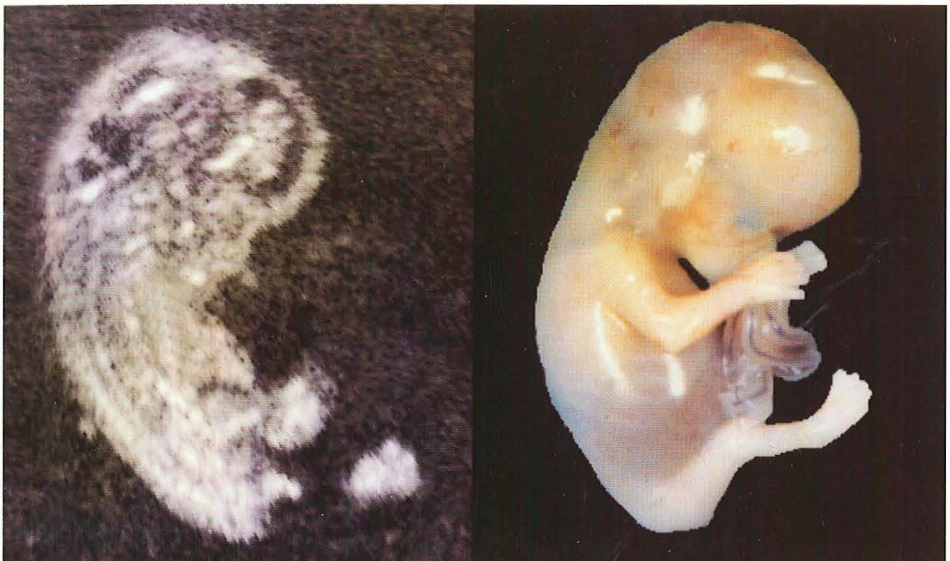
Christina Vogt Isaksen

# PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS

## A ten year correlative study of fetuses and infants with developmental anomalies



NTNU Trondheim
Norwegian University of Science and Technology
Faculty of Medicine

*Christina Vogt Isaksen*

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## A ten year correlative study of fetuses and infants with developmental anomalies

*I dedicate this thesis to*
*my three children who*
*were given the gift of life*

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of Science and Technology
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Department of Pathology
Department of Laboratory Medicine
Trondheim University Hospital
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Trondheim, June 1999

Christina Vogt Isaksen

# ABBREVIATIONS

| | |
|---|---|
| ARS | Amniotic rupture sequence |
| ASD | Atrial septal defect |
| AVSD | Atrioventricular septal defect |
| CHD | Congenital heart defect |
| CNS | Central nervous system |
| DNA | Deoxyribonucleic acid |
| FISH | Fluorescence *in situ* hybridization |
| IUGR | Intrauterine growth retardation |
| LBWC | Limb-body wall complex |
| RNA | Ribonucleic acid |
| TOP | Termination of pregnancy |
| US | Ultrasound |
| VSD | Ventricular septal defect |

# LIST OF PAPERS

This thesis is based on the following papers:

I Christina Vogt Isaksen, Sturla H. Eik-Nes, Harm-Gerd Blaas, Sverre H. Torp. Comparison of ultrasound and postmortem findings in fetuses and infants with central nervous system anomalies. Ultrasound Obstet Gynecol 1998;11:246-53.

II Christina Vogt Isaksen, Sturla H. Eik-Nes, Harm-Gerd Blaas, Eva Tegnander, Sverre H. Torp. Comparison of prenatal ultrasound and postmortem findings in fetuses and infants with congenital heart defects. Ultrasound Obstet Gynecol 1999;13:117-26.

III Christina Vogt Isaksen, Sturla H. Eik-Nes, Harm-Gerd Blaas, Sverre H. Torp. Fetuses and infants with congenital urinary system anomalies: correlation between prenatal ultrasound and postmortem findings. Accepted for publication in Ultrasound Obstet Gynecol.

IV Christina Vogt Isaksen, Sturla H. Eik-Nes, Harm-Gerd Blaas, Sverre H. Torp. A correlative study of prenatal ultrasound and postmortem findings in fetuses and infants with an abnormal karyotype. Accepted for publication in Ultrasound Obstet Gynecol.

V Christina Vogt Isaksen, Borgny Ytterhus, Sølvi Skarsvåg. Detection of trisomy 18 on formalin-fixed and paraffin-embedded material by fluorescence *in situ* hybridization (FISH). Accepted for publication in Pediatric and Developmental Pathology.

# SUMMARY

### Introduction

Congenital anomalies affect about 3% of births and constitute over 30% of perinatal deaths. Detection of fetal developmental anomalies by ultrasound examination of pregnant women has become a specialized field of medicine. For quality control of this practice, a detailed postmortem examination is necessary.

### Purpose

The study was designed to evaluate the concordance of prenatal ultrasound findings with the postmortem examination in fetuses and infants with congenital anomalies. The population consisted of fetuses and infants of both referred and non-selected pregnant women.

### Material and Methods

The study comprised 408 fetuses and infants with congenital anomalies. Criteria for inclusion were a prenatal ultrasound examination at the National Center for Fetal Medicine, Trondheim University Hospital, and an autopsy performed during the ten year period 1985 to 1994. The postmortem examinations were performed at the Department of Pathology, Trondheim University Hospital (365) and at hospitals cooperating with the center (43). Anomalies of the central nervous system, heart and urinary system, and fetuses with chromosome aberrations, were analyzed separately. Results from the ultrasound and autopsy examinations were analyzed and categorized according to the degree of concordance.

### Results

Central nervous system (CNS) anomalies were found in 140 (34%) of the 408 fetuses and infants. In 120 of these the CNS anomaly was the main diagnosis, in the rest the CNS anomaly was associated with other important anomalies and chromosome aberrations. Congenital heart defects (CHD) were found in 106 (26%) of the cases, with ventricular septal defect (VSD) as the most prevalent diagnosis. Urinary system anomalies were present in 112 cases (27%), and 98 fetuses (24%) had a chromosome aberration with trisomy 18 as the most frequent abnormal karyotype, and CHD as the most prevalent diagnosis.

When all anomalies were included, the *main diagnosis* was correct in 90%. For central nervous system anomalies, the concordance was 94%; for urinary system anomalies, 91%; and for congenital heart defects 91%. C*omplete agreement* was obtained in 89% of the central

nervous system anomalies and 87% of the urinary system anomalies, while congenital heart defects were significantly ($p<0.05$) more difficult to diagnose, 73%. When the study was split into two time periods, the detection of congenital heart defects rose significantly ($p<0.01$) from the first (48%) to the second (82%) time period.

**Discussion**

Anomalies of the central nervous system were the lesions most easily detectable. Bilateral renal lesions had a higher detection rate than unilateral lesions. The accompanying anhydramnios in bilateral lesions will usually trigger the suspicion of a renal anomaly. The increased detection rate of cardiac anomalies from the first time period to the second is consistent with improved experience and technical advancements. Previous studies have compared prenatal ultrasound examination with autopsy findings in cases with congenital anomalies, though only a few have specifically addressed the discrepancies between prenatal and postmortem findings. Other studies concerning single organ anomalies have dealt with both clinical findings in live infants and autopsy findings. All these studies have shown variable results not directly comparable to our series.

**Conclusion**

The correlation between prenatal ultrasound and autopsy findings is good with the main diagnosis correct in 90%. No significant differences were found between the findings for the central nervous system, the heart and the urinary system. As for complete agreement, congenital heart defects were more difficult to detect than CNS and urinary system anomalies, though a significant improvement occurred between the first to the second time period.

**Future aspects**

There is reason to believe that improved quality of the scans and increased experience of the ultrasonographers and ultrasonologists will continue, so that in the future most structural anomalies in the fetus will be detected at an earlier gestational age. The verification of an ultrasound diagnosis is important for future progress in ultrasonography. Confirmation of the ultrasound findings by autopsy will still be necessary. Autopsies of increasingly smaller fetuses will demand experience with more sophisticated methods of examination. The collaboration between the two specialties is mutually stimulating.

# INTRODUCTION

"To investigate the causes of death, to examine carefully the conditions of organs, after such changes have gone on in them as to render existence impossible and to apply knowledge to the prevention and treatment of disease, is one of the highest objects of the physician."
"To practice pathology without books is to sail uncharted seas; to practice pathology without performing autopsies is not to go to sea at all."

*Sir William Osler*

## General Background

Prenatal ultrasound examination for term determination and detection of congenital anomalies has become widely accepted, not only in institutions but also in private practice. In 1986, a consensus conference was arranged by the Norwegian health authorities in order to better organize the use of ultrasonography in pregnancy. The panel recommended a routine ultrasound examination around the 17th gestational week in order to reduce the number of examinations, improve the quality and reach a greater part of the population (Backe and Buhaug 1986). Determination of term, number of fetuses, placental location, anomalies and the general condition of the fetus, were the main reasons for implementing the routine ultrasound examination (Backe and Buhaug 1986, Nafstad and Backe 1989). At the same time, a demand for continuous quality control of ultrasonography was put forth. According to the law concerning state control of health services of 1984, there is an extensive demand for quality control (Den norske lægeforening 1989).

Gradually, both among health personnel and in the media, a debate has evolved pertaining to the use of ultrasound in pregnancy. The ethical aspect of screening for congenital anomalies has become a target for political discussion and was addressed at another consensus conference in Oslo in 1995 (Norges Forskningsråd 1995). The quality of ultrasound examinations is continually increasing and the possibility to diagnose congenital anomalies has shifted to an earlier stage of pregnancy, continuously narrowing the gap to the gestational age when legal abortions are permitted.

After the introduction of ultrasonography in obstetrical practice, both authorities and the public have become aware of the consequences of this sophisticated diagnostic tool. The performance of a thorough autopsy with documentation of findings represents a quality control of the work performed by ultrasonographers and ultrasonologists in the field of obstetrics. A postmortem examination of aborted stillborn fetuses and infants is by no means the only correct answer to all problems, but traditionally it has been considered as the "gold standard". As we will see, this is not always the complete truth, though a conscientious autopsy by an experienced pathologist knowing what to look for and aware of the pitfalls, will provide an adequate quality control of the established ultrasonographic activity.

The increasing accuracy of ultrasonography has lead to earlier diagnosis of anomalies, thus the aborted fetuses are both younger and smaller. This implies new methods and techniques of examination by the pathologist. Correct diagnoses are important not only for the parents involved but also for health personnel and for epidemiological purposes. In Norway, information on all late induced abortions and stillborns after 16 gestational weeks is to be sent to the Medical Birth Registry of Norway. A conscientious and accurate autopsy is therefore essential in order to ensure that the epidemiological data will be correct. A continuous surveillance of congenital anomalies will allow detection of changing incidences of anomalies over time and in particular areas. This is to enable the authorities to pinpoint an eventual environmental disturbance (Medical Birth Registry of Norway 1997). An example of this type of work was the review by Irgens *et al.* on pregnancy outcome in Norway after the Chernobyl accident (Irgens *et al.* 1991).

The increasing demands in perinatal medicine require the special competence of individuals with different backgrounds and experience. Collaboration between different professions becomes increasingly important. Multidisciplinary joint meetings with ultrasonographers, ultrasonologists, midwives, nurses, obstetricians, pediatricians, pediatric surgeons and pathologists have become mutually instructive and necessary for daily work.

# Historical Background

## Congenital anomalies

### Mythology

The nomenclature used for some congenital anomalies has parallels in Greek mythology. The most severe form of alobar holoprosenecephaly is accompanied by cyclops. The cyclopes in Greek and Roman legends were fabulous creatures with a single eye in the center of their foreheads. The Uranian cyclopes were freed by Zeus from the underworld and as a sign of gratitude they fashioned thunder and lightning, enabling him to take control of the celestial throne. They were put to death by Apollo who would not forgive them for having given Zeus the lightning with which he struck and killed Asclepius, his son. The most famous Cyclopes were those described by Homer. They were brutal giants not afraid to devour an occasional human who ventured into their territory. In the eyes of the Greeks, they represented the type of savage, uncultivated race, devoid of any idea whatsover of civilization (Grant and Hazel 1973, Schmidt 1980). Thanatophoric dysplasia comes from the Greek word thanatophoros which means death-bearing. Euripides, in his tragedy Alcestis, describes Thanatos as the god of Death. He lived in the Underworld and was the son of Nyx (night) and the twin brother of Hypnos (sleep) (Grant and Hazel 1973, Schmidt 1980).

In the Icelandic saga of Njål there is a description of Iceland's conversion to Christianity (in the year 1000). The foundation of laws included a statement that children (with anomalies) no longer were to be abandoned (Paasche 1986). Though no evidence has been found that the vikings had racial hygienic measures behind their actions. In the Nordic countries, before the era of Christianity, there was no law against laying out newborns. It was an accepted practice probably related to poverty, but also for other social reasons. The custom to abandon infants who were too weak to survive seems to have been accepted in many cultures, best systematized among the ancient Greeks. Particularly in Norway, the introduction of Christianity probably did not completely abolish the tradition of abandoning infants with congenital anomalies. In several laws from the last half of the 12th century, among these Grágás from Trøndelag, were detailed descriptions of how malformed a child could be before it was permitted to be laid out. Even if these descriptions were of such a character that probably no child was sufficiently malformed to fulfill these laws, they opened up for such a practice in theory (Mundal 1987).

### Ethical considerations

In cultures all over the world the most basic wish of all parents is the birth of a healthy child. The choice to terminate a pregnancy when the fetus has a lethal anomaly or serious handicap is perhaps one of the most difficult decisions to make. It is important that such a decision be made without any pressure from the environment. Prenatal diagnosis is considered real progress when applied in strictly medical and individual terms. Expanded to a whole community to serve collective choices, new technology may disturb the essential values on which our culture is founded (Fletcher and Evans 1992, Mattei and Rauch 1997). From the turn of the century, prejudices generated a science of improving the human race by selective breeding (Hubbard 1986). The passage of eugenic laws has demonstrated the danger of not distinguishing between science and politics (Roll-Hansen 1989, Wahlstein 1997). Eugenic legislation in China gives no escape from the physician's order for sterilization or pregnancy termination, though the law is administrered differently depending on class, ethnic, political and geographical differences (Morton 1998).

## Ultrasound examination

In 1880, the Curie brothers detected the pressure-electric effect and noted the potential for creating ultrasonic waves (Curie and Curie 1880). The first practical application was for determining the position of icebergs, as a response to the Titanic tragedy. The Austrian neurologist Dussik was the first to try ultrasound for medical diagnostic purposes in 1938. However, his method failed because of the differences of ultrasonic absorption in tissues (Dussik 1942). In 1947, Howry started to perform experiments with navy sonar equipment and radar amplifiers and together with Bliss, an engineer, he developed a pulse-echo technique. The quality of the picture was improved by a compound-scanning technique and the results were reported in 1952 (Howry and Bliss 1952).

New possibilities for the use of sonar examination technique in obstetrics and gynecology were introduced by Ian Donald who, together with Brown, developed the first direct-contact two-dimensional ultrasonic scanner in 1954 (Donald et al. 1958). Improvement of imaging technique was continued by Kossoff in the early seventies (Kossoff 1972). The process of developing ultrasound as a valuable diagnostic tool came forth through the efforts of clinicians and engineers, and this cooperation continues in constant development. Ultrasonography in obstetric practice - being able to visualize a living fetus in utero - has revolutionized our concepts of life.

Since the late seventies, ultrasound has been used for determination of term, location of the placenta and diagnosis of twins. Gradually, ultrasonography became a sophisticated tool for diagnosing congenital anomalies with the consequences this has for further management of the pregnancy. One of these consequences is the unsuccessful outcome of pregnancy which has a great impact on the family involved. Their decision to terminate a pregnancy has to be founded on the knowledge of the anomalies involved.

Bertil Sundén was one of the pioneers in detecting anomalies by ultrasound. In 1964 he described 3 cases of acrania (Sundén 1964). In 1972, the therapeutic abortion of an anencephalic fetus was performed after antenatal detection by ultrasonography (Campbell *et al.* 1972). Spina bifida, hydrocephalus and other craniospinal defects were in focus a few years later (Campbell 1977). The medical and technical expertise in this area is continuously improving, though the diagnostic possibilities with modern high resolution ultrasound apparatus are probably not yet completely envisaged. High-frequency transvaginal ultrasound has increased the diagnostic accuracy of fetal anomalies and made detection in early pregnancy possible (Blaas and Eik-Nes 1996). There is reason to believe that three-dimensional ultrasound reconstructions will further contribute to improve these diagnostic possibilities (Blaas *et al.* 1998).

## Perinatal pathology

We have little information on autopsy from ancient times, but we do know that the practice of dissection was permitted in Alexandria. In the 13th century, the study of the dead body was recommended, but there were strong religious and social objections to autopsy, though there were no formal church prohibitions. Pope Sixtus IV (1471-1484) issued a bill permitting studies of human bodies by students at Bologna and Padua. Morgagni (1682-1772) connected clinical observations with pathological data, bringing new enlightment into pathology. Xavier Bichat (1771-1802) introduced a turning point in medical history by combining medical activities like anatomy, physiology and pathology on the one hand, and bedside care on the other. He focused on the function and the differences between the living and non-living, and his interest in autopsies went hand in hand with his interest in living patients (Dorsey 1978).

The correlation between clinical data and autopsy findings made tremendous progress throughout the 18$^{th}$ and first half of the 19$^{th}$ century. Virchow and the introduction of the microscope contributed further to the development of pathology. In the 20$^{th}$ century hospital standards were considered good when the autopsy rate was high (Dorsey 1978). However, with the increased burden on clinical pathology in recent years, the autopsy rates have been falling. It has been argued that the autopsy has a less important role in the quality control of medical practice because of advances in clinical knowledge with modern and sophisticated diagnostic methods and equipment. But studies still show substantial differences between major postmortem findings and clinical diagnoses (Goldman *et al.* 1983, Scottolini *et al. 1983,* Harrison and Hourihane 1989), necessitating continued autopsy evaluation as the basis for quality control of medical diagnoses. Finally, the mere performance of an autopsy does not necessarily lead to progress. It also depends on the person examining the material. It is necessary to have the perspective autopsy provides in order to achieve knowledgeable and justifiable medical care.

Pediatric pathology has slowly gained acceptance as a subspecialty. In 1900 Ballantyne was appointed as lecturer on antenatal pathology and teratology in Edinburgh and was probably the first perinatal pathologist. "Manual of antenatal pathology and hygiene", is a fine introduction to the field (Ballantyne 1902). Unfortunately, many pathologists have regarded performing perinatal autopsies as an unrewarding task contributing little to further care (Husain and O'Conor 1991). This attitude is changing. Obstetricians and perinatologists are increasingly interested in "what went wrong" and why the fetus or infant died. One area of special interest has been maternal infections and suboptimal prenatal care; these have been associated with prematurity which up to recently has accounted for a significant proportion of perinatal deaths (Naeye 1972). Various studies have shown that in about 20% of perinatal deaths the autopsy was the only means of establishing the cause of death and in an additional

20% of cases the postmortem findings influenced genetic counseling (Craft and Brazy 1986, Meier *et al.* 1986).

Improvements in obstetric and neonatal medicine have changed the epidemiology of perinatal mortality. Non-invasive techniques for fetal assessment and increasing use of ultrasonography for prenatal diagnosis of congenital anomalies leading to induced abortion, have accentuated the need for a detailed postmortem examination. Technical advancements have lead to more precise prenatal diagnoses, emphasizing the need for accurate anatomical verification of dysmorphic features and anomalies. Increased ultrasound expertise has made it possible to diagnose congenital anomalies at an earlier stage of pregnancy, and the gestational age at termination of pregnancy (TOP) has diminished over the last years. The necessity of establishing techniques that are appropriate for small fetuses is thus evident. Dissection of organs under a stereomicroscope might soon be part of the daily routine for a perinatal pathologist. The postmortem examination of late first-trimester and early second-trimester fetuses is thus evolving into a specialized field of pathology and represents a continuous challenge having to do with examinations of increasingly smaller fetuses.

