reduction defect diagnosed at 17 weeks after a CVS performed at 9 weeks' gestation (Boyd, 1990). Firth reported a cluster of 5 babies with limb reduction abnormalities in 289 pregnancies having early first trimester CVS (between day 56 and 66 of gestation) (Firth,1991). Four babies had an oromandibular-limb hypogenesis syndrome, 1 had a terminal transverse limb reduction defect. Burton observed four cases among 463 patients having CVS at less than 11 weeks (Burton, 1992). Further analysis of 75 cases of reported limb reduction defects (Firth, 1994) revealed a strong correlation between the severity of the defect and the mean gestational age at which the CVS procedure was performed; this correlation was evident for isolated transverse limb reduction defects as well as for those combined with oromandibular hypogenesis. A retrospective cohort study in five Italian obstetric centres (Mastroiacovo, 1993) concluded that CVS presents a fourfold risk of transverse limb reduction defects (1/1143) compared with the background rate in

Italy (1/4458). The rate at 9 weeks of gestation was 2.9 times higher than at 10 weeks. A correlation between the severity of limb defects and the timing of CVS was also suggested by Hsieh, who detected a significantly higher incidence of limb defects after CVS (0.294%) than in the general population (0.032%) in Taiwan. In addition, the incidence of severe limb defects was even more pronounced after CVS (0.22% versus 0.0026%) (Hsieh, 1995) Different hypotheses suggested a vascular compromise of end-arteries or compromise of the umbilical blood flow during the CVS procedure (Luijsterburg, 1997; Hibbard, 1994). Doppler flow analysis of the umbilical artery and fetal heart rate before and after CVS procedures did not reveal compromised umbilical blood flow (Hibbard, 1994). Acceleration of fetal heart rate observed after CVS may be secondary to various degrees of placental bleeding and subsequent fetal blood loss (Kofinas, 1995). Haemorrhagic lesions on the calvarium, face, thorax and distal segments of the limbs were reported by embryoscopy in a 9.5 weeks old fetus after CVS (Quintero, 1992). However, in a subsequent series of 200 CVS procedures performed between 8 and 12 weeks, no additional cases were observed. Only intentional and vigorous placental trauma with a blunt instrument was able to produce similar haemorrhagic lesions. Probably too early sampling, by operators with poor expertise and improper sampling techniques causing placental tissue disruption may explain the cluster of limb defects observed in the early years of the use of technique (Brambati, 1995).

The WHO safety report following the 7th International Conference on Early Prenatal Diagnosis of Genetic Diseases in 1994 (Kuliev, 1996) reviewed accumulated experience of over 138 000 CVS procedures. The overall incidence of limb reduction defects after CVS is 5.2-5.7/10 000, as compared to 4.8 – 5.97/10 000 in the general population. CVS was accepted as a safe procedure with no increased risk for congenital malformation in particular transverse limb defects. Analysis of the temporal relation between CVS and limb reduction defects revealed no increased risk above the expected population incidence from the 8th week onward (Kuliev, 1999). Recent recommendations (Wilson, 2000) stress the safety of CVS performed after 70 days of gestation with no increased risk for limb reduction defects.

3.2.3 Placental mosaicism

Placental mosaicism is defined as (Hahnemann, 1997): (1) the presence of at least two cells with identical chromosomal aberration, concomitant with a cell line with a normal diploid karyotype or with another chromosomal aberration in either direct CVS preparation/short term incubation, CVS culture or both; (2) the presence of a non-mosaic aberration in all mitoses analyses after direct CVS preparations/short term incubation, concomitant with a cell line with a normal, diploid karyotype or with another chromosomal aberration on CVS culture or vice versa.

Placental mosaicism raises two important questions: 1: are the genetic results confined to the placenta or is there a true fetal mosaicism? and 2: how frequent is an unfavourable pregnancy outcome associated with the finding of a mosaicism or a rare non-mosaic trisomy? The identification of placental mosaicism in short-term cultures has been reported in 1.09-2.14% of CVS performed in singleton pregnancies (Brambati, 1995). Of these, confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16 and 22 demonstrate incidences of 9-91/100 000 pregnancies, the most common being trisomy 7 (Wolstenholme,1996). This finding should prompt further long-term culture analysis to differentiate between true and placental confined mosaicism. Confined placental mosaicism was present in 77-89% of these cases. In a minority of cases an extra amniocentesis had to be performed to confirm or exclude true fetal mosaicism. A more recent update on the accuracy of cytogenetic findings in 62 865 chorionic villus samplings showed in addition to an incidence of placental confined mosaicism in 1% and true fetal mosaicism in 0.15% (Table 3.8), a false negative result in 0.03% of all cytogenetic analyses, almost exclusively related to direct preparation alone. In addition, false negative results are more common in late CVS than in first trimester CVS. However most of these cases present abnormal sonographic findings, requiring anyway a re-investigation by amniocentesis or fetal blood sampling. In 98.5% of CVS procedures, there are no difficulties in formulating the cytogenetic diagnosis (Hahneman, 1997).

Table 3.8: Simplified classification of ambiguous cytogenetic findings in CVS (after Hahneman JM, 1997).

Eucromic 1986-1992	CVS mosaics	Non-mosaic fetoplacental discrepancy	Total
False+ CVS results	656 (1.04%)	96 (0.15%)	752 (1.19%)
True+ CVS results	77 (0.12%)	12 (0.02%)*	89 (0.14%)
False – CVS results	-	19 (0.03%)	19 (0.03%)
Unclassifiable results	92 (0.15%)	-	92 (0.15%)

* including : non-mosaic abnormality on CVS confirmed in the fetus proper, however, a mosaic firm (n=9); one case of 45,X,-15+t(Y;15) on direct CVS preparation and 45,X,der(15) on amniocentesis and in the aborted fetus; one case with 48,XX,+21,+22 on direct CVS and 47,XX,+21 in the aborted fetus; one case with 70,XXX,+18 on direct CVS and 47,XX,+18 in the aborted fetus

Mosaic anomalies detected in extra-embryonic tissue involving sex chromosomes, supernumerary markers or trisomy 13 or 18 often extend into the fetal cell lines. Others like trisomy 16, are almost always confined to the placenta, but have an adverse effect on pregnancy outcome through mechanisms like either trisomic zygote rescue and uniparental disomy or placental insufficiency. An increased fetal loss rate associated with confined placental mosaicism was observed in 6.5-16.7%. Unfavourable pregnancy outcome (intrauterine growth retardation or fetal demise) is particularly related to the chromosome number involved. Fryburg (Fryburg, 1993) did not observe an increased number of pregnancy losses, congenital malformations or developmental delays in infants with confined placental mosaicism; however there was a 15% chance of intrauterine growth retardation.

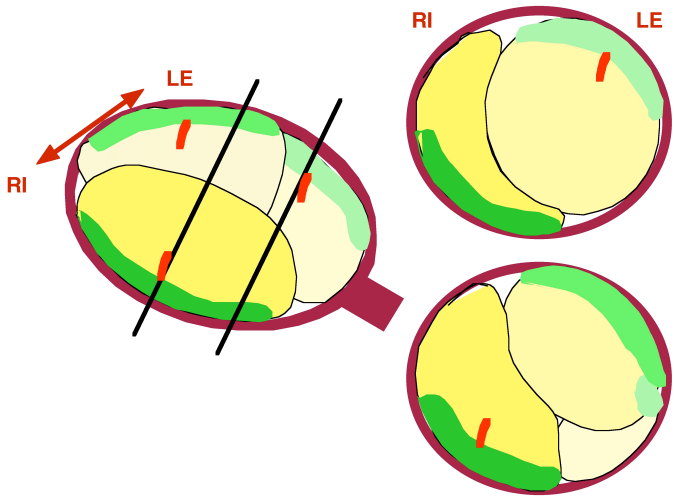
A third and smaller group, including trisomies 2, 3, 7 and 8, is usually associated with a normal outcome.

3.2.4 Practical approach of CVS in multiple pregnancies

Sonographic exploration of the first trimester multiple pregnancy before the sampling is the most time consuming task. Gestational sacs must be counted, localized and mapped in relation to the others. The number of fetuses in each gestational is counted, and the relationship between those fetuses is determined.

Localization of the chorion frondosum of each gestational sac is mapped in accordance with the insertion of the umbilical cord onto the chorionic plate. Multiple drawings of the interfetal relationships make it possible to identify each fetus at the time the genetic results become available (Figure 3.9), and facilitate identification of the genetically anomalous fetus for selective feticide.

Figure 3.9: Mapping of the different chorionic masses by ultrasound.



The approach of the chorion frondosum of each fetus depends on its localization, its interaction with placental tissue of the other fetuses, and the presence of disturbing elements like hyperstimulated ovaries, multiple abdominal scars, frozen pelvis, maternal obesity and vaginal infections. In the majority of cases a combined transabdominal-transcervical or a complete transabdominal approach will be used. CVS in multiple pregnancies in experienced centres is successful in almost 100%. Pergament reported sampling failures in 2/258 fetuses in 126 twin and 2 triplet pregnancies. This series however was a compilation of data from 4 centres, with each a relatively small number of patients. In 5/524 fetuses of 262 consecutive twin pregnancies in our centre (De Catte, 2000) an insufficient amount of chorionic villi was retrieved. Interfering factors were maternal obesity, enlarged hyperstimulated ovaries, and extreme retroversion of the uterus. In cases where it was technically impossible to sample the fetuses one intended to, a second trimester amniocentesis was proposed in the first place.

Chorionic villus sampling in monochorionic twin pregnancies had been limited to a single procedure, either systematically or in cases where the insertion of the

umbilical cords on the chorionic plate is too close to allow selective sampling (Brambati, 1991, 2001; Pergament 1992; Wapner, 1993; van den Berg, 1999). On other occasions, an area near each cord insertion is sampled (Wapner, 1993; van den Berg, 1999).
Amniocentesis of both amniotic cavities in cases of closely inserted umbilical cords, might become more appropriate since the development of new methods of selective feticide in this type of pregnancies.

3.2.5 Fetal loss rate and obstetric outcome

The total fetal loss rate in twin pregnancies varies between 3.0 % and 5.5% (Table 3.9), and is as low as 1.7 % in dichorionic pregnancies only. Compared with spontaneous fetal loss rates of 2-5% in twin pregnancies after 12 weeks of gestation (Brandenburg, 1994; Benson, 1994; De Catte, 1996; Aytoz, 1998) it seems that an early prenatal diagnostic procedure in multiple pregnancies is safe. Aytoz et al demonstrated that the fetal loss rate after CVS in 111 twin pregnancies obtained by intracytoplasmic sperm injections did not differ significantly from a control ICSI twin population (Aytoz, 1998). In addition, the fetal wastage figures are comparable with these obtained after amniocentesis in twin pregnancies. Data pertaining to 124 consecutive second trimester amniocenteses performed in our centre by the same operators over a same period of time revealed a total fetal loss rate of 3.7%. These figures are in agreement with data from the literature and reported by those Brandenburg (Brandenburg, 1994). Moreover, high spontaneous loss rates are still observed in twin pregnancies after establishing viability by ultrasound. In a 5 years prospective study of 137 twin pregnancies identified sonographically at a mean gestational age of 10.1 weeks, there was a complete pregnancy loss rate of nearly 11 % (Grobman, 1998). Furthermore, a fetal loss rate of 3.2% was still observed in twin pregnancies after a normal ultrasound examination between 14 and 20 weeks of gestation (Ghidini, 1993). Additionally, advanced maternal age has been considered a factor for increased fetal loss after CVS in singleton and twin pregnancies (Jahoda, 1991; Brandenburg, 1994). Brandenburg calculated the frequency of having no live children in a twin pregnancy without prenatal diagnosis for different maternal ages. The rate increased from 4.6% at 35 years

to 5.2% at 45 years. At the same time, the frequency of having two children both with normal karyotypes decreased from 88.9% to 79.2% (Brandenburg, 1994). Pergament and coworkers found no increased procedure-related pregnancy loss following CVS for advanced maternal age in singleton and twin pregnancies (Pergament, 1992). This confirms the observations of the Canadian collaborative CVS-amniocenteses trial (1989), which showed a non significant difference in fetal loss between first trimester cervical CVS and second trimester amniocentesis (7.6 % versus 7.0 % respectively). However, these data should be used with caution, because 11 centres carried out 1 192 CVS procedures (only about 100 procedures per centre). Wynberger (2000) elegantly demonstrated a significant decrease in fetal loss with increasing experience, regardless of whether the procedure is performed via the transcervical or via the abdominal route. In our data there is a non-significant trend towards higher rates of fetal wastage in the maternal age group (De Catte, 2000). In addition, CVS in ICSI twin pregnancies is associated with a very low risk of pregnancy complications (Aytoz, 1998). This should make CVS, even in twin pregnancies after infertility treatment, an acceptable alternative for amniocentesis.

Table 3.9: Reported outcomes of chorionic villus sampling in twin pregnancies.

	Wapner (93)	Pergament (92)	De Catte (2000)	Brambati (91)	Brambati (2001)
number	161	126	262	65	198*
fetal loss < 22 w	14/309 (4.5%)	5/244 (2.0%)	16/509 (3.1%)	2/120 (1.7%)	NA
total fetal loss rate	15/309 (4.8%)	10/244 (4.1%)	28/509 (5.5%)	2/120 (1.7%)	11/364 (3.0%)

NA: not available

*: including all sampled twin pregnancies; exclusion of the monochorionic twin pregnancies results ina total fetal loss rate of 1.7% (5/147), all after 22 weeks of gestation

Obstetric outcome after CVS in twin pregnancies has been reported sporadically (Brambati, 1991; Aytoz, 1998; De Catte, 2000; Brambati, 2001), and have been summarized in Table 3.10. No major difference in mean gestational age at delivery and low birth weight rates were observed between the published series. These data match those found in a historical control group of nearly 9000 twin pregnancies registered after 21 weeks of gestation in Flanders over nearly

the same period (SPE Yearbook, 1992-1999). Although the SPE data include our cases, this bias would be insignificant because of the great number of patients without prenatal diagnosis. The prematurity rate of nearly 70% in our series is unexpectedly high, but it is most likely related to the higher number of pregnancies being induced for pre-eclampsia and pregnancy associated hypertension disorders (70 %) (28 – 36 weeks) and preterm prelabour rupture of membranes (11 %) (23 – 35 weeks). Nevertheless, the mean gestational age at delivery matches the reported data. Case control investigation (De Catte, 1996; Aytoz, 1998; Brambati, 2001) could not demonstrate significant differences in perinatal outcome associated with the performance of first trimester CVS in twin pregnancies.

First trimester prenatal diagnosis in all three fetuses of triplet pregnancies has been reported sporadically. Usually these pregnancies are under consideration for fetal reduction, and hence usually only two CVS procedures are performed.

Table 3.10: Obstetric outcome after CVS in twin pregnancies compared with SPE-data.

	De Catte (96)	Aytoz (98)	De Catte (2000)	Brambati (2001)	SPE data (91-99)
number	104	111	262	169	8964
GA at deliv (SD)	35.7 (3.3)	NA	35.9 (2.9)	35.8 (2.9)	35.9 (2.9)
<37w (%)	51	53	69.6	43,0	49.1
< 33 w (%)	10	NA	13.8	9.3	10.5
<2500g (%)	50	52	51.9	44.6	55.2
<1500g (%)	10	6.4	6.3	6.4	7.9

A review of the literature revealed only three papers where the investigators had some degree of experience in the performance of CVS in all three fetuses of a triplet pregnancy (Eddleman, 2000; Brambati, 2001; De Catte, 1998, 2002). The absence of fetal loss is probably due to the small number. However, we found that even after reduction to twin pregnancies, a high number of patients develops hypertensive disorders during pregnancy (De Catte, 1998).

3.2.6 Implications on cytogenetic, biochemical and DNA results.

Uncertain results in one of both samples, requiring further investigation, are more frequent after CVS than after amniocentesis (5 % versus 0.3 % respectively; van den Berg, 1999), and relate to sampling problems (erroneous sampling, mixed sampling, cross contamination, maternal cell contamination) and laboratory issues (confined placental mosaicism and pseudomosaicism).

Selective sampling of all placental sites easily by transcervical aspiration is more difficult (De Catte, 1996 and 2000). Failure to sample each placenta separately has been reported when using both transcervical and transabdominal CVS only (van den Bergh, 1999; De Catte, 2000; Wapner, 1993). In fetuses of the same sex, it is impossible to spot these errors by routine analysis of the sampled material. The incidence of non-selective sampling therefore is estimated at 3- 5.5% (Pergament, 1992; Brambati, 1991; van den Berg, 1999; De Catte, 2000), which is twice the rate found in fetuses of diffferent sex. Brambati carried out additional investigations in cases of dichorionic fetuses of the same sex, and tried to confirm sampling reliability by studying the cytogenetic or DNA polymorphisms (fingerprinting) (Hill, 1985), or by performing amniocentesis. Christiaens (1994) confirmed the need of additional testing in cases where samples of fetuses of the same sex in a dichorionic twin pregnancy show concordant normal or abnormal results.

Van den Berg et al (1999) summarized their experience with genetic analysis of chorionic villi and amniotic fluids of 500 multiple pregnancies. Assuming that twin A in a dichorionic pregnancy has a normal karyotype established by CVS, then the probability for twin B to have a chromosomal malformation is 3 %, to present a confined placental mosaicism is 1.2% and to show a generalized mosaicism is only 0.19%. If the same fetus A has been subjected to an amniocentesis to confirm a normal karyotype, then the probability for twin B to have a karyotype anomaly is 2%, and the chance of an uncertain result requiring further investigation is 0.2% (van den Berg, 1999).

The impact of placental mosaicism can largely be reduced by the use of both short term and long term villi preparations. Since in long term preparations mesenchyme cells of the villous core are cultured, the results are not influenced by the higher incidence of numerical chromosomal aberrations in trophoblastic

tissue (Table 3.11). In the minority of cases in which inconclusive results after long term culture persist, amniocentesis must be performed. Whenever maternal cell contamination is responsible for the mosaic results, long-term cultures are not very helpful.

Table 3.11: Compilation of abnormal cytogenetic findings in CVS samples.

	Wapner 1993	Pergament 1992	Brambati 1991	De Catte 2000
Failure rate	0/322 fet	2/258 fet	0/122 fet	5/524 fet
Indication				
Maternal age (%)	76.4%	81%	69%	31.3%+12.6%#
Icsi (%)	-	-	-	43.5%+12.6%#
Chrom abn (n)	15	8	2	24
Aneuploidy	7	6**	2	17
Mosaicism total	8	2	-	3
Placental confined	6	1	-	3
True mosaicism	2	-	-	0
False negative	1	0	0	0
Selectivity errors	2$	2	1	2
XX/XY cultures (cell contamination)	6	6	0	3
Additional amnio's performed	9 (5.6%)	1	8	5
Fetal loss	15/309	10/250*	2/98	28/509

$: one sampling error and one processing error

* : excluding 8 fetuses undergoing selective abortion or therapeutic abortion

** : 7 cases have been reported, although one case was a fetus with a mosaicism not confirmed by additional examination but selectively reduced.

: 12.6% of the cases were sampled because of advanced maternal age and ICSI

Rates of follow-up sampling for quality reasons after cytogenetic analysis of amniotic fluid cells and chorionic villi after short term or combined short and long term cultures were comparable. However, the number of follow-up cytogenetic investigations for representativity reasons was significantly higher for chorionic villi then for amniotic fluid (1.99 %, 1.33 % and 0.1 % for short term cultured villi, for short and long term cultured villi and for amniocytes respectively (Los, 2001).

Combining procedures has been shown to increase the risk of adverse pregnancy outcome. In 9 out of 161 twin pregnancies in which CVS was followed by amniocentesis, 4 fetuses died in the perinatal period (Wapner, 1993). Neither

Brambati's nor our data could confirm this observation (Brambati, 1991; De Catte, 2000). Multiple needle insertions in either CVS or amniocentesis have been associated with a higher post procedure abortion rate (Rhoads, 1989; Simpson, 1976). Nevertheless, repetitive amnioreductions performed for polyhydramnios –e.g. in fetuses with oesophageal atresia or in twin-twin transfusion syndrome - are rarely complicated by inadvertent outcome due to the needling itself.

It has been argued that for reasons of erroneous sampling, uncertain results in one or both samples, and subsequent required further investigation, the use of CVS should be restricted to pregnancies at high cytogenetic risk (≥ 3 %) or at risk for monogenetic conditions (Los, 2001).

Summary

The risen incidence of multiple births since the mid-seventies highly coincides with the unrestricted use of artificial reproductive technology and ovulation induction. The increase in maternal age over the last three decades accounts only partially for the epidemic rise in twin pregnancies, but is probably not significantly related to the rise in higher order multiple deliveries. Strangely, ART causes a significant increase in monochorionic pregnancies.

Screening for fetal aneuploidy in multiple pregnancies will no longer rely on advanced maternal age but, comparable to singleton pregnancies, progressively change towards risk determination by nuchal translucency measurements in the first trimester. Hence, there will be a considerable shift of invasive procedures towards the first trimester.

Assessment of chorionicity in multiple pregnancies must be done in the first trimester. The composite approach, using the number of gestational sacs, the number of yolk sacs and amniotic cavities and the number of fetal poles, has a sensitivity and specificity of 100%. Later in gestation these parameters become less discriminating. The lambda sign, the thickness of the dividing membrane and its number of layers are less powerful tools to establish chorionicity.

First trimester CVS has become a valid alternative for prenatal diagnosis in multiple pregnancies. It should be performed only be experienced operators, taking into account the selectivity rules, the back-up long term cultures or additional investigational tools in cases of doubtful results. Fetal loss rates are comparable with those reported after amniocentesis. However, results from chorionic tissue require more frequently further investigation than from amniotic fluid; in particular, uncertain results relate to sampling problems and placental mosaicism. In cases of doubtful concordant results, zygosity testing should clarify the issue.

Amniocentesis in twin pregnancies can be accurately performed under ultrasound guidance, without the instillation of a dye. The transmembraneous approach facilitates the selective aspiration of each amniotic cavity. The introduction of FISH on uncultured amniocytes significantly shortens the cytogentic investigation. Nevertheless, in case of a selective feticide, pregnancy loss rates may still be higher.

In monochorionic pregnancies, prenatal diagnosis is often restricted to one of the fetuses. However, monochorionic fetuses are rarely discordant for genetic abnormalities. Selective feticide in these pregnancies can now be achieved by cord occlusion techniques. Techniques of prenatal diagnosis should therefore be highly selective, and for this purpose, aspiration of each amniotic cavity may be the best choice.

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Chapter 4

Multifetal pregnancy reduction and (s)elective reduction

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1 Multifetal pregnancy reduction

1.1 General considerations and technical aspects.

Aberg introduced fetal reduction as a new technique to selectively eliminate a fetus affected by a genetic disorder in a twin pregnancy (Aberg, 1978). Fetal reduction in twin and higher order multiple pregnancies may be carried out (1) to improve the chances of survival and health of the remaining fetus(es) and to reduce maternal morbidity, (2) to selectively eliminate a malformed fetus or a fetus affected by a genetic disease, or (3) to accomplish the patient's needs for planned parenthood according to her social and psychological strength.

Fifty-two percent of the twin pregnancies and 80 % of the triplet pregnancies are established after ART (*SPE, Yearbook, 1990-1999*). Nearly all of higher order multiple pregnancies (> 3) are related to ovulation induction or IVF techniques.

The best treatment for multiple pregnancies remains prevention, which can be achieved by either reducing the number of transferred embryo's (Gerris, 1999), by stringent stimulation protocols, or by canceling hCG administration at midcycle in cycles in which ultrasound demonstrated the presence of multiple mature ova. However, inevitably some multiembryonic gestations will still occur. In addition to a high degree of diligence exercised by those providing fertility treatments, physicians should inform the couples that fetal reduction is a cause of fetal loss in the remaining fetuses, and that lower birth weights and higher prematurity rates in twins reduced from triplets are of concern (*ACOG Committee Opinion 215, 1998*).

When counseling infertile women, physicians should be aware of the eagerness of infertile women to take irrational risks to achieve their goal (Goldfarb, 1996). Investigation on the attitudes towards multiple births among couples with infertility problems showed an increased willingness to have twins, although the majority the couples concerned were aware of the increased medical risks. There was a lesser desire for triplet pregnancies, but a higher order multiple pregnancy was rejected. This correlation was stronger with

advancing maternal age (Gleicher, 1995; Goldfarb, 1996). Moreover, the level of acceptance of triplet or even quadruplet pregnancies in women under fertility treatment without children, was higher than in those with children. In this infertile population, multifetal reduction was accepted by 51% (Gleicher, 1995) to 72 % of the patients (Goldfarb, 1996).

Many practitioners currently performing multifetal reductions consider this technique a temporary solution until fertility treatment regimes will improve. Yet a substantial number of fetal reductions are carried out yearly, but the number of higher order multiple pregnancies progressively decreases so that relatively more triplet and twin pregnancies are submitted to a reduction procedure nowadays both in the United States and in Europe (Evans, 2001). Data from our centre show that fetal reductions are more frequently requested by the increasing number of older couples expecting twins and triplets.

The technique of multifetal pregnancy reduction and the post-reduction outcome are determined by the chorionicity and amnionicity of the pregnancy. Usually, and especially after fertility treatment, multifetal pregnancies are multichorionic, and since there are no placental interfetal vascular communications, each gestational sac can be considered individually. Fetal reduction will therefore not harm the evolving co-fetus(es). In monochorionic pregnancies, however, this classical approach will result in simultaneous loss of or damage to the identical co-twin. Other approaches to achieve fetal reduction must be considered; these latter will be described later.

Multifetal reductions are performed either transabdominally or transvaginally between 10 and 13 weeks of gestation. The transcervical suction and aspiration technique has been abandoned because of the high pregnancy loss rates related to infection (Salat-Baroux, 1988). MFR before 10 weeks is not recommended as it is technically more difficult because of the smaller fetal size, and because of the possibility of spontaneous fetal loss that would alleviate the need for a fetal reduction. Besides, after 10 weeks detailed sonographic exploration of the fetuses may reveal early structural defects, an increased nuchal translucency or a smaller fetal size, changing the procedure from a merely technically driven one to a selective feticide.

No major differences in perinatal outcome have been observed with either transvaginal or transabdominal intracardiac or intrathoracic injection of potassium

chloride, although the transabdominal procedure is most widely used (Evans, 2001(a)). The transvaginal approach is usually favoured by ART specialists, but it has some minor inherent disadvantages: the needle has a larger diameter (17-19 gauge), it can be moved only in a restricted area, and one is forced to reduce the nearest fetus(es). There is a greater need for analgesia, but the use of a spring loaded needle device may facilitate the procedure (Timor-Trish,1993). Transabdominal procedures may be more difficult or even impossible due to maternal obesity, large ovarian cysts following ovarian hyperstimulation, or intestinal loops covering a retroverted uterus and obscuring the view (Brambati, 1995).

Transabdominal fetal reductions are carried out under local analgesia and continuous ultrasound monitoring with a free hand technique with a 22 G spinal needle. Nowadays, an intracardiac or intrathoracic injection of a KCl solution (0.5 – 1ml; 1-2 meQ/ml) quickly results in fetal asystoly. Injection of an air bolus or pure alcohol have been abandoned.

There is however a lengthy learning curve (Evans, 2001(a); Berkowitz, 2001) to perform these procedures, but after some time in outcome improves probably due to improved operator skills and better sonographic exploration of the chorionicity of multiple pregnancies. In general, fetal loss rates and early prematurity rates have dropped by about 50 % over a decade. However, there is also a sharp decrease in the incidence of quintuplet or higher order pregnancies, which may partially account for the better results (Table 4.1).

Table 4.1: Evolution in obstetric outcome in relation with time (Evans, 2001).

Number of pregnancies: 3513	< 1991	1991-1994	1995-1998
Number of fetuses ≥5	23.9%	15.9%	12.2%
Pregnancy loss≤ 24w	13.2%	9.4%	6.4%
Total pregnancy loss	17.7%	9.7%	6.6%
Preterm delivery ≤ 28 weeks	10%	2.8%	4.3%

Multifetal pregnancy reduction in higher order multiple pregnancies (≥3) is almost exclusively performed in the first trimester, between 10 and 13 weeks. Moral and ethical conflicts are usually tempered by knowledge of adverse pregnancy outcome in cases without MFR, especially in higher order multiple

pregnancies. However, both parents and some physicians are reluctant to reduce triplet pregnancies (Benshushan, 1993). According to some authors, modern obstetric management of triplet pregnancies achieves acceptable outcomes. However, a high morbidity still affects the offspring (Yokoyama, 1995(a,b); Kok, 1998; Bergh, 1999). Hence, it is mandatory for health care professionals to counsel the couple objectively about the obstetric, neonatal, psychological and financial burdens involved in either keeping or reducing the multifetal pregnancy. In addition, once it has been decided to carry out a reduction - often a difficult decision after a long fertility treatment - couples should receive adequate psychological support.

1.2 MFR in higher order multifetal pregnancies (≥ quadruplets).

Obviously, the most important contribution of fetal reduction to pregnancy outcome for both the remaining fetuses and the mother has been observed in higher order multiples pregnancies. Although there are no case control studies, the outcome can be compared with historical cohorts.

Early reports by Berkowitz and Evans led to the assumption that multifetal pregnancy reduction was ethically justified in conditions where the ability to carry the pregnancy to viability was very small (Berkowitz, 1988; Evans, 1988). In the early nineties, several groups reported their initial experiences (Tabsh, 1990; Lynch, 199O; Wapner, 1990; Boulot, 1990; Benshushan, 1993). These data were critically reviewed by Benshushan (1993). Overall pregnancy loss rates after multifetal reduction in 108 quadruplet, 40 quintuplet and 93 sextuplet pregnancies from different publications were 7.4%, 27.5% and 12.9% respectively. These figures should not be taken for granted as they reflect the initial experience of the centres concerned and pertain to only small numbers of patients per centre (Table 4.2).

In a collaborative study on more than 1000 cases in the world's leading centers, Evans showed that multifetal reduction had become a relatively safe and efficient method for improving outcome in multifetal pregnancies (Evans, 1994(a)). Pregnancy loss rates were related to the type of the procedure, and to the starting and finishing numbers of fetuses (Table 4.3). Transvaginal reductions

carried a non-significantly lower pregnancy loss rate (12.5%) than transabdominal procedures (16.2%).

Table 4.2: Multifetal reduction in higher order pregnancies.

Author	Number	% pregnancy loss**	% delivery ≤ 32 weeks $	Mean gest age at delivery
Wapner 1990	Quad: 11 Quint: 4	9% -	NA	NA
Tabsh 1990	Quad: 13 Quint:4	0 0	NA	NA
Boulot 1993	Quad: 18 Quint: 5 Septupl: 1	11.1% 20% 100%	NA	NA
Groutz 1996	Quad: 10	0	NA	33.3w
Evans 2001	Quad: 1127 Quint: 312 ≥Sextup: 171	12.3% 16.1% 22.8 %	14.2% 15.7% 16.9%	NA

$ Pertaining to delivered patients only

** Pregnancy loss rates only include complete pregnancy failures, not pregnancies with an extra fetal loss after the procedure. Hence total fetal loss rates are at least as high, and probably higher.