Autopsy aids in the identification of specific diseases and iatrogenic disorders, and may result in altered management of subsequent pregnancies. Furthermore, in identifying congenital anomalies and inherited diseases, it is equally important to be able to exclude their presence. Every perinatal autopsy has thus a prognostic significance (Cartlidge *et al.* 1995). On the other hand, it is important to realize that an inadequately performed autopsy can be thoroughly misleading (Rushton 1994).

In conclusion, fetal and perinatal pathology is important for surveying perinatal mortality, analysis of causes of death, diagnosis of develomental anomalies, genetic counseling with information to parents regarding risks in future pregnancies, either pertaining to genetic inheritance or the risk of future abortions or intrauterine deaths and, finally, as a quality control of prenatal diagnosis of congenital anomalies by ultrasound examination (Rutledge *et al.* 1986, Manchester *et al.* 1988, Shen-Schwarz *et al.* 1989, Clayton-Smith *et al.* 1990, Grant *et al.* 1993, Weston *et al.* 1993, Chescheir and Reitnauer 1994, Julian-Reynier *et al.* 1994).

## Fluorescence *in situ* hybridization (FISH)

Of the various modern techniques applicable in perinatal pathology, fluorescence *in situ* hybridization (FISH) is a method that can be a useful supplement in the diagnosis of chromosome aberrations. It is a sensitive method for detection of structural and numerical chromosome aberrations. Hybridization is the ability of single-stranded DNA or RNA to anneal to its complementary regions, while failing to anneal to an unrelated sequence (Gelehrter and Collins 1990). Probes have been developed from specific chromosomal regions and modified for sensitive and specific detection (Klinger *et al.* 1992). Fluorescence *in situ* hybridization permits the visual localization of a specific DNA segment to either condensed DNA during metaphase or to elongated chromatin present in interphase nuclei (Wilkins-Haug *et al.* 1996). The method confers information only on the specific chromosome/gene studied.

FISH with chromosome-specific DNA probes can be used to study cells from various tissues, either in metaphase nuclei if cell culture is available, or on interphase nuclei when cell culture is not possible. Locus-specific probes can detect microdeletions in congenital disorders and probes for the centromeric regions of chromosomes can detect aneuploidy (Dewald *et al.* 1997). The use of region-specific DNA probes to chromosomes 13, 18, 21, X, and Y on uncultured amniocytes has gained wide acceptance as a rapid and preliminary prenatal diagnosis of aneuploidies (Christensen *et al.* 1992, Klinger *et al.* 1992, Ward *et al.* 1993). Protocols for the use on formalin-fixed (Fletcher and Evans 1992) and paraffin-embedded material have been developed (Kuchinka *et al.* 1995, Köpf *et al.* 1996) rendering retrospective analysis of abortuses and stillborn fetuses with multiple anomalies possible. The impact this technique can have on genetic counseling is thus evident, though never to forget the limitations of the method.

# General Considerations

## Terminology and definitions (Spranger *et al.* 1982, Wigglesworth 1991)

**Reproductive loss or pregnancy wastage:** mortality among human conceptuses before, during or shortly after birth

**Spontaneous abortion, miscarriage or early fetal death:** loss of a fetus before it is sufficiently mature to survive

**Stillbirth or late fetal death:** delivery of a potentially viable dead fetus (intrapartum death = fresh stillbirth; antepartum = macerated stillbirth) (Alberman and Creasy 1977)

**Fetal mortality:** intrauterine death irrespective of gestational age

**Neonatal mortality:** death of a liveborn child during the first 4 weeks of life

**Perinatal mortality:** liveborn and stillborn infants after gestational week 28; includes late fetal deaths and early neonatal deaths (first week of life)

Mortality is indicated by the number of deaths per 1000 births.

**Morbidity:** illnesses in the perinatal period are most often related to anomalies, prematurity, hypoxic brain damage and infections

**Congenital anomaly:** significant definable structural and/or developmental abnormality observed at birth (Gilbert-Barness *et al.* 1989).

**Malformation:** intrinsic defect which occurs very early during the initiation and organization of one developmental field (i.e. cleft lip and palate)

**Disruption:** secondary change in an otherwise normal developmental field (i.e. amniotic rupture sequence)

**Deformation:** normal anatomy deformed by external forces (i.e. foot deformity because of oligohydramnios)

**Sequence:** cascade of secondary malformations as a result of a focal primary defect (i.e. posterior urethral valves)

**Syndrome:** intrinsic alterations of several developmental fields by one etiologic agent (i.e. chromosome disorder)

**Dysplasia:** abnormal cellular organisation within tissues and its morphologiocal result (i.e. osteogenesis imperfecta)

**Association:** non-random occurrence of several malformations consistently observed together; unknown etiologic agent (i.e. VACTERL - vertebral, anal, cardiac, tracheo-esophageal, renal, limb disorder)

## Etiology

The causes of congenital anomalies can be considered in five broad groups: 1. chromosome anomalies, 2. mutant genes, 3. multifactorial disorders which are considered the result of interaction between genetic predisposition and presumed environmental factors, 4. teratogenic agents, 5. unknown. Single gene defects account for 6-8% (Gelehrter and Collins 1990, Keeling and Boyd 1993) and chromosome aberrations for about 6% of anomalies at birth, disturbed interactions between different genetic factors about 20% and for over half of all congenital anomalies, no specific cause can be found (Kalter and Warkary 1983).

### Chromosome aberrations

A chromosome aberration is an abnormality of chromosome number or structure resulting in the addition or deletion of entire chromosomes or parts of chromosomes. A polyploid cell can have 3 (triploid), 4 (tetraploid) or more complete sets of chromosomes instead of the normal 2 (diploid). Aneuploidy is any chromosome number that is not an exact multiple of the haploid number, i.e. the chromosome number of a normal gamete, with one member of each chromosome pair. It can refer to an extra copy of a single chromosome (trisomy) or the absence of a single chromosome (monosomy) resulting from nondisjunction during meiosis or mitosis.

Most major chromosome disorders are characterized by growth retardation, mental retardation, and a variety of somatic abnormalities. The loss or gain of whole chromosomes, except sex chromosomes, is often incompatible with survival. Major chromosome abnormalities are found in almost half of spontaneously aborted fetuses (Gelehrter and Collins 1990). Since as many as 50% of all conceptions, also those unrecognized, end in spontaneous abortion, 25% of all conceptions have a major chromosome anomaly. According to Jacobs (Jacobs 1977), no less than 10% of all clinically recognizable conceptions in the human species have been estimated to be chromosomally abnormal. As only 5% of stillborn infants have a chromosomal abnormality, most of the chromosomal loss occurs early in gestation (Alberman and Creasy 1977, Kajii *et al.* 1980, Hassold 1986, Gelehrter and Collins 1990). Before the widespread use of ultrasonography in pregnancy, chromosome abnormalities affected more than 0.5% of newborns (Jacobs *et al.* 1974, Alberman and Creasy 1977, Gelehrter and Collins 1990). Today, the practice of prenatal ultrasound examination, amniocentesis and chorionic villi biopsy, with subsequent therapeutic measures, has reduced the incidences of unexpected perinatal anomalies (Chitty 1995).

The most common abnormal karyotypes seen in spontaneously aborted fetuses are 45X, triploidy and trisomy 16. Trisomy 16 is not seen in liveborn infants, while 45X, before the era of ultrasound, occurred in liveborn females with a frequency of about 1/7000. Of autosomal abnormalities, trisomy 21 is the most common in liveborns. (Jacobs *et al.* 1974, Warburton *et al.* 1991). The overall incidence of all karyotypes is difficult to know because of the great variation in individual viability.

**Inherited genetic diseases**

Monogenic inheritance

Single gene disorders are due to mutations on a single genetic locus. These can have a great effect on the phenotype. They are inherited in a simple Mendelian fashion as autosomal dominant, autosomal recessive, or X-linked. Examples are Meckel-Grüber syndrome and infantile polycystic renal disease.

Polygenic inheritance

These disorders result from the interaction of multiple genes, each of which may have a relatively minor effect. These diseases are the most common of human genetic disorders. They are not inherited in a simple Mendelian fashion nor associated with chromosome abnormalities, but genetic factors do play an important role in their manifestation. Examples of polygenic inheritance are diabetes mellitus and hypertension, in addition to a variety of congenital defects such as cleft lip, cleft palate and most congenital heart defects. The additive or interactive effects of multiple genes create a predispostion to disease which is manifested in the presence of appropriate environmental triggers (Gelehrter and Collins 1990).

**Non-genetic causes**

Environmental toxins

***Teratogens***

A teratogen is a chemical or physical agent that produces or raises the incidence of congenital anomalies. The dose is not necessarily related to the damage produced. Demonstration of causal relationship in respect to an agent can be difficult because of the multiplicity of variables. Apart from the dose, they include timing of the insult, interaction with other potential teratogens and individual susceptibility (Keeling and Boyd 1993).

***Genetic toxicology***

A mutagen is a chemical or physical agent that increases the mutation rate by causing changes in DNA (deoxyribonucleic acid). A mutation is any permanent heritable change in the sequence of genomic DNA. Mutagenesis includes induction of DNA-damage and all types of genetic changes, ranging from one or a few basepairs, to gross changes where the inherited effects can be dominant or recessive. A mutation can occur spontaneously or as a result of interactions with physical or chemical mutagens. Radiation-induced mutations represent an increase in the frequency of the same mutations that occur spontaneously (Nilsen 1997).

***Chemical changes caused by radiation***

Particles causing ionizing radiation will be absorbed in biological material and react either directly with cell components initiating a chain of reactions (mostly $\alpha$-particles), or indirectly by reacting with atoms or molecules in the cell (particularly water) producing free radicals that can harm crucial molecules in the cell itself. Free radicals are caused by loss or gain of an electron; this is a very reactive condition where the atom tries to pair the electron by interacting with other elements. The condition is caused mostly by $\beta$ and $\gamma$ radiation. It does not make any difference whether the crucial molecule is damaged directly or indirectly. Since most cells and tissues are composed of 70-90% water, it is likely that much of the radiobiological damage is a consequence of an indirect impact of radiation (Nilsen 1997).

***Chromosome effects***

Ionizing radiation will affect a chromosome differently depending on the phase of cell cyclus during which it occurs. Some aberrations are stable, i.e. they can be transferred through repeated cell divisions. Chromosomal rearrangements transferable to cell populations are deletions, duplications, inversions and symmetrical translocations (Carrano and Natarajan 1988). Assymetrical rearrangements such as dicentric translocations and ring chromosomes have often been observed in connection with radiation and in experiments have increased with the radioactivity (Bauchinger *et al.* 1983). Such rearrangements are unstable and result in cell death because of loss of vital genetic material (Carrano and Natarajan 1988). The duration of unrepaired - or wrongly repaired - damage constitutes the background for mutations and chromosome aberrations, which can result in cell death or altered characteristics (Nilsen 1997).

Ionizing radiation has two different effects on biological material: stochastic and non-stochastic. The first is defined as damage where the effect is a function of the dose itself; for the second, the damage is dose-dependent. Mutations and chromosome aberrations are probably responsible for stochastic effects while cell death is responsible for non-stochastic effects. Distinguishing between these two processes in cases where cell death is achieved by low dosage can be almost impossible (Reitan 1989). Somatic cells respond differently to radiation than reproductive cells do. Mutations in somatic cells disturb normal development and will increase the frequency of congenital anomalies, cancer and infectious diseases. Mutations in reproductive cells will give increased genetic instability in later generations and cause genetic diseases, reduced fertility, dominant lethality, spontaneous abortions and congenital anomalies (Nilsen 1997).

***Ionizing radiation and congenital anomalies***

In connection with the Chernobyl accident in April 1986, a meticulous mapping of the radioactive fallout in Norway took place (Lie *et al.* 1992, Reitan 1994). A detailed calculation of population doses per muncipality and per month was done. These data were used as a measure of uterine doses to the fetus during the second month of gestation and correlated with data from the Norwegian Medical Birth Registry (Lie *et al.* 1992). No associations were found for conditions previously reported to be associated with radiation, i.e., microcephaly, cataracts, anencephaly, spina bifida or growth retardation (Lie *et al.* 1992). A positive association was found between hydrocephaly and total dose, including both external and internal (food based) radiation. Defining relevant doses can be difficult, and the latency from exposure until deleterious health effects occur will vary. Interaction with other carcinogens and environmental pollutants must also be taken into account. Moreover, the mean internal dose from nuclear contamination may not be comparable to a dose of the same magnitude from external radiation. Biological systems are complex and it is unlikely that they follow physical equations (Reitan 1994).

### Drugs

Reliable prediction of drug teratogenicity is difficult. The effect of a drug in animals is not direct evidence of its effect in humans. A review of teratogenic drugs is not in this scope, so only a few examples will be mentioned. Teratogenic effects have been observed with a variety of chemotherapeutic agents used in the management of malignant diseases. Folic acid antagonists are among those with highest risk (Keeling and Boyd 1993). Several anticonvulsant drugs produce recognized defects, Carbamazepine and Valproate can cause craniofacial defects and myelomeningocele. Retinoic acid prescribed for acne can cause anomalies involving craniofacial, cardiac, thymic and central nervous system structures. Contrary to vitamin A (retinol), retinoic acid has a short half-life and is not stored in tissues, and therefore does not represent a risk to pregnancies conceived after cessation of treatment (Lammer *et al.* 1985). Adverse effects of alcohol were described by Ballantyne already in 1902 (Ballantyne 1902), though the dysmorphic features and anomalies of the fetal alcohol syndrome were recognized much later (Jones *et al.* 1973). It is less known that even small amounts of alcohol (80g per day) consumed during the first month of pregnancy (particularly the third week) can cause holoprosencephaly (Larsen 1993).

### Infections

Certain infections affecting the mother during pregnancy are tranferred to the fetus and can produce a range of effects varying from structural anomalies to immunological disorders. Some of the more important infectious agents recognized as causing organ anomalies detectable by ultrasound are presented in the following text.

***Rubella virus*** is teratogenic during embryonic development and since the association with cataract was established by Gregg in 1941 its role as a cause of a variety of congenital anomalies has become well established. Prospective studies have been undertaken in order to investigate the risk of fetal injury and timing of maternal infection. In a follow-up study of children born after an epidemic of rubella in Sweden, the risk of major defects following first trimester maternal infection was found to be 10% (Lundström 1962). In a study by Miller *et al.*, heart defects were seen in infants when the infection had occurred before the 11th gestational week, while deafness was most frequently seen in those infected between 13-16 weeks (Miller *et al.* 1982).

***Cytomegalovirus (CMV)*** is endemic worldwide and is the most frequent cause of congenital infection (Holzel 1993). Primary infection during pregnancy occurs from 0.7%-4% (Alford *et al.* 1990) with fetal infection in 30%-40% of cases (Stagno *et al.* 1986). Fetal infection can also follow recurrent infection.The risk of fetal disease appears to be higher when infection occurs in the first half of pregnancy (Stagno *et al.* 1986). Serious cytomegalovirus infection is a multiorgan disease and causes tissue destruction with calcifications; in the CNS the affection is preferably in the subependymal region with resulting microcephaly and hydrocephaly (Squier 1993), detectable by ultrasonography.

***Parvovirus B19*** infection in pregnancy was first described in 1984 (Brown *et al.* 1984). The virus replicates in late erythroid precursors, inhibiting maturation, and the resulting anemia produces cardiac failure and hydrops (Andersen 1990, Berry *et al.* 1992). Infection can be diagnosed by intranuclear inclusions in red blood cell precursors, serological and molecular methods are also available. Parvoviruses are teratogenic in animals; there is no evidence for this in humans (Berry *et al.* 1992). Parvovirus infection may account for up to one third of cases of non-immune fetal hydrops and can be detected by *in situ* DNA hybridization (Porter *et al.* 1988). Since blood transfusions may alleviate the anemia in affected fetuses, establishing the diagnosis is critical (Soothill 1990).

***Toxoplasmosis*** caused by the parasite Toxoplasma Gondii follows consumption of infected undercooked meat or exposure to oocysts in cat excreta or infected soil. Antibody studies show marked differences, from 15%-95%, in different populations (Squier 1993). Infection during pregnancy results in transmission to the fetus in approximately half of the cases, with varying rates of transmission and severity of disease depending on the stage of pregnancy during which the infection occurs (Desmonts and Couvreur 1974). Toxoplasmosis results in tissue destruction with calcifications; the CNS manifestations affect the ependyme in such a way that may obstruct the spinal canal causing hydrocephaly, less often microcephaly (Squier 1993).

Mechanical damage

***Oligohydramnios*** was first described by Potter (Potter 1946) in association with renal agenesis, though Potter's facies is similar whatever the cause. Low-set ears, small chin, flattened nose and talipes can simulate a chromosomal aberration, but it is the lack of amniotic fluid that causes this postural deformation (Scott and Goodburn 1995).

***Uterine deformities or tumors***, f.ex. large submucosal leiomyomas can also affect a fetus, depending on the deformity and the position of the fetus.

# Survey of Anomalies

The following descriptions are limited to anomalies relevant for the comprehension of this thesis.

## Central nervous system anomalies

Anomalies of the central nervous system are among the most common prenatally diagnosed anomalies (Sabbagha *et al.* 1985, Rutledge *et al.* 1986, Grant *et al.* 1993, Chescheir and Reitnauer 1994). In perinatal autopsy studies, CNS anomalies are the most frequently encountered developmental anomalies, varying from 30-40% (Shen-Schwarz *et al.* 1989, Clayton-Smith *et al.* 1990, Weston *et al.* 1993, Swain *et al.* 1994, Julian-Reynier *et al.* 1994, Isaksen *et al.* 1998).

### Neural tube defects

Neural tube defects are second only to congenital heart defects as a cause of perinatal mortality due to birth defects. They are major malformations of the central nervous system in which the central canal of the malformed brain or spinal cord is persistently open to the outside environment (Copp *et al.* 1990). During development, the exposed nervous tissue degenerates leading to absence of the cranial vault or local disruption of the vertebrae. Neural tube defects result from locally defective neural tube closure during the 3rd to 4th week of gestation, based on conceptional age (Nicolaides and Campbell 1987, Larsen 1993). They can arise from failure of neurulation itself or failure of development of adjacent structures necessary for neurulation to occur. Cranial defects seem to arise from failure of *de novo* closure initiation events while caudal defects result from failure of completion of closure that might be due to imbalance of growth rates within the posterior neuropore region (Copp *et al.* 1990). There has been some discussion as to whether some lumbosacral myelomeningoceles might result from reopening of a previously closed neural tube (Seller and Kalousek 1986, Copp *et al.* 1990). When the neural tube fails to close, the natural induction of other structures like bones, membranes, muscle, fat and skin does not occur (Keeling 1994). Neural tube defects are seen in chromosome aberrations such as trisomy 18 and triploidy, also in syndromes such as Meckel-Gruber Syndrome. Most have a multifactorial etiology with greatly differing prevalences in different countries. The incidence has gone down since the introduction of folic acid to the diet of pregnant women (Seller and Nevin 1984). In a study of 170 birth defect necropsies from Brasil, the incidence of neural tube defects was lower than expected in a European population (Peres *et al.* 1998); it has been assumed this is connected with a diet rich in folic acid.

Anencephaly

In anencephaly, coexistent defects in the basal part of the occipital bone and the vertebral bodies suggest that the primary abnormality may be in the rostral end of the notochord rather than in the neural tube itself (Marin-Padilla 1991, Kjær *et al.* 1994). Anencephaly, the absence of most of the brain and cranial vault, encompasses about 60% of neural tube defects (Keeling 1994) (Fig. 1). In craniorachischisis the defect includes the vertebral column, often limited to the cervical region but may extend caudally with an open spinal canal all the way to the sacrum. Partial development of the cranial vault with unorganized vascular and glial tissue is called meracrania, if the defect continues to the foramen magnum it is called holocrania. Present in the first trimester, most of the glial tissue disappears in the second trimester. The eyes are prominent, the nose flat and cleft lip/palate often present. A short neck is caused by missing or fusioned cervical vertebrae. Agenesis or hypoplasia of the pituitary gland causes adrenal hypoplasia. In 1964, Sundén described a case of acrania detected by ultrasound at 31 weeks gestational age, later confirmed by x-ray (Sundén 1964). The diagnosis of anencephaly by ultrasound in 1972, followed by therapeutic abortion, was still a major achievement eight years later (Campbell 1972).