NA: Data are not available, or not specifically allocated to a given subclass

However, transvaginal procedures were usually carried out at an earlier stage in pregnancy (9.2 versus 11.3 weeks), and in pregnancies with lower starting and ending number of fetuses (3.5±1.1 versus 3.7±1.0. and 1.7±0.5 versus 2.0±0.5 respectively). These differences may well account for the observed differences in pregnancy losses. Although the data do not allow a reliable analysis of very early prematurity rates for the different subgroups, the viability rate in these reduced high order multiple pregnancies was much better than in historical controls (Table 4.3).

In the latest cumulative report in 2001, Evans stressed the importance of increasing experience on the improved pregnancy outcome after MFR. Pregnancy loss rate in 1610 higher order (≥4) multiple pregnancies was 13.7% overall, but it differed significantly depending on the starting number: in quadruplets, quintuplets and sextuplets or higher order pregnancies loss rates were 12.2%, 15.1% and 21.6% respectively. Differences related to the route of

reduction were not discussed. However, in the most recent period of 1995-1998, fetal loss rates were 7.3%, 11.4% and 15.4% respectively. Accordingly, a steep decrease in very early preterm delivery rate was observed. Overall, MFR in quadruplet pregnancies resulted in an early preterm delivery rate (≤33weeks) of 14.2%, compared with 15.7% and 16.9% in quintuplets and sextuplets or higher. Fetal reduction especially in higher order multiple pregnancies (≥5) results in a slower intrauterine growth and in earlier deliveries (Luke, 2001). An inflammatory response to reabsorbed dead feto-placental tissue with subsequent release of cytokines and stimulation of prostaglandin synthesis is the most likely cause of pregnancy loss and very preterm delivery following reduction. Moreover, there is a sustained decrease in maternal serum concentrations of placental hormones (hCG, progesteron and oestriol) starting 2 weeks after the reduction procedure and lasting for several months (Sebire, 1996). Both mechanisms may have an additive effect.

Table 4.3: Outcome after transabdominal MFR in high order multiple pregnancies (Evans, 1994).

Transabdominal reduction	Starting number of fetuses			
(number of patients)	4 (351)	5 (82)	6 (24)	≥7 (11)
Pregnancy loss ≤ 24w: overall (%)	11.7	22.0	50	45.5
Viability (%)	88.3	78.0	50	54.5
	Starting number of fetuses			
	4		≥5	
Pregnancy loss ≤ 24w: reduced to 1 (%)	36.4		67.7	
Pregnancy loss ≤ 24w: reduced to 2 (%)	11.5		25.5	
Pregnancy loss ≤ 24w: reduced to 3 (%)	10.5		36.8	

The expected gestational age at delivery can roughly be estimated taking into account that every additional fetus decreases gestational age at delivery by approximately 3.6 weeks, and each reduced fetus increases that gestational age by 3.0 weeks (Haning RV, 1996). The number of weeks at delivery is expressed by: 41.6 – 3.63x(number of fetuses at 8 weeks) + 3.01x (number of fetuses reduced), in which the number of fetuses reduced represents the fetuses that became non-viable after pregnancy reduction or through natural causes (Haning RV, 1996). Although this formula is only an approximation derived from the experience of one group of investigators, it can be particularly useful at the time

of counseling for fetal reduction. Other pregnancy complications like intrauterine growth retardation and pregnancy-induced hypertension occurred at frequencies consistent with twin pregnancies.

1.3 MFR in 72 high order multiple pregnancies at the AZ VUB

Between 1990 and 2001, 72 patients with higher order multiple pregnancies (quadruplets or higher) were referred to us for fetal reduction. Three patients were excluded from the analysis since the pregnancy was still in progress at the time of this writing. Although the total number of patients referred for MFR increased significantly over that period (from 14 to 69), the percentage of quadruplet or higher order pregnancies decreased from 50% to 7% (Figure 4.1).

Material and methods were comparable to those reported earlier (De Catte, 1998(a), 2002). There were 50 quadruplet, 9 quintuplet, 7 sextuplet, 2 septuplet and 1 octuplet pregnancies (Table 4.4). Mean maternal age was 29.0±3.5 years, and mean gestational age at the time of the reduction was 10.5±0.9 weeks. Four pregnancies were lost completely (4/69 or 5.8%) of which two at 23 and 28 weeks, and 65 patients had at least one live born child (94.2%). There were two additional fetal losses in two quadruplet pregnancies reduced to triplets. The total fetal loss rate was 11/130 or 8.5%. Delivery occurred at a mean gestational age of 35.4±3.0 weeks; 16/67 patients (24%) delivered at ≤ 33 weeks and 2 (3.0%) at ≤ 28 weeks. Of all live born children, 88 (74%) had a low birth weight (< 2500 g), and 14 (12%) a very low birth weight (< 1500 g). The starting and finishing numbers of fetuses did not significantly influence pregnancy outcome, although there was a trend towards a lower gestational age at delivery, higher preterm delivery rates and higher very low birth weight rates with increasing starting and fininishing numbers (Tables 4.4, 4.5 and 4.6). The lack of statistical significance was related to the small sample size of our study.

Fetal and pregnancy losses in our data correlate well with these reported by Evans (Evans, 2001(a)). The number of patients we studied is six times smaller than in the collaborative data set, which encompasses data from 11 different centres. The individual contribution of the majority of centres to the data set is much smaller than the figures we present, and this might account for their higher pregnancy loss rates (13.7 versus 5.8%). The low fetal and pregnancy loss rate is

most probably related to the continuous efforts of only two operators, carrying out all invasive procedures in our centre. With growing experience (1995-2001), only 1/37 pregnancy was lost (2.7%) compared with 3/32 (9.4%) during the preceding period 1990-1994 (NS). Fetal loss rates over the same periods were 2/65 and 9/65 respectively (p:0.054). There was no improvement in the numbers of early preterm deliveries and very low birth weight infants with time.

Figure 4.1: Number of fetal reductions performed between 1990 and 2001 in our centre, subdivided in high order multiple pregnancies (>3), triplet pregnancies and twin pregnancies.

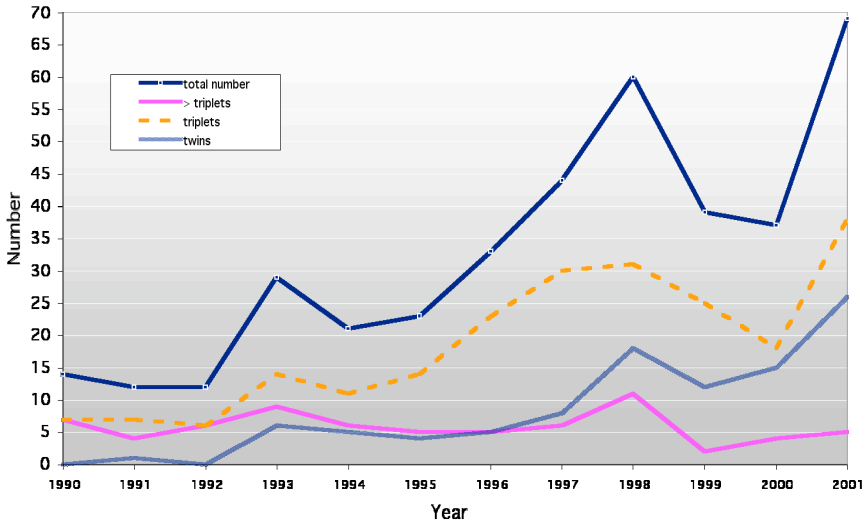

Table 4.4: Outcome of higher order multiple pregnancies according to the number of fetuses before MFR.

Starting number	N	Fetal loss (%)	Pregnancy loss (%)	Neonatal loss (%)	Mean GA ±SD deliv (w)	n≤33w (%)	n<2500g (%)	n<1500g (%)
4	50	6 (6.7)	2	0	35.9±3.0	10 (21)	56 (67)	8 (10)
5	9	3 (15)	1	0	34.1±2.6	2 (25)	17 (100)	3 (18)
6	7	2 (14.3)	1	2 (1.7)	34.3±3.1	2 (33)	11 (92)	3 (25)
7	2	0	0	0	32.5±0.7	2 (100)	4 (100)	0
8	1	0	0	0	-	-	0	0
TOTAL	69	11 (8.5)	4 (5.8%)	2 (1.7)	35.4±3.0	16 (24)	88 (74)	14 (12)

Table 4.5 : Outcome of higher order multiple pregnancies according to the number of fetuses after MFR.

Finishing number	N	Fetal loss (%)	Pregnancy loss (%)	Neonatal loss (%)	Mean GA ±SD deliv (w)	n≤33w (%)	n<2500g (%)	n<1500g (%)
3	4	5 (41)	1	0	32.3±2.3	2 (67)	7 (100)	3 (43)
2	53	6 (5.7)	3	2(2.0)	35.1±2.6	13 (26)	78 (78)	10 (10)
1	12	0	0	0	37.5±3.4	1 (8)	5 (42)	1 (8)

Table 4.6: Outcome of MFR to twin pregnancies according to the starting number.

Starting number	N	Fetal loss (%)	Pregnancy loss (%)	Mean GA ±SD deliv	n≤33w (%)	n<1500g (%)
4	36	4 (5.6)	2	35.4±2.6	8(22.9)	6 (8.8)
5	7	0	0	34.5±2.4	1 (14.3)	1(14.3)
≥6	10	2(10)	1	34;3±3.0	3(33.3)	3 (16.7)
TOTAL	53	6 ((5.7)	3 (5.7)	-	12(23.5)	10 (10)

1.4 Fetal reduction in triplet pregnancies

Over the last decade, fetal reduction in triplet pregnancies has been the object of an intense debate in the literature. The improved obstetric and neonatal management of triplet pregnancies, especially in tertiary care facilities, gave rise to a malaise among health care professionals regarding fetal reduction. They argue that when compared with non-reduced triplet pregnancies, pregnancy loss rates, gestational age at delivery and mean birth weight did not improve significantly after fetal reduction (Leondires, 2000, Papiernik, 1998; Angel, 1999; Kadhel, 1998; Benhushan, 1993). Moreover, compared with non-reduced twin pregnancies, the pregnancy outcome is worse (Depp, 1996; Sebire, 1997(a); Silver, 1997). However, even if pregnancy loss rates are not lower in reduced triplet than in non-reduced triplet pregnancies, other improvements such as an increased gestational age at delivery, decreased low and very low birth weight rates, less neurodevelopmental disorders, lower neonatal and postneonatal mortality rates (Luke, 2001) and lower number of maternal complications are of

such magnitude that the procedure should be proposed to all women with triplet pregnancies. In addition, population based data (Papiernik, 1996; *In Newman and Luke, 2000; SPE data 1992-1999*) continue to show, despite considerable obstetric and neonatal improvements, that the overall outcome is worse and the long term handicaps in triplet pregnancies are more frequent than in twin or singleton pregnancies.

Table 4.7 summarizes the overall outcome of triplet pregnancies reduced to twin or singleton pregnancies reported in the literature from 1990 to 2001.

Table 4.7: Pregnancy outcome after fetal reduction in triplet pregnancies.

Author	Number	% pregnancy loss**	% delivery ≤ 32 weeks	Mean gest age at delivery
Porreco 1991	13	1 (7.7%)	1	35.7±2.5
Melgar 1991	5	0		34.8
Tabsh 1990	23	0	NA	NA
Lynch 1990	13**	3(23.1)	0	36
Bollen 1993	33	3(11%)	1(3.3%)	34/36.9
Boulot 1990	37	4(13.3%)	-	37.7£
Boulot 1993	32	4(12.5%)	2(7.1%)	36.7±2.3
Macones 1993	47	0$	(3/43) 7%	35.6±2.8
Lipitz 1994	34	4(11.8%)°	3(9.7%)°°	36.7±3.7
Evans 1994	445	39(8.8%)	NA	NA
Berkowitz 1996	179	7.3%	10(6%)''	35.9
Groutz 1996	30¥	-	NA	35.9
Sebire 1997	66	5/66 (7.6%)	5/61 (8.2%)	36
Evans 1998	759	58 (7.6%)	82 (10.8%)	35.5
Yaron 1999	143	9 (6.2%)#	NA	35.6±3.1
Leondires 2000	46*	6 (13.0)#	NA	33.2±1.03
Evans 2001	1749*	6.5%	10.1%	55.1% ≥ 37w

**: completed pregnancies/ ¥: pregnancies reaching 24 weeks or more weeks/ °: ≤ 26 weeks/$: < 28 weeks/ °° <32 weeks/ # ≤ 24 weeks/ *:fetal reductions to twin and singleton pregnancies/ £: For 24/30 pregnancies reduced to twins

The percentage of fetal loss, very early preterm delivery and mean gestational age at delivery cannot be calculated since some of the patients are mentioned in different papers. In series with more than 30 procedures performed, the fetal loss rate varied between 0 (Macones 1993) and 13.0% (Leondires, 2000), whereas in 1749 triplet pregnancies reduced in the leading centres (Evans, 2001(a)) the loss rate amounted to 6.5%. A significant drop in pregnancy

loss rates has been observed from 8.8% in 1994 to 7.6% in 1998 and to 6.5% in 2001. Considering only reductions of triplet and quadruplet pregnancies to twins, 5.1% and 13.0% of pregnancies respectively, were lost in the early years compared with 4.4% and 6.6% respectively, in the most recent period. The mean gestational age at delivery (Table 4.7) ranges between 33.2 and 37.7 weeks. More than 55% of the 1749 triplets reduced (Evans, 2001(a)) delivered after 37 weeks. Early preterm delivery (≤ 32 weeks) occurred in approximately 10% in the largest series.

Comparison of obstetric and perinatal outcome between reduced triplet pregnancies (to twins), non-reduced triplet pregnancies and twin pregnancies show in general a more favourable outcome in reduced triplet pregnancies than in non-reduced ones (Yaron, 1999; Smith-Levitin, 1996; Evans, 2001(a;b); Lipitz,1996; Boulot, 1993; Lipitz, 1994(a); Macones, 1993), and in the majority of cases an outcome comparable to that of a control twin population (Table 4.8). However, there has been some concern about higher numbers of preterm delivery, lower mean birth weights and higher pregnancy complication rates in triplet pregnancies reduced to twins than in non-reduced twin pregnancies (Viscarello, 2000; Groutz, 1996; Angel, 1999; Leondires, 2000; Kadhel, 1998; Silver, 1997; Depp, 1996; Sebire, 1997(a)). In some of these papers the reduced twin group consisted of pregnancies with a greater starting number of fetuses (more than 3) (Sebire, 1997(a); Depp, 1996; Silver, 1997; Angel, 1999), probably accounting for some of the discrepancies in obstetric outcome. Luke (2001) found in twin pregnancies resulting from MFR that with an increasing number of fetuses before reduction the frequency of lower birth weights and preterm deliveries was significantly greater than in non-reduced twin pregnancies. However, after adjusting birth weights for gestational age however, no differences in growth restriction were observed between spontaneous twin pregnancies and higher order multiple pregnancies reduced to twins (Smith-Levintin, 1996; Torok, 1998). Competition early in pregnancy at the time of implantation may account for a smaller materno-placental exchange surface, and this effect may become more important with increasing number of fetuses. Even after MFR, there is no rapid change in the area of materno-placental exchange. In addition, the higher the number of reduced fetuses, the greater the amount of non-viable remains, and the more important the resulting inflammatory reaction, probably responsible for

the increased preterm delivery rates in reduced higher order multiple pregnancies (Groutz, 1997;Evans, 2001(a)).

Table 4.8: Comparative outcome in reduced triplets, non-reduced twin and triplet pregnancies.

		Reduced triplet pregnancies		Non-reduced twin pregnancies		Triplet pregnancies	
Author	Nr	% pregnancy loss**	Mean GA at delivery	% pregnancy loss	Mean GA at delivery	% pregnancy loss	Mean GA at delivery
Porreco 1991	13	1	35.7±2.5	-	-	0/11	35.2±2.3
Melgar 1991	5	0	34.8	-	-	0/20	33.1
Bollen 1993	31	3	34/36.9	-	-	2/32 (6.3%)	32.5
Boulot 1993	32	4(12.5%)	36.7±2.3	-	34.4	3/48 (6.3%)	34.4±2.3
Macones 1993	47	0$	35.6±2.8	NA	34.8±4.5	NA/14	31.2±4.9
Lipitz 1994	34	4(11.8%)°	36.7±3.7	-	-	27/106 (25.4%)°	33.5±3.6
Lipitz 1996	43¥	-	36.0±3.8	-	36.9±3.4 (n=134)	-	-
Groutz 1996	30	-	35.9±2.7	_	36.9±2.8	2210 ± 467	2361 ± 505
Sebire 1997	66	5/66 (7.6%)	36	-	-	1/47 (2.6%)	34
Yaron 1999	143	9/143 (6.2)#	35.6±3.1	40/605 (6.3%)#	34.4±3.6	3/12 (25%)#	32.9± 4.7
Leondires 2000	46*	6 (13.0)#	33.2±1.0	-	-	8/81 (9.9%)#	32.0± 0.6
Evans 2001	1749*	6.5%	NA	10.1%			

**: completed pregnancies/¥: pregnancies reaching 24 weeks or more weeks// # ≤ 24 weeks/ °: ≤ 26 weeks/ $: < 28 weeks/ °°<32 weeks /*:fetal reductions to twin and singleton pregnancies; GA: gestational age

It is much harder to evaluate the benefit of fetal reductions on neonatal morbidity. Neonatal intensive care admission, the number of days of stay in the unit, the incidence and duration of mechanical ventilation, and the incidence of necrotizing enterocolitis are some of the parameters to evaluate. Melgar (1991), Macones (1993), Angel (1999) and Viscarello (2001) found a smaller number of neonates admitted to the neonatal intensive care unit after MFR. In addition, the number of neonates needing ventilatory support or developing RDS was lower in the reduced triplets compared with the non-reduced ones (Boulot, 1993; Lipitz, 1994), and comparable to that in non-reduced twin pregnancies (Lipitz, 1996(a)).

1.5 Fetal reduction in 191 triplet pregnancies in AZ VUB

Our database of 402 patients with multiple pregnancies undergoing fetal reduction or selective feticide included 229 triplet pregnancies (57%), of which 191 are completed. Outcome is available for 188 patients (98.4%). Outcome parameters were compared and analysed statistically using Chi-square test and Fisher exact test. In 152 patients, the pregnancy was reduced to a twin pregnancy, in the remaining 36 to a singleton pregnancy. Outcome is shown in Tables 4.9,4.10, and 4.11.

Table 4.9: Overall outcome in 188 reduced triplet pregnancies.

Pregnancy outcome	N (%)
Fetal loss < 24 w	14/340 (3.8)
Fetal death ≥ 24w	1/326 (0.3)
Early neonatal loss	15/325 (4.6)
<28 w	6/180 (3.3)
28-32 w	19/180 (10.6)
33-36w	71/180 (39.4)
≥ 37w	72/180 (40)
< 1500g	38/320* (11.9)
1500-2499g	167/320* (52.2)
≥ 2500g	113/320* (35.3)

*: 6 birth weights unknown

The differences in outcome, that are quite remarkable, are related to the number of remaining fetuses after the reduction procedure; they include a higher mean gestational age at delivery, a lower preterm delivery rate, and a lower low birth weight rate in pregnancies reduced to singletons (Table 4.10). The total fetal loss rate was 4.4% (15/340). These data confirm the safety of the reduction procedures in triplet pregnancies, but also indicate a different obstetric outcome than expected compared with non-reduced twin and singleton pregnancies. Some of these differences are caused by the high number of maternal complications even after fetal reduction, among which preterm induction of labor for hypertensive disorders is the most frequent one (De Catte L, 1998(a)). Table 4.11 shows the obstetric outcome of triplet pregnancies reduced to twin and singleton pregnancies, compared with the outcome in non-reduced triplet, twin and singleton pregnancies in Flanders.

Evaluation of neonatal morbidity was not possible with our data due to the lack of complete information in the majority of patients.

Table 4.10: Obstetric outcome in 188 triplet pregnancies after MFR in relation with the finishing number.

	Fetal reduction to 2	Fetal reduction to 1	P
Number of pregnancies	152	36	
Mean maternal age (SD)	31.3±4.3	30.3±3.9	0.2
Mean GA at reduction (SD)	11.1±1.0	11.2±1.2	0.8
Number of fetuses reduced	152	72	-
Fetal loss < 24 w	11/304	3/36	NS
Fetal death ≥ 24w	1/293	0	NS
Early neonatal loss	15/292	0	NS
Mean GA at delivery (SD)	35.1±3.1	37.5±3.7	0.0006
Delivery <28 w	6/147	0	NS
Delivery 28-32 w	19/147	0	0.06
Delivery 33-36w	68/147	5/33	0.002
Delivery ≥ 37w	54/147	28/33	0.0001
Birth weight < 1500gr	37/287*	1/33	NS
Birth weight 1500-2499gr	167/287*	2/33	0.0001
Birth weight ≥ 2500gr	83/287*	30/33	0.0001

287 : 304-11(fetal loss) – 6 (unknown weight)

Table 4.11: Comparative outcome in singleton, reduced triplet to singleton, twin, reduced triplet to twin, and triplet pregnancies.

Parameter	Singleton SPE data	Triplet reduced to singleton	Twin SPE data	Triplet reduced to twin	Triplet SPE data
Number	125 429	36	8964	152	313
Mean GA delivery	-	37.5±3.7	35.9±2.9	35.1±3.1	32.9±2.9
< 28 w	0.4%	0	2.5%	4.1%	10.1%
< 33 w	1.1%	0	10.5%	17.0%	48.2%
< 1500 g	0.8%	3.0%	7.9%	12.9%	29.7%
< 2500 g	4.4%	9.1%	55.2%	71.1%	94.1%

1.6 Fetal reduction to singleton pregnancies for psychological or social reasons.

Reducing the number of fetuses in high order multiple pregnancies to a smaller number to improve obstetric outcome has been widely accepted. In these cases the number of fetuses after the procedure is exceptionally 3 but usually 2. Fetal reduction to singleton pregnancies has been accepted in cases of twin pregnancies discordant for structural malformations, triplet pregnancies with a monochorionic component, obstetric history of a congenitally malformed uterus or cervical incompetence, or an obstetric condition with fatal outcome for one of the fetuses.

More recently, pregnant women in their 40s or 50s, and pregnant after years of infertility treatment or after donor egg implantation, only want a singleton pregnancy for social rather than for medical reasons (Evans, 1997 and 2001). In addition, women conceiving twins or triplets the natural way, can in view of their social or financial status accept fetal reduction to a singleton pregnancy as valid alternative to interruption of the entire pregnancy. Evans' (2001(a)) multicentre report on 154 twin pregnancies reduced to singletons for psycho-social indications showed a pregnancy loss rate of 2/145 or 1.3% (< 24 weeks) and a preterm delivery (<28 weeks) rate of 1.4%.

In 38 multiple pregnancies reduced to singleton pregnancies for social reasons in our centre, the pregnancy loss rate was 2/38 or 5.3% (De Catte, 2002(a)). Mean gestational age at delivery was 38.1±2.6 weeks and the mean birth weight 2895±668g, which is smaller than in low-risk singleton pregnancies but slightly better than in twin pregnancies. These observations fit those reported by Lynch (1996), Evans (1999) and Berkowitz (1997).

Fetal reduction for psycho-social reasons can be considered as a valid and medically justified alternative to terminate the entire pregnancy. However one should not recommend fetal reductions in twin pregnancies as a standard procedure.

2. (S)elective fetal reduction

2.1 In multichorionic pregnancies

Over the years, ultrasound has become a highly sensitive tool for prenatal diagnosis of structural malformations in multiple pregnancies in specialized antepartum care centres (Edwards, 1995; Malone, 1996; Allen, 1991). The anomalous fetus was not always identified because of the early gestational age at the time of the scan (Edwards, 1995), indicating the importance of repeat ultrasound examination in these pregnancies. In total, nearly 90% of the anomalies were found. In addition, prenatal diagnosis for chromosomal abnormalities or Mendelian disorders in multiple pregnancies by first trimester CVS or second trimester amniocentesis has become more widely available. Twin gestations complicated by a single anomalous fetus showed a significantly lower gestational age at delivery (33.6 versus 35.6 weeks; p: 0.008), a lower birth weight (1864 g versus 2318g; p: 0.001), and a higher rate of perinatal mortality and caesarean delivery (64.3% versus 26.9%; p: 0.01) than normal twin pregnancies (Malone, 1996). However it is not clear whether the obstetric advantage of selective termination is applicable to all fetal malformations. Favourable outcome for the unaffected twin without selective termination of the affected fetus has been reported for twin pregnancies with anencephaly (Sebire, 1997(b); Lipitz, 1995) or lethal chromosomal abnormalities (Sebire, 1997(c)).

A second group of patients that might benefit from (s)elective feticide are those having suffered from preterm delivery because of cervix incompetence, Mullerian tract malformations (Ginsberg, 1997), multiple fetuses in a previous pregnancy, or the occurrence of PPROM at less than 24 weeks in one of the fetuses in a multiple pregnancy. Expectant management after PPROM in multifetal pregnancies reduces survival of the normal co-fetus(es) to less than 55% (Debbs, 1999; De Catte, 1998(b)). Selective feticide of the anhydramnic fetus increases mean gestational age at delivery, reduces the frequency of chorioamnionitis, and augments fetal survival rates to 90% (Debbs, 1999).

A third indication is the presence of a monochorionic twins as a part of a dichorionic triplet pregnancy (Sebire, 1997; Chasen, 2002). Thirty percent of

dichorionic triplet pregnancies are complicated by a twin-twin transfusion syndrome so that selective termination of the monochorionic twin should be encouraged (Chasen, 2002).

Late elective termination of abnormal fetuses in twin pregnancies has been associated with a favourable perinatal outcome of the healthy twin in several studies (Berkowitz, 1996; Hartoov, 1996; Lipitz, 1996(b) and 1997; Evans, 1999; Eddleman, 2001) (Table 4.12).

Table 4.12: Selective fetal reduction and pregnancy outcome.

Author	Number	Major indication	Mean GA reduction (w)	Pregnancy loss rate ≤24 w	Mean GA at delivery
Chitkara 1989	17 (all twins)	82% chrom 11% struct 6% Mendelian	20-23.5	12%	35.1w 80% >32 w
Evans 1994	183 (169 twins)	52% chrom 42% struct 6% Mendelian	80% > 16	12.6%	NA 73.2% > 32w
Berkowitz 1995	73	46.5% chrom	85% > 17	4%	36.6w 84% > 32w
Lipitz 1996	36 (all twins)	64% struct 36% chrom	> 24	-	36.9±2.9w 92% >32w
Berkowitz 1997	100 (69% twins)	60% chrom 36% struct	84% > 17	3%	36.8 85.4% ≥32w
Hartoov 1998	28	29% struct 14% chrom 57% ≥ triplet	20.2±3.9	3.4%	36.6±2.2
Evans 1999	402 (345 twins)*	56.1% chrom 40.3 struct 3.1% Mendelian	NA	7.5% (7.0%) *	NA 78% > 33 w
Shalev 1999	23	43.5% struct 56.5% chrom	28-33	-	36.9±2.1 all > 32w
Eddleman 2001	200 (164 twins)*	50% chrom 43.5% struct 3.5% Mendelian	19.2	4% (2.4%) *	36.1 w 84.2% ≥ 32 w

NA : not avaible

* : data refer to twin pregnancies in the studied population

Pregnancy loss rates vary between 3 and 12%, mean gestational age at delivery between 35.1 and 36.9 weeks; more than 73% deliveries occur after 32 weeks. Increased pregnancy loss rates were observed in association with procedures performed after 16 weeks (Evans, 1994(b)), with a higher starting number of fetuses (Eddlemann, 2001), and with reduction of the fetus located in

front of the cervix. However, in our small series (De Catte, 2002) gestational age at which the procedure was carried out was the single most important factor determining fetal loss after reduction to singleton pregnancies. Performing the MFR/SF before the 15th week of gestation led to a pregnancy loss of 3.3%. In one of the two patients concerned, fetal death was related to a congenital CMV infection. Procedures which took place after 14 completed weeks resulted in a significantly higher pregnancy failure rate (31.6%; p: 0.001). This observation was also made by other authors previously (Lynch, 1996, Evans, 1999) and it was considered a strong argument for improving prenatal detection of congenital malformations in the first trimester. Compiled data (Evans, 1999) on selective feticide for congenital malformations in 402 multiple pregnancies demonstrated a progressive rise in fetal loss in relation with the gestational age at which the procedure was performed: fetal loss rates amounted to 5.4%, 8.7%, 6.8%, and 9.1% for procedures carried out between 9-12, 13-18, 19-24 and ≥25 weeks, respectively. Berkowitz and coworkers (1997) demonstrated that selective feticide for congenital malformations in 100 consecutive cases was accompanied by a spontaneous loss rate of only 3%, and resulted in the birth of healthy infants at or near term in the vast majority of cases.

In our series and independent of the indication, mean gestational age at delivery (35.8-38.1 weeks) and mean birth weights after fetal reduction (2875-2897g) were smaller than in low-risk singleton pregnancies, but slightly better than in twin pregnancies (De Catte, 2002). These data fit with those reported for FR/ST to singleton pregnancies (Lynch, 1996; Sebire, 1997; Silver, 1997). Fetal reduction after 14 weeks significantly lessens mean gestational age at delivery. This observation was also made by Yaron (1998), who reported a decrease of 3 weeks in mean gestational age at delivery, and a mean drop of 700 g in birth weight.

Although a substantial number of these procedures are performed at a later gestational age, no maternal coagulation disorders were noticed.

2.2 In monochorionic pregnancies

2.2.1 (S)elective reduction in 10 monochorionic pregnancies at the AZ VUB.

Material and Methods

Ten cases of monochorionic twin/triplet pregnancies required fetal reduction either for a severe congenital malformation (n=3), a severe twin-twin transfusion syndrome (n=4), an acardiac twin (n=1), or psychological reasons (n=2) over a period of 5 years. There were 8 monochorionic biamniotic twin pregnancies and 2 monochorionic triamniotic triplet pregnancies. Chorionicity was determined by identification of like-sex fetuses, the absence of the twin peak sign, the presence of a thin wispy interfetal membrane, and the finding of malformations characteristic for monochorionic pregnancies.

Selective feticide in monochorionic twin pregnancies by one of the techniques described was approved by the institutional review board of our institution. Patients' written consent was obtained on all occasions after detailed counseling about the maternal and fetal risks and side effects of the procedures, including pregnancy loss rates reaching 25 %, preterm rupture of the membranes, preterm delivery, and the development of neurological and nephrological sequellae in the surviving co-twin in cases of incomplete vascular obstruction.

Fetal reduction was performed by means of injection of histoacryl in 5 cases, and by thermocoagulation in the next 5 cases. The ultrasound guided injection of the histoacryl was done under local anaesthesia through 22 gauge needles, inserted respectively in the umbilical vein and the left cardiac ventricle. Histoacryl was then injected first into the umbilical vein, leading to a fetal bradycardia, and immediately thereafter in the left ventricle resulting in an instantaneous cardiac arrest.

Electrocoagulation was resorted to in the remaining five cases, under general anaesthesia. In four of them, a disposable bipolar coagulation forceps with a diameter of 3 mm (Everest Medical, Minneapolis, Minn) was used. Three adjacent sites of the cord were coagulated. In the fifth case a monopolar intrafetal coagulation procedure was carried out because of an acardiac fetus with a short umbilical cord syndrome. Power settings were between 20 and 60 watt for 10 to 30 seconds per coagulation. The absence of umbilical cord flow was assessed by pulsed and colour Doppler flow mapping, after removing the instruments from the coagulated site.

Sonographic follow-up examination took place within 24 hours, one week later, and every two weeks thereafter.

Outcome measures were fetal-neonatal death, the procedure-delivery time interval, gestational age and birth weight at delivery, and obstetric complications observed after the invasive procedures.

Results

Indications for selective termination included four cases of early and complicated TTS. Two of these patients presented with early onset TTS with huge polyhydramnios and at least two amnioreductions before 20 weeks of gestation. In the two remaining TTS cases, the direction of the shunt had abruptly changed, leading to ventriculomegaly and cerebral hypoplasia, renal hyperechogenicity and minor hydropic fetal changes. A fifth pregnancy was complicated by a TRAP sequence and a short umbilical cord syndrome (1.5cm) and a body stalk malformation. Three additional patients had a biamnionic twin pregnancy with one fetus presenting a severe congenital malformation: one fetus had an exencephaly in association with a persistent cloaca; the second one showed a severe growth restriction, a bilateral ventriculomegaly, a short umbilical cord, and a thickened myocardium; the third one had a dilatation of the lateral and third ventricles. Two patients with a monochorionic triamniotic triplet pregnancy requested a fetal reduction for psychological reasons.

All histoacryl procedures took less than 15 minutes, without major technical difficulties. Once the histoacryl injected, the needle had to be withdrawn immediately. In all cases absent blood flow was observed on colour and pulsed Doppler by the end of the procedure. The coagulation procedures required between 30 and 50 minutes. In all bipolar coagulation procedures, the umbilical cord was coagulated at three different sites to decrease the possibility of incomplete occlusion. The mean time from insertion of the trocar needle to the first coagulation attempt was less than 5 minutes in all instances. Adjusting coagulation power settings was very time consuming in our hands on each occasion. In addition, although blood flow ceased completely as observed by pulsed and colour Doppler flow, fetal heart rate did not stop immediately. One of the cases was complicated by an important intra-amniotic bleeding, caused by a non-transplacental insertion of the trocar. In another case, an intra-abdominal

haemorrhage and maternal anaemia necessitated a laparotomy and placement of a haemostatic suture on the site of the insertion of the trocar in the uterine wall.

Table 4.13: Fetal reduction in 10 monochorionic pregnancies: indications, procedures, procedure-delivery interval and outcome.

Case	Indication	Technique	P-D interval	Outcome
1	Exencephaly/persistent cloaca	Tissue col 17w	0	Fetal death within 24h
2	IUGR; hydrocephaly, short umbilical cord	Tissue col 19w	16w	Normal outcome 35w, 2260g
3	Early TTS 19w Amniodrainage 4100ml	Tissue col 23w	3w	Preterm delivery 26w, neonatal death 1 week later
4	Early TTS 19w ventriculomegaly, renal echogenicity Amniodrainage 4000ml	Tissue col 21w	16w	Normal outcome 37.5w, 3190g
5	G1, 20 weeks Dilatation of the lateral and third ventricles	Tissue col	18w	Normal outcome 38w, 3160g
6	G1 Psychological	Bipolar coagulation 18w	16w	Normal outcome 35w, 1545g and 1640g
7	G1 Psychosocial	Bipolar coagulation 19w	18w	Normal outcome 37w, 2700g and 2650g
8	Acardiac twin Limb-body-wall complex	Monopolar coagulation	3w	PPROM Chorioamnionitis 24w, neonatal loss
9	G1 Early TTS	Bipolar coagulation 21 w	6w	Preterm delivery 27w Neonatal death
10	Early TTS/ 17w amniodrainage 1200ml	Bipolar coagulation 18w	0	Maternal haemorrhage Fetal death

TTS: twin-twin transfusion syndrome

IUGR: intrauterine growth restriction

P-D interval: procedure – delivery interval

Monopolar coagulation in the acardiac fetus with an abdominal wall defect and short umbilical cord was difficult because of the absence of tissue near the abdominal aorta. The monopolar probe was inserted and coagulation was effected at the level of two different paravertebral locations in the abdomen. Blood flow ceased completely. However, ultrasound control performed the next

day revealed a persistent small blood flow towards the acardiac fetus, and a worsening of the general condition of the co-twin. A second intervention succeeded in stopping the flow completely.

The outcome of the 10 procedures is summarized in Table 4.13. Two pregnancies (20%) were lost within 24 hours after the procedure. Although selective termination was achieved by different techniques, the death of the co-fetus was caused by exsanguination, as shown by signs of severe fetal anaemia on post-mortem examination. Histologic examination of the coagulated cord revealed the incomplete occlusion of the treated zones. In the second case, occlusion of the umbilical vein by histoacryl appeared to be incomplete in the terminated fetus. No sign of abnormal intravascular coagulation was found in the normal co-twin.

Of the eight remaining pregnancies, three ended three to six weeks after the procedure. PPROM occurred in one pregnancy two weeks after the intervention, and one week later the patient delivered at 26 weeks; the neonate died one week after birth because of prematurity related complications. A second patient was admitted at 23 weeks (4 weeks after the procedure) in preterm labour due to chorioamnionitis which led shortly thereafter to a preterm delivery. This infant also died due to extreme prematurity. In a third patient, preterm labour and subsequent delivery occurred at 27.5 weeks, and the neonate succumbed within 24 hours. There were no immediate complications related to the procedures in these three cases. Chorioamnionitis was the underlying condition of the fast deteriorating condition.

Five patients (50%) delivered between 34 and 38 weeks of seven healthy babies. None of the neonates showed neurological complications. The mean procedure-delivery interval in the eight ongoing pregnancies was 12.0±6.7weeks (SD; 6.7; range: 3-18).

2.2.2 Techniques: general discussion.

Fetal reduction or selective termination in monochorionic pregnancies cannot be performed safely by intracardiac injection of KCl. Via placental vascular anastomoses the chemical may reach the normal-cofetus(es) and harm this (those) later or even lead to fetal death and loss of the entire pregnancy

(Benson, 1998). It has been suggested that trombogenic material of the dead fetus reaches the monochorionic co-twin, resulting in a diffuse intravascular coagulopathy leading to fetal death or cortical necrosis of the kidney and brain. An other hypothesis favours the idea of a rapid decrease in vascular resistance after the fetus dies, causing an acute feto-fetal transfusion towards the deceased co-twin resulting in a hypovolemic shock and hypoxaemia with subsequent organ failure. Of course, one might speculate on the transfer of potassium chloride to the other fetus, but rarely a large amount is used, and rarely all of the KCl is injected into the fetal circulation. Therefore, a direct effect of potassium chloride is doubtful.

Several techniques have been advocated over the past decade to bypass the inherent risk of damaging the normal co-fetus. Most of the techniques were either too invasive for mother and fetus, or too complex to perform. Few case reports and small series described the potential benefit of hysterotomy and ligation of the umbilical cord during fetoscopy or by an ultrasound guided technique. Although these procedures have been occasionally successful, they have been abandoned because of their complexity, high complication rate, and low survival rates of the co-twin. Endoscopic procedures either by bipolar coagulation or by laser coagulation of the cord vessels were a direct spin-off of the endoscopic laser coagulation of the intertwin vascular anastomoses on the chorionic plate in twin-twin transfusion syndrome (Ville, 1998; De Lia, 1999). However, two major obstacles were encountered. Access to the umbilical vessels by fetoscopy is possible by broad 5mm fetoscopes or the combination of a smaller diagnostic fetoscope and a second trocar to guide the coagulation device, hence increasing the risk of preterm rupture of the membranes. In addition, expertise in fetoscopy is difficult to achieve.

Alternative less invasive approaches have emerged: injection of histoacryl or alcohol, and the ultrasound guided mono- and bipolar coagulation procedures. Absolute alcohol is known as a vessel sclerosant used in adult medicine to interrupt blood flow in different organs without causing tissue damage in distant organs. The injection of pure alcohol into the umbilical cord vessels has been reported for the first time in association with acardiac twinning (Holzgreve, 1994; Sepulveda, 1995). Injection of the alcohol causes an immediate retraction of the

vessel wall. Only few cases have been reported, some of which resulted in an incomplete occlusion of the vessel (Denbow, 1999).

The injection of histoacryl has been reported in a selected number of cases (Table 4.14)(Dommergues, 1995; Denbow, 1999; Donner, 1997). Although access to the different vessels was technically easy, only 10 out of 18 (56 %) of the fetuses that ought to have been spared, survived and evolved further. Complications associated were related to preterm rupture of membranes and preterm delivery.

Table 4.14: The use of histoacryl/enbucrilate gel for selective feticide in monochorionic multiple pregnancies.

Author	Number of cases	Site of injection	Survival	Complication rate**
Dommergues 1995	4	Umbilical vein+ heart	3/4	1/4
Dumler 1996	3	Intracardiac	1/3	3/3
Schild 1998	1	blood vessel anastomosis	1	Acardiac in triplet PPROM, preterm delivery, survival 1/3
Denbow 1999	3		1/3	2/3
Donner 1999	2	Umbilical vein+ heart	2/2	0?
Deprest 1999	4	NA	2/4	3/4
Present study 2002	5	Umbilical vein+ heart	3/5	2/5
Total	18		10	

**/ premature rupture of membranes or preterm labour < 30 w (Nicolini, 2001).

In some cases, the clot that formed after injection of histoacryl did not completely block the umbilical vein, which led to a quickly deteriorating condition of the remaining fetus because of subsequent exsanguination. Most authors preferred blocking the fetal circulation both in the umbilical vein and at the fetal heart, requiring the insertion of two needles. In the five cases we treated, time required for needling the umbilical cord vein and the left ventricle of the heart was less than 5 minutes. No difficulties were encountered during the nearly simultaneous injection of the histoacryl in the two different sites. There was no immediate demise of any of the normal co-twins. However, in one patient, intra-uterine death of the co-twin supervened within 24 hours. Pathologic examination revealed an incomplete occlusion of the cord vein, and a severely anaemic co-twin. Another

case was complicated by PRROM and preterm delivery followed by early neonatal death at 27 weeks. Of the five procedures performed, four were succesful in isolating the fetus from the placental circulation. However, the rather poor overall success and the supposed risk of trombogenic material reaching the circulation of the normal co-twin and initiating a diffuse intravascular coagulopathy leading to subsequent fetal death or severe handicap made this technique less popular.

Monopolar thermocoagulation of the fetal thoracic or abdominal aorta has been reported almost exclusively in association with twin reversed arterial perfusion sequence (Table 4.15). A unique series of 12 cases shows a high survival rate of 73% (Holmes, 2001). A 1mm diameter insulated wire electrode is introduced into the fetal abdomen close to the selected vessels with the aid of an 18 gauge needle. After connection to a standard monopolar diathermy machine, coagulation of the vessels was effected starting at 10 W for 10 to 15 seconds, and increasing with 5-10 W until an echogenic area, representing the coagulated tissue, appears around the tip of the electrode. The cessation of blood flow in the acardiac fetus is confirmed by colour Doppler ultrasound. The technical simplicity, the low cost of the equipment and the negligible maternal morbidity make it the technique of choice for this rare condition when growth of the acardiac fetus and progressive deterioration of the normal co-twin become manifest. Fetal death of the co-twin in three cases within two weeks of the procedure was probably caused by cardiac failure. In one case, the coagulation procedure needed to be repeated. We performed a monopolar coagulation in a monochorionic diamniotic twin pregnancy because of an acardiac twin with a body stalk malformation, a very short umbilical cord, and an oligohydramnios. Bipolar thermocoagulation was considered technically impossible. Monopolar coagulation of the abdominal blood vessels was attempted, the insertion of the guiding needle and of the coagulation wire were uneventful. Because of the body stalk malformation, there were almost no viscera in the vicinity of these vessels, and therefore it was difficult to obtain the correct power setting for the coagulation. Cessation of blood flow was finally observed by colour Doppler and pulsed Doppler having coagulated twice. Preterm labour and delivery occurred as a result of a chorioamnionitis three weeks later. This complication is most likely procedure related.

Table 4.15: Monopolar thermocoagulation in monochorionic multiple pregnancies complicated by TRAP sequence.

Author	Number	Localization	Survival rate
Holmes, 2001	12	Intra-abdominal Umbilical cord	73%

Thermocoagulation using a bipolar forceps can be effected through a small 3 to 4mm trocar and under ultrasound guidance (Table 4.16). Most cases of bipolar thermocoagulation have been reported in association with severely deteriorated twin-twin transfusion syndrome, twin reversed arterial perfusion sequences or in cases of monochorionic twin pregnancies in which one fetus was affected by a severe structural malformation. The bipolar coagulation forceps has a diameter of 2.7, 3 or 5mm, and are introduced through a trocar needle under ultrasound guidance and local or general anaesthesia. Survival rates vary from 76% (Nicolini, 2001) to 85% (Johnson, 2001).

The initial step consisting of gaining access to the amniotic cavity, directing the forceps and grasping the umbilical cord under ultrasound guidance was achieved within 5 minutes in all our cases. However, coagulation procedures took too long because of the low power settings for fear of transecting the cord. This observation was shared by others (Nicolini, 2001), but as experience grows optimal power settings and coagulation time for different gestational ages and cord sizes will be better known. Complications of the procedure are preterm delivery, PPROM, chorion-amnion separation, subchorionic haematoma and brachycardia of the co-twin (Deprest, 2000; Nicolini, 2001; Johnson, 2001); they are encountered in 30 to 50 percent of cases.

As Denbow (1999), we fear that in this initial phase selective feticide in monochorionic pregnancies by any of the different techniques causes a much higher complication rate than appearing in the literature. Various communications at scientific meetings reporting initial and often unsuccessful experiences do not reach scientific journals. As a rule, these techniques have been applied in a small number of pregnancies, usually because of a deteriorating condition of one of the fetuses. Although these procedures are performed by highly skilled operators with a considerable experience in ultrasound guided prenatal invasive diagnostic and therapeutic procedures, complications are rarely related to the insertion of

the instruments. In addition, the introduction of smaller instruments like 18-22 gauge needles does not always prevent complications like PPROM and chorioamnionitis.

Table 4.16: Bipolar thermocoagulation of the cord under ultrasound guidance for selective feticide in monochorionic multiple pregnancies.

Author	Number	Complication rate	Survival rate
Deprest, 2000	10	5/10 4/10: PPROM (2 transient) 1/10: abruptio placentae	8/10
Nicolini, 2001	17	6/17	13/17
Johnson, 2001	21	4/21: PPROM 2/21:amnion-chorion separation 1/21: subchorionic haematoma	Fetal loss 3/20
Present data, 2002	4 (10 fetuses)	2/4	4/10

Summary

Multifetal reduction in higher order multiple pregnancy increases survival rates and lessens obstetric complications for the remaining fetuses at the cost of an acceptably small pregnancy loss rate. The lowest preterm delivery rates were achieved after reduction to singletons, the highest survival rates after reduction to twin pregnancies.

Fetal reduction in triplet pregnancies carries a low pregnancy loss rate, and results in an improved pregnancy outcome. However, reduced twin and singleton pregnancies do not progress as uneventfully as non-reduced ones. Fetal reduction to a singleton pregnancy for social indications remains controversial; but the risk of adverse pregnancy outcome is low. Multiple multichorionic pregnancies complicated by congenital malformations or presenting obstetric risk factors may benefit from selective reduction. In most cases, the risk for pregnancy loss is acceptably low, and the outcome for the remaining fetus better than in non-reduced twin pregnancies discordant for congenital malformations.

Selective feticide in monochorionic twin pregnancies under ultrasound guided injection of histoacryl or monopolar/bipolar thermocoagulation requires less experience and less expensive equipment than fetoscopic approaches. Nevertheless, complication rates and pregnancy loss rates remain much higher than after injection of potassium chloride for feticide in dichorionic pregnancies.

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Chapter 5

Combined procedures

De Catte L, Camus M, Bonduelle M, Liebaers I, Foulon W. Prenatal diagnosis by chorionic villus sampling in multiple pregnancies prior to fetal reduction. Am J Perinatol 1998;15:339-43.

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1. Introduction

Future parents of children to be born out of multiple pregnancies are often overwhelmed by perinatal morbidity and mortality risks figures. Some problems emerged when, in addition to fetal reduction, the issue of prenatal diagnosis was discussed by referring physicians. Eddleman et al (Eddleman, 2000) clearly highlighted the dilemmas encountered in ART pregnancies. The fear of losing the pregnancy after fetal reduction is further increased by the possibility that one of the fetuses left after reduction might carry a chromosomal abnormality. The birth of a chromosomally abnormal child in series of MFR has been reported occasionally been reported (Evans, 2001(a)). In Flanders, respectively 37.5% and 35% of women pregnant of twins and triplets are over 30 years old, and 7.3% and 5.1% over 35 years (*Studie Centrum voor Perinatale Epidemiologie, Birth Register Flanders, 1991-1999*). The calculated risk of at least one fetus presenting an aneuploidy in a trichorionic triplet pregnancy when the gravida is about 30 – 31 years old equals that of singleton pregnancies in women 35 years old. So a high number of multiple pregnancies would benefit from prenatal diagnosis.

Classical mid-trimester amniocentesis after first trimester fetal reduction needs only a limited training to perform and is safe and accurate (McLean, 1998, Selam, 1999; Stephen, 2000; Antsaklis, 2000). However, on discovery of an abnormal karyotype, an additional selective feticide is required. Information on first trimester chorionic villus sampling before fetal reduction, and subsequent reduction to ensure that the fetuses that remain are chromosomally normal is based on limited numbers (De Catte, 1998; Eddleman, 2000; Brambati, 2001). Most series consist of different types of multiple pregnancies, and comparison with multifetal pregnancy reduction without prenatal diagnosis is not available.

2. Fetal reductions and amniocentesis

Only five studies evaluate the risk of amniocentesis after multifetal reduction (Tabsh, 1995; McLean, 1998, Selam, 1999; Stephen, 2000; Antsaklis, 2000) (Table 5.1). The populations studied are very heterogeneous. Starting

numbers and finishing numbers of fetuses in relation to multifetal reduction vary respectively from 6 to 2, and from 3 to 1. Different protocols and approaches have been resorted to as experience increased. Most of the fetal reductions were performed transabdominally. A variety of control groups were used : pregnancies reduced to twins but without amniocentesis, non-reduced twin pregnancies with mid-trimester amniocentesis, or a control group compiling data from a collaborative study on multifetal pregnancy reduction.

Surprisingly, overall fetal loss rate in patients undergoing MFR and amniocentesis is generally lower than in the patients having had MFR alone. One would expect a greater pregnancy wastage after the increased number of invasive procedures performed on a same pregnancy. However, comparison of pregnancy loss rates is biased by several other factors:

1: Mode of conception
2: The time of enrolment of the patient into one or the other group
3: The number of fetuses reduced
4: Differences in the number of fetuses left after reduction
5: Differences in MFR protocol
6: Maternal age

It is difficult to account for all these variables because the numbers of patients studied is rather small (range: 52 to 127), and the population studied heterogeneous. The retrospective nature of the data and the enrolment of the patients into the group of MFR or MFR/amniocentesis indicates that fetal loss rates might be higher in the MFR group only, since some of these pregnancies abort before genetic amniocentesis is performed (Antsaklis, 2000). Fetal loss rates increase with the number of fetuses left after reduction, whether or not amniocentesis is performed afterwards. Fetal loss rate was three times higher in twin than in singleton pregnancies after MFR/amniocentesis, but on all occasions much lower than in the control group of pregnancies that were only reduced (Stephen, 2000). Nevertheless, pregnancy outcome does not seem affected by the combination of MFR and amniocentesis. Overall fetal loss rates after the combined procedure (5.7%) compare with these of MFR to twins without amniocentesis (4.-7.2%), and with these of non-reduced twin pregnancies with amniocentesis (3.3%). However, the very early preterm delivery rate before 28

completed weeks was significantly higher in the MFR/amniocentesis group (Antsaklis, 2000) leading to an increased neonatal morbidity and mortality, and a higher risk of impaired neurological development in childhood.

Table 5.1: Obstetric outcome after post-reduction amniocentesis.

Author	Nr	Fetal reduction to	Fetal loss (%)	GA at delivery			Fetal loss Control group
				25-28	29-32	>32	
Tabsh 1995	53	twins	5/53 (9.4)	-	-	-	5/123 (4%)
McLean 1998	79	twins	< 24w 4/79 (5.1)	4 (5%)	8 (10.2%)	63 (79.7%)	11.9% 152/1358
Selam 1999	127	singletons, twins and triplets	< 24 w 4/127 (3.1)	-	-	-	12/167 (7.2%)
Stephen 2000	91	singletons, twins and triplets	<24 w: 8/87 (9.0)	4 (5%)	8 (10%)	69 (85%)	57/295 19.3%
Stephen 2000 triplets only	52	singletons:11 twins :41	3/52 (5.8)	2 (4%)	7 (14%)	40 (82%)	19/127 15%
Antsaklis 2000	73	twins	3/73 (4.1)	12 (17.1%)	6 (8.6%)	52 (71.2%)	13/392 (3.3%)

3 First trimester CVS before fetal reduction

3.1 Triplet pregnancies and pre-reduction CVS at the AZ VUB

3.1.1 Material and Methods

Between 1993 and 2000, 188 consecutive patients with triplet pregnancies requested fetal reduction after extensive counseling concerning the fetal, neonatal and maternal risks, the long term complications and the psychological and financial implications of giving birth to three or two children, and the procedure related risks of fetal reduction (In Newman, Luke, 2000, Chapter 10; Lipitz, 1989; Gonen, 1990; Robin, 1991; Luke, 1992; Yokoyama, 1995; Hardardottir, 1996; Garel, 1997; Senat, 1998; Chasen, In Blikstein and Keith, 2001; Devine, 2001; Evans, 1993; Evans, 1994(a)). Prenatal diagnosis by first trimester CVS or second trimester amniocentesis was discussed and offered to

all women of advanced age and to those having become pregnant after intracytoplasmic sperm injection (ICSI). Couples not desirous of prenatal diagnosis or preferring amniocentesis underwent the multifetal reduction procedure immediately following the counseling. Patients deciding to have a pre-reduction CVS, had this latter after counseling about the procedure. Fetal reduction was postponed one week in order to obtain the results of the CVS. All technical procedures were performed by one of two operators, and there was only one operator involved in each individual pregnancy. Sonographic anatomical evaluation of all fetuses was carried out with a 5 and 7.2 MHz transabdominal probe, and crown-rump-length and nuchal translucency (since 1996) were measured to establish early growth discordance and to estimate the individual fetal aneuploidy risk. Detailed mapping of all fetuses and their relationship to the placentas in longitudinal and transverse sections was done to ascertain selectivity both of villi sampling and of fetal reduction. CVS procedures were transcervical or transabdominal, depending on the placental localization. Details on the techniques of sampling and on fetal reduction are described in previous papers (De Catte, 1996, 1998, 2000). Briefly, CVS was performed initially in all three fetuses as part of an investigational study in pregnancies established after ICSI, and later on in only two or one of the fetuses depending on the number of fetuses that would remain after fetal reduction, thus diminishing the number of unnecessary invasive procedures. If a chromosomal abnormality would be detected, that particular fetus would be reduced, and in the same session, a CVS of the non-sampled fetus would be carried out. CVS was done transabdominally using a double18 and 20 gauge needle system; when performed transcervically, an aspiration catheter was used. An amount of 10 mg and 25 mg of villi was required for adequate cytogenetic and DNA or metabolic analysis respectively. Multifetal reductions were carried out transabdominally by injecting potassium chloride into the fetal thorax near to or into the fetal heart. The procedure was considered completed if fetal asystoly was observed for at least 2 minutes. Antibiotics were given in all cases of combined CVS and reduction, and in the majority of the patients submitted to MFR only (operator dependent).

A procedure was considered successful if chorionic villus sampling performed on the fetuses to be left after fetal reduction, resulted in a fetal karyotype within one week after the sampling. For the purpose of comparison, fetal loss was defined

as all fetal deaths before 24 weeks. Fetal deaths after 24 weeks, were defined as late fetal deaths, and neonatal death or the demise of all live born infants during the first week following birth. Data on the perinatal outcome of triplet pregnancies after fetal reduction with (group 1) or without pre-reduction CVS (group 2) were compared. Statistical analysis included chi-square test and Student's T test at a significance level of 0.05 when appropriate.

3.1.2 Results

Fetal reduction was performed in 188 consecutive triplet pregnancies. Group 1 consisted of 71 patients in whom a first trimester prenatal diagnosis by chorionic villi sampling was performed before fetal reduction. In the 117 patients of group 2, fetal reduction was done without prenatal diagnosis. Mean maternal age was 33.7±4.1 years in group 1 and 29.6±3.5 years in group 2 (p: <0.0001). Mean gestational age at the time of the CVS was 10.9 ±2.6 weeks. In 20 triplet pregnancies, the three placental sites were sampled (4 times transabdominally only, once transcervically only, and 15 times a combined transabdominal (2)-transcervical (1) approach was used). Of the remaining 51 patients, 46 had two placentas sampled (1 transcervically only, 18 transabdominally only, and 27 combined), and 5 women had only one CVS procedure per pregnancy (2 transabdominally, 3 transcervically). The number of placental sites sampled in these 51 patients corresponded to the number of fetuses left after the fetal reduction procedure. There were no sampling failures, and all 157 procedures resulted in a fetal karyotype. Chromosomal abnormalities on short term culture analysis were found on 4 occasions: a complete trisomy 20 (47,XX,+20), a mosaicism for a chromosome of the C group (46 XX/47,XX,+C), a trisomy 21(47,XX,+21), and a 46,XX der(20). These fetuses were electively reduced. Long-term culture cytogenetic analysis of the villi and of amniotic fluid aspirated at the time of the reduction revealed a normal female karyotype for the first two cases, which meant that the chromosomal abnormalities were confined to the placenta; the two other chromosomal aberrations were confirmed (47,XX,+21; 46,XX.ish(20)(wpc20+2)). No fetal sexing errors were reported at birth.
Fetal reduction was performed at a mean gestational age of 11.5 ± 0.7 weeks in group 1, and 10.9 ± 1.2 weeks in group 2 (p: <0.0001). Table 5.2 summarizes the

pregnancy outcome of triplet pregnancies after CVS/MFR (group 1) and MFR (group2).

Table 5.2: Pregnancy outcome after pre-reduction prenatal diagnosis and fetal reduction, compared with multifetal reduction only.

	Group 1	Group 2	p
Nr of pregnancies/nr of fetuses	71/213	117/351	-
Mean maternal age (SD)	33.7 (4.1)	29.6 (3.5)	<0.0001
Mean GA at reduction (SD)	11.5 (0.7)	10.9 (1.2)	<0.0001
Number of fetuses reduced	79	145	
Mean number of fetuses reduced per pregnancy (%)	79/71 (1.1)	145/117 (1.2)	NS
Total number of insertions	239	145	
Mean number of insertions per pregnancy (SD)	3.4	1.2	
Number of fetuses after MFR	134	206	NS
Fetal loss < 24 w (%)	3/134 (2.2)	11/206 (5.3)	NS
Fetal death ≥ 24w (%)	1/131 (0.8)	0	NS
Early neonatal loss (%)	3/130 (2.3)	12/195 (6.2)	NS
Mean GA at delivery (SD)	35.1 (3.9)	34.8 (5.2)	NS
Delivery <28 w (%)	1/69 (1.5)	5/111 (4.5)	NS
Delivery 28-32 w (%)	7/69 (10.1)	12/111 (10.8)	NS
Delivery 33-36w (%)	30/69(43.5)	43/111 (38.7)	NS
≥ 37w (%)	31/69 (44.9)	51/111 (45.9)	NS
Birth weight < 1500g	15/131	23/189*	NS
Birth weight 1500-2499g	75	94	0.03
Birth weight ≥ 2500g	41	72	NS

* Birth weight not known on 6 occasions.

Overall fetal loss before 24 weeks of gestation was 2.2 % (3/134) in group 1 compared with 5.3% (11/206) in group 2 (p:0.3). One fetal death at 20 weeks of gestation in group 1 was related to a congenital CMV infection, reducing the alleged technique related risk to 2/134 (1.5%). There was one additional fetal death after 24 weeks in a pregnancy with CVS/fetal reduction to twins. The co-twin evolved normally. Additional neonatal losses related to extreme preterm delivery supervened in 3/130 of the cases in group 1 (2.3%), and in 12/195 of the cases (6.2%) in group 2. None of these 12 fetuses (7 pregnancies, showed characteristics of a chromosomal malformation, and therefore were not karyotyped. One neonate presented a severe hydrocephaly; 5 neonates suffered from a type IV cerebral haemorrhage, and 6 neonates succumbed because of

severe RDS. Mean gestational age at delivery in the remaining 6 pregnancies was 26.3 w (SD 1.97w).

Mean gestational age at delivery in the CVS/MFR group was 35.1 ± 3.9 weeks, compared with 34.8 ± 5.2 weeks in the fetal reduction group (p: NS), and early preterm delivery (≤ 33 weeks) occurred in 8/69 (11.6%) and 17/111 (15.3%) pregnancies respectively (p: NS). Mean birth weights for triplet pregnancies reduced to twins with and without pre-reduction CVS and without were respectively 2196±625 g and 2209±533 g for twin A (p: 0.9) and 2132±554gr and 2179±529gr for twin B (p: 0.6), respectively. Mean birth weights in pregnancies reduced to singletons were 2840±840 g in group 1 and 2938±398 g (p: 0.8) in group 2. There was no difference between the number of low birth weight infants (<2500g) after CVS/MFR (90/131: 68.7%) and that after MFR alone (117/189: 61.9%)(p: 0.2).

In group 1, 63 of the 71 pregnancies (89%) were reduced to twins and the remaining 8 to singletons (11%), compared with 89 (76%) and 28 (24%), respectively, in group 2 (p: 0.051)(Tables 5.3 and 5.4).

Table 5.3: Obstetric outcome in patients undergoing pre-reduction CVS/MFR and MFR only to twin pregnancies.