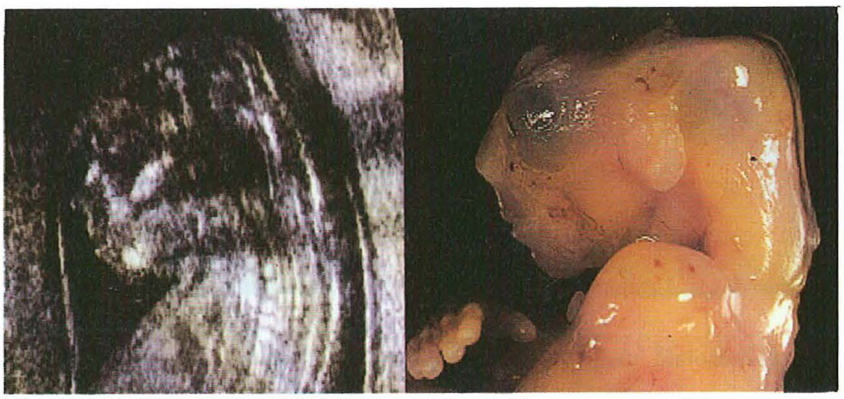

Figure 1. Ultrasound image and autopsy photograph of a 13 week old fetus with anencephaly.

Myelomeningocele

Myelomeningocele may present itself at any level of the spinal canal but is most common in the lumbar or lumbosacral region (Fig. 2). Neural tissue lies on the posterior surface of the vertebral bodies which lack development of the arches. The defect is closed by a thin membrane that often ruptures during delivery. A myelomeningcele in the lumbosacral region is almost always accompanied by the Arnold-Chiari malformation with traction on the medulla oblongata so that it comes to lie in the cervical part of the spinal canal. The cerebellum may be partly herniated through the foramen magnum. Cerebellar hypoplasia is the rule. Some degree of hydrocephaly is commonly encountered and can be ultrasonographically visible by the 18$^{th}$ week. The relationship between myelomeningocele, Arnold-Chiari malformation og hydrocephaly is still unclear. Deformation of joints, especially ankles, is common because of defect innervation. The intracranial sonographic findings with scalloping of frontal bones (lemon sign) and downward traction of the cerebellum (banana sign) found in fetuses with myelomeningocele were demonstrated in 1986 (Nikolaides *et al.* 1986) and proved to be a major breakthrough in the diagnosis of myelomeningocele (Cohen and Haller 1994). At the National Center for Fetal Medicine (NCFM), University Hospital of Trondheim, three cases with spina bifida have been detected at 9 weeks' gestational age after last menstrual period (LMP) (20-28mm CRL) using two-dimensional and three-dimensional ultrasound, with delay of termination of pregnancy (TOP) until week 13 in order to ensure the diagnosis by ultrasound and autopsy.

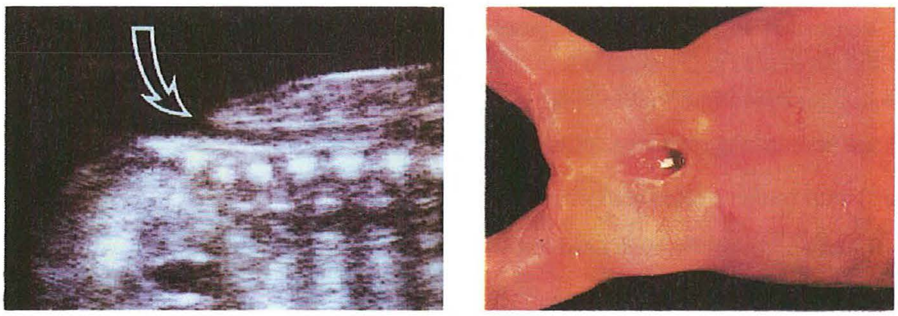

Figure 2. Left: ultrasound image with arrow pointing at open defect in a myelomeningocele. Right: autopsy photograph demonstrating the defect in the lumbosacral region.

Encephalocele

Herniation of the brain and meninges through a bony defect of the skull is most common in the frontal and occipital region. Geographical differences exist, occipital defects are most common in Europe (Lundar and Nornes 1991, Keeling 1994). Encephaloceles are usually skin covered and alfa-foeto-protein in the amniotic fluid might therefore not be elevated. The connection can be broad or narrow based. If the cerebral ventricles are compressed, hydrocephaly may develop. A bony defect is always present and may be in connection with the foramen magnum. Meckel-Gruber syndrome is regularly accompanied by an occipito-encephalocele (Ahdab-Barmada and Claassen 1990). Prenatal diagnosis of encephalocele was first described by Campbell (Campbell 1977) and has been described during the first trimester (Blaas and Eik-Nes 1996).

**Hydrocephaly**

If there is any obstruction to the circulation of the cerebrospinal fluid, increased pressure will ensue and the cerebral ventricles dilate. Wastage of cerebral tissue can also be a cause of hydrocephaly. Communicating hydrocephaly, caused by an extraventricular obstruction, is the most frequent type of hydrocephaly in fetuses. The most common associated anomaly is myelomeningocele with Arnold-Chiari malformation (1/3 of cases) (Chervenak *et al.* 1985). Isolated hydrocephaly is frequently caused by obstruction of the aqueduct of Silvius. Stenosis and forking without gliosis can be caused by a developmental anomaly, i.e. familial cases with X-linked pattern of inheritance. Septum formation and gliosis with exfoliated fibrin and cells obstructing the narrow aqueduct are most probably acquired lesions caused by congenital infections (CMV, toxoplasmosis, rubella) or ischaemic injury to cerebral tissue with or without hemorrhage. Abnormal development of other brain structures can also result in hydrocephaly, i.e. Dandy-Walker malformation, agenesis of the corpus callosum, and holoprosencephaly. Dandy-Walker malformation consists of a cystic dilatation originating from the roof of the 4$^{th}$ ventricle pushing the cerebellum upwards and forward. The vermis is absent or hypoplastic and the hemispheres atrophic. Lateral displacement and compression of the cerebellar hemispheres have been described by ultrasonography (Cohen and Haller 1994). Hydrocephaly can be striking during the second trimester (wide anterior fontanelle and suture lines). Intracranial tumours are exceptional, but hydrocephaly is a common complication because of obstruction of the cerebrospinal fluid. Teratomas occur most often, but primitive neurectodermal tumours have been observed. A method for reliable prenatal diagnosis of hydrocephaly based on the width of the atrium of the lateral ventricles, obtained at the level of the biparietal diameter, was introduced by Cardoza *et al.* in 1988 (Cardoza *et al.* 1988).

### Holoprosencephaly

Holoprosencephaly is a defect development of the prosencephalon with incomplete division of the cerebral hemispheres, often accompanied by midline facial anomalies. The least severe form is arhinencephaly, thereafter agenesis of the corpus callosum, while alobar holoprosencephaly/aprosencephaly as the most severe form can be associated with proboscis and cyclops. The prechordal mesenchyme rostral to the notochord is thought to be responsible for inducing cleavage of the prosencephalon (O'Rahilly and Müller 1994). Holoprosencephaly, with or without facial defects, is seen in trisomy 13 (50%) (Leech and Schuman 1986, Keeling 1994, Ming and Muenke 1998), but also in trisomi 18, triploidy and as a monogenic syndrome associated with Meckel Gruber syndrome (Cohen 1982, Ahdab-Barmada and Claassen 1990, Nicolaides *et al.* 1993). Teratogens may be a cause, and also alcohol in small amounts at an unfavourable point of time during the pregnancy (Larsen 1993). Maternal diabetes, viral infections, toxoplasmosis and various drugs have also been reported in connection with holoprosencephaly (Cohen 1982). The division of the hemispheres becomes ultrasonographically visible during the 7$^{th}$ week and alobar holoprosencephaly should thus be detectable as early as the end of week 7 (Blaas and Eik-Nes 1996). A case of holoprosencephaly at 9 weeks' gestational age based on the last menstrual period (crown rump length 22mm) has been detected at the NCFM, confirmed by autopsy after TOP at 12 weeks of gestational age.

### Microcephaly

Microcephaly is generally associated with a small brain and mental retardation. It is observed in many syndromes and is common in trisomy 4p, 13, 18 and in partial deletions such as 18p-, 18q- and 13q- (Jones 1997). It occurs also in infections such as rubella, toxoplasmosis and cytomegalovirus. Brain development can be disturbed by a wide range of environmental factors, including drugs and ionizing radiation (Irgens 1991). The detection by prenatal ultrasound is dependent on the extent of size reduction. To diagnose fetal microcephaly, several measurements (biparietal diameter (BPD), occipitofrontal diameter, head perimeter and head perimeter/abdominal perimeter) confirming the discrepancy between the head and abdominal size must be used (Chervenak *et al.* 1987).

## Congenital heart defects

Congenital heart defect (CHD) has been the most common congenital anomaly encountered in newborns with an incidence of 8-10 per 1000 (Tegnander *et al.* 1995, Mitchell *et al.* 1971, Achiron *et al.* 1992). The incidence is up to five times higher in abortuses and stillbirths (Richards *et al.* 1955, Mitchell *et al.* 1971, Bound and Logan 1977, Hoffman and Christianson 1978, Hoffman 1990). Over 50% of fetuses with CHD have anomalies in other organs and they are frequently associated with chromosome aberrations. CNS anomalies have been easier to detect by prenatal ultrasound than CHD (Rutledge *et al.* 1986, Chescheir and Reitnauer 1994, Saari-Kemppainen *et al.* 1994). The introduction of the 4-chamber view in the routine fetal examination has greatly improved the detection rate (Allan *et al.* 1986, Tegnander *et al.* 1994, Tegnander *et al.* 1995).

Ventricular septal defect (VSD) (Fig.3) is the most common anomaly and strongly associated with trisomy 18 (Hyett *et al.* 1995). Most VSDs are perimembranous and located near the valves. Almost 70% of the muscular defects tend to close spontaneously during the first year of life (Meberg *et al.* 1994). Isolated VSDs can be difficult to visualize on prenatal scans. VSDs can also occur in combination with other defects, particularly atrial septal defects (ASD), aortic coarctation and hypoplastic left ventricle (Isaksen *et al.* 1999a). An endocardial cushion defect/atrioventricular septal defect (AVSD) involves the lower part of the atrial septum and upper part of the ventricular septum producing a range of defects related to the timing of the insult (Silverman *et al.* 1986). It is the most common CHD in fetuses with trisomy 21 (Hill 1996).

In ventricular hypoplasia, underdevelopment of the ventricular chambers usually implies a decrease in the size of the cavities. A hypoplastic left heart involves atresia or hypoplasia of the mitral and/or aortic valves. It can be prenatally diagnosed even when the examination is limited to the four-chamber view. Prenatal diagnosis leads to better management at birth, ensuring ductal patency with prostaglandin therapy, thus leading to diminished morbidity (Petrikovsky 1998). Without treatment, survival with the hypoplastic left heart syndrome beyond one month is rare (Eapen *et al.* 1998). Increased survival rate has been observed with the Norwood repair method, though follow-up data beyond 10 years are not available (Petrikovsky 1998).

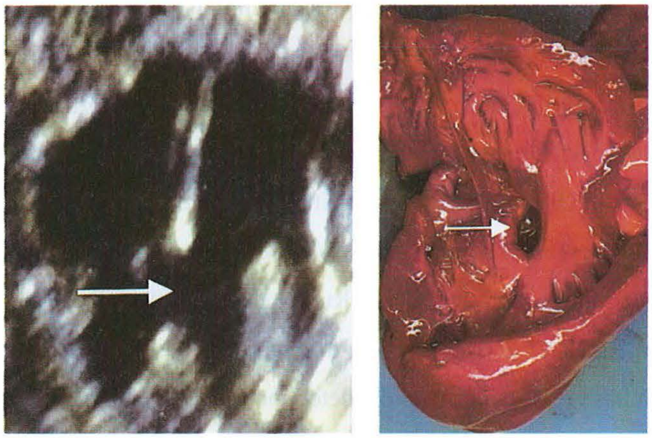

Figure 3. Ultrasound image and autopsy photograph demonstrating a ventricular septal defect. Arrows point at the defect.

Isolated pulmonary stenosis is relatively common, while pulmonary atresia with an intact ventricular septum has a much lower incidence. Ultrasonographically, it is easier to detect a pulmonary atresia when the ventricular septum is intact and accompanied by a hypoplastic right ventricle. In truncus arteriosus, the single great vessel responsible for the outflow supplying the systemic, coronary and pulmonary circulation is always overriding a VSD. Antenatally, truncus arteriosus may be missed by the four-chamber view of the heart, necessitating outflow views (Petrikovsky 1998). Truncus arteriosus is a conotruncal defect in which 30% of liveborn infants have major noncardiac anomalies (Jones 1997). Transposition of the great arteries involves the aorta arising from the right ventricle and the pulmonary artery from the left ventricle. A transposition will be missed at ultrasound with the four-chamber view only. An outflow tract image is necessary to disclose this anomaly (Petrikovsky 1998). Coarctation of the aorta is an obstruction in the aortic arch that may be preductal or ductal, ranging in severity from mild to critical narrowing. Prenatal visualization can be difficult (Isaksen *et al.* 1999a) and associated findings can sometimes trigger a suspicion. Fallot's tetrade (VSD, overriding aorta, pulmonary stenosis and right ventricular hypertrophy) are among the lesions that in large series frequently are missed on the initial ultrasound scan (Yagel *et al.* 1997). This association of anomalies is the result of a single embryologic defect due to malalignment with anterior deviation of the conus septum, which creates infundibular narrowing, a perimembraneous VSD, and dextroposition with overriding of the aorta above the VSD. Epstein's anomaly involves tricuspid valve dysplasia causing stenosis and insufficiency. The septal and/or posterior leaflets are displaced inferiorly and it is this downward displacement that makes it possible to diagnose by the four-chamber view (Petrikovsky 1998). This anomaly has been associated with maternal lithium ingestion during the first trimester of pregnancy (Cohen *et al.* 1994).

## Urinary system anomalies

Renal anomalies are usually discovered either because of reduced or deficient urine production with anhydramnios or oligohydramnios and/or abnormal ultrasound findings. The overall frequency of urinary tract abnormalities is approximately 2-3 per 1000 pregnancies (Helin and Persson 1986, Ville *et al.* 1998). The incidence of bilateral renal agenesis is low, 0.1-0.3/1000 births (Woolf 1995); renal cystic dysplasia/renal agenesis (renal adysplasia) and upper urinary tract dilatation being the largest group of urinary tract anomalies (Ahmed *et al.* 1988, Daneman and Alton 1991, Kim and Song 1996, Kubota *et al.* 1996).

### Obstructive uropathies

The urinary tract can be obstructed at various levels encompassing a wide variety of conditions characterized by dilatation of part or all of the urinary tract. When the obstruction is complete and occurs early during gestation, the normal growth of the kidneys will be disturbed and renal hypoplasia/dysplasia will develop (McVary and Maizels 1989, Chevalier 1995, Bierkens *et al.* 1996, Ville *et al.* 1998). Intermittent or late obstruction during the second half of pregnancy results in hydronephrosis with the severity of renal damage depending on the degree and duration of the obstruction (Blane *et al.* 1991, Ville *et al.* 1998).

Hydronephrosis is the most frequent of fetal renal anomalies and accounts for over 80% of urinary system anomalies (Mandell *et al.* 1991). Mild fetal pyelectasis detected in early pregnancy is frequently transient (Podevin *et al.* 1996, Guariglia and Rosati 1998). In approximately 20% (Abramowicz and Jaffe 1996, Seeds 1998) of the cases with dilation of the renal pelvis, association with other anomalies, including trisomy 21, has been found (Benacerraf *et al.* 1994, Seoud *et al.* 1999). Urinary tract anomalies resulting in hydronephrosis include ureteropelvic junction obstruction, ureterovesical junction obstruction and posterior urethral valves. Ureteropelvic junction obstruction is the most common (Abramowicz and Jaffe 1996), and is usually sporadic (Ville *et al.* 1998). Deficiency of muscle fibers at the ureteropelvic junction as well as failure of recanalization of the ureter may be the cause (Abramowicz and Jaffe 1996). In some cases ureteral valves can be found (Ville *et al.* 1998).

In ureterovesical junction obstruction, hydroureter is also present, while the bladder is normal. Lower urinary tract obstruction with urethral hypoplasia/atresia or posterior urethral valves will cause dilation of the bladder with varying degrees of hydroureter and hydronephrosis. Posterior urethral valves occur only in males and are the most common causes of bladder outlet obstruction. There is usually incomplete or intermittent obstruction of the urethra with an enlarged and hypertrophied bladder, varying degrees of hydroureter and hydronephrosis, and a spectrum of renal hypoplasia and dysplasia (Ville *et al.* 1998).

**Renal agenesis**

The complete absence of kidneys is due to failure of the mesonephric duct to give rise to the ureteric bud which induces the development of the metanephros ($24^{th}$ - $32^{nd}$ day of development) (Larsen 1993). When bilateral renal agenesis is present, the adrenals can easily be misinterpreted as kidneys since they assume an elongated and discoid shape, occupying the renal bed (Daneman and Alton 1991, Bronshtein *et al.* 1994). Limited movements because of oligohydramnios will be apparent during the second trimester with deformed extremities and facial dysmorphism like micrognathia and low-set ears (Potter's facies). The lungs will usually become hypoplastic because of loss of amniotic fluid and external compression of the thorax. In certain cases it may be difficult to distinguish renal agenesis from growth retardation as the cause of oligohydramnios, and Romero reported cases of renal agenesis diagnosed by ultrasound, while at autopsy, the kidneys were present (Romero *et al.* 1985). Unilateral renal agenesis will often remain undetected.

**Renal cystic dysplasia/renal agenesis (renal adysplasi) (Potter type II)**

Multicystic renal dysplasia can be uni- or bilateral. Contralateral renal agenesis or hypoplasia is common in unilateral dysplasia (Fig.4). Bilateral renal dysplasia is usually incompatible with life and is the most frequent form of cystic renal disease seen in a perinatal autopsy material. Renal dysplasia has traditionally been considered as a sporadic occurrence but in some families it is dominantly inherited. It can be part of syndromes like Meckel-Gruber and Fraser and also occurs in connection with chromosome aberrations, especially autosomal trisomies. Renal dysplasia occurring in connection with lower urinary tract obstruction is frequent and was called Potter type IV cystic kidney in Potter's classification. The structural abnormalities of these two variants of cystic dysplasia (multicystic dysplasia and peripheral cortical cystic dysplasia) are essentially identical with the severity of morphological changes being the only difference (Chevalier 1995).

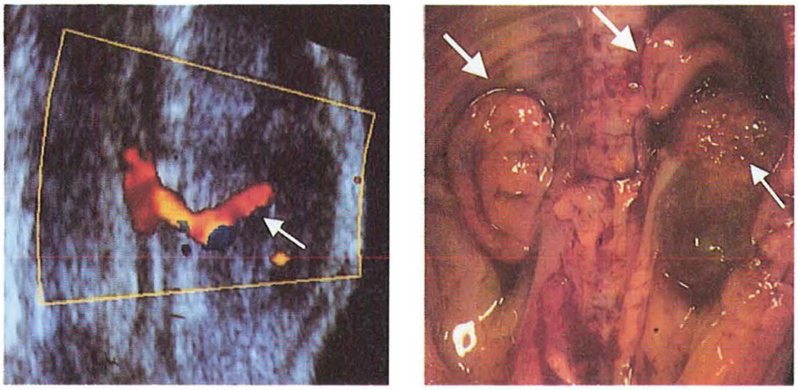

Figure 4. Unilateral renal dysplasia with contralateral renal hypoplasia.
Left: color Doppler of abdominal aorta with arrow pointing at the left renal artery.
Right: autopsy photograph demonstrating elongated adrenal on the right side (top left arrow) and dysplastic kidney on the left side (top right arrow pointing at adrenal gland and bottom arrow pointing at kidney).