	CVS and fetal reduction to 2	Fetal reduction to 2	p
Number of pregnancies	63	89	
Mean maternal age (SD)	33.6(4.3)	29.8(3.6)	<0.0001
Mean GA at reduction (SD)	11.4 (0.7)	10.9 (1.2)	0.003
Number of fetuses reduced	63	89	
Fetal loss < 24 w (%)	2/126 (1.6)	9/178 (5.1)	NS
Fetal death ≥ 24w	1/126	0	NS
Early neonatal loss (%)	3/123 (2.4)	12/169 (7.1)	NS
Mean GA at delivery (SD)	35.3 (2.8)	34.9 (1.1)	NS
Mean BW Twin A (SD)	2196 (635)	2209 (533)	0.9
Mean BW Twin B (SD)	2132 (554)	2179 (529)	0.6
Delivery <28 w	1/62	5/85	NS
Delivery 28-32 w	7/62	12/85	NS
Delivery 33-36w	29/62	39/85	NS
Delivery ≥ 37w	25/62	29/85	NS
Birth weight < 1500g	14/124	23*/163	NS
Birth weight 1500-2499g	75	92*	NS
Birth weight ≥ 2500g	35	48*	NS

*Birth weight not known on 6 occasions.

In 63 patients in whom fetal reduction was preceded by a CVS, the numbers of fetal and early neonatal losses were comparable with those in 89 patients having a reduction to twins without CVS (respectively 2/126 and 9/178, p: O.20 and 3/123 and 12/169, respectively, p: 0.13). In addition mean gestational age at delivery and mean birth weight were comparable in both subgroups. For patients having had their pregnancy reduced to singletons, fetal and neonatal loss rates, mean gestational age at delivery and mean birth weight did not differ significantly whether a CVS was carried out before reduction or not (respectively 1/8 and 2/28, p: 0.8; 0/7 and 0/26, p: NS; 38.0±3.7 and 38.1±1.5, p: 0.6; 2840±840 g and 2938±398 g, p: 0.8).

Table 5.4: Obstetric outcome in patients undergoing pre-reduction CVS/MFR and MFR to singleton pregnancies.

	CVS and fetal reduction to 1	Fetal reduction to 1	p
Number of pregnancies	8	28	
Mean maternal age (SD)	34.9(2.4)	29.1(3.4)	<0.0001
Mean GA at reduction (SD)	12.1 (0.4)	10.9 (1.2)	0.02
Number of fetuses reduced	16	56	
Fetal loss < 24 w (%)	1/8* (12.5)	2/28 (7.1)	NS
Fetal death ≥ 24w	0	0	NS
Early neonatal loss	0	0	-
Mean GA at delivery (SD)	38 (3.7)	38.1 (1.5)	NS
Mean BW (SD)	2840 (840)	2938 (398)	0.8
Delivery <28 w	0/7	0/26	NS
Delivery 28-32 w	0	0	NS
Delivery 33-36w	1/7	4/26	NS
Delivery ≥ 37w	6/7	22/26	NS
Birth weight < 1500g	1/7	0/26	NS
Birth weight 1500-2499gr	0	2	NS
Birth weight ≥ 2500gr	6	24	NS

*: fetal loss because of congenital CMV infection

The differences in perinatal outcome are related to the number of fetuses after fetal reduction (Table 5.5), irrespective of pre-reduction CVS. Mean gestational age at delivery after fetal reduction to singleton pregnancies (37.5±3.7 weeks) was significantly greater than after reduction to twin pregnancies (35.1±3.1 weeks; p: 0.0006). There was a significantly higher preterm delivery rate (93/147

versus 5/33; p: 0;0001) and a higher frequency of low birth weight infants after MFR to twin pregnancies (52/287 versus 3/33; p: 0.0001).

Table 5.5: Obstetric outcome in patients with MFR only in relation with the ending number of fetuses after MFR.

	Fetal reduction to 2	Fetal reduction to 1	P
Number of pregnancies	152	36	
Mean maternal age (SD)	31.3±4.3	30.3±3.9	0.2
Mean GA at reduction (SD)	11.1±1.0	11.2±1.2	0.8
Number of fetuses reduced	152	72	-
Fetal loss < 24 w	11/304	3/36	NS
Fetal death ≥ 24w	1/293	0	NS
Early neonatal loss	15/292	0	NS
Mean GA at delivery (SD)	35.1±3.1	37.5±3.7	0.0006
Delivery <28 w	6/147	0	NS
Delivery 28-32 w	19/147	0	0.06
Delivery 33-36w	68/147	5/33	0.002
Delivery ≥ 37w	54/147	28/33	0.0001
Birth weight < 1500g	37/287*	1/33	NS
Birth weight 1500-2499g	167/287*	2/33	0.0001
Birth weight ≥ 2500g	83/287*	30/33	0.0001

* 287 : 304-11(fetal loss) – 6 (unknown weight)

2.3 General considerations.

Until recently, counseling patients about first trimester CVS followed by fetal reduction (De Catte, 1998(a); Eddleman, 2000; Brambati, 2001) was based on experience gained in a limited number of cases. Most series consisted of different types of multiple pregnancies, and comparison with multifetal pregnancy reduction without prenatal diagnosis was not available.

In our centre, first trimester prenatal diagnosis in triplet pregnancies established after ICSI has been encouraged in order to evaluate the possible increased risks of chromosomal abnormalities (In't Veld, 1995; Van Opstal, 1997; Govaerts, 1998; Aboulghar, 2001; Bonduelle, 2002; Hansen, 2002). Therefore, CVS was performed initially for each of the three fetuses in 20 triplet pregnancies. All fetuses were sampled successfully, and karyotypes were available for each of them. However, this policy caused two problems. Couples requesting multifetal reduction for their triplet pregnancy now were falsely

reassured about the outcome of their pregnancy by the presence of three normal karyotypes and subsequently declined the fetal reduction procedure. Besides, some couples took the sex of the fetus into account for the reduction procedure. Hence, a new policy was adopted so that only those fetuses to be left after fetal reduction were sampled. Our management evolved in the opposite direction of that by Eddleman (2000) who as experience grew, started sampling more fetuses than those intended to remain after reduction. Although we agree on the safety of CVS in multiple pregnancies, we assume that a greater number of needle insertions, augments the risk of adverse pregnancy outcome (Brambati, 2001).

Fetal loss after reduction in triplet pregnancies without CVS (5.3%) in our hands corresponds to the pregnancy loss rates after multifetal reduction reported by the leading experts in the field (6 to 8%)(Evans, 2001(a)). Our series demonstrated an additional early neonatal loss rate of 6.2%, possibly related to inadvertent delivery and inadequate neonatal resuscitation in primary care hospitals. Death was related to RSD (6/12) and periventricular bleeding type IV (5/6) in very prematurely born infants. The overall outcome in triplet pregnancies after fetal reduction was not affected by the fact that CVS for prenatal diagnosis had been done or not, but low birth weight was more frequent after CVS/MFR. The fetal loss rate observed after CVS/fetal reduction in our hands (3/134) is comparable to those reported by Brambati (2/72) (2001) and Eddleman (2/82) (2000). But, unlike Eddleman (2000) we did not observe a significantly lower fetal loss rate after CVS/fetal reduction after fetal reduction only. However, fetal reduction of chromosomally abnormal fetuses may improve the outcome in multiple pregnancies, and therefore neutralize the risks associated with pre-reduction CVS. If fetal reduction had been performed without prenatal diagnosis in those four triplet pregnancies with chromosomal abnormalities, leaving the chromosomal abnormal fetuses to evolve, and assuming that all these pregnancies would have been lost completely, the benefit of pre-reduction prenatal diagnosis would have been demonstrated (3/126 versus 19/122; p: 0.04). However, since two chromosomal abnormalities were confined to the placenta, and since spontaneous reduction of the fetus with chromosomal abnormalities not necessarily jeopardizes the complete pregnancy, these assumptions are highly speculative. More data will be needed to clarify this issue.

Chorionic villus biopsy did not influence the obstetric outcome of triplet pregnancies reduced to twins and singletons (Table 5.6). Significant differences in gestational age at delivery, preterm delivery and low birth weight rates, irrespective of pre-reduction CVS were only related to the ending numbers of fetuses after reduction, and the best results were seen after reduction to singleton pregnancies. Fetal losses assumed to be higher in pregnancies reduced to singletons, did not differ significantly in our data. These results support the introduction of pre-reduction prenatal diagnosis in order to prevent the birth of a severely handicapped child, since this can be achieved without increasing the risk of pregnancy complications.

Table 5.6: Pre-reduction CVS in triplet pregnancies.

	Eddleman 2000	De Catte 2001	Total
Nr of triplets	52	71	123
Nr of CVS	108	157	265
Nr of fetuses reduced	74	79	153
Fetal loss	2	3	5/216
Perinatal loss	0*	4	4*

* incomplete data, since some pregnancies were still in progess

Summary

Prenatal diagnosis before with fetal reduction does not increase nor reduce the risk of adverse pregnancy outcome compared with fetal reduction alone. Patients requesting fetal reduction may benefit from pre-reduction prenatal diagnosis by chorionic villus sampling. Additional cytogenetic information reassures the couple about the absence of chromosomal abnormalities in the fetuses remaining after reduction. As previously mentioned, sampling should only be done by experienced operators who perform both the CVS and the reduction procedure in the same patient.

Amniocentesis after first trimester fetal reduction is safe. There is less expertise needed, but in combination with fetal reduction the benefit of the selection is lacking. Pregnancy loss rates are comparable with those of CVS in combination with fetal reduction.

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Chapter 6

Psychological implications of multifetal pregnancy reduction

Mental health risks have been observed in parents with multifetal pregnancies. One year postpartum, 67% of the mothers having triplets reported psychological difficulties and 25% of the women were treated with antidepressants (Garel M, 1992). Moreover, infants of higher order multiple sets are at risk for neglect and developmental delays.

Multifetal pregnancy reduction lowered the incidence of prematurity, reduced maternal health risks and improved the “quality of life” for the twins of reduced pregnancies. But does it restore mental and psychological stability? And how do couples cope with the irony of a multifetal pregnancy with poor perinatal prognosis after years of infertility and the decision to eliminate long-desired fetuses to enhance the chance of taking home healthy babies? Are emotional reactions more severe and prolonged than experienced by infertile women who spontaneously miscarry because it is self-initiated? Little research on the psychological impact of multifetal pregnancy reduction has been done.

Follow-up of 20 families in The Netherlands indicated the unawareness of the risks and consequences of infertility treatment (70%), and the lack of information received related to the risk of multifetal pregnancies after ovulation induction. The majority of couples being able to discuss their uncertainties and problems related to the multifetal pregnancy and the reduction procedure experienced those conversations as positive and supportive (78%). Although the majority of couples were religious, their moral state did not influence their decision about fetal reduction. In addition, most couples were able to enjoy their pregnancy after the reduction procedure. About half of the couples experienced some conflicting emotions at the time of birth and the weeks thereafter. However, none of them showed permanent regrets or distress (Kanhai, 1994). These data seemed reassuring since fetal reduction procedures were lengthy in time and showed a high pregnancy loss rate (18.1%). Garel concluded after a 2 years follow-up study that mothers of triplets were more anxious and depressed, and showed more acute difficulties in their relationship with their children than mothers from reduced pregnancies, who accepted multifetal pregnancy reduction to achieve their parental goals. After a period of two years the majority of women had overcome the emotional pain associated with the reduction. One major

drawback is the lack of information on a great number of women who had miscarriage or who refused to participate in the study (Garel, 1997).

A semistructured questionnaire was used to gather information by telephone from 91 women; they were asked about their immediate and persistent psychological effects of the multifetal pregnancy procedure and of the decision to submit to the reduction. 84% of the women experienced the reduction procedure as stressful, 68% as emotionally painful, and very frightening. Mourning for the reduced fetuses was experienced by 70 % of the women, but lasted for a relatively short period. The majority of patients had little difficulty to detach emotionally from the reduced fetuses. Those women feeling more sadness and guilt were younger and more religious, desired larger families, and reacted more emotionally to the reduction procedure. Emotional distress was also related to the number of ultrasound scans performed before the reduction procedure. More frequent ultrasound scans intensified the emotional attachment to the pregnancy and made coping with the reduction procedure more difficult emotionally in 56 % of the women. In our centre, the number of ultrasound scans is limited; at this stage in pregnancy, it should be used predominantly to obtain the information necessary to correctly counsel couples about all aspects of multifetal pregnancies, with or without invasive procedures. The degree of emotional pain recalled by the couple was intensified if they had been watching the ultrasound monitor while the reduction procedure was carried out (Schreiner – Engel, 1995). Therefore, in our practice, the patient's television monitor is turned off during the procedure. Only 7 % of patients with good pregnancy outcome would reconsider the fetal reduction procedure. Those patients suffering a pregnancy loss after fetal reduction rated the procedure as physically more painful, stressful, emotionally difficult and they grieved considerably longer. However, 70% would elect to have the reduction again (Schreiner-Engel, 1995).

McKinney examined 42 patients after a multifetal pregnancy reduction and compared their emotional and psychological reaction to that of 44 women with singleton and twin IVF pregnancies. She observed no differences in the reactions at the time of delivery or in the immediate postpartum period, in those women with a successful pregnancy outcome. However, in patients suffering a pregnancy loss, more than 60% showed major depressive disorders in the reduction group as well as in the control group. Patients suffering a pregnancy

loss after fetal reduction were 17 times more likely to experience an episode of depression than patients with normal pregnancy outcome. In addition, women who already had a living child at the time of the multifetal pregnancy were more likely to develop depressions. In contrast, neither the religious or moral conviction nor their reproductive history was related to their mental health after fetal reduction. Fear to loose the entire pregnancy after fetal reduction was the greatest concern for 78% of the women, and 69% reported sadness and depression on the day of the reduction procedure. Surprisingly, significantly more patients experienced the fertility treatment as more stressful than the reduction, and 47% felt relieved not trying to raise three of four infants (McKinney, 1995).

Bergh showed that although the overall psychological well being of the parents after fetal reduction was good, the events around the reduction were experienced as chaotic and emotionally disturbing. She recommended that reduction procedures should be performed by a limited number of physicians in experienced centres with emphasis on the psychological support of the couple (Bergh, 1999). The aim of emphasizing bonding and coping with fetal loss at the time of fetal reduction is to reduce the anxiety that surrounds the multifetal pregnancy reduction procedure and to refocus the parents' attention towards the surviving fetuses. It is therefore important to get the couple to view the surviving fetuses. In addition, to strengthen the bond with the surviving fetuses, ultrasound pictures should be taken and shared with the couple. At last, the couple should be stimulated to send some "baby pictures" or be prepared to share information about their delivery and babies later, once again to reinforce normality of the pregnancy. However, at several points, this bonding process might fail. In 50% of the failing cases, time pressure brakes down the coping/bonding process (Britt, 2001).

Summary

Fetal reduction in patients with multifetal pregnancies after long years of infertility treatment is unquestionably a major paradox. Although pregnancy outcome benefits from the procedure, the emotional pain and the anxiety at the time of the reduction and in the weeks thereafter are tremendous. However, with normal pregnancy outcome, couples having had fetal reduction show emotional and psychological reactions identical to those of IVF couples with twins and singletons. To facilitate the coping process, one should actively reinforce the bonding with the remaining fetus(es) in a way that parents will experience their pregnancy as normal. Training and time may be the key factors in this process.

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Chapter 7

Conclusion and Future research

The high incidence of multiple births since the mid-seventies is related to the unrestricted use of artificial reproductive technology and ovulation induction. The increase in maternal age over the last three decades accounts only partially for the epidemic rise in twin pregnancies, but is probably not significantly related to the rise in higher order multiple deliveries. Unexpectedly, ART causes a significant increase in monochorionic pregnancies.

Early fetal loss rates in multiple pregnancies are high. At least 26% of gestational sacs disintegrate spontaneously, and after fetal heart activity is established, only 70% of triplet and 90% of twin pregnancies reach viability. Perinatal mortality rates in twin and triplet pregnancies are respectively about 5 and 8 times higher in population derived data. The gestational age at delivery and the mode of delivery determine fetal and neonatal losses in 313 triplet and 8964 twins pregnancies that we examined in Flanders. Early preterm delivery (<33weeks) is 8 times more frequent in twin and 24 times higher in triplet pregnancies. Very low birth weight infants are born respectively 9 times and 29 times more often in twin and triplet pregnancies. Long-term morbidity consists of mental and physical disabilities. At least one handicap affects 7.4% of twin, 21.6% of triplet and 50% of quadruplet pregnancies. Cerebral palsy has been found respectively 5 times and 17 times more frequently in twin and triplet survivors than in singletons.

Maternal risks include anaemia, post-partum haemorrhage (10%), hypertension and pre-eclampsia (30%), gestational diabetes (10%), and maternal death (10/100 000). Psychological and psychiatric decompensation within 2 years after delivery of healthy triplets affected 20% of the mothers. Fetal death in multiple pregnancies is associated with a higher morbidity and mortality in the remaining fetus(es). Particularly in monochorionic pregnancies there is a high rate of neonatal death and sequellae in the survivors. Expectant management is recommended until the surviving fetus(es) can be delivered safely. Congenital malformations are more frequently observed in multiple pregnancies and particularly after fertility treatment including ICSI. These malformations may jeopardize pregnancy outcome. Specific complications related to monochorionicity – TRAP sequence, twin-to-twin transfusion syndrome,

monamnionicity and cord entanglement, and conjoined twins - carry a tremendously high risk of pregnancy failure.

Chorionicity in multiple pregnancies must be determined in the first trimester; it is the corner stone of prenatal diagnosis and fetal reduction procedures. The composite approach, using the number of gestational sacs, the number of yolk sacs and amniotic cavities, and the number of fetal poles, has a sensitivity and specificity of nearly 100%. Later on in gestation these parameters become less discriminative.

Prenatal diagnosis has become more solicited in multifetal pregnancies because of the steep rise in numbers, the aging of the pregnant population and the introduction of different first trimester screening policies for fetal aneuploidy. Amniocentesis has been shown to be accurate and safe. The use of a dye has become obsolete. Transmembraneous procedures are not widely used, but can decrease inadvertent sampling of the same amniotic cavity. The introduction of FISH on uncultured amniocytes significantly fastens the cytogenetic investigation. But, in case of a selective feticide, pregnancy loss rates may be higher. Unpublished data from amniocenteses performed in our centre reveal a low sampling failure rate, a total fetal loss rate of 3.3%, and sporadic culture problems. First trimester CVS has become an accurate and valuable technique of prenatal diagnosis in multiple pregnancies, and suits the new screening policies for fetal aneuploidy well. However, it should be performed only be experienced operators, taking into account the selectivity rules, the back-up long term cultures or additional investigational tools in cases of doubtful results. Our data show that, after an appreciable learning curve, CVS in twin and triplet pregnancies is almost 100% successful, has an acceptable low loss rate, and provides correct genetic information in nearly all instances. Nevertheless, compared with amniocentesis, it is associated with a higher number of additional diagnostic investigations, mainly because of sampling errors and placental mosaicism. CVS enables selective feticide in an early stage of gestation probably resulting in a more favorable pregnancy outcome and a less traumatic experience.

Multifetal reduction in higher order multiple pregnancies increases survival rates and reduces obstetric complications for the remaining fetuses at an acceptable small pregnancy loss rate. The lowest preterm delivery rates were observed after reduction to singletons, the highest survival rates after reduction to

twin pregnancies. Our unpublished series of fetal reduction in 72 high order multifetal pregnancies confirms a consistent reduction in fetal loss rate, a increase in gestational age at delivery and a decrease in both very early preterm deliveries and very low birth weight infants compared with expected values for non-reduced high order pregnancies.

Fetal reduction in triplet pregnancies carries a low pregnancy loss rate, and results in an improved pregnancy outcome. However, reduced twin and singleton pregnancies do not evolve quite as well as non-reduced ones. In 188 consecutive fetal reductions in triplet pregnancies to twins and singletons, we found that although the outcome was not identical to that of non-reduced twin and singleton pregnancies, a substantial benefit of the reduction procedure was noticeable. Fetal reduction to a singleton pregnancy for social indications remains controversial; nevertheless there is a low risk of adverse pregnancy outcome as demonstrated by the 38 cases we performed. Therefore, psychosocial indication for fetal reduction should be accepted as a valuable alternative to interruption of pregnancy. Multichorionic pregnancies complicated by congenital malformations or presenting obstetric risk factors may benefit from selective reduction. In most cases, risk for pregnancy loss is acceptably low, and the outcome for the remaining fetus better than in non-reduced twin pregnancies discordant for congenital malformations. In our small series, the outcome after the selective feticide was better if performed before the 14th week of gestation, and for chromosomal malformations rather than for structural defects. Selective feticide in monochorionic twin pregnancies under ultrasound guided injection of histoacryl or monopolar/bipolar thermocoagulation necessitates less experience and less expensive equipment than fetoscopic approaches. Nevertheless, complication rates and pregnancy loss rates remain much higher than after feticide with potassium chloride in dichorionic pregnancies. Our limited experience suggests at least the presence of a steep learning curve in the fine-tuning of these procedures.

Prenatal diagnosis prior to fetal reduction does not increase the risk of adverse pregnancy outcome compared with fetal reduction alone. Patients requesting fetal reduction may benefit from pre-reduction prenatal diagnosis by chorionic villus sampling as shown by our series of 71 triplet pregnancies. Additional cytogenetic information reassures the couple about the absence of

chromosomal abnormalities in the remaining fetuses post-reduction. As previously mentioned, should experienced operators perform both the CVS and the reduction procedure in the same patient. Amniocentesis can safely be done after first trimester fetal reduction. There is less expertise needed, but in combination with fetal reduction the benefit of the selection is lacking. Pregnancy loss rates are comparable with CVS in combination with fetal reduction.

To carry out a fetal reduction in patients with multifetal pregnancies who submitted during long years to infertility treatment is very paradoxical. Although pregnancy outcome benefits from the procedure, the emotional pain and the anxiety at the time of the reduction and in the weeks thereafter are tremendous. However, with normal pregnancy outcome, couples having had fetal reduction show emotional and psychological reactions identical to those of IVF couples with twins and singletons. To facilitate the coping process, one should actively reinforce the bonding with the remaining fetus(es) in a way that parents experience their pregnancy as normal. Training and time may be the key factors in this process.

We feel that much more attention should be paid to prevention of multiple births, better instruction of the couples about the risks of fertility treatment and obstetric risk involved with multiple pregnancies, correct diagnosis of chorionicity with centralizing obstetric care for monochorionic pregnancies, the promotion of first trimester prenatal diagnosis by chorionic villus sampling, and demystifying fetal reduction procedures for triplet and twin pregnancies. Prevention of multiple births without reducing the pregnancy success rate in fertility programmes is one of the major goals. Preliminary data on single embryo transfer show the potential benefit of intrauterine insertion of one top quality embryo for younger infertility patients. Although sonographic criteria for the detection of chorionicity in the first trimester have been published for more than a decade, it remains a major challenge to instruct all obstetricians to routinely check for monochorionic pregnancies. A nationwide screening program early in the first trimester would allow identification of nearly all monochorionic pregnancies. Since this is a small population at high risk, one should study the benefit of obstetric follow-up in tertiary care centres only using uniform guidelines. Prenatal diagnosis in patients with long standing infertility is often considered dangerous and is therefore avoided by parents and physicians. Systematic implementation of fetal

aneuploidy screening by nuchal translucency thickness and the degree of nasal bone development in multichorionic pregnancies could reduce the need for invasive diagnostic procedures. Further research needs to clarify the sensitivity and false positive rate of this screening method in the different fertility treatment regimes. A prospective randomized trial comparing CVS and amniocentesis in multiple pregnancies in centres equally experienced in both techniques would elucidate many of the controversies concerning differences in pregnancy outcome and malaises with cytogenetic investigation. It would also be interesting to study the psychological impact of fetal reductions on a larger scale, and related to the mode of conception, the duration of fertility treatment, and the immediate and long-term outcome of the offspring. Future investigations should focus on the potential advantages of fetal reduction procedures on the short and long term morbidity of the remaining infants. Furthermore, there are no data on the neurological and cognitive development of children born after fetal reduction procedures, and information about improved parent-child(ren) interaction after fetal reduction is lacking. Moreover, we question the impact of parental age and careers on raising children of multiple births.

Chapter 8

Published papers

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FIRST TRIMESTER CHORIONIC VILLUS SAMPLING IN TWIN GESTATIONS

Luc De Catte, M.D., Inge Liebaers, M.D., Walter Foulon, M.D., Maryse Bonduelle, M.D., and Elvire Van Assche, M.Sc.

ABSTRACT

First-trimester prenatal diagnosis was offered to 104 twin pregnancies mainly for advanced maternal age and cytogenetic evaluation of a new fertilization technique. Chorionic villus sampling (CVS) was performed transcervically (35%), transabdominally (23%), or by combination of these two techniques (42%). Although no placental biopsy failures occurred, two errors in fetal sexing were recorded due to non-selective placental sampling. In these two cases, both fetuses were sampled transcervically. Cytogenetic results were available for all fetuses; six of them showed an abnormal direct chromosomal pattern, but long-term villi culture analysis or additional amniocentesis ($n = 1$) reduced the number to four. Early fetal loss (3.4%) and perinatal mortality (6.3%) after CVS were comparable with a control group of 101 consecutive twin pregnancies without prenatal diagnosis (respectively 6.9% and 5.3%). Perinatal loss in the CVS group was associated in 10 of 12 fetuses with preterm premature rupture of the membranes and consequent preterm delivery. Mean gestational age at delivery, mean birthweight and the frequency of preterm delivery, and low birthweight infants were nearly identical in both groups. This study shows that CVS in the first trimester of pregnancy is an accurate and fast approach for prenatal diagnosis in twin gestations with an acceptable risk of adverse pregnancy outcome. However, a transcervical approach for both fetuses is not recommended.

Keywords: Prenatal diagnosis; twin gestation; first trimester; chorionic villus sampling

INTRODUCTION

Chorionic villus sampling (CVS) in single pregnancies has become a widely accepted technique for prenatal diagnosis in the first trimester.[1] Although the estimated fetal loss rate compared with second-trimester amniocentesis is slightly higher, genetic analysis is reliable and accurate.[1,2] The need for prenatal diagnosis in twin pregnancies emerges from the increase in multiple gestations, partly because of an advanced maternal age at conception, and partly because of extended use of assisted procreation techniques.[3] In addition, the probability of at least one twin having a clinically relevant chromosomal abnormality is about 1.6 times higher compared to a singleton at the same maternal age.[4,5] Usually these chromosomal anomalies are discordant in dizygotic twins, and, surprisingly, even occasionally in monozygotic pairs.[6] Prenatal diagnosis in the second trimester may therefore raise the need for late selective feticide, and hence compromise the outcome of the normal twin.[7] Although multifetal reduction in the first pregnancy trimester bears a significantly lower risk,[8] only a few papers describe the efficacy and reliability of early prenatal diagnosis through CVS in twin gestations.[9,10] Pergament and associates collected 128 multiple gestations undergoing CVS at four different centers, of which two performed 15 cases or less. In the study by Wapner and associates, the outcome after 161 CVS and 81 amniocenteses in one center were compared.

In this study we analyzed the feasibility and accuracy of CVS in twin pregnancies according to the method of sampling. Perinatal outcome was compared

Department of Obstetrics and Gynecology, and Medical Genetics, Akademisch Ziekenhuis Vrije Universiteit Brussel, Brussels, Belgium

Reprint requests: Dr. De Catte, Division Maternal–Fetal Medicine, Department of Obstetrics and Gynecology, Akademisch Ziekenhuis V.U.B., Laarbeeklaan 101, 1090 Brussels, Belgium

with a group of twin pregnancies followed in our prenatal unit over the same period of time, from the first trimester of pregnancy onward, but without prenatal interventions.

MATERIAL AND METHODS

Prenatal diagnosis was offered to 104 twin pregnancies (mean maternal age: 33.2 years; SD: 3.7) from January 1988 to December 1993. Genetic counseling in the early first trimester instructed the couple about the available diagnostic techniques, the present knowledge of the risks involved, the difficulties related to the selectivity in sampling, and the likelihood of discordant cytogenetic results.

CVS was performed under ultrasound guidance (Toshiba, SSA 250, SSA 270, curved linear probe, 3.75 and 5 MHz) from 9 weeks of gestation onward. Detailed sonography prior to the chorion sampling was used to identify the placental localization and its chorionicity, to clearly map all fetuses and placentas, and to discuss the technical approach for each fetus individually. Chorionic villi were aspirated transcervically by a Portex catheter or transabdominally, using a double needle system (outer needle 18G, inner needle 20G). Selective sampling of the chorion frondosum of each fetus may necessitate the combination of both aspiration methods. Short-term chromosomal analysis was obtained within 72 hours;[11] in all cases, a cultured chromosomal preparation was attempted[12] to differentiate, if appropriate, between true fetal and placental confined mosaicism.

The efficacy of the procedure and the selectivity in sampling were analyzed and compared. The safety of the procedure was evaluated by the number of spontaneous abortions (< 500 g), fetal death (> 500 g or 22 weeks) and total pregnancy loss. Obstetric outcome was assessed by the frequency of preterm delivery, low birthweight, and the perinatal mortality rate and compared with that of a control group of 101 consecutive twin pregnancies (mean maternal age: 30.0 years; SD: 4.3) without prenatal diagnosis.

Statistical analysis, at a significance level of $p < .05$, included chi-square test and Student's t test when appropriate.

RESULTS

Chorionic villus biopsy was performed in 104 consecutive twin gestations at a mean gestational age of 10.9 weeks (SD: 0.83; range 9 to 13 weeks). Nearly 80% of the samples were taken in weeks 10 and 11 (80 of 104). Indications for CVS included advanced maternal age ($n = 48$) (mean age: 36.4 years; SD:1.9), cytogenetic evaluation of pregnancies established by intracytoplasmic sperm injection (ICSI; $n = 42$), psychological comfort ($n = 7$), parental chromosomal abnormalities ($n = 4$), and other ($n = 3$).

CVS resulted in a sufficient amount of villi in all cases to perform a short- and long-term chromosomal analysis, except for 18 samples, of which only a short-term analysis was available. In 36 twin pregnancies (35%), CVS was performed transcervically for both fetuses at a mean gestational age of 10.8 weeks (SD: 0.82). The mean amount of chorionic villi retrieved for twin A and twin B was respectively 15 mg (SD: 11.0) and 14 mg (SD: 6.3). Twenty-four patients had a transabdominal CVS in both fetuses (23%) at a mean gestational age of 11.3 weeks (SD: 0.69). A sufficient amount of villi was sampled in all cases, with a mean of 14 mg (SD: 4.2) for twin A and 13 mg (SD: 4.0) for twin B. A combined transcervical–transabdominal approach enabled a first-trimester prenatal diagnosis in 44 twin pregnancies (42%). Mean gestational age at the time of the procedure was 10.8 weeks (SD: 0.89). The transcervical and the transabdominal route yielded, respectively, a mean amount of 15 mg (SD: 7.4) and 13 mg (SD: 4.5) of chorionic tissue.

The mean number of instrumental insertions (Portex catheter or guide needle) per pregnancy, presented in Table 1, was significantly less in the transabdominal CVS group (2.0; SD: 0) compared with the transcervical group (2.36; SD: .67; $p = .01$) and with the combined transcervical–transabdominal group (2.18; SD: .39; $p = .03$). The mean number of aspirations performed did not significantly differ in the three groups, although in the transabdominal CVS group, the mean number of aspirations was slightly higher [2.54 compared with 2.36 (transcervical group) and 2.39 (combined group)].

Cytogenetic analysis was available for all twin pairs. Two errors in fetal sexing occurred in two twin

Table 1. Technical Aspects of First-Trimester CVS in Twin Pregnancies in Association with Fetal Wastage

	Insertions per Pregnancy Mean ± SD	*Aspirations per Pregnancy Mean ± SD*	*Total Fetal Loss*	*Corrected Fetal Loss*
Transcervical ($n = 36$)	2.36 ± 0.67[a]	2.36 ± 0.67	5/72[†]	3/72
Transabdominal ($n = 24$)	2.0 ± 0[a,b]	2.54 ± 0.96	8/48#	6/48
Combined ($n = 44$)	2.18 ± 0.39[b]	2.39 ± 0.38	6/86*	6/86

[†] Including one early twin-to-twin transfusion syndrome.
Including two cases with major congenital malformations.
* Two multifetal reductions were performed in this group.
[a] $p = 0.01$
[b] $p = 0.03$

Table 2. Pregnancy Losses after First-Trimester Chorionic Villus Sampling, Compared with a Twin Control Population

		CVS Group 104 Pregnancies 208 Fetuses	*Control Group 101 Pregnancies 202 Fetuses*	*P Value*
Elective termination	TP	—	—	—
	S	2	—	—
Early pregnancy loss	TP	3	7	NS
	S	1	—	
Late pregnancy loss	TP	3	1	—
	S	2*	1	—
Neonatal loss	TP	2	3	NS
	S	—	1	
Total fetal loss		15	17	NS

* Fetal death due to congenital malformations.
TP = total pregnancy
S = single fetus
NS = not statistically significant

pregnancies. Both gestations were sampled transcervically early in our experience with CVS in multiple gestations. In the first case, chromosomal analysis revealed a normal female karyotype twice. However, amniocentesis performed after sonographic suspicion of one male fetus revealed a normal male and female karyotype. In the second case, a mixed male–female karyotype was found in both fetuses. A normal male and female fetus were observed on second-trimester ultrasonography. No further investigation was carried out. Chromosomal abnormalities were found in five pregnancies, involving six fetuses. In one twin pregnancy, both fetuses presented a marker chromosome (47,XX, +mar). Other chromosomal aberrations included a 45,X, a balanced de novo translocation *t*(10;14), and two cases of tetraploidy on short-term analysis. Long-term culture analysis in one of these two tetraploidy cases revealed a normal 46,XX karyotype. In the second case, amniocentesis was necessary to show a normal female fetus.

In two twin pregnancies, an embryo reduction to a singleton pregnancy was performed for obstetric reasons. Hence, pregnancy outcome was studied in 104 twin pregnancies or 206 fetuses (Table 2). The rate of spontaneous abortions (< 500 g) in twin pregnancies after first-trimester CVS (7 of 206 = 3.4%) was comparable with that of the control group (14 of 202 = 6.9%). In three patients, the total pregnancy was lost after CVS, and once a cytogenetically abnormal fetus (45,X) aborted spontaneously. Only one pregnancy was lost within 72 hours following the CVS procedure, after an episode of maternal fever. A fast developing oligopolyhydramnions sequence resulted in the demise of both fetuses before 20 weeks of gestation in a second pregnancy. No etiology was found in the third total pregnancy loss. Two out of the three cases of total pregnancy loss occurred after a transabdominal approach of both fetuses. Perinatal mortality in the CVS group involved seven pregnancies, of which five were lost completely. The perinatal mortality rate was 12 of 197 (6.1%), compared with 10 of 188 (5.3%) in the control group. Fetal death in the CVS group was associated with a major congenital malformation in two pregnancies (one polymalformed fetus, one corpus callosum agenesis with cardiac defect). Five twin pregnancies suffered from premature preterm rupture of the membranes (PPROM) at a mean gestational age of 22 weeks. Expectant management resulted in poor obstetric outcome since delivery in only one pregnancy could be delayed until 29 weeks. Nevertheless, both neonates died. PPROM occurred three times after a combined transcervical–transabdominal approach. The total fetal loss rate was 15 of 206 or 7.3%.

Obstetric outcome (Table 3) was available for 197 neonates after first trimester CVS and 188 twins from the control group. Mean gestational age at delivery in the CVS group was 35.7 weeks (SD: 3.3) and did not differ significantly from the control group (mean: 36.0 ± 3.1). A mean birthweight of 2392 g ± 632.1 g and 2348 g ± 620.0 g was observed respectively in the CVS and control group. Fifty-one pregnancies ended preterm (50%) in the CVS group compared with 49 (52%) in the control group. No differences in delivery rates before 33 weeks were observed in both groups (respectively 10 of 101 and 9 of 94). Low birthweight (LBW) rates were identical in the two groups: 100 of 199 in the CVS group, and 92 of 188 in the control group. Analysis of perinatal outcome according to the sampling route did not reveal significant differences in mean birth weight, LBW rates, mean gestational age at delivery, and preterm delivery rates.

DISCUSSION

Amniocentesis is an accepted, accurate, and reliable technique of prenatal cytogenetic diagnosis in twin gestations.[4] Selectivity of sampling is assured by injecting a dye in the first punctured amniotic cavity

Table 3. Obstetric Outcome after First-Trimester Chorionic Villus Sampling, Compared with a Twin Control Population

	CVS Group	*Control Group*
Gestational age at delivery: mean ± SD	35.7 ± 3.3 wk	35.6 ± 3.5 wk
Preterm delivery < 37 wk: number (%)	51 (51)	49 (53)
Preterm delivery < 33 wk: number (%)	10 (10)	9 (10)
Birthweight: mean ± SD	2392 ± 632 g	2348 ± 620 g
LBW* < 2500 g: number (%)	100 (50)	92 (49)
LBW* < 1500 g: number (%)	20 (10)	16 (9)

* Low birthweight

after aspiration of the amniotic fluid. Total pregnancy loss following amniocentesis in twin gestations is about 5.8%,[4,13] which is three times the rate in singletons.[14] In addition, nearly 6% of twin pregnancies experience the demise of one fetus. The detection of a chromosomal abnormality in one twin fetus often ends in the termination of the entire pregnancy. Selective feticide of the abnormal twin fetus in the second trimester of gestation is associated with a higher morbidity and mortality of the normal twin.[8] The increased incidence of twinning in recent years, partially because of advanced maternal age at procreation, and partially because of assisted procreation programs, implies an increased demand for prenatal diagnosis.

First-trimester prenatal diagnosis by CVS in singleton pregnancies has been available for 10 years and is widely accepted.[1] The use of this technique in twin pregnancies has only been described in a few hundred cases.[9,10] It implies great care in selectivity of sampling and sample processing.[15] To optimize a selective approach in every fetus, the type of CVS procedure is chosen only after careful analysis of the placental localization. In our study, chorionic villi were obtained from both fetuses in all twin pregnancies. Selectivity was successfully managed in all cases of a combined transcervical–transabdominal or a transabdominal approach only. However, chromosomal analysis after transcervical CVS in two twin pregnancies did not reveal the appropriate karyotypes. Both cases occurred early in our experience with CVS in twin gestations. An admixture of chorionic villi from the more proximal lying chorion frondosum occurred during aspiration of the most distal placenta. Diagnostic errors were mentioned respectively in 2 of 161 and 2 of 256 multiple gestations undergoing CVS in the series of Wapner et al. and Pergament et al.[9,10] The sampling technique in none of these four cases was described. However, to minimize this contamination problem, we recommend the use of transabdominal and transcervical samplings, the sampling at the level of the umbilical cord insertion, and meticulous demarcation of each implantation site and its borders.

The number of instrument insertions in CVS in singletons has been associated with an increase in procedure-related fetal losses.[16] This might be of importance since, in twin gestations, at least two instrumental insertions are necessary. Our data show that the number of instrumental insertions in the transabdominal CVS was significantly lower than in the other two applied techniques, because of the application of a guide needle. Nevertheless, the number of aspirations performed per gestation and the number of associated fetal losses were comparable in the three groups.

Direct cytogenetic analysis revealed an abnormal karyotype in 6 fetuses (3%); on long-term culture, only 5 abnormal results remained. All of the anomalies were of lesser importance. The 45,X fetus aborted spontaneously. None of the chromosomal abnormalities were related to neonatal impaired outcome. The low frequency of cytogenetic abnormal fetuses is probably related to younger age of the investigated population (33.2 years). Advanced maternal age was the indication for the CVS in only 46% of the cases, at a mean maternal age of 36.4 years. In our series, additional amniocentesis was performed in 2 twin pregnancies (2%), once for erroneous fetal sexing, a second time for a tetraploidy in both the direct preparations and long culture method. Both pregnancies evolved uneventfully. Amniocentesis following first-trimester CVS occurred respectively in 1 of 126 and 9 of 161 (6%) patients in the series of Pergament et al. and Wapner et al. In the latter group, fetal loss rate associated with two consecutive diagnostic procedures increased to 22%.[10] Fetal loss rates after the first-trimester CVS in twin gestations are comparable with those reported in the collaborative study on genetic amniocentesis in twin pregnancies.[4,9,10,13] Total fetal loss rate after CVS in twin pregnancies varies from 3.7 to 4.9%.[9,10] In our series, a slightly higher fetal loss rate of 7.3% was calculated, including the fetal demise of two congenitally malformed fetuses, the spontaneous abortion of a 45,X fetus, and the loss of a pregnancy complicated by a twin-to-twin transfusion syndrome. Exclusion of these non-procedure-related losses decreases the fetal loss

rate to 4.8%. Neonatal death was reported in 7 out of 294 live born infants (2.3%) in the group of Wapner, and in 4 out of 192 neonates in this series (2.1%). These figures match the neonatal death rates reported following amniocentesis in twin pregnancies and in our control population. Our data could not demonstrate a particular influence of the different sampling procedures in relation to the pregnancy loss rates. We encountered 7 cases of PPROM, of which 5 occurred in the midsecond trimester. In 4 cases a combined transcervical–transabdominal approach was performed. However, microbiological investigation of all fetuses and placentas denied the presence of an infectious agent. Even the mean number of instrumental insertions and villus aspirations in this subgroup was not significantly increased.

The overall obstetric outcome in twin gestations after first trimester CVS equals that of a control twin population, under prenatal control from the first trimester of pregnancy, over the same period of time and in the same institution. However, we do realize that conclusions drawn from these two small groups have to be taken with caution. Indeed, significant differences in outcome measures would have to be unrealistically high, with just over 100 patients in each group, not allowing the performance of CVS in twin pregnancies in the first place. To address these important measures of outcome, much larger data sets and multivariate analysis are needed, as differences in mean maternal age, parity, previous pregnancy history, (in)fertility history, and socioeconomic factors may easily influence pregnancy course and outcome. Selectivity in placental tissue sampling should preferentially be established by a combined transcervical–transabdominal or a dual transabdominal approach only. Prenatal diagnosis at this stage of pregnancy enables a safer selective feticide in cases of a chromosomal abnormality in one of the fetuses.

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PRENATAL DIAGNOSIS BY CHORIONIC VILLUS SAMPLING IN MULTIPLE PREGNANCIES PRIOR TO FETAL REDUCTION

Luc De Catte, M.D., Michel Camus, M.D.,† Maryse Bonduelle, M.D.,‡ Inge Liebaers, M.D.,‡ and Walter Foulon, M.D.**

ABSTRACT

Ovulation induction and assisted-reproduction techniques have dramatically increased the incidence of high-risk multiple pregnancies over the past 10 years. Perinatal outcome may be improved by the use of multifetal reduction. The fetus to be reduced used to be selected only on technical grounds. We report on the results of prenatal diagnosis by chorionic villus sampling (CVS) during the first trimester in 32 multifetal pregnancies in which fetal reduction was requested. The mean gestational age at CVS was 10.5 weeks. Chromosomal analyses were available for all sampled fetuses, three of which were chromosomally abnormal. In 24 couples, fetal reduction to twin pregnancies was successfully carried out within 1 week after the CVS. In seven cases, the couples elected not to proceed with fetal reduction after receiving information that the chromosomal analysis was normal in all fetuses. Mean gestational ages at delivery were, respectively, 34.6 and 31.8 weeks in the reduced and the nonreduced groups ($p = 0.04$). No fetal losses occurred in either group; one neonatal death was observed after a preterm delivery because of preeclampsia in a twin pregnancy.

Prenatal cytogenetic diagnosis during the first trimester in multiple pregnancies prior to fetal reduction appears to be feasible, accurate, and safe. Abnormal chromosomal results indicate the fetus(es) to be reduced. The parents' decisions not to proceed with the fetal reduction procedure, where chromosomal results in all the fetuses were normal, were unexpected.

Keywords: Chorionic villus sampling; triplet gestation; fetal reduction; prenatal diagnosis

The incidence of multiple gestations has increased dramatically in recent years mainly as a consequence of assisted-fertilization techniques and ovulation-induction procedures. The increased medical risks associated with multiple-gestation pregnancies and their associated costs cannot be ignored.[1] The benefits of fetal reduction in high-order multiple gestations ($n \geq 4$ fetuses) are obvious, as improvement in perinatal outcome exceeds the risks of pregnancy loss associated with the technique.[2,3] However, fetal reductions in triplet pregnancies are still subject to debate. Several recent articles report on improved perinatal outcome in terms of decreased perinatal morbidity after reduction of triplet pregnancies to twins.[4–6] So far, the fetus to be reduced has been selected merely on the basis of ease of access to the gestational sac so as to inject potassium chloride into the fetal heart or the pericardial space. In a number of such pregnancies, however, prenatal diagnosis might have to be considered because of advanced maternal age[7,8] or because of cytogenetic evaluation of pregnancies established with the introduction of newly developed fertilization techniques such as intracytoplasmic sperm injection (ICSI).[9,10] Fetal reduction should then of course be guided by the cytogenetic results of the analysis. As fetal pregnancy reduction in the second trimester carries a significantly higher risk of

*Department of Obstetrics and Gynecology, Division of Feto-Maternal Medicine, †Centre for Reproductive Medicine, and ‡Centre of Medical Genetics, University Hospital Free University Brussels, Belgium

Reprint requests: Dr. De Catte, Division Maternal-Fetal Medicine, Dept. of Obstetrics and Gynecology, Akademisch Ziekenhuis V.U.B., Laarbeeklaan 101, 1090 Brussels, Belgium

pregnancy wastage,[3] prereduction prenatal diagnosis should be considered early in gestation by chorionic villus sampling (CVS).

The aim of this study is to demonstrate that cytogenetic investigation in multiple gestations by CVS can be combined successfully with first-trimester fetal reduction to twin pregnancies. The technical aspects of CVS and the cytogenetic results are discussed. Perinatal outcome of the pregnancies after this double invasive procedure in the first trimester is reported.

MATERIALS AND METHODS

From 1993 to 1996, 32 consecutive couples with multiple pregnancies (31 triplet and 1 quadruplet pregnancy) requested a fetal reduction proceeded by a prenatal diagnosis through chorionic villus sampling after extensive counseling. The indications for prenatal diagnosis were advanced maternal age in 14 patients, cytogenetic evaluation of in vitro fertilization (IVF) with ICSI in 16 women, and simply for the purposes of reduction in 2 cases. Counseling included extensive discussion of perinatal outcome in triplet- and reduced triplet pregnancies.[4–6] Difficulties with the interpretation of cytogenetic results derived from chorionic tissue (mosaicism and pseudo-mosaicism) as experienced after prenatal diagnosis in singleton pregnancies were discussed, as well as the possibility of a confirmatory amniocentesis in the second trimester in such cases. The additional risk of fetal loss associated with the CVS procedure was estimated to be < 1% per fetus. This risk was derived from data on CVS in twin gestations.[11–13] The technique of fetal reduction was explained, and the couple was informed of the 10% risk of total pregnancy loss.[2,3,14] Sonographic anatomical exploration was performed in all fetuses (Toshiba SSA 270, curved linear transducer, 5 MHz, Nasu, Japan) and crown-rump length was measured to test for early growth discordance. Detailed mapping of all fetuses and placentas in longitudinal and transverse sections was carried out to ascertain selectivity in villi sampling as well as in the fetal reduction. CVS was performed under ultrasound guidance at between 9 and 12 completed weeks of gestation. A double-needle system (18-gauge outer needle and 20-gauge inner needle) was used for the transabdominal CVS; transcervical aspiration was carried out by a Portex catheter (Portex ®, Kent, England). A minimum of 10 mg of chorionic tissue was collected for each fetus. The chorionic tissue was approached by transcervical, transabdominal, or a combination of both CVS techniques to ascertain the selectivity in retrieving villi. Direct and long-term cytogenetic analyses were carried out.[15,16] Direct cytogenetic results were reported to the obstetrician as normal or abnormal without reference to the sex of the fetus. Sonographically guided fetal reduction was performed transabdominally (22-gauge spinal needle) or transvaginally (20-gauge needle) within 1 week of the CVS procedure and on the basis of the direct chromosome preparations only. Potassium chloride (1 mEq/mL) was injected intracardially or as near as possible to the fetal heart. The needle tip was kept in situ until asystoly was observed for at least 2 min. Follow-up sonographic evaluation was performed 3 to 4 hr after the procedure. Oral amoxycilline treatment was started for 5 days, 500 mg t.i.d.

Data on the obstetrical outcome were collected for all completed pregnancies and include early fetal wastage, fetal death, neonatal death, gestational age at delivery, birth weight, pregnancy, and neonatal complications.

RESULTS

In 32 consecutive multichorionic multiple pregnancies referred for fetal reduction, a first-trimester prenatal diagnosis was performed for advanced maternal age in 14 patients (mean age at the time of the CVS: 36.4 years; *SD:*1.07; range 35 to 38 years), and for evaluation of ICSI as a new assisted-fertilization technique in 16 patients (maternal age < 35 years). Two couples insisted on having prenatal cytogenetic analysis performed before fetal reduction with no other indication.

In 23 triplet pregnancies, CVS was performed in all 3 fetuses at a mean gestational age of 10.5 weeks (*SD:* 0.7 weeks; range: 9 to 11 weeks). In 16 of these pregnancies, villi were retrieved by a combined transcervical (1 fetus) transabdominal (2 fetuses) procedure, in 6 cases by transabdominal sampling only, and in 1 case by transcervical route only. The mean number of aspirations per pregnancy to collect a sufficient amount of villi was 3.2 ± 0.41 (range: 3 to 4). Because the number of instrumental insertions per CVS and the fetal reduction of the precervical sac have been associated with an increased risk of adverse pregnancy outcome, CVS was limited to the two lowest precervical implanted gestational sacs in the remaining eight triplet and one quadruplet pregnancies. The procedures were performed at a mean gestational age of 10.5 weeks (*SD:* 1.1 week; range: 9 to 12 weeks). Seven out of these pregnancies were sampled transabdominally only, whereas in the two remaining pregnancies chorionic villi were retrieved by a combined approach. The mean number of aspirations to collect the required amount of villi was 2.6 ± 0.48 (range: 2 to 4).

Direct and long-term chromosomal preparations were available for all fetuses (n = 87). In two triplet pregnancies, one of the three direct preparations showed an abnormal karyotype: one short-term analysis revealed a complete trisomy 20 (47,XX,+20), while the other displayed a mosaicism for a chromosome of the C group (46 XX/47,XX,+C). These fetuses were selectively reduced. Long-term culture cytogenetic analysis of the villi and of amniotic fluid aspirated at the time of the reduction revealed a normal female karyotype for both cases. In one other triplet pregnancy, karyotyp-

Table 1. Perinatal Outcome in Triplet Pregnancies After CVS and/or Multifetal Reduction

	All Completed Pregnancies n = 25	*Twin Pregnancies n = 20*	*Triplet Pregnancies n = 5*
Gestational age at birth Mean weeks ± *SD*	34.0 ± 2.7	34.6 ± 2.5*	31.8 ± 1.9*
Birth weight Mean g ± *SD*	1952 ± 520	2063 ± 482†	1655 ± 518†

*p = 0.036.
†*p* = 0.008.

ing of the precervical fetus revealed a 47,XX,+18 on both short-term and long-term analyses. The other sampled fetus was cytogenetically normal. One week after CVS, the precervical fetus was selectively reduced, while a CVS procedure was performed in the third fetus, which was found to be chomosomally normal. Prenatal sex predictions corresponded with postnatal observations in all cases, and no clinical signs of chromosomal abnormalities were present.

Among the 32 pregnancies initially scheduled for fetal reduction, 7 couples cancelled the procedure after having been informed of the normal chromosomal analysis in all fetuses. In three triplet pregnancies, fetal reduction was performed transvaginally at a mean gestational age of 10.6 weeks (*SD:* 0.5 weeks). In all cases the fetus in the precervical sac was approached, and the reduction was accomplished in one single attempt. In the remaining 22 triplet pregnancies, the transabdominal fetal reduction occurred at 11.2 weeks (*SD:* 0.67 weeks; range: 10 to 13 weeks). In only three cases was the precervically situated fetus reduced because of an abnormal chromosomal result. A second attempt was required in one case in which fetal heartbeat resumed after more than 2 min of asystoly. In none of the 22 reduced triplet pregnancies did major complications occur in terms of fever, infection, vaginal bleeding, or partial or complete pregnancy loss.

Obstetrical outcome was studied in 20 completed twin and 5 completed triplet pregnancies (Table 1). Neonatal data have been collected for all 55 neonates. Five twin and two triplet pregnancies are still ongoing uneventfully. All but two have evolved beyond 30 weeks.

The overall mean gestational age at delivery was 34.0 weeks (*SD:* 2.7; range 29 to 38 weeks), with a mean birth weight of 1952 g (*SD:* 520; range 820 to 2930 g). In the reduced triplet pregnancies, mean gestational age at delivery was 34.6 weeks (*SD:* 2.5; range: 29 to 38 weeks), compared with 31.8 weeks (*SD:* 1.94; range: 29 to 34 weeks) in the nonreduced triplets ($p = 0.04$) The mean birth weight in both groups was, respectively, 2063 g (*SD:* 482; range: 1050 to 2930 g) and 1655 g (*SD:* 518; range: 820 to 2560 g) ($p = 0.008$). Perinatal complications (Table 2) included eight cases of hypertensive disorders (pregnancy-induced hypertension/preeclampsia), six cases of preterm premature rupture of membranes (PPROM), two cases of preterm labor before 30 weeks of gestation, and one neonatal death after a preterm delivery at 31 weeks in a preeclamptic twin pregnancy. No fetal losses were encountered.

DISCUSSION

Perinatal morbidity and mortality can be efficiently reduced in triplet pregnancies by fetal reduction.[4–6] Twin pregnancies reduced from triplets have better outcome than conservatively managed triplet pregnancies. Performed in the first trimester of pregnancy, fetal reduction of triplets to twins is associated with a lower risk of total pregnancy loss and a decreased morbidity for the ongoing fetus(es).[3] The fetus to be reduced is usually selected because of technical accessibility, rather than the presence of sonographically depicted malformations or markers for chromosomal aberrations. At least two recent articles deal with the increased risk of chromosomal anomalies in twin pregnancies,[7,8] observations that are not completely confirmed by Pergament et al.[11] and Wapner et al.[12] Furthermore, some concern has arisen about a possible higher rate of sex-chromosome aneuploidies and structural defects in pregnancies conceived after IVF with ICSI.[9,10] In light of the foregoing, is it still ethically justifiable to perform fetal reductions in multiple gestations without prior cytogenetic investigation of the fetuses?

In the absence of major obstetrical risks (e.g., uterus unicornis or cervical incompetence), triplet pregnancies are usually reduced to twins. Because the number of invasive procedures performed in the first trimester of pregnancy correlates with the

Table 2. Perinatal Complications in 25 Pregnancies After Prenatal Diagnosis by CVS and/or Multifetal Reduction

	Reduced Pregnancies n = 20	*Triplet Pregnancies n = 5*
Hypertension	2	0
Preeclampsia	4	2
PPROM	5	1
Preterm labor	2	1
No complications	7	1

rate of pregnancy loss,[17] CVS might be limited to two of the three fetuses, so that the nonsampled third one can be selectively reduced if the cytogenetic results of the first two fetuses are normal.[18] This policy would reduce the number of invasive procedures by at least one. Diagnosis of a chromosomal abnormality in one of the investigated fetuses would result in its selective reduction, followed by chromosomal analysis of the noninvestigated fetus. Initially, however, our policy was to collect data on all fetal karyotypes before embryo reduction because this provided additional objective information on this recently developed, highly invasive method of fertilization.

Although all couples were extensively counseled in the same way, fetal reduction is often a psychological burden because most couples have been involved in infertility treatment for years. As shown from our data, seven couples initially requesting a fetal reduction decided to keep the triplet pregnancy after they were informed of the normal cytogenetic results for all the fetuses. More recently, the number of CVS procedures has been limited to the number of fetuses desired after fetal reduction. This protocol reduced the number of insertions per pregnancy in our series significantly from 3.2 to 2.6. Although the number of instrumental insertions has been associated with increased fetal loss rate, no differences were demonstrated in our small series.

CVS was performed only after a detailed sonographic study of the pregnancy. All fetuses were explored anatomically to exclude major structural defects and the related chorion frondosum was mapped in longitudinal and transverse planes. This comparatively time-consuming exploration is one of the cornerstones in establishing selectivity in aspiration of the villi and consequently in the feticide of a fetus possibly carrying a chromosomal abnormality or a genetic disorder.[19] For obvious reasons, we strongly recommend that a single operator should perform both invasive procedures.

The optimal timing for CVS is set at 9–12 weeks. The incidence of spontaneous embryo reduction in triplets has dropped by then because about 90% of such reductions occur during the first 7 weeks of gestation.[20] Furthermore, limb-reduction defects in fetuses after CVS have been associated with early (before 9 weeks of gestation) first-trimester placental aspiration.[21] CVS in triplets has been described only sporadically.[18] Among the 32 multifetal pregnancies we sampled, the combined procedure (transabdominal and transcervical) was applied in 18 cases. To combine the two CVS procedures, the operator should have extensive experience of both sampling methods. A complete transabdominal procedure was performed in 13 cases. The transcervical-only approach, as performed in one case, can be applied exceptionally to all three fetuses without compromising the selectivity of the samples.

Chromosomal analysis was successful for all fetuses. In total, a karyotype anomaly was observed in 3 of 87 (3.4%) short-term chromosome analyses, but in only one (1.1%) long-term chromosome study. Two of the three short-term abnormalities were confined to the trophoblast. Confined placental mosaicism,[22,23] and more particularly placental trisomy 22,[24] has been associated with third-trimester placental insufficiency, intrauterine growth retardation, and fetal demise. After reduction of these two fetuses, subsequent analysis from amniotic cells aspirated at the time of the feticide showed a normal karyotype. In a third pregnancy, where CVS was performed for maternal age (39 years), a trisomy 18 was found and selectively reduced. The presence of a severe chromosomal aberration in the precervical fetus led to a different approach and pregnancy outcome, as it is our policy to maintain the precervically situated fetus in multifetal reduction procedures without prior prenatal diagnosis.

Only two techniques for fetal reduction are currently used: the transabdominal and the transvaginal route. Preference for the use of one of either techniques is related mainly to the field of expertise of the operator. In our study, 87.5% of the reductions were performed transabdominally in accordance with the majority of the prenatal diagnostic techniques performed in our center. Physicians involved in infertility treatment are more familiar with the transvaginal technique, which resembles the transvaginal ovum pick-up procedure. Although some authors advocate the use of a spring-loaded device to perform the reduction procedure,[25] we used a free-hand single-operator technique. In all cases except one, the reduction was completed after one needle insertion; although the needle was withdrawn after 2 min of fetal asystole, normal fetal cardiac activity required a second intervention 3 hr later. A maximum of 2 cc potassium chloride (1 mEq/mL) was injected into or nearby the fetal heart. The precervical sac was avoided in cases of a transabdominal reduction procedure because the presence of dead tissue adjacent to the internal os might be responsible for ascending infection and subsequent fetal loss. There is no striking evidence as to the benefit of antibiotic treatment after multifetal reduction. However, we felt that the number of invasive procedures over a short lapse of time and the retention of the dead fetus presented a higher risk of subsequent infection, spontaneous abortion, or early PPROM. Six pregnancies ended preterm because of a detoriating preeclamptic condition, and two more were complicated by pregnancy-induced hypertension. This explains partially the rather low mean gestational age at delivery in the reduced triplet pregnancies. Preterm labor was easily managed beyond 30 weeks with bed rest and tocolytics in 3 other patients. Although multiple instrumental insertions were performed in the first trimester of pregnancy, this was not associated with early or late fetal loss. We believe that experience in combining different sampling techniques may contribute to lowering the complication rate in these pregnancies.

Multifetal reduction should not be considered a primary way to avoid high-order multiple gestations. Elective transfer of two good-quality embryos results in pregnancy rates nearly identical to those for the transfer of three embryos.[26] Moreover, in ovulation induction, overstimulated cycli should preferably be cancelled. However, if necessary, first-trimester prenatal diagnosis prior to the reduction procedure can be a way of selecting the fetus to be reduced. Our data support the technical feasibility, safety, and selectivity of first-trimester prenatal diagnosis in multiple gestations, and the cytogenetic accuracy to be relied on for fetal reduction.

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SELECTIVE FETICIDE IN TWIN PREGNANCIES WITH VERY EARLY PRETERM PREMATURE RUPTURE OF MEMBRANES

Luc De Catte, M.D., Monika Laubach, M.D., Adel Bougatef, M.D., and Carine Mares, M.S.

ABSTRACT

Nine consecutive multichorionic multiple gestations with early second-trimester (≤20 weeks) preterm premature rupture of the membranes (PPROM) of the lower gestational sac were managed expectantly. Mean gestational age at PPROM was 17.5 weeks (13–20 weeks), and the mean PPROM delivery time interval was 6.2 weeks (0–11 weeks). A fetal loss of 63% (12 of 19), and a subsequent neonatal loss of 57% (4 of 7) were observed. Of the four pregnancies evolving beyond 25 weeks, three delivered before 30 weeks. The baby take-home rate was 16% (3 of 19). Histologic evidence of chorioamnionitis was present in 5 of 7 (71%) investigated pregnancies.

Three other consecutive twin pregnancies were complicated by PPROM of the precervical gestational sac at 13 to 16 weeks of gestation (mean: 15 weeks). In the absence of clinical chorioamnionitis and amniotic fluid, selective feticide with potassium chloride was performed. Pregnancy was successfully prolonged beyond 33 weeks in two cases. The overall PPROM delivery time interval was 21 weeks (20–22 weeks). No neonatal losses were encountered. The baby take-home rate was 66% (2 of 3).

Selective feticide of the fetus with early midtrimester PROM in the absence of maternal signs of infection may improve the former unfavorable pregnancy outcome.