**Autosomal recessive polycystic kidney disease (ARPKD); Infantile polycystic disease of liver and kidneys (Potter type I)**

Infantile polycystic kidney disease is rare (0.02/1000 births). It is inherited as an autosomal recessive condition with a wide spectrum of renal and hepatic involvement (Ville *et al.* 1998).

The sonographic combination of a large echogenic renal mass, oligohydramnios and nonvisualized bladder raises the suspicion of ARPKD (Petrikovsky 1998), though these features may not be visible during the second trimester. Morphologically the kidneys are enormously enlarged and the cysts are usually visible through the capsule with a radial arrangement on the cut surface. The liver is enlarged with dilated portal tracts, the lungs are hypoplastic because of oligohydramnios.

### Meckel-Gruber syndrome

This is an autosomal recessive syndrome with CNS anomalies and cystic kidneys. Median cleft lip and palate, postaxial polydactyly and dwarfism are frequent anomalies. The liver is sometimes enlarged with proliferation of bile ducts in the portal tracts. The CNS anomaly is usually an occipital encephalocele, although anencephaly, hydrocephaly and Dandy-Walker malformation can occur.

### Fraser syndrome

Fraser syndrome is also an autosomal recessive syndrome with cryptophthalmos, cutaneous syndactyly, laryngeal obstruction, genital hypoplasia, and renal anomalies in its most serious form. Renal agenesis is the most common, but cystic dysplasia or lower urinary tract obstruction can also occur. In cases with laryngeal atresia or severe stenosis, the lungs will be expanded because of fluid retention.

## Body wall defects

### Omphalocele

The umbilical cord is always involved when the defect occurs in the exomphalos. The sac consists of the amniotic membrane fused with the parietal peritoneum. Small defects consist of a sac at the umbilicus with the umbilical cord inserted at the apex. This is considered a failure of the physiological herniation of the midgut to return to the abdominal cavity in the $10^{th}$ week (Kalousek *et al.* 1990). Large defects involve the abdominal wall above the umbilicus while the umbilical cord has its course in the inferior part of the sac. The liver and other abdominal organs can be contained in the sac. Anomalies in other organs are common, particularly the heart and central nervous system with rates from 30-75% (Gilbert and

Nicolaides 1987, Hughes *et al.* 1989, Torfs *et al.* 1990). When other anomalies and/or dysmorphic features are present, the possibility of a chromosomal aberration must be considered. Of prenatally diagnosed omphaloceles, about 50% have an abnormal karyotype (Gilbert and Nicolaides 1987). Omphalocele is common in trisomy 13, 18 and in chromosome 9-syndrome, in other malformation syndromes and sometimes in triploidy. A large omphalocele can be difficult to distinguish from a limb-body wall defect.

### Diaphragmatic hernia

Diaphragmatic hernia is most common on the left side. When the defect is large, most of the abdominal viscera are present in the thorax and the mediastinum is displaced. Diaphragmatic hernia can be a manifestation of chromosome disorder. In those cases it is often associated with other anomalies, particularly CHD and dysmorphic features (Isaksen *et al.* 1999b). Polyhydramnios is a common finding after 24 weeks and is also a predictor of poor prognosis (Benacerraf and Adzick 1987). Large defects with compression of the lungs can be a serious medical and surgical challenge in the perinatal period.

## Fetal hydrops

Fetal hydrops is a generalized increase in and accumulation of body fluid in subcutaneous tissues and serous cavities. Fetal hydrops can be either immune type, due to blood group incompatibility, or nonimmune type, the latter representing 75-90% of cases of fetal hydrops (Abramowicz and Jaffe 1996, Ville *et al.* 1998). Intrauterine anemia, heart failure and hypoproteinemia are the three main mechanisms involved. Structural abnormalities interfering with the fetoplacental circulation, chromosome abnormalities and skeletal dysplasias may also be associated with fetal hydrops. Intrauterine infection and twin transfusion syndromes are the most common causes of intrauterine anemia (Kalousek *et al.* 1990). Effusions will often accumulate in body cavities parallel with the development of hydrops. Hydrops is usually encountered at the routine ultrasound examination during the second trimester. The spontaneous resolution of fetal hydrops has not been reported and the prognosis is poor with mortality rates of 80-95% (Ville *et al.* 1998). Even after a postmortem examination, the cause of the abnormality may remain unexplained.

## Cystic hygroma

Lymphatic vessels are derived from venous walls and eventually lose their connection and form a separate lymphatic system. The connection is maintained in the juguloaxillary sacs which drain the lymph to the venous system (Kalousek *et al.* 1990). The lymphatic system in this region develops along the aortic arch, and anomalies of the aortic arch are often associated with anomalies of the thoracic duct system (van der Putte and Van Limborgh 1980). Nuchal cystic hygroma is an abnormal development of lymphatic vessels resulting in accumulation of lymphatic fluid in the tissues of the neck. The endothelium lined cavities in the nuchal area can vary in size and may either be subdivided by thin septa or be multiloculated. The incidence of chromosome defects is 75%, with Turner syndrome as the most common. Association with fetal hydrops and CHD is found to a large extent (Nicolaides *et al.* 1992, Nicolaides *et al.* 1993). Accurate prenatal ultrasonographic detection has been possible for many years (Chervenak *et al.* 1983) and has been described from 10 weeks' gestation onwards (Keeling and Boyd 1993).

## Nuchal edema

Nuchal edema has been defined as a soft-tissue thickening >5mm in the dorsal cervical region (Benacerraf *et al.* 1987). Nuchal edema must be distinguished from nuchal cystic hygroma, but it may constitute one end of the spectrum of fetal hydrops (Nicolaides *et al.* 1992). It is associated with multisystem fetal malformations and chromosome abnormalities, mainly trisomy 21, but also trisomies 13 and 18, triploidy, deletions and translocations (Nicolaides *et al.* 1992, Pandya *et al.* 1995, Snijders *et al.* 1996). Chromosomally normal fetuses with nuchal edema have a poor prognosis because of an underlying infection, syndrome or anomaly, particularly cardiac defects (Nicolaides *et al.* 1992). In the first trimester, the term nuchal translucency is used because of the ultrasonographic feature observed; in the second trimester it might evolve into either nuchal edema or cystic hygroma (Snijders *et al.* 1996). Increased fetal nuchal tranlucency thickness at 10-14 weeks of gestation can potentially identify more than 80% of trisomies 21, 18, 13, Turner syndrome and triploidy, with a false positive rate of less than 5% (Pandya *et al.* 1995). By combining maternal age and fetal nuchal translucency thickness, this sensitivity would increase to at least 85% (Nicolaides *et al.* 1994).

## Chromosome aberrations

Most fetuses with major chromosome abnormalities have structural anomalies (Wladimiroff *et al.* 1995) detectable by detailed ultrasonographic examination (Nicolaides *et al.* 1992, Nicolaides *et al.* 1993). A wide range of phenotypic expressions exists for the different types of chromosome abnormalities, but no single anomaly is pathognomonic for a given chromosome defect (Hill 1996). Specific anomalies appear at different gestational ages and knowledge of the natural evolution is essential for further assessment and counseling. Bilateral, large, late-appearing and persistent choroid plexus cysts appear to be more common in association with aneuploidy (Bar-Hava *et al.* 1993). Nuchal edema during the first and second trimester, will often resolve at a later stage (Landwehr *et al.* 1996). Trisomies for all chromosomes except chromosome 1 have been found in spontaneous abortions (Hassold 1986). Trisomy 16 is the most common, but has never been reported in a liveborn. Trisomy 13, 18 and 21 account for 67% of all fetuses born with a karyotypic abnormality (Snijders *et al.* 1994). The antenatal diagnosis of a chromosome abnormality is important for several reasons: termination of pregnancy can be offered if the diagnosis is made early in pregnancy; late diagnosis may influence the mode of delivery and avoid a caesarian section for fetal distress (Twining and Zuccollo 1993).

**Trisomy 21 (47,XY,+21 or 47, XX,+21)**

Down syndrome was originally described by John Langdon Down in 1866 and nearly 100 years passed before the discovery in 1959 of the presence of an extra chromosome 21 (Jorde *et al.* 1994). Down syndrome is the most common autosomal chromosome abnormality with an occurrence of 1 in 800 livebirths. Ninety-five percent of the cases are due to an extra chromosome 21 caused by nondisjunction during meiosis. Three percent are due to translocations and 2% are mosaics. The most common cause of mosaicism in a trisomic conception is loss of the extra chromosome in some of the cells during mitosis. Mosaicism usually results in a milder clinical expression (Hill 1996). The portion of chromosome 21 responsible for the major anomalies is band 22 of the distal long arm (q 22) Karyotyping because of advanced maternal age will identify about 30 % of fetuses with trisomy 21 (Nyberg *et al.* 1990, Snijders *et al.* 1998). Benacerraf *et al.* found that prenatal ultrasound examination of second-trimester fetuses with measurement of nuchal thickness and ratio of expected/actual femur length could identify 75% of fetuses with trisomy 21. When other anomalies such as AVSD and meconium peritonitis were added, the sensitivity of sonographic detection rose to 82% (Benacerraf et *al.* 1987). Nuchal thickening, cystic hygroma and hyperechogenic bowel are anomalies more frequently detected before 20 weeks gestational age (Nyberg *et al.* 1990). CHD, duodenal atresia, omphalocele, mild cerebral ventricular dilatation, and growth retardation can all be observed during the second trimester; of these, cardiac defects are the most easily missed. One or more of these sonographic abnormalities have been observed in 33% of fetuses with trisomy 21 (Nyberg *et al.* 1990).

**Trisomy 18 (47,XY, +18 or 47, XX, +18)**

In 1960, J. H. Edwards described a girl with peculiar facies, webbing of the neck, CHD and other minor abnormalities. Through chromosome study he found an extra chromosome apparently identical to the 17th pair (Edwards *et al.* 1960). Edwards syndrome is the second most common autosomal trisomy with an estimated incidence between 1 in 4-8000 livebirths, and with a higher prevalence during the first and second trimester (Hill 1996). It is the most common chromosome abnormality among stillborns with congenital anomalies (Jorde et al 1994). The median life expectancy for liveborns with trisomy 18 is 5 days. About 50% of children with trisomy 18 die in the first week of life and about 5-10% survive the first year (Carter *et al.*) The mortality rate is higher in males (Weber 1967). There is a bimodal maternal age distribution curve suggesting a genetic tendency to nondisjunction independent of maternal age (Moerman *et al.*1982). Eighty-five percent have an additional chromosome 18, 10% are mosaics and 5% result from a translocation (Hill 1996). Neonates with mosaic trisomy 18 are less severely affected (Hill 1996), though there are reports of long-term survival without mosaicism (Baty *et al.* 1994). It appears that the long arm of chromosome 18 is responsible for the characteristic phenotype. More than 130 abnormalities have been reported with trisomy 18 (Hill 1996). In a review by Nyberg *et al.* the abnormalities most frequently detected by prenatal ultrasound before 24 weeks included cystic hygroma, nuchal thickening and myelomeningocele, while intrauterine growth retardation (IUGR), CHD, and an enlarged cisterna magna were detected more frequently after 24 weeks than before. Intrauterine growth retardation was the single most common abnormality, more prominent during the second and third trimester (Bar-Hava *et al.* 1993, Nyberg *et al.* 1993). A slower rate of cell growth and division in trisomic cells explains the growth restriction (Paton *et al.*

1974). The combination of IUGR together with polyhydramnios is unusual and should evoke the possibility of a chromosome abnormality, specifically trisomy 18 (Nyberg *et al.* 1993, Hill 1996). Omphalocele, renal abnormalities, rocker bottom feet, clenched hands (Fig. 5) and single umbilical artery are common ultrasound detectable anomalies (Nyberg *et al.* 1993). Choroid plexus cysts are also associated with trisomy 18 (Gray *et al.* 1996) and can be easier to detect by ultrasound than at autopsy (Isaksen *et al.* 1999b). The "strawberry-shaped" head described ultrasonographically is caused by brachycephaly and a narrow frontal cranium, possibly due to facial and frontal cerebral hypoplasia (Nicolaides *et al.* 1992). The most common pathologic features of trisomy 18 are CHD (96%) with VSD (85%), rocker bottom feet, omphalocele and horseshoe kidney (each 33%), dysmorphic facial features (approximately 30%), and clenched fingers (50%) (Kalousek *et al.* 1990, Isaksen *et al.* 1999).

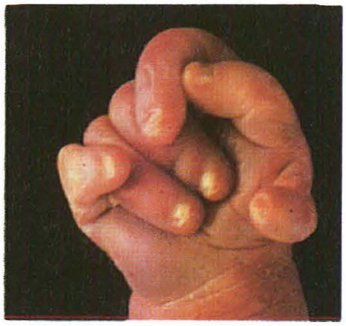

Figure 5. Ultrasound image and autopsy photograph demonstrating clenched fingers and polydactyly.

**Trisomy 13 (47,XY,+13 or 47,XX,+13)**

Trisomy 13 was first described by Patau in 1960 (Patau *et al.* 1960). It is the least common of the autosomal trisomies because of its higher intrauterine mortality and has an incidence between 1-4 of 20 000 births (Hill 1996). About 80% have full trisomy 13, most of the remaining cases have trisomy of the long arm due to a translocation (Jorde *et al.* 1994). Survival beyond one year has been reported (Redheendran *et al.* 1981). Fetuses with trisomy 13 mosaicism may show a less severe phenotype with variation from near-normal to the full pattern of anomalies (Jones 1997). Compared to the other autosomal trisomies they have more severe craniofacial and cerebral anomalies and approximately 50% of fetuses with alobar holoprosencephaly have trisomy 13. Post-axial polydactyly, primarily of the hands, is present in up to 80% of the cases (Hill 1996).

**Triploidy (69,XXX or 69,XXY)**

Triploidy is among the most frequently observed of the chromosome abnormalities. It occurs in 1% of conceptions, the majority of these ending in spontaneous abortion during the first trimester (Boue *et al.* 1982, Crane *et al.* 1985). Second and third trimester stillbirth is also common and very few are liveborn (Jacobs *et al.* 1982, Royston and Bannigan 1987). Cases with full triploidy and multiple anomalies, surviving up to 7 months, have been described (Arvidsson *et al.* 1986, Niemann-Seyde *et al.* 1993). The absence of significant organ anomalies or presence of mosaicism may contribute to a prolonged survival time (Pettenati *et al.* 1986, Niemann-Seyde *et al.* 1993). The most constant findings are severe growth retardation, head/trunk asymmetry and syndactyly. In a prenatal study by Jauniaux *et al.*, the most frequent combination of anomalies was malformation of the hands and ventriculomegaly (Jauniaux *et al.* 1996). Paternal origin accounts for ¾ of triploid conceptions, with fertilization of a haploid ovum by two haploid sperm as the most prevalent mechanism (Crane *et al.* 1985, Niemann-Seyde *et al.* 1993). Paternal origin is almost always accompanied by placental enlargement and partial mole (Jacobs *et al.* 1982), partial mole frequently accompanied by a higher fetal mortality. Relatively normal intrauterine growth and microcephaly have been described in cases with paternal origin, while maternal origin seems to be accompanied by severe intra-uterine growth retardation and macrocephaly (Niemann-Seyde *et al.* 1993). Prenatal sonographic features include intrauterine growth retardation and body asymmetry with relative macrocephaly, oligohydramnios and an abnormally enlarged and/or hydropic placenta (Crane *et al.* 1985).

**Turner syndrome (45,X)**

The phenotype associated with one X chromosome was originally recognized by Henry Turner in 1939. The 45,X karyotype accounts for 15-20% of the chromosome abnormalities seen among sponataneous abortions, while only 1 in 2500-5000 liveborn females have the disorder.The great majority of conceptions are therefore lost prenatally (Jorde *et al.* 1994). Slightly more than 50% are monosomic, about 15% have structural abnormalities of the X chromosome and the rest are mosaics (Gelehrter and Collins 1990, Jorde *et al.* 1994). This variation in chromosome abnormality helps to explain the considerable phenotypic variation seen in this syndrome (Jorde *et al.* 1994). Seventy-five percent of fetuses with cervical cystic hygromas have Turner syndrome (Azar *et al.* 1991). Most cases of lethal Turner syndrome also present with generalized edema, including pleural effusions and ascites (Isaksen *et al.* 1999b). Some are accompanied by horseshoe kidney that can be suspected by the ultrasonographic appearance of bilateral mild hydronephrosis (Ville *et al.* 1998).

## Summary

Almost forty years with ultrasound in obstetrical practice have passed. Dysmorphic features and anomalies not considered detectable 10-15 years ago are obvious today. There is reason to believe that the improved quality of the scans and the increased experience of the ultrasonographers and ultrasonologists will continue, so that in the future most structural anomalies in the fetus can be and will be detected. Confirmation of the ultrasound findings by autopsy will still be necessary. Future collaboration between the specialists in ultrasonography and perinatal pathology will be mutually stimulating, contributing to increased knowledge about the etiology and pathogenesis, and to the detection of developmental anomalies.

# AIM OF THE STUDY

The principle aim of the study was to compare prenatal ultrasound findings with postmortem findings in fetuses and infants with congenital anomalies. The main purposes were to evaluate the diagnostic accuracy of prenatal ultrasound in relation to autopsy results, investigating the degree of accordance between the two methods and to assess the impact of prenatal diagnosis on subsequent management. The study included the following challenges: to analyze the quality of the ultrasound examination pertaining to single organs, to compare these with each other and to look at the quality of the examination in fetuses with multiple organ anomalies. It was thus meant to be a quality control of ultrasound examination and an assessment of the quality of the postmortem examination. The final diagnosis based on ultrasound findings and autopsy results constitute the basis for parental counseling and death statistics.

**The aims of the individual papers are summarized below:**

1. To compare the prenatal ultrasound diagnoses of central nervous system anomalies with the autopsy diagnoses.
2. To focus on ultrasonographic and postmortem findings in fetuses with congenital heart defects and evaluate the concordance of prenatal ultrasound findings with the postmortem examination in order to estimate the diagnostic accuracy.
3. To evaluate the correlation between ultrasound and postmortem findings in fetuses and infants with urinary system anomalies.
4. To register the ultrasound and postmortem findings in fetuses and infants with an abnormal karyotype and compare these in relation to the different chromosome aberrations.
5. To enumerate chromosome 18 by fluoresence *in situ* hybridization (FISH) on autopsy material from fetuses and infants with unknown karyotype with suspicion of trisomy 18.

# MATERIAL AND METHODS

## Study population

All fetal, perinatal and neonatal autopsy reports performed at the Department of Pathology, University Hospital of Trondheim between January 1985 and December 1994, were reviewed. Infants with a congenital anomaly or fetuses aborted because of an anomaly suspected by ultrasound examination were further analyzed. Additional information from maternal charts and sonographic records were collected and only the cases where prenatal ultrasound had been performed at the National Center for Fetal Medicine (NCFM) were included in the study, altogether 365 cases. In an additional 44 cases, referred women underwent a sonography scan at the NCFM, but gave birth at their local hospital. The autopsy reports were obtained with permission from the respective pathology departments and included when the given information was sufficient; one case was excluded because of missing data. The total material thus comprised 408 cases.

## Ultrasound examination

The routine scans at the center were performed by specially trained midwives. If any abnormality was suspected, the woman was referred to highly qualified obstetricians for further examination. A targeted ultrasound scan was performed in cases with hereditary risk factors or abnormal development of the pregnancy. The cases referred to the center were either sent from other hospitals or from gynecologists in private practice. The scan included a survey of the fetal anatomy, biometric measurements of the fetus and location of the placenta. The fetal biparietal diameter measured at the routine examination was the basis for assessment of gestational age and used for determination of possible growth retardation. When necessary, Doppler examination was used to evaluate hemodynamic alterations. In any case of doubt the fetus was examined by another doctor or the examination was repeated. In fetuses having a congenital heart defect a pediatric cardiologist was usually consulted during the first part of the study (1985-89) and regularly during the second part (1990-94). Any available information about karyotype and/or biochemical analysis of fetal blood and/or amniotic fluid was also registered. All data were stored in a computer database and the comparisons are based on the recorded findings in the ultrasound report.