Keywords: Preterm premature rupture of membranes; PPROM; feticide; twin pregnancies

The management of singleton pregnancies with ruptured membranes before 26 weeks of gestation often consists in termination of pregnancy to prevent maternal and perinatal morbidity and mortality.[1] Recently, a number of studies have demonstrated that the overall survival rate in singleton pregnancies with preterm premature rupture of membranes (PPROM) before 26 weeks is about 40%.[1] However, before 20 weeks of gestation, the probability of pulmonary hypoplasia is over 50%,[2] and neonatal survival rates reach only 12%.[3] Independent risk factors for the development of pulmonary hypoplasia are the gestational age at PROM and the presence of severe oligohydramnios for more than 2 weeks.[4,5]. Maternal chorioamnionitis occurs in 46% of the cases.

Data on PPROM in twin gestations are scarce. Mercer and co-workers[6] reported the outcome in 101 cases of early third-trimester PPROM. No difference in infant survival rate was observed compared with a control group. Montgomery[7] reported similar observations in 80 twin pregnancies with PPROM between 25–36 weeks of gestation. However, information on the occurrence, management and outcome of early second-trimester PPROM prior to 20 weeks of gestation in twin gestations is lacking. One might speculate that the early onset and the chronic leakage of amniotic fluid puts the fetuses and the mother at an increased risk of infection and unfavorable pregnancy outcome. To preserve this pregnancy, and to eliminate chronic fluid leakage, Dorfman and co-workers[8] described successful outcome in one twin pregnancy after selective termination of the oligohydramnic fetus early in the second trimester.

To establish the obstetric and neonatal outcome after PPROM before 20 weeks of gestation, we analyzed the data of 12 consecutive multiple multi-

Department of Obstetrics and Gynecology, and Neonatology, University Hospital, Vrije Universiteit Brussel, Brussels, Belgium

Reprint requests: Dr. De Catte, Division Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University Hospital V.U.B., Laarbeeklaan 101, 1090 Brussels, Belgium

chorionic gestations. Nine pregnancies were managed expectantly. In three twin gestations a selective termination of the oligohydramnic fetus was attempted to preserve the normohydramnic co-twin.

MATERIALS AND METHODS

Data charts from all multiple gestations between January 1991 and June 1996 were analyzed for the occurrence of early PPROM before 20 weeks of gestation. All patients were listed for fertility treatment, and all except one became pregnant after assisted procreation. Gestational age and multichorionicity were documented sonographically in the first trimester of pregnancy in our center. Nine consecutive pregnancies presenting with a severe oligo- or anhydramnion of the precervical gestational sac, and with a clear diagnosis of ruptured membranes, were managed expectantly. The diagnosis of PPROM was made by the observation of vaginal liquid pooling by speculum examination, or the presence of ferning in association with an oligo-anhydramnion. Initial investigation consisted in maternal leucocyte count, cross-reacting protein (CRP), and ultrasound examination to exclude major malformations. No antibiotic or tocolytic treatment was installed routinely. Enhancement of fetal lung maturition was initiated at 24 weeks, and continued weekly thereafter with 2 × 12 mg of dexamethasone, 12 hr apart. After 24 weeks of gestation, delivery was performed in a tertiary care center with an attending neonatologist, responsible for resuscitation.

In all cases, but two, the placenta was submitted for pathological examination in search for histologic evidence of funisitis and/or chorioamnionitis. In cases where fetal pathological examination was approved by the parents, fetal lungs were examined for signs of pneumonia.

Three consecutive pregnancies, in which PPROM occurred before week 20 of gestation, were managed by selective feticide. In all cases, clinical and laboratory signs of chorioamnionitis were ruled out. The patients were counselled regarding the outcome of multiple gestations after multifetal reduction without intervening PPROM, and the outcome of early PPROM in our obstetric unit. Transabdominal selective feticide was performed by intracardiac injection of potassium chloride (1 meQ/mL) with a 22-gauge spinal needle under ultrasound guidance. Ampicillin, 500 mg orally 3 times a day, was started for 5 days. Ultrasonographic surveillance occurred every 2 weeks, and maternal inflammatory parameters were checked weekly.

Obstetric outcome was collected in terms of PPROM delivery time interval, fetal loss rate, premature delivery rate, neonatal loss rate, and baby take-home rate.

RESULTS

Analysis of the multiple gestations' data charts from 1991 until 1996 revealed nine multiple gestations with a PPROM ≤ 20 weeks (one triplet, eight twin pregnancies) (Table 1). Mean gestational age

Table 1. Expectant Management and Perinatal Outcome in Twin Pregnancies After Early Second-Trimester PROM

Case No.	*G.A. at PROM (Weeks)*	*Time Interval Until Delivery (Weeks)*	*Outcome BB1*	*Outcome BB2*	*Histology: Placenta Fetal Lungs*	*Remarks*
1*	20	4	fetal death†	fetal death† fetal death†	pneumonia	preterm labor
2	20	2	fetal death† expulsion at 20 weeks	fetal death† expulsion at 22 weeks	no chorioamnionitis	
3	20	6	neonatal death	neonatal death	chorioamnionitis; funisitis	
4	15	1	expulsion 17 weeks	36 weeks, normal outcome	‡	PPROM after amniocentesis
5	16	9	neonatal death; potter-like sequence	survival; normal development	no chorioamnionitis; pneumonia	
6	19	10	neonatal death	survival; normal outcome	‡	
7	13	8	fetal death†; expulsion at 21 weeks	PPROM 21 weeks; expulsion	chorioamnionitis; pneumonia; U. Urealyticum	spontaneous pregnancy
8	19	0	fetal death†	fetal death†	chorioamnionitis; pneumonia	
9	15	8	fetal death†	PPROM at 23 weeks; fetal death†	chorioamnionitis	

*Triplet pregnancy.
†Intrapartal fetal death.
‡Placental pathology not available.

Table 2. Outcome After Selective Feticide in Early Second-Trimester PPROM in Twin Pregnancies

Case No.	G.A. at PPROM (Weeks)	Time Interval Until Delivery (Weeks)	Outcome BB1	Outcome BB2	Pathology	Invasive Procedures
1	16	3	selective feticide	19 weeks, PPROM	no chorioamnionitis	–
2	13	20	selective feticide	33 weeks, 1760 g	no chorioamnionitis	–
3	16	22	selective feticide	38 weeks, 3320 g, PROM	no chorioamnionitis	CVS

at the occurrence of the PPROM in these nine pregnancies was 17.5 weeks, with a range from 13 to 20 weeks. PPROM presented in the lower gestational sac in all the cases. The mean interval of time between the rupture of membranes and the delivery was 7.4 weeks. In one case, however, delivery took place within hours following the amniotic fluid leakage. In five pregnancies, delivery was postponed for ≥8 weeks. In one twin pregnancy, PPROM occurred after amniocentesis. After early expulsion of the oligohydramnic fetus 2 weeks later, pregnancy continued for another 18 weeks. Four pregnancies evolved beyond 25 weeks. All of these patients delivered prematurely, and 3 of 4 pregnancies ended before 30 weeks. Fetal loss was observed in 12 of 19 (63%) fetuses. Of the seven live born fetuses, four died in the immediate neonatal period because of severe prematurity (57%). The baby take-home rate was 3 of 19 or 16%. Histologic proof of chorioamnionitis and funiculitis was present in five of the seven submitted placentas (71%). In four pregnancies, histologic evidence of fetal pneumonia was found in either one of the fetuses.

In three most recent bichorionic twin pregnancies complicated by early second-trimester rupture of membranes, selective feticide was performed (Table 2). Mean gestational age at the time of the PPROM was 15 weeks (range 13 to 16 weeks). There were no signs of chorioamnionitis present as maternal body temperature, leukocyte count and CRP were within normal ranges. In the third case, PPROM occurred 4 weeks after chorion villus sampling, revealing normal cytogenetic analysis for both fetuses. Severe oligohydramnios was observed in all the cases. Selective feticide was performed within 3 days after the diagnosis of PPROM. In all cases the procedure was successful at the first attempt, and <1 mL of potassium chloride was needed to achieve fetal asystoly. The mean time interval between PPROM and delivery was 15 weeks (range: 3 to 22 weeks). One pregnancy ended at 19 weeks after PPROM of the second gestational sac and subsequent spontaneous delivery. There were no histologic signs of chorioamnionitis. In the two other cases, the pregnancy evolved beyond 32 weeks. One pregnancy went into labor at 33 weeks after 4 weeks of parenteral β-mimetic tocolysis. The patient delivered spontaneously a healthy male infant weighing 1760 g, with an apgar score of respectively 6/9/10 after 1, 5, and 10 min. Placental weight was 350 g and pathological investigation showed no signs of chorioamnionitis. Twenty-two days later the baby was discharged from the neonatal ward. The third pregnancy evolved uneventfully, and repeated cervical swabs were negative. At 33 weeks of gestation, a sharp intermittent intravaginal pain was caused by the presence of a flat triangular shaped bony structure in the cervical os. By pathological examination it was characterized as a compressed fetal thoracic cage. PROM occurred at 38 weeks of gestation. The patient delivered vaginally a healthy girl weighing 3320 g, with an apgar score of 9/9/10. The intrauterine retention of placental fragments and fetal parts (lithopedion) had to be removed by aspiration. Antibiotic treatment was implemented. The infant is thriving well. Pathological investigation of the placenta revealed no signs of chorioamnionitis.

DISCUSSION

Conservative management in singleton pregnancies after early preterm PROM leads to lung hypoplasia, facial and limb deformations, chorioamnionitis, early preterm delivery, and fetal or neonatal death.[5,9] The stage of lung development at the time of rupture of membranes and the subsequent amount of amniotic fluid left determines the probability of fetal pulmonary hypoplasia.[2,4,5] Moreover, there is a strong relationship with the development of skeletal compression deformities, the development of lung hypoplasia and the degree and duration of the oligohydramnios.[2] The perinatal survival rate in patients with PROM before 23 weeks is about 13%.[2] Therefore, management by termination of pregnancy is an accepted policy in these immature singleton pregnancies with oligohydramnios.

However, if second-trimester amniotic fluid leakage occurs as a complication of prenatal invasive procedures, a more favorable perinatal outcome is expected at least in singleton pregnancies. Although Crane[10] cautions that a conservative management of postamniocentesis amniotic fluid leakage implies an increased risk for preterm delivery and fetal skeletal deformity, Gold et al[11] suggests that the same conservative approach could lead to reaccumulation of a normal amount of amniotic fluid and a term delivery. Even in a few cases without amniotic fluid reaccumulation, a normal outcome was encountered.[12] Amniotic fluid leakage related to amniocentesis may therefore represent a fundamentally different etiological entity.

Early second-trimester rupture of the membranes complicating twin pregnancies creates an even greater dilemma in obstetric management. Often, these pregnancies are established after several attempts of various assisted procreation techniques in couples with advanced maternal age. Because early rupture of membranes is not invariably associated with immediate fetal death, the couple experiences severe psychological stress. A conservative management frequently leads to high morbidity and mortality rates for the fetus with ruptured membranes. In our series, all fetuses presenting with PPROM died, whereas only three of the fetuses with intact membranes survived. All pregnancies delivered prematurely. We suggest that an ascending intra-amniotic infection may result from chronic amniotic fluid leakage and jeopardize the perinatal outcome of the normal co-twin. Of the six completely lost pregnancies in our group, five demonstrated signs of placental or fetal infection on pathological examination. In only one of these five pregnancies delivery followed within hours after the PPROM. In the remaining four, the PPROM delivery time interval was 4 to 8 weeks, supporting the hypothesis of secondary ascending infection.

Amniotic fluid leakage from one of the gestational sacs in twin pregnancies may be the result of prenatal diagnostic procedures. Information regarding the outcome of this complication in multiple gestations is hardly available.[13–16] However, 10 to 20% of fetal loss rates after amniocentesis are caused by early rupture of membranes, in which cases both fetuses are lost.[15,16] Expectant management of mid trimester rupture of the membranes in twin gestations after chorionic villus sampling (CVS) results in a high perinatal death rate.[15,17] In none of these cases a chorioamnionitis was present.[17] In our conservatively managed series only one out of nine PPROM cases occurred after an amniocentesis. Pregnancy outcome was favorable although that fetus was lost soon after the rupture of the membranes. Selective reduction of the fetus with the CVS related midtrimester PPROM led to the first successfully managed case of amniotic fluid leakage after chorion biopsy in multiple gestations in our experience.

Our data prove that, although their limited number, conservative management of PPROM in multiple gestations results in an extremely high fetal loss rate, high prematurity figures, and in a baby take-home rate of only 16%. The more aggressive approach in early preterm PROM complicating one of the twins, is termination of pregnancy. The risks of fetal and maternal complications, and late second-trimester fetal loss are avoided; however, for the couple it is a psychologically devastating experience.

Selective feticide may offer an alternative.[8] Selective feticide in the second trimester of pregnancy has been used predominantly in cases of fetal malformations and chromosomal abnormalities. Compilation of data from the largest series demonstrates the feasibility and the relatively low risk of pregnancy loss for the normal co-twin.[18] The decision to selectively interrupt the anhydramnic fetus may improve the prognosis of the normal co-twin. The cessation of urine production and long-term amniotic fluid leakage, may prevent early ascending intra-amniotic infections, and hence prolong pregnancy into the viable period. Maternal medical complications associated with the demise of one fetus in the early second trimester are rarely observed. Our data suggest indeed that this aggressive approach results in a prolongation of pregnancy far into the viable period in two of three pregnancies. Moreover, no signs of chorioamnionitis were present in these cases. Nevertheless, this second-trimester intervention may induce feelings of grief and anxiety so that psychological support should be provided during gestation and delivery.

Conservative management of early second-trimester PPROM in multiple gestations results in bad obstetric outcome. Although only a limited number of cases have been reported, selective feticide in cases of early second-trimester preterm PROM may favor the perinatal outcome of the normal co-twin. However, multicentric data analysis is needed to evaluate the usefulness of selective feticide for this indication.

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Obstetric outcome after prenatal diagnosis in pregnancies obtained after intracytoplasmic sperm injection

Ayse Aytoz[1,4], Luc De Catte[2], Michel Camus[1], Maryse Bonduelle[3], Elvire Van Assche[3], Inge Liebaers[3], Andre Van Steirteghem[1] and Paul Devroey[1]

[1]Centre for Reproductive Medicine, [2]Department of Obstetrics and Prenatal Medicine and [3]Center for Medical Genetics, University Hospital and Medical School, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium

[4]To whom correspondence should be addressed

In this study we compared the pregnancy outcome of 576 pregnancies after prenatal diagnosis with that of 540 pregnancies without prenatal diagnosis in our micro-injection programme. Amniocentesis was suggested for singleton pregnancies (n = 465) and chorionic villus sampling (CVS) was proposed for twin pregnancies (n = 111 pregnancies, 222 fetuses). A total of 365 patients with singleton pregnancies and 175 patients with twin pregnancies who did not undergo prenatal diagnosis were selected as controls. Compared with the controls, the odds ratios in the amniocentesis group for preterm delivery, low birthweight, very low birthweight and fetal loss were 0.97 [95% confidence interval (CI): 0.60–1.57], 1.27 (95% CI: 0.78–2.06), 1.57 (95% CI: 0.53–4.66) and 0.86 (95% CI: 0.32–2.37) respectively. Compared with the controls, the odds ratios in the CVS group for preterm delivery, low birthweight, very low birthweight and fetal loss were 0.89 (95% CI: 0.61–1.30), 1.03 (95% CI: 0.74–1.45), 0.79 (95% CI: 0.41–1.53) and 0.47 (95% CI: 0.17–1.30) respectively. We concluded that, in this series of intracytoplasmic sperm injection (ICSI) pregnancies, prenatal testing did not increase the preterm-delivery, the low-birthweight, or the very low-birthweight rates as compared with those of the controls. In the prenatal diagnosis group, the fetal loss rate was comparable to that of the control group. Larger prospective controlled studies are needed in order to inform patients reliably about the risks and the advantages of prenatal testing in ICSI pregnancies.

Key words: amniocentesis/chorionic villus sampling/intracytoplasmic sperm injection/pregnancy outcome

Introduction

Mid-trimester amniocentesis and first-trimester chorionic villus sampling (CVS) are now offered routinely to women at increased risk of having a child with a chromosomal abnormality, particularly to women over the age of 35. Since its introduction in 1991, the safety of intracytoplasmic sperm injection (ICSI) has been of particular interest. Consequently, a prospective follow-up study of pregnancies and children was carried out in our centre, including agreement to genetic counselling and prenatal diagnosis, and physical examination of the children born after ICSI (Bonduelle *et al.*, 1996). As part of the study, all patients were asked to undergo a prenatal diagnosis if they became pregnant. The question has been raised as to whether routine prenatal testing involves additional risks for the patients undergoing ICSI. In this study, we report on our experience with prenatal diagnostic techniques, i.e. mid-trimester amniocentesis for singleton pregnancies and first-trimester CVS for twin pregnancies after ICSI.

Materials and methods

The obstetric outcomes of 576 ICSI pregnancies after prenatal diagnosis were compared with the obstetric outcomes of 540 ICSI pregnancies without prenatal diagnosis at similar ages and parity. Obstetric outcomes of 904 pregnancies and prospective follow-up study of 877 children born after ICSI have been reported previously (Bonduelle *et al.*, 1996; Wisanto *et al.*, 1996). As part of the follow-up study, all patients were asked to undergo a prenatal diagnosis. The risks and benefits of the different types of prenatal diagnosis were discussed in detail at ~6 to 8 weeks of gestation. In our ICSI programme, 54.5% of couples agreed to undergo prenatal diagnosis. The main reason for not agreeing to prenatal diagnosis was fear of losing the pregnancy; a smaller group refused because of religious reasons. All ICSI patients had an ultrasound examination at the 7th week of gestation. The patients who agreed to prenatal testing had a second detailed structural ultrasound examination prior to the diagnostic procedure. Amniocentesis was suggested for singleton pregnancies and CVS was proposed for multiple pregnancies (De Catte *et al.*, 1996). The prenatal diagnosis was performed mainly by two experienced operators.

Before amniocentesis, the abdomen was disinfected with a polyvidone chloride solution. Amniocentesis was carried out transabdominally under ultrasound guidance using a 22 G spinal needle. A sample of 20 ml of amniotic fluid was removed from the amniotic cavity. A sample of 18 ml of amniotic fluid was sent to the cytogenetics laboratory for long-term culture and 2 ml was used to determine the concentration of α-fetoprotein. Chromosome preparations were obtained from cultured amniocytes according to a modified technique by Verma and Babu (1989).

The technique of CVS has been described previously (De Catte *et al.*, 1996). CVS was performed under ultrasound guidance (Toshiba, SSA 250, SSA 270, curved linear probe, 3.75 and 5 MHz). Detailed sonography prior to the chorion sampling was used to identify the placental localization and its chorionicity, to clearly map all fetuses and placentas, and to discuss the technical approach for each fetus individually. Chorionic villi were aspirated transcervically by a Portex catheter or transabdominally, using a double-needle system (outer needle 18G, inner needle 20G). Short- and long-term chromosomal

Table I. Cytogenetic diagnosis after prenatal procedure

Diagnosis	Prenatal procedure Amniocentesis	CVS
46,XY	240	103
46,XX	206	112
Cytogenetic abnormality	8 (1.72%)	5 (2.25%)
Failure	4 (0.86%)	2 (0.90%)
Missing results	7 (1.51%)	–
Total	465	222

CVS = chorionic villus sampling.

preparations from cultured chorionic villi were obtained by procedures previously reported (Yu *et al.*, 1986; Gibas *et al.*, 1987).

Obstetric outcomes after prenatal diagnosis were assessed by the frequencies of preterm delivery (<37 weeks), low birthweight (<2500 g), very low birthweight (<1500 g) and total pregnancy loss as compared with those of a control group. Patients who had two consecutive prenatal tests in order to confirm previous karyotyping or who had selective feticide for triplet or quadruplet pregnancies were not included in the study.

The χ^2 test or Fisher's exact probability test was used to compare the percentages in different groups and the independent risk effect from different prenatal diagnosis techniques was analysed using odds ratios computed with 95% confidence intervals (CI) (Medcalc, Medcalc Software, Ghent, Belgium).

Results

Amniocentesis was performed in 465 singleton ICSI pregnancies at a mean gestational age of 15.8 weeks (range 14–18). The mean maternal age was 32.9 years (range 22–45). The mean maternal age in the control group was 31.84 years (range 19–43). Chorionic villus biopsy was performed in 111 twin ICSI pregnancies at a mean gestational age of 11.1 weeks (range 11–14). The mean maternal age was 31.98 years (range 23–40) in the study group and 31.46 years (range 23–40) in the control group. The cytogenetic results of 222 CVS samples and 465 amniotic fluid samples are shown in Table I. The mean numbers (± SD) of needle insertions in the CVS group for the first and second fetus were 1.1 ± 0.3 and 1.1 ± 0.2, respectively. In one case the karyotype of one fetus was misdiagnosed after CVS. There were 343 males and 318 females with normal karyotypes. Six of 687 samples were not suitable for cytogenetic analysis. The results from seven prenatal diagnosis procedures performed in other centres could not be obtained (Table II). Thirteen out of 674 samples available for analysis were abnormal (1.93%). Of these seven were *de novo* (four sex-chromosomal aberrations and two reciprocal translocations) and six were inherited chromosomal aberrations (one reciprocal, one Robertsonian, two inversions and two supernumerary) as listed in Table II.

In the amniocentesis group, five pregnancies including four terminations (two for abnormal karyotypes, two for congenital malformations detected by ultrasound examination) ended before 20 weeks gestation, and five pregnancies were lost after 20 weeks of gestation. The total post-procedural loss rate, including one spontaneous abortion (loss before 20 weeks) and five stillbirths (loss at 20 weeks or later), was 1.30%

Table II. Cytogenetic abnormalities found after prenatal diagnosis

Abnormality	Prenatal Procedure	TOP
De novo		
47,XY,+21	CVS	+
47,XXY	Amniocentesis	+
47,XXY	Amniocentesis	+
47,XXY	CVS	–
47,XYY	Amniocentesis	–
46,XX,t(2;13)(p12;q32)	Amniocentesis	–
46,XY,t(4;5)(q21;q13)	CVS	–
Inherited structural		
46,XX,der(14;15)(q10;q10)	Amniocentesis	–
46,XX,t(2;5)[a]	Amniocentesis	–
46,XY,inv(5)(p13;q13)	Amniocentesis	–
46,XY,inv(1qh)	Amniocentesis	–
47,XX,+mar	CVS	–
47,XX,+mar	CVS	–

[a]Break points unknown.
TOP = termination of pregnancies; CVS = chorionic villus sampling.

(6/461). In the control group, 10 out of 365 pregnancies were lost (2.74%) beyond 15.5 weeks of gestation, which was the mean intervention date for amniocentesis. Five pregnancies were lost spontaneously before 20 weeks. Five fetuses died after 20 weeks gestation The total pregnancy-loss rates were not statistically different between the 2 groups (P = 0.22) (Table III). In patients <35 years, the pregnancy-loss rates were 1.69% (5/295) and 2.92% (8/274) in the amniocentesis and in the control groups respectively (not significant; χ^2 test), while in patients ⩾35 years the pregnancy-loss rates in the study group and in the controls were 0.60% (1/166) and 2.20% (2/91) respectively (not significant; Fisher exact test).

A total of 460 pregnancies after amniocentesis were evaluated beyond 20 weeks of gestation. Forty-one of these 460 pregnancies ended before 37 weeks gestation (8.91%). There were 46 infants (10.00%) weighing <2500 g and 10 infants (2.17%) weighing <1500 g at birth. In the control group, 360 pregnancies were evaluated beyond 20 weeks gestation. The prematurity, low-birthweight and very low-birthweight rates were 9.17%, 8.06% and 1.39% respectively (Table IV). The risk of having preterm delivery, low birthweight and very low-birthweight did not increase after amniocentesis. Compared with pregnancies without prenatal diagnosis, the odds ratios for preterm delivery, low-birthweight and very low-birthweight were 0.97 (95% CI: 0.60–1.57), 1.27 (95% CI: 0.78–2.06) and 1.58 (95% CI: 0.53–4.66) respectively, in the amniocentesis group.

A total of 222 chorionic villus samples were analysed in 111 twin pregnancies. One twin pregnancy was reduced to a singleton because of the presence of trisomy 21 in one of the fetuses. There were six spontaneous losses (2.71%) among women who underwent CVS and 11 losses (3.14%) in the controls. This difference was not significant (P = 0.97) (Table III). In the CVS group, the pregnancy-loss rate was 3.07% (5/163), where maternal age was younger than 35 years, and it was 1.72% (1/58), where maternal age was ⩾35 years. In the control group, the pregnancy-loss rate was 3.68% (10/272), where the mothers were younger than 35 years, and it was 1.28% (1/78) where they were ⩾35 years. The pregnancy-

Table III. Pregnancy losses after prenatal diagnosis and in the controls (number in parentheses are percentages)

Pregnancy losses[a]	Amniocentesis (singletons) (n = 461)	Control (singletons) (n = 365)	CVS (twins) (n = 221)	Control (twins) (n = 350)
Fetal loss <20 weeks	1 (0.22)	5 (1.37)	4 (1.81)	2 (0.57)
Fetal loss ≥20 weeks	5 (1.08)	5 (1.37)	2 (0.90)	9 (2.57)
Total losses	6 (1.30)	10 (2.74)	6 (2.71)	11 (3.14)

[a]In the study group, losses after chorionic villus sampling or amniocentesis procedure were considered. In the control group, for chorionic villus sampling, losses after 11 weeks gestation were considered and, for the amniocentesis group, losses after 15.5 weeks gestation.
Differences between groups are not significantly different.
CVS = chorionic villus sampling.

Table IV. Late pregnancy outcome (≥20 weeks) and birth characteristics of babies in the prenatal diagnosis group and in the controls (numbers in parentheses are percentages)

	Amniocentesis (n = 460) 460 fetuses	Control (n = 360) 360 fetuses	Odds ratio	CVS (n = 109) 217 fetuses	Control (n= 174) 348 fetuses	Odds ratio
Preterm delivery	41 (8.91)	33 (9.17)	0.97 CI: 0.60–1.57	58 (53.21)	101 (58.05) CI: 0.61–1.30	0.89
Low birthweight	46 (10.00)	29 (8.06)	1.27 CI: 0.78–2.06	114 (52.53)	180 (51.72) CI: 0.74–1.45	1.03
Very low birthweight	10 (2.17)	5 (1.39)	1.58 CI: 0.53–4.66	14 (6.45)	28 (8.05)	0.79 CI: 0.41–1.53

CVS = chorionic villus sampling; CI = confidence interval.

loss rates in the controls and in the CVS group were similar in the different age groups.

Pregnancy outcome in the CVS group (Table IV) was studied in 109 twin pregnancies or 217 fetuses beyond 20 weeks gestation. Fifty-eight pregnancies delivered preterm (53.21%). The low-birthweight (n = 114) and the very low-birthweight (n = 14) rates were 52.53% and 6.45%, respectively. In the control group, 174 twin pregnancies or 348 fetuses were followed beyond 20 weeks gestation. Preterm deliveries occurred in 101 pregnancies (58.05%). The low-birthweight (n = 180) rate and the very low-birthweight (n = 28) rate were 51.72% and 8.05% respectively. Compared with pregnancies without prenatal diagnosis, the odds ratios for prematurity, low-birthweight and very low-birthweight were 0.89 (95% CI: 0.61–1.30), 1.03 (95% CI: 0.74–1.45) and 0.79 (95% CI: 0.41–1.53) respectively in the CVS group (Table IV).

Discussion

There have been few studies to assess spontaneous abortion rates after normal first-trimester ultrasound examination in the general population (Gustavii, 1984; Wilson *et al.*, 1984). Such studies have reported a pregnancy-loss rate varying from 2.13% (≤20 weeks gestation) to 7.2% (≤28 weeks gestation) after a normal ultrasound examination at the 10th week of gestation. Gustavii (1984) also reported that the spontaneous-abortion rate was 2.3% (≤28 weeks gestation) after a normal ultrasound examination at the 14th week of gestation. In another study where only patients with threatened abortion were evaluated, a 12% pregnancy loss rate has been reported (Mantoni and Pedersen, 1982). We believe that these figures are very important in estimating the added risk of spontaneous abortion after prenatal diagnosis. So far there have been no published data assessing pregnancy losses after ICSI. In the amniocentesis group in our series the pregnancy-loss rate, including spontaneous abortions and stillbirths, was 1.30%. In the CVS group, the pregnancy-loss rate was 2.71% in twin pregnancies. These results are compatible with the previously reported loss rates in the general population (Gustavii, 1984; Wilson *et al.*, 1984).

In our group of ICSI pregnancies, the pregnancy-loss rate after amniocentesis (1.30%) was also comparable to that of our control group (2.74%) in singleton pregnancies. In the control group, five spontaneous abortions occurred, while in the amniocentesis group there was only one spontaneous abortion. Terminations following the diagnosis of a cytogenetic abnormality might have removed some cases from the study group which would have aborted spontaneously. Because of lack of ultrasound examination in the control group at the corresponding date of amniocentesis, dead fetuses might have been included in the control group. The spontaneous-abortion rate in the control group might therefore have been overestimated. In the literature, two case-controlled studies have reported similar pregnancy-loss rates after amniocentesis and in control groups (NICHD, 1976; MRC, 1978). The only randomized case-controlled study including women younger than 35 years without genetic risk has shown that, in the amniocentesis group, the spontaneous-abortion rate (from 16 to 28 weeks gestation) (1.7%) was significantly higher than that in the control group (0.7%) (Tabor *et al.*, 1986). In this

study, the intervention was performed by five clinicians with different experience and with an 18-gauge needle. In our study group, two experienced operators performed the amniocenteses with a 22-gauge needle. In this series of ICSI pregnancies, amniocentesis did not emerge as a risk factor for prematurity, low-birthweight or very low-birthweight as compared with that in controls. Tabor *et al.* have also reported similar preterm-delivery rates after amniocentesis and in control groups (Tabor *et al.*, 1986). However, in ICSI pregnancies the preterm-delivery rate, the low-birthweight rate and the very low-birthweight rate remain higher than in the general population (Bekaert *et al.*, 1997).

It has been reported that selective feticide of an abnormal twin fetus in the second trimester increases the morbidity and the mortality of the normal twin (Evans *et al.*, 1994). In our practice, CVS is proposed for twin ICSI pregnancies because of the advantage it has of providing an earlier diagnosis than amniocentesis. Thus, a selective feticide can be carried out at an earlier gestational age. In our study group, the fetal-loss rate after CVS (2.71%) was comparable to the pregnancy-loss rate in controls (3.14%). In the CVS group, four pregnancies (eight fetuses) were lost up to 20 weeks after chorionic villus biopsy. The earliest loss was 5 weeks after the procedure. The loss rate in our study population is in line with that in the study published by De Catte *et al.* (1996) which evaluated the pregnancy outcome after CVS in twin pregnancies. Large-scale studies have confirmed the safety of CVS, finding no statistically significant difference in fetal-loss rates as compared with those of controls undergoing second-trimester amniocentesis (Canadian Collaborative CVS-Amniocentesis Clinical Trial Group, 1989; Rhoads *et al.*, 1989).

In our twin pregnancies, the preterm-delivery rate, the low-birthweight rate and the very low-birthweight rate after CVS were comparable to those of the controls who did not undergo prenatal testing. Similar results have been reported previously (De Catte *et al.*, 1996).