The ultrasound machines employed were Hitachi EUB 565, Dornier AI 3200 and Vingmed Sound CFM 750. They were equipped with transducers with frequencies ranging from 3.5-7.5 Mhz.

## Autopsy

The results of the sonographic examination with the prenatal diagnosis was available to the pathologist prior to the postmortem. From the year 1985 to 1989, no standardized autopsy protocol was adhered to, and the autopsies were performed by doctors in training, with the supervision of a consultant pathologist. The quality of these autopsy protocols is variable. After 1990, one pathologist was in charge of all the perinatal postmortems. A standardized autopsy protocol was employed, including full body radiology and photographic documentation when necessary. All organs were examined, including *in situ* examination of the heart and removal of the brain under water in order to minimize trauma. The morphological diagnoses given in the autopsy report were the basis for the comparison with ultrasound findings.

The data registered included mother's age, residence, referring practice/clinic, outcome at delivery and, for liveborns, how long the infant lived. When feasible, gestational age according to Naegele, ultrasound and postmortem examination was noted. Body measurements, organ weights and femoral radiographic measurements were included in order to indicate developmental age. Also included was information on eventual chromosome aberration, dysmorphic features, hydrops, and placental changes. Anomalies were registered and classified according to organ. In cases with multiple anomalies, the organs or organ systems were classified according to the most serious defect, taking into account the clinical outcome if the pregnancy was continued. The diagnoses were organised under the following categories: central nervous system anomalies, congenital heart defects, urinary system anomalies, gastro-intestinal anomalies, skeletal dysplasias, diaphragmatic hernias/abdominal wall defects, arthrogryposis/muliple lethal pterygium syndrome, cystic hygroma/fetal hydrops and miscellaneous anomalies. All information was collected and systematized in Microsoft Excel from where necessary data was further processed with the Statistical Package for Social Sciences (SPSS).

The correlations between the ultrasound and autopsy findings were categorized as follows (Isaksen *et al.* 1998, Isaksen *et al. 1999a,b).*

1) Full agreement between the ultrasound and autopsy findings.
2) Minor autopsy findings not detected or not recorded at the ultrasound examination.
3) Major autopsy findings not detected at the ultrasound examination, although other ultrasound findings indicated termination of pregnancy.
4) None of the autopsy findings suspected at the ultrasound examination. In these cases the fetus/infant died naturally *in utero* or shortly after birth.
5) Minor ultrasound findings not confirmed at autopsy. These unverified ultrasound findings did not precipitate unjustified management, as they were supplementary to other detected anomalies confirmed at autopsy.
6) Major ultrasound findings not confirmed at autopsy. This category includes false positives, as well as cases in which the ultrasound findings were not verified at autopsy because of technical difficulties (traumatisation/maceration of the fetus) at the postmortem, making a morphological diagnosis difficult.

## Fluorescence *in situ* hybridization (FISH)

FISH was performed on selected cases with unknown karyotype and anomalies suggestive of trisomy 18. The criteria were either a congenital heart defect, dysmorphic facial features or a CNS anomaly. Cases with known karyotype were used as controls. The procedure is described elsewhere (Köpf *et al.* 1996). Briefly, formalin-fixed tissue was treated with protease. Nuclei were hybridized with a chromosome-18-specific centromere probe. Material from the thymus was preferred as this generally was better preserved than tissue from other organs.

# RESULTS

Of the total material consisting of 408 cases of fetal and infant deaths, 206 (51%) came from all over Norway, 120 (29%) came from the city of Trondheim and 82 (20%) from neighboring communities. Fifty-three percent were female. It was not possible to determine the sex in one case. The mean age of the mothers at the time of termination of pregnancy or at delivery was 28 years (range 17-44). The distribution of anomalies is shown in Table 1.

**Table 1. Congenital anomalies in 408 fetuses and infants**

| Anomaly/organ involved | Main diagnosis | % | Secondary diagnoses | % | Total | % |
|---|---|---|---|---|---|---|
| Normal morphology | 13 | 3.2 | - | - | 13 | 1.9 |
| CNS anomalies | 120 | 29.4 | 20 | 7.6 | 140 | 20.8 |
| CHD | 63 | 15.4 | 43 | 16.2 | 106 | 15.8 |
| Urinary system anomalies | 50 | 12.2 | 62 | 23.4 | 112 | 16.6 |
| Lung anomalies | 1 | 0.2 | 3 | 1.1 | 4 | 0.6 |
| Genital anomalies | 1 | 0.2 | 9 | 3.4 | 10 | 1.5 |
| Musculoskeletal system | 6 | 1.5 | 36 | 13.6 | 42 | 6.[illegible] |
| Arthrogryposis/ lethal pterygium syndrome | 13 | 3.2 | 3 | 1.1 | 16 | 2.4 |
| Skeletal dysplasias | 17 | 4.2 | 2 | 0.8 | 19 | 2.8 |
| Gastro-intestinal anomalies | 8 | 2.0 | 33 | 12.4 | 41 | 6.0 |
| LBWC/ARS | 15 | 3.7 | 2 | 0.8 | 17 | 2.[illegible] |
| Diaphragmatic hernia/ omphalocele | 24 | 5.9 | 18 | 6.8 | 42 | 6.[illegible] |
| Cystic hygroma/fetal hydrops | 66 | 16.2 | 27 | 10.2 | 93 | 13.8 |
| Miscellaneous | 11 | 2.7 | 7 | 2.6 | 18 | 2.[illegible] |
| Total | 408 | 100 | 265 | 100 | 673 | 100 |

CNS, central nervous system; CHD, congenital heart defect; LBWC, limb-body wall complex; ARS, amniotic rupture sequence

Not unexpectedly, CNS anomalies were the most frequent of the congenital anomalies, the majority 120/140 (86%) having the CNS anomaly as the main diagnosis. Of the 106 cases with CHD, 63 (59%) had this as the main diagnosis. Urinary system anomalies occurred in 112 cases and in 50 cases (55%), this was the main diagnosis (Table 2).

**Table 2. Survey of CHD, CNS and urinary system anomalies**

| | Organ system | | | |
|---|---|---|---|---|
| | **CNS anomalies** | **CHD** | **Urinary system anomalies** | **Total** |
| Main diagnosis | 120 | 63 | 50 | 233 |
| Additional findings | 20 | 43 | 62 | 125 |
| Total | 140 | 106 | 112 | 358 |

In 39 of the 106 cases with CHD, the CHD was the sole manifestation, 15 of these were combined with fetal hydrops. In 67 cases the heart defect was associated with anomalies in other organs. Combinations with urinary system and CNS anomalies were the most frequent. Altogether 33 were combined with urinary system anomalies. In twenty-five of the 33 cases with the combination of CHD and urinary system anomalies a ventricular septal defect (VSD) was present. Eight of these cases with VSD had a horseshoe kidney, 5 were trisomy 18, another three had anomalies strongly suggestive of trisomy 18 and were verified as such by fluorescence *in situ* hybridization (FISH). The only other combination horseshoe kidney/CHD was a case with Turner syndrome that had a primum atrial septal defect (ASD). Unilateral renal agenesis and bilateral renal dysplasia were the other main diagnoses combined with VSD, otherwise the combinations of urinary system anomalies and CHD were randomly distributed. In 38 of the 50 cases with the urinary system as the main diagnosis, the urinary system was the only organ system affected; the rest were combined with CHD, gastro-intestinal and skeletal anomalies. Of the 27 cases with combined CHD and CNS anomalies, 17 had an abnormal karyotype, trisomy 18 being the most frequent with 12 cases. The combination of CNS anomalies, CHD and urinary system anomalies occurred in 9 cáses, in 6 of these 9 cases a chromosome aberration was present (Table 3).

**Table 3. Combination of CNS anomalies, CHD, urinary system anomalies and abnormal karyotype**

| | Diagnosis | Sex | Gestational age in weeks | Chromosome aberration |
|---|---|---|---|---|
| 1 | IUGR, Arnold-Chiari malformation, VSD, horseshoe kidney, claw hand, club feet | M | 29 | trisomy 18 |
| 2 | IUGR, holoprosencephaly, spina bifida, omphalocele, renal agenesis left side, dysplastic right kidney, VSD, syndactyly right hand | F | 22 | triploidy |
| 3 | IUGR, duplex kidney with ectopic ureterocele, hypoplastic left ventricle with VSD, CNS dysplasia | F | 37 | trisomy 18 |
| 4 | IUGR, dysmorphic features, horseshoe kidney, VSD, polymicrogyria | F | 36 | trisomy 18 |
| 5 | IUGR, horseshoe kidney, hypoplastic left ventricle with VSD and aortic coarctation, CNS dysplasia | F | 41 | trisomy 18 |
| 6 | Dysplastic left kidney, hydrocephaly, diaphragmatic hernia right side, ASD, VSD, bilateral syndactyly, micrognathia | F | 21 | partial trisomy 13 |

IUGR, intrauterine growth retardation

## Skeletal abnormalities

Skeletal anomalies are common in cases with chromosome aberrations, in these cases the skeletal anomaly is only part of a syndrome of multiple anomalies. Eighteen of the 42 cases with skeletal abnormalities had an abnormal karyotype (43%).The chromosome status is shown in Table 4.

**Table 4. Karyotype in cases with skeletal abnormalities**

| | Number | Percent |
|---|---|---|
| Normal karyotype | 16 | 38.0 |
| Unknown karyotype | 8 | 19.0 |
| Trisomy 13 | 1 | 2.4 |
| Trisomy 18 | 12 | 28.6 |
| Trisomy 21 | 1 | 2.4 |
| Triploidy | 3 | 7.2 |
| Partial trisomy 5 | 1 | 2.4 |
| Total | 42 | 100 |

Three of 8 syndactylies were detected at the ultrasound examination. Four abnormalities of the spinal column and one radial aplasia were not detected. The detection rate for skeletal anomalies was thus 76% (32/42).

**Diaphragmatic hernias and body wall defects**

Altogether 59 cases had either a diaphragmatic hernia, omphalocele or body wall defect, 3 of these cases had both a diaphragmatic defect and an omphalocele (Table 5). Six cases from the first time period primarily interpreted as an abdominal wall defect or omphalocele, were reclassified as limb-body wall complex. Of the cases with a diaphragmatic defect and/or omphalocele, 83% (35/42) were karyotyped. Twenty of the 26 cases with omphalocele were karyotyped, 75% (15/20) had an abnormal karyotype, 67% 10/15) had trisomy 18. Twelve of the 13 cases with diaphragmatic hernia were karyotyped, 50% (6/12) had a chromosome aberration, of these, 50% (3/6) were trisomy 18. All three cases with both diaphragmatic hernia and omphalocele were karyotyped, 2 had trisomy 18, the third had normal chromosomes. Fourteen of the 17 cases with LBWC were karyotyped, all had normal chromosomes (Table 5).

**Table 5. Karyotyping in diaphragmatic defects and body wall defects**

| Chromosome status | Omphalocele | Diaphragmatic hernia | Omphalocele and diaphragmatic hernia | LBWC | Total |
|---|---|---|---|---|---|
| Trisomy 13 | 3 | 2 | 0 | 0 | 5 |
| Trisomy 18 | 10 | 3 | 2 | 0 | 15 |
| Trisomy 21 | 0 | 0 | 0 | 0 | 0 |
| Triploidy | 2 | 0 | 0 | 0 | 2 |
| Miscellaneous | 0 | 1 | 0 | 0 | 1 |
| Normal karyotype | 5 | 6 | 1 | 14 | 26 |
| Not known | 6 | 1 | 0 | 3 | 10 |
| Total | 26 | 13 | 3 | 17 | 59 |

Diaphragmatic and abdominal wall defects are often seen in combination with CNS anomalies, anomalies of the urinary system and CHD. Such cases have a high incidence of chromosome aberrations. Of 8 cases with combined omphalocele and/or diaphragmatic hernia, CNS anomaly and CHD, 6 were trisomy 18, and of 8 cases with the same anomalies except CNS anomaly, another 6 had trisomy 18. When omphalocele and/or diaphragmatic hernias were combined with CHD and renal anomalies, 5 of 7 had an abnormal karyotype, 3 trisomy 18 and 2 partial trisomy 13.

**Fetal hydrops and cystic hygroma**

Altogether 93 cases were afflicted with hydrops and/or cystic hygroma, 25 had a CHD, 14 of these a chromosome aberration. The chromosome status is shown in Table 6.

**Table 6. Chromosome status in fetal hydrops and/or cystic hygroma**

| Chromosome abberation | Number |
|---|---|
| Trisomy 13 | 3 |
| Trisomy 18 | 5 |
| Trisomy 21 | 13 |
| Turner syndrome | 14 |
| Miscellaneous | 2 |
| Normal karyotype | 34 |
| Not known | 22 |
| Total | 93 |

In one case of intrauterine fetal death, the infant of 31 gestational weeks was hydropic; this was not noted at the ultrasound examination shortly before birth. In 3 cases, hydrops/cystic hygroma diagnosed at the ultrasound examination was not possible to confirm at autopsy, either because of fragmentation or maceration of the fetus.

**Normal morphology**

Thirteen cases with normal morphologic features are included in the material, 5 of these were trisomy 21 without manifested anomalies, 2 had Klinefelter's syndrome, 2 were 47,XXX (triple X), one was a case with hydrothorax and missing arm of chromosome 9, one had a cytomegalovirus infection with ascites and one was intrauterine growth retarded with pericardial effusion. In these cases the ultrasound and autopsy diagnoses were in agreement. One case with anhydramnios was monitored over a period of several days with no urine production. The instillation of fluid (glucose) in the amniotic cavity did not result in any urine production and the pregnancy was terminated with the diagnosis of non-functional urinary system. This case is retrospectively being evaluated for possible renal tubular dysgenesis.

### Abnormal karyotype

Looking at CHD, CNS and urinary system anomalies, it is evident that the incidence of chromosome abnormalities varies from one type of anomaly to another. This must also be seen in relation to the number of cases in the different diagnostic categories that are karyotyped. It is evident from Table 7 that a major CHD is by far the anomaly most often encountered in fetuses with an abnormal karyotype, next in frequency are urinary system anomalies while CNS anomalies are not so frequently associated with chromosome aberrations: only half as often as with CHD. The percentage of cases karyotyped are identical in CNS anomalies and CHD. In urinary system anomalies, fewer cases are karyotyped; thus, the results are not quite as reliable.

**Table 7. Cases with abnormal karyotype**

| | Cases karyotyped | Rate of total | | Normal karyotype | | Abnormal karyotype | |
|---|---|---|---|---|---|---|---|
| | N | n/N | % | N | % | N | % |
| CNS | 108 | 108/140 | 77 | 83 | 77 | 25 | 23 |
| CHD | 83 | 83/106 | 78 | 30 | 36 | 53 | 64 |
| Urinary system anomalies | 75 | 75/112 | 67 | 47 | 63 | 28 | 37 |

### Fluorescence *in situ* hybridization (FISH)

Ten cases with unknown karyotype and 7 control cases with known karyotype were selected for FISH using a chromosome-18-specific centromere probe. Of the unknown cases, 2 showed 3 signals in the majority of the nuclei and were thus considered as trisomy 18; in these fetuses characteristic findings of trisomy 18 (omphalocele, VSD and horseshoe kidney) were present. In one case a mixture of 2 and 3 signals were found, indicative of mosaicism for trisomy 18. All 15 cases with horseshoe kidney ended thus with known karyotype (9 with trisomy 18, 3 with Turner syndrome and 3 normal). In one case triploidy was suspected because of morphological findings. Hybridization was therefore performed with a chromosome-8-

specific probe, in addition to the chromosome-18-specific probe, and since both showed a mixture of 2 and 3 signals, the fetus was suspected of having a triploid mosaicism.

**Overall discrepancies**

Of all the 408 cases analyzed during the ten year period, there was complete agreement between prenatal ultrasound and postmortem findings in 305 (75%) (Table 8). The main diagnosis was correct in 85% during the first time period (1985-89) and in 92% during the second time period (1990-94). The difference between the two time periods is non- significant ($p>0.05$ and $p<0.1$).

**Table 8. Correlation between prenatal and postnatal findings in fetuses/infants with congenital anomalies (n=408)**

| Category | 1985-89 | | 1990-94 | | Total 1985-94 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| 1) Full agreement | 94 | 68 | 211 | 78 | 305 | 75 |
| 2) Minor autopsy findings not found by ultrasound | 23 | 17 | 37 | 14 | 60 | 15 |
| 3) Major autopsy findings not found by ultrasound | 10 | 7 | 4 | 1 | 14 | 3 |
| 4) No autopsy findings suspected by ultrasound | 9 | 6 | 5 | 2 | 14 | 3 |
| 5) Minor ultrasound findings not confirmed at autopsy | 1 | 1 | 7 | 3 | 8 | 2 |
| 6) Major ultrasound findings not confirmed at autopsy | 1 | 1 | 6 | 2 | 7 | 2 |
| Total | 138 | 100 | 270 | 100 | 408 | 100 |

**Comparison between CNS, CHD and urinary system anomalies**

Differences in detection rate between the different anomalies are shown in Table 9, the whole time period 1985-94 included.

**Table 9. Fetuses and infants with CNS anomalies, CHD, and urinary system anomalies: correlation between prenatal ultrasound and autopsy findings**

| Category | CNS | | CHD | | Urinary system anomalies | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| 1) Full agreement | 125 | 89 | 74 | 73 | 97 | 87 |
| 2) Minor autopsy findings not found by ultrasound | 7 | 5 | 18 | 18 | 5 | 4 |
| 3) Major autopsy findings not found by ultrasound | 1 | 1 | 4 | 4 | 4 | 3.5 |
| 4) No autopsy findings suspected by ultrasound | 3 | 2 | 3 | 3 | 4 | 3.5 |
| 5) Minor ultrasound findings not confirmed at autopsy | 2 | 1.5 | 1 | 1 | 2 | 2 |
| 6) Major ultrasound findings not confirmed at autopsy | 2 | 1.5 | 1 | 1 | 0 | 0 |
| Total | 140 | 100 | 101 | 100 | 112 | 100 |

For the categories full agreement and minor autopsy findings not detected at ultrasound, CNS anomalies were the most easily detectable (94%), thereafter urinary system anomalies (91%) and CHD (91%). For CHD, the proportion of autopsy findings (both minor and major) not detected at ultrasound examination diminished significantly ($p<0.001$) from the first to the second time period. For CNS anomalies and urinary system anomalies the decrease in percentage of autopsy findings not detected at ultrasound examination was non-significant. Overall, there were slightly more ultrasound findings not confirmed at autopsy during the second time period than during the first time period. Some of these were transitory choroid plexus cysts and some were macerated fetuses difficult to diagnose properly at autopsy.

Differences in detection rate between the different anomalies are shown in Table 10. The difference in detection rate between CNS anomalies (89%) and CHD (73%) was significant ($p<0.01$), likewise between urinary system anomalies (87%) and CHD (73%) ($p<0.05$). The difference between CNS anomalies and urinary system anomalies was not significant.

**Table 10. Comparison of detection rate in the different organ systems**

| **Organ system** | **Cases with full agreement/total cases** | **%** |
|---|---|---|
| CNS anomalies | 125/140 | 89 |
| CHD | 74/101 | 73 |
| Urinary system anomalies | 97/112 | 87 |

# GENERAL DISCUSSION

## Introduction

Ultrasound examination of pregnant women is a diagnostic method that has been in use since the 1960's (Sundén 1964), with increased use during the 1970's, aided by better techniques (Kossoff 1972). Routine prenatal ultrasound examination was officially recommended in 1986 in Norway, 1980 in Germany, 1987 in Iceland, 1988 in Austria and 1995 in Switzerland. In most European countries and in the US, between 70 and 99% of pregnant women have had prenatal ultrasound examinations (The Swedish Council on Technology Assessment in Health Care 1998). A continuous development of equipment and of knowledge has taken place. Since the mid-eighties, an increased focus on developmental anomalies in addition to term determination has ensued. In 1985, the ultrasound laboratory was founded in Trondheim and in 1990 it was established as a national referral center (NCFM) at the Department of Gynecology and Obstetrics, Trondheim University Hospital. From then on, pregnant women with suspected fetal anomalies were referred from the whole country of Norway.

At the Department of Pathology, the proportion of perinatal autopsies with a diagnosis of congenital anomalies increased and has constituted almost half of all fetal and infant postmortem examinations since 1990. This shift in the epidemiology of perinatal autopsies prompted an interest in ultrasonography and congenital anomalies, particularly to investigate the extent to which prenatal ultrasound diagnosis correlated with the results of the postmortem examination. During the last 20 years, obstetrical ultrasonography has been brought to a high level of experience and expertise. At the same time, technical improvements of the sonographic equipment continue to further improve the diagnostic possibilities (Blaas *et al.* 1998). As a consequence, the diagnoses of congenital anomalies have become more accurate and it is possible to detect anomalies earlier in gestation. The need to look at a comprehensive autopsy material, analyzing single organ systems, emerged as a challenge.

This study is based on the curiosity prompted by the above mentioned development. The gathering of data took particular consideration of detailed information about the different organs, evaluated whether the ultrasound diagnoses were in agreement with the pathological diagnoses, and registered the results of karyotyping. To see if the results of this investigation changed over time, the comparison was split into two time periods with a natural division in 1990 when the NCFM was established. We are comparing the results of two different procedures, since some of the pregnant women had been to routine ultrasound during week 17-18 and some were examined either because of complications in pregnancy or were referred from other centers to a highly specialized ultrasound examination. The number of fetuses/infants where all the anomalies were missed at the routine ultrasound examination are few in number.

## Value of perinatal autopsy

Postmortem examination of fetal and perinatal deaths has been a priority at the Department of Pathology and the autopsy rate of fetuses with congenital anomalies has been high. In most cases parents are willing to give the necessary permission. In general, the frequency and quality of autopsies varies (Hågerstrand and Lundberg 1993, Julian-Reynier *et al.* 1993, Cartlidge *et al.* 1995, Chiswick 1995, Waldron 1995) and depends on the routine adopted for these examinations (Gilbert-Barness 1994).

Among pathologists, the importance of performing a perinatal autopsy has frequently been underestimated scientifically; it has also been regarded as contributing little to patient care (Husain and O'Conor 1991, Chambers 1992). This has been unfortunate. Every perinatal autopsy has a prognostic importance. However, a high autopsy rate loses its value if the quality of the postmortem examination is poor. The identification of anomalies, inherited disorders, and environmental or maternal factors relevant to the death may affect subsequent pregnancies. The exclusion of certain disorders can provide reassurance for future pregnancies and knowledge of the cause of death is thus essential for future parental counseling (Rushton 1994, Saller *et al.* 1995, Ramsing *et al.* 1991). Since perinatal mortality is an indicator of a nation's health, it is evident that both accuracy and completeness of information are necessary for reliable statistics (Porter and Keeling 1987, Sharma 1994).

## Perinatal mortality and incidences of congenital anomalies

Up to the nineties, congenital malformations have continued to be the most frequent cause of infant death in western countries (Husain and O'Conor 1991). The contribution of congenital anomalies to perinatal mortality in liveborn infants under 1 year of age in Norway was 31% in 1995 (Statistisk årbok 1998). According to Young and Clarke, the contribution of lethal malformations to perinatal mortality almost doubled from 1976 to 1985 (Young and Clarke 1987) and it was not expected that an enthusiastic prenatal diagnostic program would have an effect on the overall perinatal mortality. The introduction of routine prenatal ultrasonography during the second trimester was expected to detect only about half of these cases; those with major structural abnormalities of the brain, heart, kidneys or skeleton (Campbell and Smith 1984). The incidence of major congenital anomalies in children born in Norway was constant during the ten year period 1985-94, with a yearly average rate of 23 per 1000, excepting only the years 1990 and 1991 which had slightly higher rates of 25 and 27. If all anomalies are included there was an increase from 28 in 1985 to 33 in 1994, also with a maximum during the years 1990 and 1991 (Medical Birth-Registry of Norway 1997). The yearly anomaly rate in the county of Sør-Trøndelag was on the average 33 per 1000 during the same 10-year period (Medical Birth Registry of Norway 1997). The rate of infants born with congenital anomalies seems therefore not to have changed appreciably after the introduction of routine ultrasound examination.

## Comparison of prenatal ultrasound and postmortem findings: general remarks

Several studies have compared ultrasound findings with autopsy diagnoses (Rutledge *et al.* 1986, Shen-Schwarz *et al.* 1989, Clayton-Smith *et al.* 1990, Wilson *et al.* 1992, Grant *et al.* 1993, Weston *et al.* 1993, Julian-Reynier *et al.* 1994, Chescheir and Reitnauer 1994) but only a few address specific organ discrepancies (Rutledge *et al.* 1986, Weston *et al.* 1993, Chescheir and Reitnauer 1994, Julian-Reynier *et al.* 1994).

Until the late eighties, few studies had evaluated the overall accuracy of real-time ultrasound (Sabbagha *et al.* 1985, Rutledge *et al.* 1986, Manchester *et al.* 1988, Shen-Schwarz *et al.*

1989). Since then, the detection of fetal anomalies has become an important part of the routinely performed ultrasound examination. Manchester *et al.* found a false positive rate of 1.5% and a false negative of 2%. In 37% of the infants born with anomalies there were additional problems not detected prenatally by ultrasound. Manchester's study comprised a referred population including both living infants and perinatal deaths, with a mean gestational age of 29 weeks at the time of referral (Manchester *et al.* 1988). Clayton-Smith *et al.* confirmed this with a revised diagnosis in 40% of the cases (Clayton-Smith *et al.* 1990). Shen-Schwarz *et al.* found that in 46% of fetal anomalies detected by targeted ultrasound examination (because of elevated maternal serum alpha-fetoprotein, intrauterine fetal death, abnormal routine US screening, advanced maternal age, etc.), autopsy provided additional information that assisted in making a specific diagnosis and/or evaluating the severity of an anomaly (Shen-Schwarz *et al.* 1989).

In the beginning of the nineties, the situation had still not changed dramatically. In a series by Grant *et al.* the prenatal diagnoses were confirmed in 100 of 196 cases (51%) with 37 (19%) having significant additional pathology (Grant *et al.* 1993). As in the study by Manchester *et al.* this series also included living infants. Weston *et al.* did an autopsy study of 153 fetuses and in 44% additional anomalies were found or the ultrasonographic diagnosis was altered. In 25% the autopsy diagnosis affected genetic counseling and altered the management of future pregnancies (Weston *et al.* 1993). In a low-risk population, Chitty *et al.* found an overall sensitivity of 74.4% in detecting anomalies in 125 fetuses with US diagnoses in the second trimester (Chitty *et al.* 1991).

From 1994, there seemed to be an abrupt change in this situation. Julian-Reynier *et al.* found that in a geographically based study of 158 pregnancies, the prenatally detected and post-termination anomalies were identical in 90%, though in 57% of the polymalformed cases, the ultrasound missed at least one other diagnosable anomaly and the risk of recurrence of the anomaly was revised in about 30% of all the cases (Julian-Reynier *et al.* 1994). At the same time Chescheir *et al.* did a similiar study: of 133 fetuses and neonates, approximately 87% of autopsy-demonstrated major abnormalities had been detected by prenatal ultrasonography, with 61% of all malformations detected (Chescheir and Reitnauer 1994).

## Comparisons of the different organ systems

### Central nervous system anomalies

Congenital CNS anomalies were the first to be detected by ultrasound (Sundén 1964, Campbell 1972, 1977). There have been major advancements during the nineties, and as of today, they are easier to detect than malformations in other organs (Cohen and Haller 1994). CNS anomalies are being detected at an increasingly earlier age (Blaas and Eik-Nes 1996). CNS anomalies are also the most common prenatally diagnosed anomalies (Gowland 1988, Grant *et al.* 1993, Weston *et al.* 1993). Sabbagha reports that of 31 fetuses with CNS anomalies, 5 cases with anencephaly were correctly diagnosed, one case of spina bifida was missed and one was false positive (Sabbagha *et al.* 1994). In the series of Rutledge *et al.*, 20 of 27 CNS anomalies were diagnosed prenatally, though of 5 neural tube defects, only one was diagnosed prenatally (Rutledge *et al.* 1986). Chescheir and Reitnauer reported 3 false-positive CNS diagnoses that did not affect management (Chescheir and Reitnauer 1994). There was a high degree of agreement between prenatal ultrasound and autopsy findings in our study without significant discrepancies between the two time periods. It is interesting to note that major anomalies were not missed at ultrasound examination during the second time period.

### Congenital heart defects

For congenital heart defects, the situation is a little different. Previous studies comparing prenatal ultrasound and postmortem findings have usually included living fetuses in addition to fetuses that have undergone a postmortem examination. They are therefore not fully comparable to our study. Over a period from August 1986 to January 1991 Tegnander *et al.* found a detection rate of 39% of critical heart defects in a non-selected population (Tegnander *et al.* 1995). In a referred population, Benacerraf reported identification of 50% of heart defects in 49 fetuses confirmed either at physical examination or at autopsy (Benacerraf *et al.* 1987). In a group of fetuses at high risk for congenital heart disease Copel *et al.*, utilizing the 4-chamber view, detected 96% of the heart defects (Copel *et al.* 1987). In 1992 Achiron *et al.*, using the 4-chamber view alone, found a sensitivity of 48% in a low-risk population. The sensitivity increased to 78% when extended echocardiography was employed. The defects missed were aortic coarctation, persistent truncus arteriosus, tetralogy of Fallot, VSD and pulmonary stenosis; these missed defects parallel our results (Achiron *et al.* 1992). Other studies have shown that small VSDs, ASD secundum, aortic coarctation and arterial valve stenosis could be incorrectly diagnosed or undetectable prenatally (Crawford *et al.* 1988, Benacerraf *et al.* 1987, Bromley *et al.* 1992). Davis *et al.* found that the prenatal diagnosis was fully or partly correct in 96% of 111 cases where it was possible to verify the diagnosis by postmortem or postnatal diagnosis, though only 29 of these pregnancies were terminated (Davis *et al.* 1990). Vergani *et al.* reports that from 1985 to 1986 the sensitivity was 43%, while the introduction of the 4-chamber view (which was obtained in 95%), increased this to 81% from 1987 to 1989 (Vergani *et al.* 1992). In a combined high and low risk population, heart defects were prenatally detected in 63% by using the 4-chamber view alone; when including the outflow tracts, 83% of the defects were detected (Bromley *et al.* 1992). In our study, a significant difference in detection rate was found comparing the two time periods, 48% versus 82%. False-positive prenatal diagnoses of congenital heart lesions seem to be uncommon, though have been recorded (Copel *et al.* 1987, Allan *et al.* 1994, Rustico *et al.* 1995). In some centers, diagnosis of fetal cardiac defects in the first trimester is followed by termination of pregnancy by dilatation and curretage. Pathologic confirmation has been possible in 62% of these cases, with small size of the heart probably the main reason for the missed diagnoses (Achiron *et al.* 1994).

**Urinary system anomalies**

The degree of deleterious effect an obstruction in the urinary tract will have on the kidneys depends not only on the level and degree of obstruction, but also on the timing in gestation and on individual response to the different factors involved in the process (Blane *et al.* 1991). Of these, the time of onset seems to be the most important determinant of severity (Chevalier 1995). Microscopic examination of the kidneys, especially in the early stages of development of renal dysplasia, is important in order to be able to evaluate how early these changes occur. Experimental ureteral obstruction influences expression of growth factors impairing renal growth and development (Chevalier 1995, Coimbra 1996, Chung 1996). Considering the variety of etiological factors governing renal cystic lesions, the morphological diagnosis classifying them into nonhereditary and hereditary forms is of utmost importance for the genetic guidance of the parents (Rapola 1991).

As for CNS anomalies, previous comparisons of prenatal ultrasound and postmortem findings of urinary system anomalies have been done as part of a general analysis of all types of anomalies without a specific focus on the kidneys and urinary tract. The detection rate for renal anomalies in these autopsy studies varies somewhat, from 60% to over 90% (Sabbagha *et al.* 1985, Rutledge *et al.* 1986, Manchester *et al.* 1988, Clayton-Smith *et al.* 1990, Chescheir and Reitnauer 1994, Saari-Kemppainen *et al.* 1994, Julian-Reynier *et al.* 1994). The interpretation of the ultrasound findings is more difficult in the presence of anhydramnios or oligohydramnios which therefore can be responsible for inaccuracies (D'Ottavio *et al.* 1989, Shen-Schwarz *et al.* 1989, Scott and Goodburn 1995). Unilateral renal anomalies have been more difficult to discover than bilateral anomalies. In our study the detection rate of unilateral lesions was 83%, while for bilateral it was 95%.

Follow-up studies of ultrasonography of urinary system pathology, as in CHD, are for the most part a combination of clinical and postmortem cases. Postnatal confirmation of the prenatal diagnoses is described in 50-78% of cases from the late eighties (Scott and Renwick 1988, Sholder *et al.* 1988). Of 55 cases Kubota *et al.* found 81% agreement between antenatal and postnatal diagnoses. The major discrepancies consisted of difficulties in discriminating dysplastic kidneys from hydronephrosis, the rest were regional variations in the affected sites which also might be due to variations over time (Kubota *et al.* 1996). In an early second-trimester sonographic screening, 21 anomalies consisting of unilateral renal agenesis, pelvic kidney and double collecting system were all confirmed postnatally or at postmortem examination (Bronshtein *et al.* 1995).

## Comparison of detection rate between CNS anomalies, CHD, and urinary system anomalies

The overall detection rate was better for CNS (89%) and urinary system anomalies (87%) than for CHD (73%). When considering the main diagnoses there were no substantial differences between the detection rate of CNS anomalies (94%), urinary system anomalies (91%) and CHD (91%). The most striking difference was the improvement in the detection of CHD from the first to the second time period, signalizing major advances in the overall antenatal sonographic detection of CHD. The more diligent diagnosis of VSD has greatly contributed to this enhancement. Other studies differ somewhat as to the detection rate of anomalies in the different organ systems. In earlier publications comparing ultrasound and autopsy findings, CNS malformations were easier to detect than urinary tract anomalies (Rutledge *et al.* 1986, Shen-Schwarz *et al.* 1989). In later publications the same was true but to a lesser extent; CNS anomalies were correctly detected in over 90% while 60 to 75% of renal anomalies were found (Chescheir and Reitnauer 1994, Julian-Reynier *et al.* 1994).

At autopsy, the situation is a little different. Renal anomalies, if not subtle, are usually not difficult to diagnose. Dilatations of the urinary tract are usually easily discernable, but the level of obstruction may be more difficult. Urethral obstruction, specifically posterior uretral valves, can be difficult to see macroscopically, even with careful dissection. Examination of the brain at autopsy is dependent on its preservation; maceration and autolysis can make this almost impossible. Cystic lesions such as choroid plexus cysts and Dandy-Walker malformation may be easier to diagnose by ultrasound. Choroid plexus cysts are often transitory, this can also in some cases explain their absence at autopsy (Nicolaides *et al.* 1994). Small VSDs in small fetuses (12-14 weeks) can be extremely difficult to find even by careful dissection, though a stereomicroscope may be of help. With ultrasonography, magnification of the image and Doppler color visualisation can often render the detection of VSD easier. On the other hand, aortic coarctation has been difficult to detect by ultrasound (Crawford *et al.* 1988), while the diagnosis is usually straightforward at autopsy. Horseshoe kidneys can also be difficult to find by ultrasound examination (Danemann and Alton 1991). These examples demonstrate that even if both methods of examination are optimal, some lesions remain difficult to diagnose either by ultrasound or by autopsy. Non-detected minor anomalies, associated with more easily detectable anomalies that are discovered prenatally, will probably neither alter the management of the patient nor affect genetic counseling. These anomalies can be important as markers of more serious derangements, therefore all discrepancies, however small, should be registered.

## Detection of anomalies over time

Splitting the material into two time periods 1985-89 and 1990-94, was done to see how the correlation may have changed over time. Apart from being two 5-year periods, this was also a natural division since the NCFM was established in 1990. It is not surprising to see certain patterns evolve. The overall agreement increased significantly for CHD, but not for CNS and urinary system anomalies. The detection of minor alterations increased during the second time period, as a sign of increased ultrasound expertise. Except for urinary system anomalies, the percentage of cases where none of the autopsy findings were detected at ultrasound decreased during the second period, signifying that routine ultrasound has become better at picking up fetal anomalies. In some fetuses anomalies were detected at ultrasound that were not confirmed at autopsy. These were anomalies where the fetus was too macerated or traumatized for a proper diagnosis, or the ultrasound finding was transitory. In one case, the pregnancy was terminated because of anhydramnios and suspicion of renal dysplasia. The reanl dysplasia was not confirmed, but retrospectively the microscopical changes in the kidneys are suspect of a renal tubular dysgenesis as the reason for the non-functioning kidneys. Concerning the main diagnosis which lead to clinical consequences, no false positives have been found.

## Markers of chromosome aberrations

CNS anomalies, CHD and urinary system anomalies are the most common anomalies found in connection with chromosome aberrations. In our study, the distribution of cases with abnormal karyotype does not differ from that of other studies (Gagnon *et al.* 1992). The reason the correlation between ultrasound and autopsy findings in trisomy 18 was lower than other trisomies, probably relates to the large spectrum of dysmorphic features and anomalies present in trisomy 18; when certain findings indicate trisomy 18, the need for finding further proof may seem unnecessary. The detection by ultrasonography of so-called "chromosome markers" indicative of a chromosome aberration, will continue to be a major challenge in the years to come. It is most probable that detection of subtle dysmorphic features will continue to be one of the targets of ultrasound examination, necessitating a postmortem for verification.

## Fluoresence *in situ* hybridization (FISH)

Some of the cases in our study had anomalies indicating the possibility of a chromosome abnormality, but karyotyping was not performed. FISH performed on paraffin-embedded formalin-fixed material is a fairly recent method (Cobben *et al.* 1994, van Lijnschoten *et al.* 1994, Hyytinen *et al.* 1994, Slagel *et al.* 1995, Kuchinka *et al.* 1995, Köpf *et al.* 1996) and has so far not gained wide acceptance for use on autopsy material. The reasons for this are probably varied. The method can be cumbersome when the material is autolyzed (Slagel *et al.* 1995). Differences in the duration of formalin-fixation necessitates variable protease treatment in each case leading to repeated hybridizations. Signal detection can also be difficult, and the process of counting signals is time consuming. Evaluation of the results has to be done taking into account the clinical course. The method is specific for the probe tested, and thus only practical for use on a limited number of conditions (Ward *et al.* 1993, Evans *et al.* 1994, Hume *et al.* 1995). The abnormal karyotypes tested are therefore the ones most commonly encountered in perinatal pathology (Evans *et al.* 1994). In our laboratory, the method was performed on a limited number of cases since working with macerated, formalin-fixed material turned out to be time consuming and repeated hybridizations were necessary in a number of cases. The advantage of such a method is primarily in abortuses and stillbirths with anomalies where a traditional karyotyping is not possible because of macerated material.

## Quality assurance

A postmortem examination has traditionally been considered the "gold standard" and quality control of autopsy has therefore been limited to assessing the accuracy and completeness of the examination. The conclusions drawn by the pathologist are usually not questioned by the clinician. Advances in diagnostic technology have not reduced the value of the autopsy (Goldman *et al.* 1983) which remains the final word in diagnostic quality control (Scottolini and Weinstein 1983). Both pathologists and clinicians have questioned the utility of the perinatal autopsy. Meier *et al.* compared information obtained by clinical review with information obtained from the autopsy report and concluded that the autopsy frequently was the only means of establishing the exact cause of death (Meier *et al.* 1986). Porter and Keeling observed important differences between clinical and pathological diagnoses in both stillbirths (36%) and neonatal deaths (44%) (Porter and Keeling 1987). With the increasing use of ultrasonography in pregnancy surveillance the importance of properly conducted perinatal autopsies has become more evident (Chambers 1992). The confirmation of prenatal diagnoses are important for the monitoring and refinement of various diagnostic techniques (Saller *et al.* 1995).