It is well known that the rate of aneuploidy increases with maternal age (Hook *et al.*, 1984). Prenatal diagnosis is therefore of the utmost importance for women over 35 years. Questions still remain as to whether prenatal diagnosis is needed for women younger than 35 and whether prenatal diagnosis involves additional risks for such patients undergoing ICSI. We evaluated our data in this respect for two different age groups, i.e. $\geq$35 and <35 years. In women younger than 35 years, the pregnancy-loss rates after CVS and amniocentesis were comparable to the pregnancy-loss rates in women of the same age who did not undergo prenatal diagnosis.

An earlier prospective follow-up study of 877 children born after ICSI has shown a slight increase in de-novo chromosomal aberrations (1.2%) and a higher frequency of transmitted chromosomal aberrations (Bonduelle *et al.*, 1996). This present study evaluating pregnancy outcome after ICSI shows that, compared with those of the controls, the pregnancy-loss rate, the preterm-delivery rate, the low-birthweight rate and the very low-birthweight rate did not increase in the prenatal-diagnosis groups. We believe that the safety of microinjection treatment should be confirmed by collaborative studies. In the meantime, and as part of counselling, prenatal diagnosis should be offered to all patients undergoing ICSI.

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Outcome of Twin Gestations After First Trimester Chorionic Villus Sampling

LUC DE CATTE, MD, INGE LIEBAERS, MD, PhD, AND WALTER FOULON, MD, PhD

Objective: To tabulate genetic results and obstetric outcomes of twin pregnancies after first-trimester chorionic villus sampling (CVS).

Methods: The study included 262 consecutive women with twin pregnancies who had first-trimester CVS between 1988 and 1998.

Results: Major indications for prenatal diagnosis included maternal age (n = 82), pregnancies after intracytoplasmic sperm injection (n = 114), or both (n = 33). Among 524 fetuses, 519 were sampled adequately. Cytogenetic results were incorrect because of sampling the same fetus twice in two pregnancies. In three pregnancies, contamination caused by mixed sampling made cytogenetic results uncertain. Correct genetic diagnoses were obtained in 509 fetuses, 24 of which had chromosomal abnormalities on direct preparations and four of which had monogenetic conditions. Additional invasive procedures were done on five occasions. Fifteen fetuses were terminated selectively. The total fetal loss rate was 5.5% (28 of 509). The indication for the procedure did not significantly determine the fetal loss rate. The mean ± standard deviation (SD) gestational age at birth was 35.9 ± 2.9 weeks, and the mean ± SD birth weights for twins A and B were 2429 ± 589.1 g and 2378 ± 588.5 g, respectively.

Conclusion: First-trimester CVS is an accurate means of prenatal genetic diagnosis in twins, offering early selective termination in cases of abnormal genetic results in one of the fetuses. (Obstet Gynecol 2000;96:714–20. © 2000 by The American College of Obstetricians and Gynecologists.)

Assisted reproductive technologies and ovulation induction have increased markedly the rates of twin pregnancies, frequently in women 35 years or older at delivery, which is an indication for prenatal diagnosis. The likelihood of at least one twin having a chromosomal abnormality is 1.5 times higher than in singleton pregnancies.[1]

Second-trimester amniocentesis is the standard for prenatal diagnosis in twins[2–4]; however, chorionic villus sampling (CVS) allows selective termination with a lower complication rate and less psychologic burden than if an abnormal result is encountered in one fetus of a multiple pregnancy.[5,6] Since 1985, experienced use of CVS has proved effective, accurate, and safe for first-trimester prenatal diagnoses in singleton pregnancies.[7] Only a few studies evaluated CVS in multiple gestations [8–11] because of concerns about the selectivity of accessing different placental sites and processing sampled villi for reliable genetic results. Also there is the fear of incorrectly identifying genetically abnormal fetuses in cases of discordant genetic results in the first trimester. We evaluated the accuracy of the sampling procedure and genetic analysis in twin pregnancies in the first trimester and compared them with earlier data to determine feasibility and risks of CVS in twin pregnancies.

From the Feto-Maternal Medicine Unit, Department of Obstetrics and Gynecology and Center for Medical Genetics, Academisch Ziekenhuis VUB, Vrije Universiteit, Brussels, Belgium.

Materials and Methods

We studied 262 twin pregnancies in which CVS was done prospectively between 1988 and 1998. Follow-up was complete in all cases. Genetic counseling preceded the invasive procedures. Women were informed about the risks of chromosomal abnormalities in their pregnancies and the possibility of detecting fetal aneuploidy with CVS or amniocentesis. Patients chose whether CVS was done. Procedures were done consecutively between 9 and 13 weeks' gestation, after sonographic examination to determine viability and chorionicity of the pregnancy. In cases of monochorionic twins, the placentas were sampled twice near the cord insertion of each fetus.

Indications for CVS were chromosomal evaluation for advanced maternal age (35 years or older) (n = 82), pregnancies established after intracytoplasmic sperm injection (n = 114), pregnancies established after intracytoplasmic sperm injection in patients with advanced maternal age (n = 33), and chromosomal evaluation or DNA analysis for other indications, including history of

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monogenetic disease, presence of sonographic markers for fetal aneuploidy or fetal structural defects, chromosomal malformations in parents or previous pregnancies, and the psychologic indications ($n = 33$).

Chorionic villus sampling was done using ultrasound guidance with a curved linear probe, after sonographic mapping of placental implantations and cord insertions, by two operators experienced in invasive prenatal techniques. Transabdominal, transcervical, or a combination of both methods were used to obtain sufficient placental tissue. Chorionic villi were aspirated transcervically with a Portex Throphocan catheter (Laboratorie Portex 5A, Berck-Sur-Mer, France) or transabdominally with a double-needle system (outer needle 18 gauge, inner needle 20 gauge). The insertion of the outer 18-gauge trocar needle allowed for multiple chorionic aspirations in case of insufficient aspiration on the first attempt. Samples of more than 10 and 20 mg per fetus were considered appropriate for karyotyping and biochemical or DNA analysis, respectively. When discordant genetic abnormalities in dizygotic twin pregnancies were detected, a transabdominal selective termination was done within 1 week after CVS following counseling and acceptance by the parents. Under local anesthesia, potassium chloride (0.5–2 mL of 1 mEq/mL solution) was injected into or near the fetal heart until asystole was established.

Short-term chromosomal analysis was done within 72 hours.[12,13] Cultured chromosomal preparation and amniocentesis were proposed, if necessary, to differentiate true fetal from placental mosaicism. One to 2 weeks were required for results from biochemical or DNA studies.

Information collected on each pregnancy included indications for prenatal diagnosis, maternal age, sampling route, number of instrument insertions and number of aspirations per pregnancy, results of genetic analyses, selective termination in cases of abnormal test results, preterm prelabor rupture of membranes, gestational age at delivery, mean birth weight, and neonatal outcome. Loss of one or two fetuses at less than 500 g or before 22 weeks' gestation was considered early fetal loss or spontaneous miscarriage. Fetal mortality rates included fetuses of more than 500 g or fetal death after 22 weeks' gestation, according to definitions of the Flemish Birth Registry (Studiecentrum voor Perinatale Epidemiologie) and based on World Health Organization and International Federation of Obstetrics and Gynecology criteria.[14,15] Perinatal mortality was considered the sum of fetal and neonatal deaths in the first week of life.

Fisher exact tests were used to compare the four groups of indications for categorical variables. In cases of globally significant differences (at a significance level of .05), two-by-two comparisons were done with a Bonferonni correction at the significance level. According to that correction, $P < .008$ was considered significant. For continuous variables, an analysis of variance model was used to investigate overall differences of the four groups, with the Tukey method for multiple comparisons to compare groups two by two.

Results

Sonographic determination of placental chorionicity found four monochorionic diamniotic (four of 262, 1.5%) and 258 dichorionic twin pregnancies (98.5%) Mean maternal ages ± standard deviation (SD) in the indication groups (chromosomal evaluation, intracytoplasmic injection, intracytoplasmic injection with advanced maternal age, and chromosomal evaluation for mixed indication) were 37.0 ± 1.8 years, 30.3 ± 2.5 years, 36.5 ± 1.8 years, and 31.3 ± 2.5 years, respectively.

Among 262 pregnancies (524 fetuses) we retrieved sufficient villi from 519 fetuses (99%). In one dichorionic pregnancy, the posterior position of the placenta and retroflexion of the uterus made a second targeted sampling impossible and the woman refused additional amniocentesis. In two other dichorionic pregnancies, maternal obesity or enlarged stimulated ovaries obstructed an aligned transabdominal approach of the placenta and resulted in aspiration of less than 10 mg of chorionic villi. A final karyotype was not established because of lack of interpretable metaphases. Only one woman opted for amniocentesis, which showed normal karyotypes for both fetuses.

The mean number ± SD of instrument insertions per pregnancy was 2.1 ± 0.42, the mean number of aspirations was 2.5 ± 0.74. There was no significant difference in technical aspects of the procedures between the four indication groups.

Chorionic villus sampling was done with combined transabdominal and transcervical approaches in 111 twin pregnancies (42.4%). Both placentas were sampled transabdominally in 93 cases (35.5%) and transcervically in 55 cases (21.4%).

Genetic analysis was available for 519 fetuses. Genetic diagnosis was correct in 509 cases (98.1%). In five pregnancies, prenatal cytogenetic results were incorrect or doubtful. In two cases, both biopsies were taken from the same placenta, leading to false determination of identical fetal sexes. In one of those cases, the difference in fetal sex was discovered by a second-trimester ultrasound and confirmed by additional amniocentesis. In the other case, the misdiagnosis was detected at birth when the patient delivered two healthy children of the opposite sex. In one other case, both samples contained

Table 1. Evaluation of Abnormal Cytogenetic Analysis on Direct and Long-Term Chromosomal Preparations

Type of abnormality on direct preparation	Number of abnormal direct preparations	Number of confirming long-term cultures	Number of additional invasive procedures	Outcome
Pericentric inversions	2	2/2	0	Normal
Balanced translocations	4	4/4	1	Normal
Marker chromosome	2	2/2	0	Normal (monochorionic pregnancy)
Mosaicism	3	0/3	0	Normal
Unbalanced translocation	1	1/1	1	Termination
Trisomy	7	7/7	2*	1 early fetal loss, 6 terminations
Sex chromosome abnormality	3	3/3	0	1 early fetal loss, 1 termination, 1 normal
Tetraploidy	2	1/2	1	1 termination, 1 normal
Total	24	20	5	13 normal, 2 early fetal losses, 9 terminations

* Amniocentesis at the time of the selective termination.

a mixture of villi from the two placentas resulting twice in 46,XX/46,XY fetal karyotypes. Ultrasound examination showed normal male and female infants. In each of the two remaining pregnancies 46,XX/46,XY mosaicism was detected in one of the fetuses. Normal ultrasound findings in those two pregnancies lessened the need for amniocentesis. In all cases, women delivered healthy children with normal karyotypes. Review of the ultrasound charts of those five women did not show any particular risk factor for nonselective sampling; however, all pregnancies were dichorionic and had fused placentas.

On direct chromosomal preparations, an abnormal fetal karyotype was detected in 22 twin pregnancies, affecting 24 of 519 fetuses (4.6%) (Table 1). Three cases of placental mosaicism in the direct preparation resulted in a normal karyotype in long-term culture analysis. Two additional cases of tetraploidy in the direct preparation were confined to the placenta and were normal either by long-term chorionic villus culture ($n = 1$) or by additional amniocentesis ($n = 1$). An abnormal fetal karyotype in direct and long-term preparations was observed in 17 cases (3.3%). There were four balanced translocations, two pericentric inversions, and two fetuses in a monochorionic pregnancy with a marker chromosome. Six showed trisomy 21, and one had trisomy 13. Three fetuses had numerical sex chromosome abnormalities; two had Klinefelter syndrome and one had Turner syndrome. On one occasion, an apparent complex translocation between chromosomes 6, 13, and 15 necessitated parental karyotyping and an additional confirmation amniocentesis which found 45,XY,der(13;15). Seven of those abnormal results were related to advanced maternal age (seven of 164, 4.3%), whereas four conceived by intracytoplasmic sperm injections had abnormal karyotypes (four of 223, 1.8%). Two twin pregnancies had prenatal diagnoses of Charcot-Marie-Tooth disease and adult polycystic kidney disease. Both were dichorionic pregnancies, and both fetuses were affected.

Data on pregnancy losses are shown in Table 2. Selective termination of one fetus was done in 11 twin pregnancies, six of which had chromosomal abnormalities on direct and long-term preparations. Although long-term villus culture showed a normal karyotype in one fetus with tetraploidy on direct preparation, and the woman was counseled about placental polyploidy in a reassuring manner, she opted for selective termination. Early second-trimester preterm prelabor rupture of membranes occurred in the precervical gestational sac of two twin pregnancies, so the two anhydramnic fetuses were selectively terminated to achieve a better outcome for the remaining one. In two additional cases, fetal termination was done once for cervical incompetence and once for psychologic distress. Two twin pregnancies were terminated completely, in which both fetuses were affected by a chromosomal abnormality (47,XXY and 47,XX+21) or Charcot-Marie-Tooth disease.

Two women opted to continue pregnancies despite fetal abnormalities detected by CVS. In one, both fetuses were affected by adult polycystic kidney disease, and in the second, one fetus had Klinefelter syndrome.

Sixteen of the remaining 509 fetuses (nine pregnancies) were lost before the 22nd week of gestation (3.1%). Two fetuses with 45,X and trisomy 13 karyotypes miscarried within 10 days after CVS. Seven pregnancies were lost completely before the 22nd week of gestation, one of which was the result of early deteriorating twin-twin transfusion syndrome.

After 22 weeks, three pregnancies were complicated by preterm prelabor rupture of membranes and were lost completely. One fetus died in each of six other pregnancies. Two of those had severe congenital mal-

Table 2. Fetal Loss Rates in Relation to Indication for Chorionic Villus Sampling

	n (%)	Pregnancies by intracytoplasmic sperm injection (*n* = 114)	Pregnancies with advanced maternal age (*n* = 82)	Pregnancies by intracytoplasmic sperm injection in patients with advanced maternal age (*n* = 33)	Patients with other indications (*n* = 33)
Termination					
Single fetus	11	1	6	2	2
Total pregnancy	2	1	0	0	1
Early fetal demise ≤22 weeks					
Single fetus	2	0	1	0	1
Total pregnancy	7	2	3	1	1
Fetal loss after 22 weeks					
Single fetus	6	2	4	0	0
Total pregnancy	3	1	1	0	1
Total fetal loss	28 (5.5)	8 (3.6)	13 (8.2)	2 (3.1)	5 (8.0)
Neonatal loss					
Single neonate	0	0	0	0	0
Both neonates	3	1	0	0	2
Total	6 (1.2)	2 (0.9)	0	0	4 (7.0)

formations; one had a major lumbosacral neural tube defect with associated hydrocephaly, the other had agenesis of the corpus callosum and a cardiac defect. The fetal loss rate after 22 weeks' gestation was 2.4% (12 of 493).

The total spontaneous fetal loss rate, defined as fetal loss after CVS until birth was 5.5% (28 of 509). After exclusion of the congenital malformations, (twin-twin transfusion syndrome, two severe congenital malformations, 45,X, and trisomy 13) the corrected total fetal loss rate was 4.3% (22 of 509). Six neonates died because of early preterm delivery after preterm prelabor rupture of membranes at 22, 25, and 26 weeks (1.2%). The infant survival rate after first-trimester CVS in twin pregnancies was 90.6% (475 of 524). When the CVS procedures were done for advanced maternal age or other indications, total fetal loss rates were 8.2% and 8.0%, respectively. Pregnancies by intracytoplasmic sperm injection had an insignificantly lower fetal loss rate after CVS (3.6%), even with advanced maternal age (3.1%). Combining transcervical and transabdominal routes resulted in a fetal loss rate of 2.7%. Transcervical or transabdominal CVS of both fetuses resulted in miscarriage rates of 3.6% and 4.3%, respectively ($P > .05$).

Perinatal outcome was calculated for 253 pregnancies or 493 fetuses, after excluding four terminated pregnancies, 11 selective terminations, and 16 early miscarriages. The mean ± SD gestational age at delivery was 35.9 ± 2.9 weeks (range 23–41 weeks). The mean birth weights ± SD for twins A and B were 2429 ± 589 g (range 550–4030 g) and 2378 ± 589 g (range 450–3620 g), respectively. Preterm delivery (no more than 37 weeks) occurred in 176 pregnancies (69.6%), whereas early preterm delivery (no more than 33 weeks) happened in 35 twin pregnancies (13.8%). There were no significant differences in mean gestational age at delivery, preterm and early preterm delivery, and low-birth-weight neonates according to procedure indication.

Discussion

Different authors[8–11] have evaluated feasibility and risks of CVS in twin pregnancies; however, the samples in those studies were relatively small, ranging from 65[8] to 161.[10] Our findings present additional data in favor of first-trimester prenatal diagnosis by CVS showing its safety, accuracy, and advantage for early selective termination.

Indications for prenatal diagnosis in twin pregnancies are similar to those for singletons. Having two fetuses doubles the risk of aneuploidy.[1] Meyers et al[16] calculated that the risk of at least one aneuploid twin at term at the maternal age of 31 years was comparable to the risk of an aneuploid singleton term pregnancy at the maternal age of 35 years. Hence, some centers offer invasive prenatal testing in twin pregnancies at earlier maternal ages. At our center, the term advanced maternal age is applied to all gravidas who will be at least 35 years old at the expected delivery date. Cytogenetic evaluation of twin pregnancies after intracytoplasmic sperm injection was the most important indication for prenatal diagnosis in our study because of the higher frequency of sex chromosome abnormalities[17] in sperm

Table 3. Reported Outcomes of Chorionic Villus Sampling in Twin Pregnancies

	Wapner et al[10]	Pergament et al[9]	Brambati et al[8]	Present study	Total
Year published	1993	1992	1991	2000	
Number	161	126	65	262	614
Fetal loss before 22 weeks	14/309 (4.5%)	5/244 (2.0%)	2/120 (1.7%)	16/509 (3.1%)	37/1182 (3.1%)
Total fetal loss rate	15/309 (4.8%)	10/244 (4.1%)	2/120 (1.7%)	28/509 (5.5%)	55/1182 (4.6%)

of men with oligoasthenoteratospermia. A prospective follow-up study showed a slight increase in de novo chromosomal aberrations (1.66%) and a higher frequency of transmitted chromosomal aberrations in children born after intracytoplasmic sperm injection.[18]

More than 98% of the twin pregnancies in our study were dichorionic, as determined by the initial ultrasound examination. In a normal twin population about 80% would be dichorionic and the remaining 20% monochorionic. Our population was biased by the high number of pregnancies by assisted reproductive techniques (147 of 262) and advanced maternal age (82 of 262), both of which favor dizygotic twinning.

Chorionic villus sampling in twin gestations was rarely unsuccessful (five of 524 fetuses). Failure to retrieve sufficient villi was associated with extreme retroflexion of the uterus, maternal obesity, and enlarged hyperstimulated ovaries. Some of those failures could have been avoided by postponing the procedure or doing amniocentesis later in gestation. However, one of those women declined further prenatal diagnosis.

Chorionic villus sampling in twin pregnancies entails a risk of 4–6% of samples being mixed, which is higher than in amniocentesis.[9,10,19] We had two cases of erroneous sampling and three cases of tissue mixing in the first 104 twin pregnancies. All five had fused dichorionic placentas. Although operators had some experience with both sampling methods in singleton pregnancies, approaching a fused dichorionic placenta was part of a new learning curve. Wijnberger and coworkers[20] substantiated a learning curve for CVS and showed that safety and success of a procedure was strongly related to number of procedures done by an operator. That learning curve is probably more important when CVS is used for prenatal diagnosis in twin pregnancies. We initially believed that transcervical sampling of both fetuses caused a higher risk of tissue contamination,[11] but in two additional cases of cross-contamination, both fetuses were sampled transabdominally. There were no cross-contamination cases in the last 158 twin pregnancies. Individualization of each fetus with special notice of intertwin positions and relationships was the most important issue in prenatal diagnosis in multiple pregnancies. Umbilical cord insertion onto the chorionic plate might serve as a landmark. Concordant abnormal genetic results in dichorionic fetuses of the same gender are extremely rare, with the calculated probability depending on the type of abnormality and were .0001% and .017%[19] for generalized or placentally confined mosaicism, respectively. That condition should always prompt the question of erroneous or mixed sampling. Repeat sampling by CVS or additional amniocentesis is necessary to elucidate that controversy.[21,22] Additional cytogenetic and DNA polymorphism studies should be done when the sexes are identical and the twins are concordant for a karyotype anomaly.[19] Inconclusive results after first-trimester prenatal diagnosis by CVS (by direct or long-term culture analysis) must be determined by additional amniocentesis. Although combining the two procedures is rarely needed, it probably increases the risk of miscarriage.

Most genetic problems in dichorionic twin pregnancies are discordant and thus affect only one fetus. Previously they were managed by termination or continuation of the entire pregnancy without interference. Expectant management might still be preferable to invasive approaches when death of a karyotypically abnormal fetus is inevitable.[23] However, Malone and coworkers[24] found that an anomalous fetus in a twin gestation significantly increased the risk of preterm delivery, frequency of cesarean, and perinatal mortality rates compared with normal twin gestations. Therefore, couples should be informed that early selective termination of the affected fetus in cases of discordant results in dichorionic twin pregnancies carries a small risk of complete pregnancy loss compared with selective termination after diagnosis by amniocentesis in the second trimester.[5,6,23,25,26]

The fetal loss rate of 5.5% in this study matches well the data from the literature[8,9,10,11] as presented in Table 3. Aytoz et al[27] found that the fetal loss rate after CVS in 111 twin pregnancies by intracytoplasmic sperm injections did not differ statistically significantly from that of a control population. Number of fetal losses were comparable to those after amniocentesis in twin pregnancies.[2,3,4,10] There are still high spontaneous loss rates

in twin pregnancies after viability is established by ultrasound. In a 5-year prospective study of 137 sonographically identified twin pregnancies at a mean gestational age of 10.1 weeks, a complete pregnancy loss rate of nearly 11% was found.[28] A fetal loss rate of 3.2% was found in twin pregnancies after a normal ultrasound examination between 14 and 20 weeks' gestation.[3] Advanced maternal age was considered a factor in increased fetal loss after CVS in singleton pregnancies.[29] Pergament and coworkers[9] found no increase in procedurally related pregnancy losses after CVS for advanced maternal age in singleton and twin pregnancies. In our data there was an insignificant trend toward higher rates of fetal loss in the older maternal age group. Chorionic villus sampling in intracytoplasmic sperm injection twin pregnancies was associated with a low risk of pregnancy complications,[27] which should make CVS an acceptable alternative for amniocentesis even in twin pregnancies after infertility treatment.

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Obstetric outcome after fetal reduction to singleton pregnancies

L. De Catte* and W. Foulon

Unit of Feto-Maternal Medicine, Department of Obstetrics and Gynecology, University Hospital Vrije Universiteit Brussel, Brussels, Belgium

Objective To study the outcome after fetal reduction or selective termination to singleton pregnancies for various indications.

Methods Fetal reduction or selective feticide to singleton pregnancies was performed in 80 multiple gestations (congenital malformations, 17 cases; high-risk obstetric conditions, 25 cases; or social/psychological indications, 38 cases).

Results The overall pregnancy loss rate was 10%; however, pregnancy failure was significantly higher in selective reductions performed for preterm prelabor rupture of membranes (PPROM) (4/8) compared with monochorionic twin and bad obstetric history. Fetal reduction to singletons for psychological reasons resulted in a pregnancy wastage of 5.3% (2/38). Procedures performed at $\leqslant 14$ weeks showed a significantly lower fetal loss rate (2/61; 3.3%), a higher mean gestational age at delivery (38.3 ± 2.2 weeks), and a decreased prematurity rate ($p \leqslant 0.001$). The number of reduced fetuses, prenatal diagnosis by chorionic villus sampling before the reduction and maternal age did not interfere with pregnancy outcome.

Conclusion Fetal reduction to singleton pregnancies has a favorable outcome, especially when performed before 14 weeks of gestation. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: congenital malformation; fetal reduction; multiple pregnancy; selective termination

INTRODUCTION

The reduction to a single fetus is appropriate in twin pregnancies with structural or genetic fetal abnormalities, uterine malformations or significant medical disease (Evans *et al.*, 1998). Twin gestations complicated by a single anomalous fetus showed a significantly lower gestational age at delivery and birth weight, and a higher rate of perinatal mortality and caesarian delivery, than normal twin pregnancies (Malone *et al.*, 1996). Late elective termination of abnormal fetuses in twin pregnancies has been associated with favorable perinatal outcome of the healthy twin in several studies (Lipitz *et al.*, 1996, 1997; Berkowitz *et al.*, 1997; Evans *et al.*, 1999a). However it is not clear whether the obstetric advantage of selective termination is applicable to all fetal malformations. Favorable outcome for the unaffected twin without selective termination of the affected fetus has been reported for twin pregnancies with anencephaly (Lipitz *et al.*, 1995; Sebire *et al.*, 1997a) or lethal chromosomal abnormalities (Sebire *et al.*, 1997b). Other commonly accepted indications of elective fetal reduction are those to improve pregnancy outcome as in patients with cervical incompetence, uterine malformations (Ginsberg *et al.*, 1997) or a history of repetitive preterm delivery. Fetal reduction to a singleton for social/psychological indications remains controversial and is not widely performed (Evans *et al.*, 1998).

*Correspondence to: L. De Catte, Department of Obstetrics and Gynecology, Feto-Maternal Medicine, AZ VUB, Laarbeeklaan 101, 1090 Brussels, Belgium. E-mail: luc.decatte@az.vub.ac.be

The present study evaluates the obstetric outcome after multifetal pregnancy reduction (MFR) or selective feticide (SF) to singleton pregnancies in various clinical conditions.

PATIENTS AND METHODS

The present prospective study is of 84 consecutive patients in which MFR or SF to singleton pregnancies was performed. Four pregnancies were excluded from further analysis because of incomplete data. Of the 80 pregnancies studied, eight were quadruplet, 27 were triplet and 45 were twin pregnancies.

Under local anesthesia and sonographic guidance, potassium chloride (KCl) (1 mEq/ml) was injected with a 22-gauge spinal needle into the fetal thorax near to or into the fetal heart. In monochorionic twin pregnancies, both fetuses were reduced by needling each fetus separately. The procedure was considered successful after a fetal asystole of 2 min and the needle was redrawn. The patients were divided into three major groups: pregnancies complicated by the presence of a congenital malformation in one of the fetuses (Group I); pregnancies with high-risk obstetric conditions (Group II) and multiple pregnancies reduced for social/psychological indications (Group III).

Prenatal diagnosis was mainly performed by chorionic villus sampling (CVS). The technical aspects were as described previously (De Catte *et al.*, 1998a). Later in the second trimester, amniocentesis or fetal blood sampling was used for rapid fetal karyotyping.

Perinatal outcome was collected for all completed

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pregnancies. Pregnancy loss at <500 g or $\leqslant 22$ weeks of gestation was described as early fetal loss or spontaneous miscarriage. Fetal mortality rates included fetuses of >500 g or fetal death occurring after 22 weeks of gestation, according to the definitions of the World Health Organization (WHO) and Fédération Internationale de Gynécologie et Obstétrique (FIGO) criteria. For the purpose of the present study, fetal loss rate was the sum of early fetal loss and fetal death. Mean gestational age at delivery, mean birth weight and prematurity rates were calculated on all pregnancies evolving beyond 22 weeks.

All statistical tests were performed two-sided at a 5% level of significance. To investigate the influence of the indication for the procedure, the gestational age at the time of the reduction, the number of fetuses reduced, and the performance of a CVS on the pregnancy loss rate and on preterm delivery, Fisher exact tests (FE) were used for each variable separately. The effect of maternal age on pregnancy outcome was investigated by means of the non-parametric Mann-Whitney test (MW). The influence of the above factors on the gestational age at delivery and the birth weight was investigated by means of Kruskall-Wallis (KW) or Mann-Whitney tests for each categorical factor and by means of a Spearman rank correlation coefficient for maternal age. Finally, stepwise regression was performed to predict the mean gestational age at delivery and the mean birth weight by each of the possible prognostic factors, with a 10% level of significance to decide whether or not to include a variable.

RESULTS

MFR or SF to singletons was performed in 80 consecutive multiple pregnancies at a mean gestational age of 13.0 ± 3.3 weeks. Sixty-one procedures were carried out at 14 weeks or less (76.3%). The mean maternal age was 31.7 ± 4.2 years. Twenty-two patients (27.5%) were 35 years old or over.

In Group I, fetal reductions were performed for either chromosomal abnormalities (eight) or for structural defects (nine) in 17 twin pregnancies. Group II consisted of 25 high-risk obstetric patients. There was a monochorionic twin pregnancy as a part of the multiple gestation in ten cases; eight patients showed a premature rupture of membranes before 20 weeks of gestation and another seven had a bad obstetric history. In Group III, 38 patients had a reduction to a singleton pregnancy for psychological reasons. In 45 twin pregnancies one fetus was reduced. Of the remaining 35 pregnancies, two fetuses were reduced in 27 cases, and three fetuses in eight cases. Prenatal diagnosis by CVS before fetal reduction was performed in 30 patients at a mean gestational age of 11.7 ± 1.9 weeks (range 10.0–18.7 weeks). Twenty-one patients were sampled for increased risk of fetal aneuploidy, of whom 15 for advanced maternal age $\geqslant 35$ years, two for ICSI, and four for congenital malformations. In eight additional cases, chorionic villi were retrieved for psychological reasons, and in one case for fetal growth discordance.

Spontaneous early or late fetal loss occurred in 8/80 (10%) reduced pregnancies (Table 1). Early fetal demise was observed in 5/8 pregnancies (62.5%), one of which was attributed to a congenital cytomegalovirus (CMV) infection. The remaining three fetal deaths occurred between 22 and 28 weeks of gestation. In one pregnancy with congenital malformation, anhydramnion led to the misjudgment of chorionicity resulting in the death of the co-twin after KCl injection. Unfortunately there was no first trimester ultrasound to state chorionicity in this patient. The correction of fetal loss rate, taking these facts into consideration, would be reduced to 7.5%. One additional neonatal loss was encountered because of extreme preterm delivery.

Table 1—Fetal loss after reduction to singleton pregnancies in relation to the indication of the procedure, the gestational age at reduction, the number of fetuses reduced, and the performance of a CVS before the reduction process

	Group I		Group II			Group III	
	Chromosome abnormality	Structural defect	Monochorionic multiplets	PPROM	Obstetric history	Social-psychological	Total (%)
$\leqslant 14$ weeks	6	–	10	1	7	37	61 (76.3)
Fetal loss	0	–	0	0	0	2	2* (3.3)
>14 weeks	2	9	–	7	–	1	19 (23.7)
Fetal loss	0	2	–	4	–	0	6* (31.6)
1 fetus reduced	8	9	–	8	–	20	45 (56.3)
Fetal loss	0	2	–	4	–	0	6** (13.3)
>1 fetus reduced	–	–	10	–	7	18	35 (43.7)
Fetal loss	–	–	0	–	0	2	2** (5.7)
CVS	7	3	2	2	2	14	30 (37.5)
Fetal loss	0	1	0	0	0	1	2† (6.7)
No CVS	1	6	8	6	5	24	50 (62.5)
Fetal loss	0	1	0	4	0	1	6† (12.0)

*$p=0.001$, **$p=0.455$, †$p=0.703$.
CVS, Chorionic villus sampling; PPROM, preterm prelabor rupture of membranes.