The benefit and value of a perinatal autopsy, particularly when dealing with fetal anomalies, is dependent on proper dissection technique with basic knowledge of perinatal medicine and experience with congenital anomalies. The postmortem examination has traditionally been the final answer to all questions pertaining to diagnosis and/or cause of death. Pathologists are trained to describe abnormal findings, though an inexperienced pathologist may miss certain anomalies out of ignorance or lack of awareness. The saying: "you find only what you are looking for" can too easily come true. The smaller the fetus, the more difficult it is to examine. Subtle anomalies diagnosed by ultrasound can sometimes be difficult to confirm postnatally and close contact with clinicians is important in order to focus on the issues in question. This is essential for clinicians and pathologists giving them an opportunity for reciprocal learning. Such collaboration promotes better communication, more interest and renders the final diagnosis more complete and of a much better quality. Knowing the ultrasound diagnoses before performing the autopsy will trigger the pathologist to look for specific anomalies. This bias will function as a guide and in this setting it is unlikely that there is any risk of diagnosing an anomaly that is not present. A pathologist is specifically trained to describe what is actually seen and the risk of making a "fake" diagnosis is not likely. A properly conducted postmortem thus serves as a complement to the prenatal diagnosis, karyotyping included; in addition to playing an important role in genetic counseling and epidemiologic studies, it is considered important for the final quality control in fetal diagnostics. The ideal is for autopsy and ultrasonography to function as complementary examinations, therefore the cooperation in these two fields can be extremely fruitful. The ultimate aim, making the final diagnosis as complete as possible, may then be achieved.

The advantage of early induced abortion in cases with lethal anomalies is evident. During the acute phase, the psychological responses after pregnancy termination due to fetal anomalies compared to perinatal loss for other reasons, are less severe (Salvesen *et al.* 1997) perhaps indicating that the psychological trauma may be easier to bear the shorter the gestation. This must be one of the main motives for trying to diagnose congenital anomalies the earliest possible. Postmortem verification of anomalies is more difficult the earlier in gestation the fetus is aborted. This is due not only to the risk of traumatisation of the fetus associated with early TOP, but is also related to the problem of demonstrating small structural alterations in fetuses at the end of the first and beginning of the second trimester.

The future of perinatal pathology and, specifically, perinatal autopsies will depend on the ability of the pathologist to accept the challenge offered by modern ultrasonography. This can only be realized through the close cooperation between representatives of the two specialities, mutually stimulating each other to attain the increased knowledge necessary to make proper use of the available techniques. The daily experience with structural anomalies teaches the pathologist, and the feed-back offered to the ultrasonographers and ultrasonologists is of value in guiding them in further learning.

# CONCLUSIONS

A comparison between prenatal ultrasound diagnoses and postmortem examinations was performed in a study consisting of 408 fetuses and infants. For the central nervous system, the heart and the urinary system, the overall agreement between ultrasound diagnoses and autopsy findings was acceptable with the main diagnosis correct in over 90%. The diagnosis of congenital heart defects increased significantly ($p<0.01$) from the first time period (1985-89) to the second time period (1990-94). Not all ultrasound diagnoses were possible to confirm at autopsy during the last time period, either because of maceration or traumatisation of the fetus, or the ultrasound findings were transitory and therefore no longer present at the postmortem examination. Ultrasound examination of fetal structures has become a sophisticated method demanding increased expertise on the part of the pathologist in order to sustain autopsy as the necessary quality control. Retrospective karyotyping by fluorescence *in situ* hybridization (FISH) on formalin-fixed and paraffin-embedded tissue was performed in selected cases. This method can be a useful supplement in cases with congenital anomalies and unknown karyotype suspicious of an autosomal trisomy or Turner syndrome.

The gestational age at which fetuses with congenital anomalies are aborted is steadily decreasing. This will require a new approach to the perinatal autopsy with dissection under a stereomicroscope becoming increasingly necessary. Serial sectioning of small structures is also an alternative method of examination. Critical correlations between prenatal examinations and postnatal investigations will improve the diagnostic value of ultrasonography. Besides the necessary feedback to evaluate the ultrasonographic data, autopsy also provides educational and research opportunities. This is invaluable for the well-being of society as a whole, as well as for the medical community. Postmortem examinations provide additional information in a large proportion of antenatally diagnosed fetal anomalies and give a more accurate diagnosis enabling a more reliable genetic counseling. Collaboration between ultrasound experts and perinatal pathologists is essential for improving the diagnostic accuracy.

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# PAPER I

*Ultrasound Obstet Gynecol 1998;11:246–253*

# Comparison of prenatal ultrasound and postmortem findings in fetuses and infants with central nervous system anomalies

*C. V. Isaksen**†, *S. H. Eik-Nes*†, *H.-G. Blaas*† *and S. H. Torp**

*Institute of Morphology and Department of Pathology, †National Center for Fetal Medicine, Department of Obstetrics and Gynecology, Norwegian University of Science and Technology, Trondheim University Hospital, Trondheim, Norway

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## ABSTRACT

*Detection of fetal developmental abnormalities by ultrasound examination of pregnant women has become a specialized field of medicine. Quality control of this field requires detailed examination of aborted fetuses. In 408 fetuses and infants with developmental anomalies, the prenatal ultrasound findings were compared with the postmortem findings. This study focused on 140 central nervous system (CNS) anomalies. Criteria for inclusion were an ultrasound examination at the National Center for Fetal Medicine (NCFM) and an autopsy performed during the period 1985–94. Results of the ultrasound and autopsy examinations were systematized into six different categories.*

*Hydrocephaly and anencephaly were the most frequent abnormalities, together accounting for 50% of the CNS anomalies. In 20 cases (14%), the CNS anomalies were associated with other important anomalies or chromosomal aberrations. In 125 of the cases (89%), there was complete concordance between the ultrasound and autopsy diagnoses. Of the 15 CNS cases with discrepancies, seven had nearly complete concordance; if we include these, the correlation was 94%.*

*In conclusion, this study confirms that developmental anomalies in the central nervous system are frequent and that ultrasound diagnoses are in good concordance with the autopsy diagnoses.*

## INTRODUCTION

During the last 20 years, obstetric ultrasonography has been established as an integral part of antenatal care and is offered for every pregnancy in many countries. Initially, assessment of gestational age, detection of multiple pregnancies and location of the placenta were the main reasons for performing the scan. During the last decade, the detection of fetal anomalies has become an important part of the routinely performed fetal ultrasound examination[1,2]. High-frequency transvaginal ultrasound has made it possible to increase the diagnostic accuracy in early pregnancy. Even the detection of embryonic anomalies has been described recently using ultrasound[3]. Targeted ultrasound examination may therefore be performed as early as at the end of the first trimester of high-risk pregnancies, for example in women who have had offspring with developmental anomalies, chromosomal abnormalities, or genetic diseases. Early diagnosis of serious fetal anomalies makes the termination process easier, reduces maternal risks and lessens the psychological trauma of the parents[4].

Postmortem examination of aborted embryos and fetuses and examination of liveborn or stillborn infants has an important role in the quality control of the work performed by obstetric ultrasonographers. Perinatal pathology is becoming a specialized field of pathology which demands expertise and experience[5]. Since the gestational age at which congenital anomalies may be detected is decreasing, the perinatal autopsy will increasingly include embryonic and early fetal examinations.

Several studies have compared prenatal ultrasound examination with autopsy findings in cases with congenital anomalies[3,6–12]. However, few have specifically addressed the discrepancies between prenatal and postmortem findings[10–12].

The aim of this study was to compare the prenatal ultrasound diagnoses of central nervous system (CNS) anomalies with the autopsy findings, and to evaluate the diagnostic accuracy of ultrasound as part of general quality control.

Correspondence: Dr C. V. Isaksen, Department of Pathology, Trondheim University Hospital, 7006 Trondheim, Norway



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## MATERIAL AND METHODS

Included in this prospective study were fetuses and infants that underwent autopsy and that during the pregnancies had been examined with ultrasound at the ultrasound laboratory, Department of Obstetrics and Gynecology, Trondheim University Hospital and who later proved to have a CNS anomaly. Eye anomalies were considered to be CNS anomalies and were included in the study. Cases with a positive ultrasound finding without a corresponding autopsy finding were included.

Until 1990, the ultrasound laboratory served the local area and the surrounding region, covering a total population of 250 000 inhabitants. In 1990, the unit was established as a National Center for Fetal Medicine (NCFM) and has since acted as a referral center for pregnant women with suspected or verified fetal anomalies from the whole of Norway.

From January 1985 to December 1994, a total of 408 autopsies of fetuses and infants with developmental anomalies were performed, 365 at the Department of Pathology, Trondheim University Hospital, and 43 at other hospitals co-operating with the national center. CNS anomalies were present in 140 cases (34%).

The ultrasound examinations were performed by obstetricians working at the center using Hitachi EUB 565, Dornier AI 3200 and Vingmed Sound CFM 750 ultrasound machines. The machines were equipped with transducers with frequencies of 3.5–7.5 MHz. The anomalies were either diagnosed or suspected during the routine fetal ultrasound examination offered to all pregnant women at 18 weeks or at a targeted ultrasound scan performed because of hereditary risk factors or a clinically abnormal development of the pregnancy. Following the ultrasound examination, a thorough anatomical description of the findings was recorded. Any available information about karyotype and/or biochemical analysis of fetal blood and/or amniotic fluid was also registered. The medical history and sonographic data were prospectively stored in a computer database. All comparisons are based on the recorded findings in the ultrasound report.

From January 1985 to December 1990, a standardized autopsy was performed at the Department of Pathology, Trondheim University Hospital, by doctors in training, supervised by a consultant pathologist. Following the establishment of the NCFM in 1990, a perinatal pathologist became part of the center's team and performed all the autopsies from then on. The perinatal pathologist had regular meetings with the obstetricians at the center and reviewed the videotapes with the sonographic findings prior to the autopsy. In addition to the standard autopsy protocol, a special protocol was developed to include routine radiography and photographic documentation of the findings. All organs were examined, the heart was examined *in situ* and the brain removed under water in order to minimize postmortem changes.

The ultrasound and autopsy findings were correlated and categorized as follows:

(1) Full agreement (all ultrasound findings concordant with the morphological diagnoses);

(2) Minor autopsy findings not found on the ultrasound examination (main diagnosis responsible for the management correct, but additional findings overlooked). Example: overlooked mild hydrocephalus in a case of thanatophoric dysplasia;

(3) Major autopsy findings not found on ultrasound examination (one or several anomalies detected at ultrasound examination leading to an interruption of pregnancy, but at autopsy additional major anomalies were found that had been overlooked during the ultrasound examination). Example: bilateral kidney dysplasia was the reason for the termination of pregnancy, while occipital encephalocele and polydactyly were found at autopsy, confirming the diagnosis of Meckel–Gruber syndrome;

(4) None of the autopsy findings suspected on ultrasound examination (negative ultrasound examination with unexpected autopsy findings). In these cases the fetus/infant died naturally *in utero* or shortly after birth;

(5) Minor ultrasound findings not confirmed at autopsy. These unverified ultrasound findings did not precipitate unjustified management, but sometimes indicated further investigation. An example of this is a choroid plexus cyst initiating a search for a chromosomal abnormality. Since some choroid plexus cysts are transient, they may not be present when the autopsy is performed;

(6) Major ultrasound findings not confirmed at autopsy. This category included false positives as well as cases in which postmortem changes interfered with reaching a morphological diagnosis.

## RESULTS

Forty-six (33%) of the 140 cases came from the city of Trondheim, and the rest from other parts of the country. Fifty-two per cent of the cases were female. In one case it was not possible to determine the sex. The mean age of the mothers at the time of termination of pregnancy or at delivery was 28 years (range 17–44 years). Thirty-one per cent of the women had experienced a previous pregnancy loss. In 1985, the mean gestational age at abortion/birth was 26.4 weeks (range 16–40 weeks); in 1994 it was 22.1 weeks (range 11–41 weeks). Termination of pregnancy was carried out in 125 cases (89%); nine (7%) were intrauterine deaths and six (4%) were live born after spontaneous delivery or delivery induced because of fetal distress.

In 120 (86%) of the 140 cases, the CNS anomaly was the principal reason for induced abortion or cause of death (Table 1). Hydrocephaly (24%) and anencephaly (26%) were the two most common CNS anomalies, followed by lumbosacral spina bifida and myelomeningocele with or without hydrocephaly (20%) (Figure 1). In the remaining

20 cases, the central nervous system anomalies were associated with more extensive anomalies in other organs or with chromosomal aberrations (Table 2). All four cases with Dandy–Walker anomaly were associated with congenital heart diseases: tetralogy of Fallot, atrioventricular septal defect, hypoplastic left ventricle and ventricular septal defect. Three cases with verified choroid plexus cysts occurred in fetuses with trisomy 18. Three cases with limb–body wall complex had associated neural tube defects.

**Table 1** Classification of the central nervous system (CNS) anomalies found by ultrasound and/or autopsy: main diagnoses or CNS findings additional to a developmental anomaly in another organ ($n$ = 140)

| | *CNS anomalies* | | *Total* | |
|---|---|---|---|---|
| *Diagnosis* | *Main diagnosis* | *Additional finding* | *n* | % |
| Hydrocephaly | 32 | 2 | 34 | 24 |
| Anencephaly | 35 | 1 | 36 | 26 |
| Acrania | 5 | 1 | 6 | 4 |
| Spina bifida with hydrocephaly | 21 | 1 | 22 | 16 |
| Spina bifida without hydrocephaly | 5 | 1 | 6 | 4 |
| Encephalocele | 4 | | 4 | 3 |
| Agenesis of the corpus callosum | | 4 | 4 | 3 |
| Holoprosencephaly | 7 | | 7 | 5 |
| Dandy–Walker anomaly | | 4 | 4 | 3 |
| Choroid plexus alterations | | 3 | 3 | 2 |
| Microcephaly/ polymicrogyria | 3 | 2 | 5 | 4 |
| Miscellaneous* | 8 | 1 | 9 | 6 |
| Total | 120 | 20 | 140 | 100 |

*Meckel–Gruber syndrome, Krabbe's syndrome, Fraser's syndrome, microphthalmia, congenital tumors

**Table 2** Cases in which the central nervous system (CNS) anomaly was additional to a main anomaly located in another organ system ($n$ = 20)

| *CNS anomalies* | *Associated anomalies* | *Total* |
|---|---|---|
| Hydrocephaly | thanatophoric dysplasia | 2 |
| | limb–body wall complex | 1 |
| Acrania/anencephaly | distorted fetus with features consistent with amniotic rupture sequence and limb–body wall complex | 2 |
| Spina bifida with meningomyelocele | limb–body wall complex | 1 |
| Agenesis of the corpus callosum | limb–body wall complex | 1 |
| | hydrops | 1 |
| | CHD | 2 |
| Dandy–Walker anomaly | CHD | 4 |
| Choroid plexus alterations | CHD and trisomy 18 | 3 |
| Polymicrogyria | CHD and trisomy 18 | 2 |
| Tuberous sclerosis | cardiac rhabdomyomas | 1 |
| Total | | 20 |

CHD, congenital heart disease

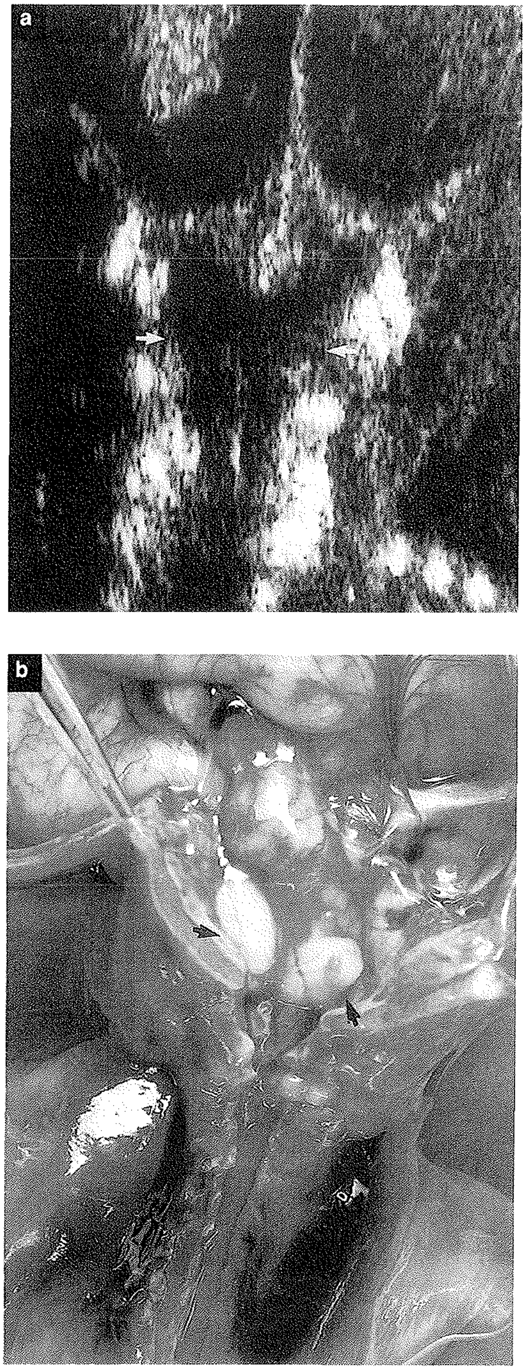


**Figure 1** Fetus, 19 weeks old. Arnold–Chiari malformation. (a) Ultrasound scan: the cerebellum is hypoechogenic. The arrows indicate the lower border of the cerebellum; (b) autopsy photograph: arrows point at the cerebellar hemispheres (light areas) partly herniated into the foramen magnum

In 125 (89%) of the 140 cases with CNS anomalies diagnosed by ultrasound, there was full agreement with the autopsy report (Table 3). In the remaining 15 cases, discrepancies between the sonographic and the autopsy findings were observed.

These 15 cases with CNS anomalies showing different degrees of accordance between the ultrasound and the autopsy diagnosis are listed chronologically in Table 4: cases 1 to 6 are from the period 1985–89, and cases 7 to 15 from the period 1990–94. In seven of the 15 cases, the main diagnosis was demonstrated prenatally (Table 4, category 2). Case 2 was the only case in category 3 where one of the major anomalies was not detected. The fetus was aborted because of anhydramnios and bilateral cystic kidney dysplasia. At autopsy, occipital encephalocele and polydactyly were also found, confirming the diagnosis of Meckel–Gruber syndrome.

Table 3 Correlation of prenatal ultrasound diagnoses and postmortem findings. Central nervous system anomalies ($n$ = 140)

| *Category* | *n* | % |
|---|---|---|
| Full agreement | 125 | 89 |
| Minor autopsy findings not found by ultrasound | 7 | 5 |
| Major autopsy findings not found by ultrasound | 1 | 1 |
| None of the autopsy findings suspected by ultrasound | 3 | 2 |
| Minor ultrasound findings not found at autopsy | 2 | 1.5 |
| Major ultrasound findings not found at autopsy | 2 | 1.5 |
| Total | 140 | 100 |

In cases 1, 3 and 4, the postmortem examination revealed anomalies not observed during the ultrasound examination (category 4). Two fetuses (cases 1 and 3) had spina bifida, one died *in utero* in the 26th week while the other was born alive but died shortly after birth. In the latter, the chromosomal analysis revealed triploidy. One infant born alive in the 32nd week (case 4) had dysmorphic features with cleft lip and palate, low-set ears and eye anomalies such as microphthalmia and cataract. A chromosomal aberration was suspected, but the culture was not successful.