There was no significant difference in fetal loss according to the three major groups of indication (FE: $p=0.393$). In Group II however, four late pregnancy losses occurred among those pregnancies complicated by early preterm prelabor rupture of membranes (PPROM), compared with none in the two other subgroups (FE: $p=0.008$) (Table 2). There was a significantly lower pregnancy loss rate associated with fetal reductions performed at $\leqslant 14$ weeks (2/61; 3.3%) compared with reductions performed at a later gestational age (6/19; 31.6%) (FE: $p=0.001$). The number of fetuses reduced (1 or >1), the performance of a CVS before the reduction or advanced maternal age did not significantly alter the pregnancy loss (FE: $p=0.455$, 0.703 and 0.930, respectively). Reduction of one fetus in 45 patients resulted in six pregnancy losses (13.3%), four of which were attributed to the PPROM group. Fetal reduction of more than one fetus in the remaining 35 cases was associated with two pregnancy losses (5.7%). A total of 2/30 pregnancies (6.7%) undergoing a CVS before the reduction were lost, compared with 6/50 (12%) in the group where prenatal diagnosis was not carried out.

The mean gestational age at delivery in 75 pregnancies evolving beyond 22 weeks of gestation was 37.3 ± 3.7 weeks (range 23–41 weeks) (Table 3). There was no difference in mean gestational age at delivery in relation to the main indication categories. However, the mean gestational age at delivery after fetal reduction for chromosomal malformations was 38.9 ± 1.4 weeks and significantly higher than the 33.1 ± 6.0 weeks for cases with structural defects (MW: $p=0.010$) (Table 2). In Group II (fetal reductions for high-risk obstetric conditions) a significantly different mean gestational age at delivery was observed according to the subgroup. Reduction of the monochorionic twin pregnancy, selective feticide of the anhydramnic twin fetus after PPROM and fetal reduction for high-risk obstetric history showed a mean gestational age at delivery of 39.2 ± 1.0 weeks, 32.8 ± 5.2 weeks and 37.8 ± 0.9 weeks (KW: $p=0.003$), respectively. The mean gestational age at delivery was 38.3 ± 2.2 weeks when fetal reduction was performed at $\leqslant 14$ weeks, compared with 33.9 ± 5.5 weeks for interventions after 14 weeks (KW: $p<0.001$). The number of reduced fetuses, the performance of a CVS before the reduction and maternal age did not significantly interfere with the gestational age at delivery ($p=0.487$, 0.725 and 0.614, respectively). Twenty-five patients delivered at or before 37 completed weeks (33%). Preterm delivery occurred significantly more often after fetal reduction for structural defects (7/9) than for chromosomal malformations (1/8) (FE: $p=0.015$), and significantly more frequently in patients with selective feticide for PPROM (4/5) than for other high-risk obstetric events (3/16) (FE: $p=0.004$).

Table 2—Detailed analysis of the pregnancy outcome in the subgroups

	Fetal loss	p	Mean GA±SD at delivery (weeks)	p	Mean BW±SD at delivery (g)	p	Preterm delivery (%)	p
Structural defects	2/9	0.471	33.1±6.0	0.010	2504±892	0.148	7/9 (78)	0.015
Chromosomal abnormalities	0/8		38.9±1.4		3299±744		1/8 (12.5)	
Dichorial triplet	0/10		39.2±1.0		3143±418		0/9	
PPROM	4/8	0.008	32.8±5.2	0.003	2428±605	0.08	4/5 (80)	0.004
High-risk history	0/7		37.8±0.9		2843±394		3/7 (43)	

GA, Gestational age; BW, birth weight; PPROM, preterm prelabor rupture of membranes.

Table 3—Mean gestational age (GA) at delivery and mean birth weight (BW) after reduction to singleton pregnancies in each group, the GA at reduction, the number of fetuses reduced and the performance of a CVS

	n[a]	Mean GA±SD at delivery (weeks)	p	Mean BW±SD (g)	p
Indication					
Group I	17	35.8±5.3		2875±897	
Group II	21	37.2±3.6	0.218	2897±512	0.965
Group III	34	38.1±2.6		2895±668	
GA					
$\leqslant 14$ weeks	56	38.3±2.2	<0.001	2964±653	0.094
>14 weeks	16	33.9±5.5		2610±718	
Fetuses reduced					
1	40	36.8±4.4	0.487	2931±742	0.592
>1	32	38.0±2.5		2844±599	
CVS					
Yes	28	37.6±3.5	0.725	2876±725	0.880
No	44	37.1±3.9		2901±652	

[a]Number of pregnancies after exclusion of early pregnancy losses ($\leqslant 22$ weeks).

The mean birth weight was 2891 ± 677 g (range 600–4800 g). Although not statistically significant, a trend towards higher birth weights was observed when the fetal reduction was performed $\leqslant 14$ weeks (2964 ± 653 g) than >14 weeks (2610 ± 718g) (KW: $p=0.094$). There was no correlation between the mean birth weight and the indication for the fetal reduction, the number of fetuses reduced, first trimester prenatal diagnosis by CVS or maternal age.

Fitting stepwise models on the mean gestational age and birth weight at delivery revealed that gestational age at reduction is the single most significant factor on pregnancy outcome and confirmed the previous findings for each factor independently.

DISCUSSION

Pregnancy outcome after fetal reduction or selective termination has been documented by the collaborative series of the world's largest centers (Evans *et al.*, 1998, 1999b). More recently, the international experience on selective termination for structural defects, chromosomal malformations or Mendelian disorders in 402 multiple pregnancies showed pregnancy loss had decreased to 8.2% (Evans *et al.*, 1999a).

Loss rates after fetal reduction to singleton pregnancies in the present study was 10%. Five of the eight losses occurred before 22 weeks of gestation. One pregnancy was lost because of a first trimester congenital CMV infection. No difference in fetal loss was observed in the three categories of patients. However, looking into the 25 high-risk pregnancies (Group II), a significant difference in pregnancy failure emerged. Four of the eight pregnancies with early second trimester PPROM were lost within 3 weeks of the procedure because of a chorioamnionitis. In one of these cases, the PPROM occurred after a cerclage for a history of cervical incompetence in a twin pregnancy. In our hands, this indication for SF still carries the highest risk of pregnancy failure and preterm delivery. Nevertheless, selective feticide of the anhydramnic fetus in multiple pregnancies complicated by a previable rupture of the membranes (Dorfman *et al.*, 1995; De Catte *et al.*, 1998b; Debbs *et al.*, 1999) has improved fetal outcome by reducing the likelihood of chorioamnionitis, increasing the number of days to delivery, and also raising the survival rate of the healthy co-fetus(es). In the remaining 17 cases, no pregnancies were lost. Among these patients, there were ten dichorionic triplet pregnancies. Monochorionic twin pregnancies carry a six-fold higher fetal loss, a two-fold increase in very preterm delivery (Sebire *et al.*, 1997c) and a 15–20% risk of twin–twin transfusion syndrome (TTTS). Therefore, if MFR is performed in dichorionic triplet pregnancies, reduction of the monochorionic twin pregnancy should be advised. In the ten cases performed in the present study, no additional losses or obstetric complications were noticed. Unfortunately, none of the patients had been correctly informed about the chorionicity and its related problems at the time of the referral.

Of the thirty-eight (47.5%) reductions performed for psychological reasons, only two were lost (5.3%). Fetal reduction to singleton pregnancies for social reasons seems a relatively safe procedure. Although MFR to singletons for psychological reasons is a policy not widely accepted, we are convinced that couples with social, financial or relationship problems might benefit from this procedure. Some ethical parallels may be drawn with first trimester termination of pregnancy for the same problems. In addition, patients suffering from longstanding primary subfertility may feel unable to cope with their multiple pregnancies and, despite being advised against it, decide to terminate their pregnancies (Marcus and Brinsden, 2000). Moreover, older couples are more inclined to want reduction to singleton gestation (Evans *et al.*, 1997), thereby reducing some problems to parenthood in their 60s and 70s. On each occasion, individual psychological and social counseling should facilitate the decision-making process.

The gestational age at which the procedure was performed was the single most important factor determining fetal loss after reduction to singleton pregnancies. Performing the MFR/SF before the 15th week of gestation led to a successful pregnancy outcome in all but two patients (96.7%), of which one was related to a congenital CMV infection. Procedures performed after 14 completed weeks resulted in a significantly higher pregnancy failure rate (31.6%; $p=0.001$). This observation was previously found by other authors (Lynch *et al.*, 1996; Evans *et al.*, 1999a) and was considered as a strong argument to improve prenatal detection of congenital malformations in the first trimester. Compiled data (Evans *et al.*, 1999a) on selective feticide for congenital malformations in 402 multiple pregnancies demonstrated a progressive rise in fetal loss ($\geqslant 24$ weeks) in relation to the gestational age at which the procedure was performed: 5.4%, 8.7%, 6.8% and 9.1% for procedures carried out between 9 and 12, 13 and 18, 19 and 24 and $\geqslant 25$ weeks respectively. In a 10-year overview, Yaron *et al.* (1998) observed a slightly higher fetal loss for fetal reductions performed before 14 weeks, which he attributed to a higher than expected rate of spontaneous abortions in the first trimester. Berkowitz and co-workers (1997) demonstrated that selective feticide for congenital malformations in 100 consecutive cases was accompanied by a low spontaneous loss rate of 3%, and resulted in the birth of healthy infants at or near term in the vast majority of cases. In addition, with increasing experience the discrepancy between fetal loss rates after first and second trimester interventions has decreased (Evans *et al.*, 1999b).

In his collaborative study on multifetal pregnancy reduction, Evans observed higher fetal loss rates (Evans *et al.*, 1998, 1999b) associated with higher starting and finishing numbers of fetuses. Fetal reduction to a singleton pregnancy showed a 13.7% risk of pregnancy loss (Evans *et al.*, 1998) compared with 10% in the present series. Procedures performed in twin pregnancies only resulted in 13.3% pregnancy loss, which seems higher than 9.0% or 7.9% in 111 and

345 twin pregnancies, respectively, reported by Evans. Some of this discrepancy can be explained by the difference in indication for the fetal reduction. Excluding PPROM as an indication for selective feticide from the present data would yield a fetal loss rate of 2/37 or 5.4% in twin pregnancies. Increasing starting numbers and numbers of fetuses to reduce did not interfere with the pregnancy loss rate in the present data. Only two pregnancies were lost out of 35 triplet and quadruplet gestations (5.7%). However, this observation could be related to the small number of patients in the present study or to the fact that these procedures were predominantly performed in the first trimester.

Mean gestational ages at delivery and mean birth weights after fetal reduction in the present series were smaller than in low-risk singleton pregnancies, but slightly better than in twin pregnancies. The present data fit with those reported for FR/ST to singleton pregnancies by Lynch (1996), Evans (1999a) and Berkowitz (1997). However, multiple pregnancies with vanishing embryos resulting in singletons tend to do better (mean gestational age: 39.1 weeks; mean birth weight: 3122 g) (Manzur *et al.*, 1995). Fetal reduction after 14 weeks significantly reduces mean gestational age at delivery. This observation was shared by Yaron *et al.* (1998), who reported a decrease of 3 weeks in mean gestational age at delivery and a mean drop of 700 g in birth weight.

Fetal reduction to singleton pregnancies implies an acceptable low risk of fetal loss, and a near to normal obstetric outcome in terms of gestational age at delivery and mean birth weight. Selective termination in multiple pregnancies with congenital malformations produces a better obstetric prognosis. Fetal reduction for psychological reasons remains a controversial issue, but carries a very low risk of pregnancy failure. We are convinced that in selected couples there are more benefits from fetal reductions in twin pregnancies than perinatal outcome.

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Monochorionic High-Order Multiple Pregnancies and Multifetal Pregnancy Reduction

Luc De Catte, MD, Michel Camus, MD, and Walter Foulon, MD, PhD

OBJECTIVE: To study the frequency and obstetric outcome of monochorionic multiple pregnancies in a population referred for fetal reduction.

METHODS: Data charts of all patients with multifetal (≥3) pregnancies referred for fetal reduction over the last 10 years were reviewed for the presence of monochorionic twin pairs or triplets.

RESULTS: Twenty-nine of 239 high-order multiple pregnancies contained a monochorionic component (12.1%), eight of which were monochorionic triplets. Half of all naturally conceived pregnancies contained a monochorionic component. High-order multiple pregnancies with a monochorionic component resulted significantly more frequently from natural conceptions (7 of 29) than multichorionic pregnancies (7 of 210) ($P = .001$). Fetal reduction of the monochorionic twin pair in 21 pregnancies resulted in eight twin and 13 singleton pregnancies; mean gestational age at delivery was, respectively, 34.3 ± 2.9 and 39.2 ± 1.4 weeks. Pregnancy loss rate was one of 21 (4.8%). In the remaining eight multiple pregnancies with a monochorionic triplet present, three were complicated by a twin reversed arterial perfusion sequence, and two couples requested a first trimester termination of pregnancy. Fetal reduction of the monochorionic triplet in a dichorionic quadruplet pregnancy resulted in a normal pregnancy outcome. In two monochorionic triplet pregnancies, fetal reduction to monochorionic twin pregnancies with bipolar coagulation of the umbilical cord resulted in a favorable pregnancy outcome.

CONCLUSION: Monochorionic twins or triplets are frequently part of naturally conceived high-order multiple pregnancies. Reduction of the monochorionic twin pairs improves pregnancy outcome. Monochorionic triplet pregnancies show a high complication rate, but may benefit from fetal reduction by cord coagulation. (Obstet Gynecol 2002;100:561–6. © 2002 by The American College of Obstetricians and Gynecologists.)

Assisted reproduction dramatically changed the incidence of multiple pregnancies in the last 15 years. In Flanders, Belgium, there is one twin pregnancy for nearly every 50 pregnancies. The estimated number of spontaneously conceived triplet pregnancies would be one in 8000–9000, yet the incidence has increased almost ten-fold. Seventy-five percent of this increase has been related to ovulation induction and the transfer of more than two embryos.[1] Only a small number is related to the rising maternal age at spontaneous conception. It could be assumed that the majority of triplet pregnancies therefore would be multizygotic and multichorionic.

According to Allen, 4.5% of all triplet pregnancies are monozygotic, but the fraction of monochorionic triplets remains unknown.[2] Assisted reproductive technologies may result in dichorionic or even monochorionic triplets if one of the embryonic disks splits after the third day after fertilization. Monochorionic twin pregnancies appear 7 to 8 times more frequently after assisted reproduction[3,4] than after spontaneous conception, and are especially associated with ovulation induction,[5] in vitro fertilization with blastocyst transfer,[6] and assisted hatching techniques.[7] However, among multiple pregnancies, the frequency of naturally conceived multiple pregnancies with a monochorionic component is much higher than in multiple pregnancies established with assisted reproductive technologies.[3,8] Between 5% and 10% of multiple pregnancies after assisted reproductive technologies have monochorionic pairs.[8,9] In triplet pregnancies, the presence of a monochorionic twin may jeopardize the perinatal outcome. Early spontaneous loss and pregnancy complications related to twin–twin transfusion syndrome rather than the number of fetuses complicate nearly 30% of dichorionic triplet pregnancies.[10]

We report the sonographic findings and obstetric outcomes in 29 monochorionic pregnancies of higher order (≥3).

METHODS

Ultrasound charts of 239 consecutive high-order multiple pregnancies (≥3) between 1990 and 2000 were reviewed. There were two octuplet, two septuplet, six sextuplets, eight quintuplet, 44 quadruplet, and 177 trip-

From the Division of Feto-Maternal Medicine and Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Academisch Ziekenhuis VUB, Vrije Universiteit Brussel, Brussels, Belgium.

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Table 1. Demographic Characteristics in 29 Monochorionic and 210 Multichorionic Multiple Pregnancies of Higher Order

	Group 1 $n = 29$	Group 2 $n = 210$	P
Maternal age (y) mean ± SD	29.4 ± 3.8	30.3 ± 4.1	.4
(range)	(24–37)	(19–43)	
Gestational age at diagnosis (wk) mean ± SD	11.3 ± 1.1	10.0 ± 1.0	.2
(range)	(10–13)	(9–15)	
White	28	206	.5

SD = standard deviation.

let pregnancies. All patients were referred for first trimester multifetal reduction with or without prenatal diagnosis. For that purpose, chorionicity and amnionicity was meticulously determined by counting the number of placental disks, evaluating the lambda sign and the thickness of the dividing membranes, and counting the number of amniotic cavities and yolk sacs per gestational sac. All pregnancies were divided into two groups: group 1 contained multiple pregnancies with a monochorionic component or pregnancies being completely monochorionic ($n = 29$), whereas group 2 consisted of pregnancies where the chorionicity matched the number of fetuses ($n = 210$).

The mode of conception was categorized in four groups: natural; by ovulation induction; by artificial reproduction techniques, including in vitro fertilization (IVF) and intracytoplasmic sperm injection; or unknown. The number of embryos transferred in utero was compared with the chorionicity of the resulting pregnancies.

Obstetric outcome was studied in terms of mean gestational age at delivery, mean birth weight, congenital malformations related to monochorionicity, perinatal loss rates, and baby take-home rate.

Statistical analysis involved Mann-Whitney test, Fisher exact test, and χ^2 test when appropriate at significance level of $P \leq .05$. Relative risks (RRs) and confidence intervals (CIs) for estimated proportions were added.

RESULTS

A total of 239 patients with high-order multiple pregnancies (≥3) was studied between 1990 and 2000. Mean maternal age and mean gestational age at the time of referral did not differ significantly between groups 1 and 2 (Table 1). Fourteen patients conceived naturally (5.9%), and of the remaining 225 patients, 105 pregnancies were established after induction of ovulation (43.9%), and 86 needed assisted reproductive technologies (36.0%). In 34 patients, the way of conception was unknown (14.2%), nine of these patients had quadruplet or quintuplet pregnancies, most likely as a result of assisted reproductive technologies. Seven of the 14 naturally conceived high-order multiple pregnancies contained a monochorionic component (0.5; 95% CI 0.23, 0.77), in contrast with 21 of 191 (0.11; 95% CI 0.07, 0.16) pregnancies established after infertility treatment ($P < .001$). Of these 21, seven were obtained after ovulation induction, and 14 after assisted reproductive technologies (Table 2). Ovulation induction resulted more frequently in a multichorionic pregnancy (28 of 105) than in a pregnancy with monochorionic component (7 of 105 ($P = .03$; RR = 0.52; 95% CI 0.27, 1.0).

The obstetric outcomes of the 29 multiple pregnancies with a monochorionic component are represented in Tables 3 and 4. In 21 patients (Table 3), including 13 bichorionic triamniotic triplet pregnancies, seven trichorionic quadramniotic quadruplet pregnancies, and one sextuplet pregnancy, the monochorionic twin pairs were selectively reduced resulting in 13 singleton pregnancies and eight bichorionic twin pregnancies. One pregnancy was completely lost at 19 weeks. Mean gestational age at delivery and mean birth weight in singleton and twin pregnancies was 39.2 ± 1.4 weeks and 3105 ± 430.6 g, and 34.3 ± 2.9 weeks, and 2083 ± 426.6 g and 2124 ± 405.4 g, respectively. The baby take-home rate was 27 of 29 or 93.1%.

Table 2. Type of Placentation According to the Way of Conception

	Total number	Group 1 (%)	Group 2 (%)	P	RR	CI
Natural	14	7 (50)	7 (50)	<.001	7.24	2.74, 19.16
OI	105	7 (6.6)	98 (93, 4)	.03	0.52	0.27, 1.0
ART	86	14 (16, 2)	72 (83.8)	1.41	1.41	0.92, 2.14
Unknown	34	1 (3)	33 (97)	.22	0.22	0.03, 1.55
Total	239	29 (12.1)	210 (87.9)			

RR = relative risk; CI = 95% confidence interval; OI = ovulation induction; ART = assisted reproductive technologies, including in vitro fertilization and intracytoplasmic sperm injection.

Table 3. Obstetric Outcome in 21 High-Order Multiple Pregnancies Containing a Monochorionic Twin

	Multiple pregnancies containing a monochorionic twin	
	Reduced to twins	Reduced to singletons
Number	8	13
TOP	0	0
Pregnancy loss	1	0
Mean GA at delivery (wk)	34.3 ± 2.9	39.2 ± 1.4
Mean BW (g)		
BB1	2083 ± 426.6	3105 ± 430.6
BB2	2124 ± 405.4	
BTHR	14 of 16	13 of 13

TOP = termination of pregnancy; GA = gestational age; BW = birth weight; BB = birth baby; BTHR = baby take-home rate.

The follow-up of the eight remaining pregnancies containing a monochorionic triplet is summarized in Table 4. Three of eight cases showed a monochorionic triplet pregnancy and a twin reversed arterial perfusion sequence, none of which reached viability. The acardiac fetus and its related pump twin were monoamniotic in two of the three cases. One patient was initially managed conservatively, but fetal death and preterm prelabor rupture of membranes at 19 weeks led to a termination of pregnancy. In the second pregnancy, a dilation and curettage was performed for a missed abortion, and in the third patient with a twin reversed arterial perfusion sequence, the couple requested a termination of pregnancy. Of the five remaining patients, two opted for a termination of pregnancy for psychologic reasons. In both cases, the monochorionicity was confirmed by pathologic examination. In one dichorionic quadramniotic quadruplet pregnancy, the selective feticide of the monochorionic triplet resulted in an uneventful pregnancy and a normal obstetric outcome. Two patients requested a bipolar coagulation of the cord of one of the fetuses at 18 weeks, resulting in a monochorionic diamniotic twin pregnancy. At 37 weeks, one patient delivered two healthy girls weighing 2700 and 2650 g, the other patient, at 34 weeks, delivered two healthy girls weighing 1545 and 1640 g. They all had an uneventful neonatal course. The baby take-home rate is five of 18 or 27.7%.

DISCUSSION

From 1980 to 1998, the triplet live births per year have increased significantly from one in 2702 to one in 517.[11] This spectacular rise in the number of triplet and higher-order multiples coincides with the rise in maternal age at which women conceive and with the more frequent use of various assisted reproductive technologies.[12] In Flanders, of a total of 340 triplet pregnancies over the past 10 years, 271 were established after infertility treatment (79.7%).[13]

Multiple pregnancies of higher order established after

Table 4. Follow-up of Eight High-Order Multiple Pregnancies Containing a Monochorionic Triplet

Case no.	GA (wk)	Number of Fet/AC	Way of conception GPA	Complication	Outcome
1	14.5	3/2	Spontaneous G2P1	TRAP	Fetal death and PPROM at 19 wk; TOP Pathology: monochorionicity confirmed
2	12	3/3	Spontaneous G2P0A1		Pathology: monochorionicity confirmed; TOP
3	10.5	3/3	Spontaneous G1P0		Pathology: monochorionicity confirmed; TOP
4	10	4/3	ET (3) G1P0	TRAP	TOP by D&C
5	11	3/2	Spontaneous G5P3A1	TRAP	Miscarriage D&C
6	12	3/3	Spontaneous G1P0	none	Bipolar cord coagulation 37 wk/2700g/2650g Pathology: monochorionicity confirmed
7	10	3/3	ET (2) G1P0	none	Bipolar cord coagulation 34 wk/1545g/1640g Pathology: monochorionicity confirmed
8	11	4/4	NA	none	Selective feticide to singleton; 39 wk/3290g

GA = gestational age; Fet/AC = fetuses/amniotic cavities; GPA = gravidity/parity/abortion; TRAP = twin reversed arterial perfusion sequence; PPROM = preterm prelabor rupture of membranes; TOP = termination of pregnancy; ET ( ) = number of embryos transferred ( ); D&C = dilation and curettage; NA = not available.

infertility treatment are usually multizygotic and multichorionic. Machin and Bamforth found six monozygotic, seven dizygotic, and two trizygotic sets of triplets among 15 consecutive spontaneously conceived triplet pregnancies.[14] Allen[2] conceived a formula by which the number of monozygotic triplet pregnancies could be calculated in a triplet population, considering the number of like-sex triplets, the number of dizygotic triplets, and the number of unlike-sex triplets, and estimated the number of monozygotic triplets pregnancies in a triplet population at 4.5%. The incidence of monochorionic multiples of higher order must even be lower. Obstetric outcome and clinical management of multiple pregnancies relies on chorionicity. Unfortunately, the few studies having evaluated the perinatal outcome in triplet pregnancies rarely mention the type of placentation, assuming trichorionicity in all cases.[15–19]

The investigation of third trimester fetal death among 89 triplet pregnancies by Berlum[20] demonstrated the association of a mono- or dichorionic placenta in ten of the 15 stillbirths, confirming the observations by Gonen et al[21] and Cherouny et al.[22] Prenatal findings on chorionicity in triplet pregnancies are scarce and almost exclusively provided by fetal reduction programs. Sepulveda et al[23] documented chorionicity in 48 triplet pregnancies by ultrasonographic examination of the epsilon zone. Although the majority of triplet gestations were trichorionic (81%), eight were dichorionic (17%), and one (2%) was monochorionic. Monteagudo et al revealed six monochorionic pregnancies of 148 patients (4%) with high-order multiples: two dichorionic triamniotic and four trichorionic quadramniotic pregnancies.[24] Only one of six pregnancies was conceived spontaneously. Of 239 consecutive high-order multiple pregnancies (at least a triplet pregnancy) amenable for fetal reduction, we found 29 patients (12%) with a monochorionic twin or triplet pregnancy. Seventy-two percent (21 of 29) of these pregnancies were the results of different fertility treatment protocols.

Of the high-order multiple pregnancies conceived in a natural way, 50% contained a monochorionic component (seven of 14). Although these results may be biased by the way the patients were recruited, they indicate that multichorionicity in naturally conceived pregnancies should not be considered obvious. Unfortunately, 33 patients in the nonmonochorionic group and one in the monochorionic group lacked data on the way of conception. Although these differences were not statistically significant, the majority of the 33 nonmonochorionic pregnancies could be naturally conceived, as only nine of them were quadruplet of higher-order multiple pregnancies. However, if we assume that all 33 cases were conceived naturally, 15% of these pregnancies still yielded a monochorionic component, which is certainly higher than the expected rate of less than 4.5%.[2] This rate has also been reported by Chasen et al;[10] in 14 cases of dichorionic triplet pregnancies, two patients conceived naturally (14.3%).

Monozygotic multichorionic triplet pregnancies result from embryonic splitting in the first 3 days after fertilization; after this, they become monochorionic. Establishing chorionicity and amnionicity in multiple pregnancies by early sonographic examination relies on counting the number of gestational sacs, identifying the number of heartbeats and yolk sacs within each gestational sac, and identifying the number of amniotic membranes.[24] The highest accuracy is obtained at 8–10 weeks' gestation. Monochorionicity is linked with a higher morbidity and mortality rate caused by complications particularly related to interfetal vascular anastomoses in the single placental disk: twin–twin transfusion syndrome, twin reversed arterial perfusion sequence, and fetal death of one fetus.[25] Feto-feto-fetal triplet transfusion syndrome with normal survival of only the fetus after early preterm cesarean delivery has been reported.[26] The incidence of the twin reversed arterial perfusion sequence in triplet pregnancies is much higher than the expected rate of one in 30.[27] Monoamnionicity would still further increase that risk. In our small series, three of eight monochorionic multiples showed a twin reversed arterial perfusion sequence, and two cases were monoamniotic.

Patients seeking reduction in multifetal pregnancies containing a monochorionic component should be advised in view of the hidden mortality of the monochorionic component to have this part of their pregnancy reduced.[25] Analysis of the outcomes of 14 dichorionic triplets by Chasen et al,[10] revealed a nearly 30% pregnancy loss rate. Two of these 14 pregnancies (14%) were terminated because of a severe twin–twin transfusion syndrome, whereas two other patients lost their pregnancy completely before viability at 21 and 22 weeks. Fetal reduction of 21 monochorionic twins and one monochorionic triplet resulted in only one pregnancy loss of 22 (4.5%). In addition, mean gestational age at birth and mean birth weight were comparable with normal twin and singleton pregnancies. Although the presence of monochorionicity was not specified in the 10-year multicenter overview of multifetal pregnancy reductions,[28] our data compare well with the reported outcome of multifetal pregnancies reduced to twins or singletons. Multifetal reduction carries a low pregnancy loss rate in experienced hands, and perinatal risk after the reduction is reduced to levels of pregnancies with the same number of fetuses.

Interfetal placental vascular anastomoses prohibit traditional fetal reduction techniques in monochorionic

fetuses. Exclusion of the fetus from the common vascular bed of the monochorionic placenta can be achieved by cord ligation, the use of histoacryl or pure ethanol, ultrasound-guided monopolar coagulation of the thoraco-abdominal vasculature, or bipolar coagulation of the umbilical cord, or even fetoscopic neodymium yttrium aluminum garnet laser cord occlusion. Although successful fetal reductions have been reported with each of these techniques, pregnancy loss rates remain much higher than for classical fetal reductions.[29] Therefore, these techniques are used only exceptionally in cases where pregnancy loss would otherwise be inevitable, or in cases where one of the fetuses presents a severe congenital malformation most likely leading to fetal death. However, as with trichorionic triplet pregnancies, couples are not always able to cope with a high-order monochorionic multiple gestation with a high perinatal morbidity and mortality. In two patients, we successfully reduced a "sonographically normal" triamnionic triplet to a biamnionic twin pregnancy. Although the reduction does not eliminate the risk for twin–twin transfusion syndrome, it is considered a valid alternative to termination of pregnancy in cases of longstanding infertility treatment. Termination of pregnancy in two of the eight first trimester uncomplicated monochorionic triplet pregnancies can be supported by the fact that these women conceived spontaneously and were primigravidas. They judged the unpredictable outcome of their pregnancy to be psychologically worse than making a new start.

In conclusion, our data indicate a high incidence of complete or partially monochorionic pregnancies among high-order multiple pregnancies, especially after a spontaneous conception. Multifetal reduction of the monochorionic component in a multifetal pregnancy leads to favorable pregnancy outcome. Patients carrying a complete monochorionic pregnancy after assisted reproductive technologies may benefit from cord coagulation techniques to reduce the twin transfusion syndrome–associated perinatal risks. In spontaneously conceived complete monochorionic gestations, a termination of pregnancy is acceptable.

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Address reprint requests to: Luc De Catte, MD, Academisch Ziekenhuis Vrije Universiteit, Feto-Maternal Medicine, Department of Obstetrics and Gynecology, Laarbeeklaan 101, 1090 Brussels, Belgium; E-mail: luc.decatte@az.vub.ac.be.

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