In categories 5 and 6 (ultrasound findings not confirmed at autopsy), there were four discrepancies involving the CNS. In case 7, a Dandy–Walker anomaly and, in case 14,

Table 4 Central nervous system (CNS) anomalies: survey of discrepancies between ultrasound and autopsy findings ($n$ = 15). Cases 1–6 from 1985–89 and cases 7–15 from 1990–94

| *Case* | *Prenatal diagnosis* | *Sex* | *GA (weeks) at autopsy* | *Mode of death/delivery* | *Final diagnosis following autopsy* | *Category* |
|---|---|---|---|---|---|---|
| 1 | none | M | 26 | IUFD | thoracolumbar myelomeningocele | 4 |
| 2 | polycystic kidneys | M | 23 | TOP | polycystic kidneys, occipital encephalocele and polydactyly consistent with Meckel–Gruber syndrome | 3 |
| 3 | IUGR, placental insufficiency | F | 34 | LB | triploidy, dysmorphism, sacral spina bifida, IUGR, placental insufficiency | 4 |
| 4 | none | F | 32 | LB | dysmorphism, microphthalmia, cataract, cleft lip/palate, ASD, trisomy 13? (karyotyping not successful) | 4 |
| 5 | thanatophoric dysplasia | M | 20 | TOP | thanatophoric dysplasia, mild hydrocephaly | 2 |
| 6 | hydrocephaly | M | 25 | TOP | hydrocephaly, micrognathia, cleft palate, vertebral anomalies, anal atresia | 2 |
| 7 | Dandy–Walker anomaly, microcephaly, dysmorphic features, IUGR | F | 21 | TOP | microcephaly, dysmorphic features with low-set ears, IUGR (1/50 cell cultures showed trisomy 18) | 5 |
| 8 | Meckel–Gruber syndrome | O | 9 | TOP | traumatized embryo, insufficient morphological diagnosis | 6 |
| 9 | anencephaly | F | 18 | TOP | anencephaly with cervical rachischisis | 2 |
| 10 | CNS anomaly, possibly Arnold–Chiari malformation, omphalocele and possibly CHD | F | 17 | TOP | Arnold–Chiari malformation, vertebral anomalies, omphalocele, ASD, VSD, tricuspid atresia | 2 |
| 11 | hydrocephaly, IUGR, placental insufficiency | F | 22 | TOP | macerated fetus, IUGR, placental insufficiency, large fontanelles and broad sutures consistent with hydrocephalus | 6 |
| 12 | cardiac rhabdomyomas | F | 34 | TOP | tuberous sclerosis with cardiac rhabdomyomas, subependymal astrocytomas and cortical tubera, hydrops | 2 |
| 13 | anencephaly | M | 20 | TOP | anencephaly with cervical rachischisis | 2 |
| 14 | trisomy 18, clenched fingers, choroid plexus cyst | F | 22 | TOP | trisomy 18, dysmorphism, slightly hypoplastic left ventricle and bicornuate uterus | 5 |
| 15 | ARS/LBWC (scoliosis and deformed left arm) | M | 11 | TOP | ARS/LBWC (acrania, scoliosis, cleft lip/palate, deformed nose and deformed left arm) | 2 |

GA, gestational age; IUFD, intrauterine fetal death; TOP, termination of pregnancy; IUGR, intrauterine growth retardation; LB, liveborn; ASD, atrial septal defect; VSD, ventricular septal defect; CHD, congenital heart defect; ARS, amniotic rupture sequence; LBWC, limb–body wall complex; O, sex not possible to determine a choroid plexus cyst were not confirmed since the brains at autopsy were too macerated to be evaluated. In both cases, chromosome analysis showed trisomy 18. Of the two fetuses with major ultrasound findings not confirmed at autopsy (category 6), one had a family history of Meckel–Gruber syndrome (case 8). The ultrasound scan of the embryo was of high quality and showed an enlarged rhombencephalic cavity, occipitoschisis and polydactyly. The embryo was damaged during abortion so that it was not possible to confirm this diagnosis at autopsy. In the other fetus (case 11), there was oligohydramnios, intrauterine growth restriction, hydrocephaly and suspicion of renal dysplasia. The intrauterine growth restriction was confirmed, the fontanelles were large and the suture lines broad, but the brain was macerated, and therefore the hydrocephaly could not be confirmed by autopsy. The placenta showed changes consistent with placental insufficiency. These two cases were not categorized as false positives.

Genuine false-positive cases were not found. Considering the cases with full agreement and the minor autopsy findings not detected prenatally, the main diagnosis was correct in 94%.

Comparing the two time periods 1985–89 and 1990–94, differences in the distribution of the categories were observed. Case 2 in category 3, and cases 1, 3 and 4 in category 4, were all from the first time period. During the more recent time period, there were no cases in which the autopsy findings were not suspected on ultrasound examination. The four cases in categories 5 and 6 were seen during the second time period. All seven cases of holoprosencephaly (Figure 2) were diagnosed after 1990.

Four of the cases with a CNS anomaly as the main diagnosis were diagnosed before 15 weeks' gestational age. These fetuses were examined within the last 5 years. Two were anencephalic fetuses and one had occipital encephalocele (Figure 3); all three were correctly diagnosed prenatally. A Meckel–Gruber syndrome was suspected at week 9 (Table 4, case 8).

In 113 (81%) of the 140 cases with CNS anomalies, amniocentesis or fetal blood sampling was carried out for chromosomal analysis. In five cases the karyotyping was not successful. In 25 cases (18%) a chromosomal abnormality was detected (Table 5). During the first time period the karyotype was not known in 21 cases (40%); during the last time period it was not known in only 11 cases (13%).

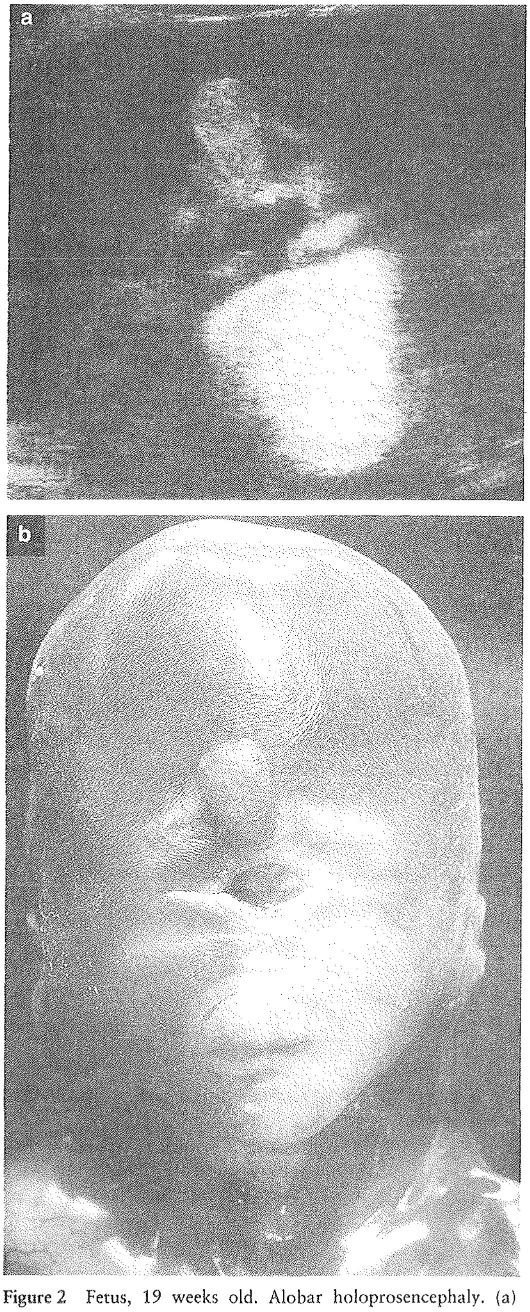


Figure 2 Fetus, 19 weeks old. Alobar holoprosencephaly. (a) Ultrasound scan of frontal section through face indicating the proboscis and cyclopia. The light area represents the lower part of the face; (b) photograph showing the proboscis and cyclopia

## DISCUSSION

Ultrasonography as a method for detecting fetal anomalies is already well established and has become an important part of routine prenatal care. The diagnostic accuracy is continuously improving, lowering the age at which anomalies can be detected.

A correct prenatal diagnosis in cases of fetal developmental anomalies is important to ensure proper management and appropriate parental counselling[8,13,14]. Proper and continuous quality control is therefore essential; this control, in turn, necessitates a detailed perinatal autopsy. In addition, autopsy in perinatology is important for epidemiological studies and may give information about

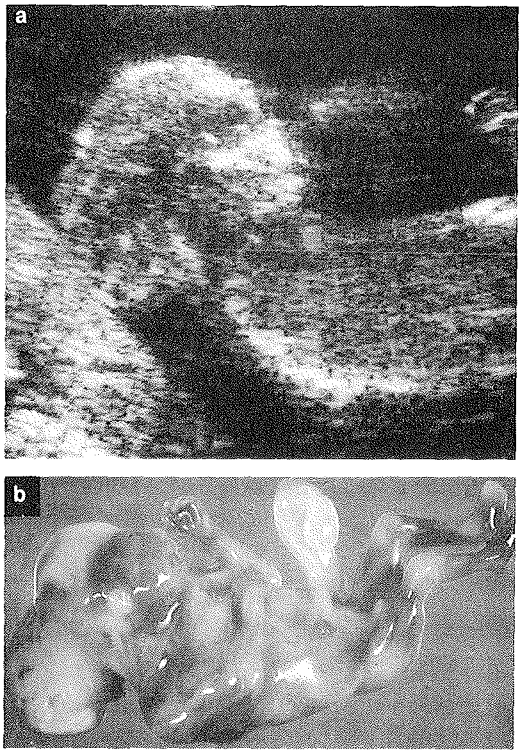

Figure 3 Fetus, 13 weeks old. Occipital encephalocele. (a) Ultrasound scan; (b) photograph

Table 5 Karyotype in 140 cases with CNS anomalies

| | *Not known* | | *Normal* | | *Abnormal* | | *Total* | |
|---|---|---|---|---|---|---|---|---|
| *Time period* | *n* | % | *n* | % | *n* | % | *n* | % |
| 1985–89 | 21 | 40 | 24 | 45 | 8 | 15 | 53 | 100 |
| 1990–94 | 11 | 13 | 59 | 68 | 17 | 19 | 87 | 100 |
| 1985–94 | 32 | 23 | 83 | 59 | 25 | 18 | 140 | 100 |

etiology and have therapeutic implications for future pregnancies[15,16].

The autopsy rate of aborted fetuses varies at different centers. The quality of the autopsies also varies[13,14,17,18] and depends on the routines adopted for these examinations[19,20]. Several studies have emphasized the need for specialized autopsy routines[5,15,21–28]. Most studies comparing ultrasound diagnoses with autopsy findings have included all types of developmental anomalies[2,6,7,9–12]. Comparative studies carried out over the last 10 years have given variable results; however, the studies tend to show that many of the major malformations are discovered at the ultrasound examination, CNS anomalies being among the most common prenatally diagnosed anomalies[6,9,10,29]. Of our 408 cases, 140 (34%) had developmental anomalies affecting the CNS; this is similar (30–40%) to other studies[2,6,8,9,11,12,16,30].

Over a 10-year period, a decrease in the mean gestational age at termination of pregnancy of the fetuses with CNS anomalies was demonstrated. The mean difference was 4 weeks 2 days between the first year of registration (1985) and the last year (1994). This is most likely to be an effect of the 18th week routine examination which was established in Norway by 1986. Because of technical improvements and the increased expertise of ultrasonographers, targeted ultrasound examinations of women at risk are increasingly being performed in the first trimester and in the beginning of the second trimester. Since the gestational age at termination of pregnancy has been reduced over recent years, the necessity of establishing appropriate autopsy techniques for small fetuses and embryos is evident. For example, dissection of organs under a stereomicroscope might soon be part of the routine for a perinatal pathologist.

Minor anomalies may be overlooked in the presence of more dominant anomalies[9]. A major issue, then, is whether the prenatal diagnosis wrongly alters the management of the patient and/or affects genetic counselling[2,6]. Concerning the CNS anomalies, 89% were correctly diagnosed. If the minor overlooked abnormalities are disregarded, this rate increases to 94%. In one case, a major CNS anomaly was not discovered at ultrasound examination and a major anomaly in another organ led to termination of pregnancy. Three cases in which none of the autopsy findings were suspected at the ultrasound examination represent a false-negative rate of 2%.

We found slight differences between the two time periods 1985–89 and 1990–94. There were no cases in the second time period in which major autopsy findings were not detected at the prenatal ultrasound examination, and no cases in which none of the autopsy findings were suspected at the prenatal scan. This probably reflects an improvement in ultrasound expertise in the course of the two time periods. There were five cases with minor findings not perceived or mentioned in the ultrasound report but registered at autopsy during the last time period. This has to do with better awareness at autopsy and a meticulous classification of certain anomalies. Cervical rachischisis in cases with anencephaly might have been seen but not recorded in the report, and the same applies to mild hydrocephaly in a case of thanatophoric dysplasia. Such additional findings had no implications for the immediate management. For genetic counselling, however, a detailed detection of developmental abnormalities is important.

In the process of analyzing the information, two additional categories were considered: transient ultrasound findings and ultrasound findings not verified at autopsy when it is technically possible to evaluate them. The examination of the CNS is often hampered by the autolytic process that readily affects the brain and may make its examination insufficient. Two cases in this study had major ultrasound findings not verified at autopsy. In both cases, the fetuses were either damaged or macerated, and therefore it was not possible to evaluate them. Based on the overall evaluation of these cases, there was no reason to believe that the ultrasound diagnosis was not correct. Thus, no true false-positive cases were confirmed.

Hydrocephaly and anencephaly were the most common CNS anomalies; this is in agreement with other studies[11,12,16,31–35]. Spina bifida with or without hydrocephaly was the third most common CNS anomaly in this study. Holoprosencephaly (seven cases) was diagnosed only during the last time period, from 1990. This might be caused by increased awareness of the condition, both ultrasonographically and at autopsy. Holoprosencephaly does not seem to be as common in other series, but the numbers are small[11,12,32,33,36,37]. The increased diagnostic accuracy in antenatal ultrasonography emphasizes the need for more experience with perinatal autopsy.

Our results confirm an overall good correlation between prenatal ultrasound and postmortem examination of major CNS anomalies[2,6,9,12,38]. No matter how efficient and correct the prenatal diagnoses become, a verification by morphological examination is desirable in order to ensure the necessary quality control[1,2,9,10,12–14,21,27,28].

Ultrasound examination and autopsy are two methods of examining embryonic and fetal anomalies and should be considered to be complementary, mutually stimulating and therefore important for the future development of perinatology.

## CONCLUSION

In a tertiary center we have compared the prenatal ultrasound diagnoses of CNS anomalies with autopsy findings. The main diagnosis was correct in 94% of the cases with no true false-positive cases and thus had a high grade of concordance with the autopsy diagnoses. Developmental anomalies in the central nervous system are frequent and were represented in 140 of 408 cases (34%) in our autopsy material.

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# PAPER II

*Ultrasound Obstet Gynecol 1999;13:117–126*

# Comparison of prenatal ultrasound and postmortem findings in fetuses and infants with congenital heart defects

*C. V. Isaksen**,†, *S. H. Eik-Nes*†, *H.-G. Blaas*†, *E. Tegnander*† *and S. H. Torp**

*Institute of Morphology and Department of Pathology, †National Center for Fetal Medicine, Department of Obstetrics and Gynecology, Trondheim University Hospital, Norwegian University of Science and Technology, Trondheim, Norway

Key words: ULTRASONOGRAPHY, AUTOPSY, CONGENITAL HEART DEFECTS, ANOMALIES

## ABSTRACT

***Objective*** *Detection of congenital heart defects by prenatal ultrasound examination has been one of the great challenges since the investigation for fetal anomalies became part of the routine fetal examination. This prospective study was designed to evaluate the concordance of prenatal ultrasound findings with autopsy examination in a population consisting of both referred women and non-selected pregnant women.*

***Design*** *Criteria for inclusion were an ultrasound examination at the National Center for Fetal Medicine and an autopsy performed during the years 1985–94. Results from the ultrasound and autopsy examinations were systematized into categories depending on the degree of concordance.*

***Results*** *Of 408 infants and fetuses with developmental anomalies, 106 (26%) had congenital heart defects. In 63 (59%) of these 106 cases, the heart defect was the principal reason for the termination of pregnancy or the cause of death. Excluding five cases with a secundum atrial septal defect, there was complete agreement between the ultrasound examination and the autopsy findings in 74 (73%) of 101 cases. In 18 cases, there were minor discrepancies between ultrasound and autopsy findings. The main diagnosis was thus correct in 92 cases (91%). From the first time period (1985–89) to the second (1990–94), the detection rate of all heart defects increased from 48% to 82%.*

***Conclusion*** *This study confirms a good correlation between ultrasound and autopsy diagnoses in fetuses and infants with congenital heart defects. A significant improvement in the detection of heart defects occurred from the first time period to the second and was probably due to increased experience and technical advances.*

## INTRODUCTION

Routine fetal ultrasonographic examination offered in the 18–19th week of pregnancy has in recent years been expanded to include evaluation of fetal anatomy[1,2]. A systematic search for congenital malformations has become an important part of antenatal surveillance. Congenital heart defects have been difficult to diagnose by prenatal ultrasound scanning, although experience and the improvement of scanning techniques and technical equipment have increased the diagnostic accuracy[3–5].

Transvaginal scanning involving high-frequency transducers enables the detection of congenital heart defects at the end of the first trimester[6–8]. This development represents a challenge to both the sonologist and the perinatal pathologist who have to examine very small fetal structures.

Congenital heart defects are responsible for 20% of neonatal deaths caused by congenital anomalies[9]. The incidence of congenital heart defects in newborns has been reported to be 8–10 per 1000; this has been the most common congenital anomaly encountered in liveborns[4,10,11]. In abortuses and stillbirths, however, the incidence of congenital heart defects has been estimated as five times higher than in liveborn infants[10,12–15]. Use of the four-chamber view in the routine fetal examination should detect the most severe heart defects, which account for two per 1000 pregnancies[3–5].

Verification of an antenatal diagnosis of cardiac anomaly in a fetus or an infant by autopsy plays an important role in the quality control of the sonographic examination. Several large autopsy studies of aborted fetuses and stillborn infants with congenital anomalies have compared prenatal diagnosis with postmortem examination, with special reference to cardiac defects[1,2,16–22]. Previous studies that specifically compared congenital heart defects detected

Correspondence: Dr C. V. Isaksen, Department of Pathology, Trondheim University Hospital, 7006 Trondheim, Norway



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prenatally by ultrasonography with postnatal examination dealt with both clinical findings in live infants and autopsy findings[4,5,23–28].

The present study focused on ultrasonographic and postmortem findings in fetuses with congenital heart defects and was designed to evaluate the concordance of prenatal ultrasound diagnoses with autopsy examination in order to estimate the diagnostic accuracy.

## MATERIALS AND METHODS

Autopsied fetuses and infants with congenital heart defects examined during pregnancy at the ultrasound laboratory, Department of Obstetrics and Gynecology, Trondheim University Hospital, were included in this study. When the ultrasound laboratory was established, it served the local area and surrounding region with a population of 250 000. From 1990, the unit was established as the National Center for Fetal Medicine (NCFM) and has served as a referral center for pregnant women with suspected or verified fetal anomalies from the whole of Norway.

A total of 408 fetuses and infants with developmental anomalies autopsied during the period of January 1985 to December 1994 were evaluated. The postmortem examinations were performed at the Department of Pathology, Trondheim University Hospital (365 autopsies, 89%) and at other hospitals co-operating with the ultrasound center (43 autopsies, 11%). Congenital heart defects were found in 106 (26%) of the 408 cases and were the basis for this study. This group with congenital heart defects did not include two acardiac acephalic fetuses.

The routine fetal examinations offered at the center or at the referring hospitals were performed by obstetricians and/or midwives specially trained in obstetric ultrasound examination. The ultrasound machines employed at the center were Hitachi EUB 565, Dornier AI 3200 and Vingmed Sound CFM 750 machines. These were equipped with transducers with frequencies ranging from 3.5 to 7.5 MHz. The scan included a survey of the fetal anatomy, biometric measurements of the fetus and location of the placenta. The fetal biparietal diameter measured at the routine ultrasound examination was the basis for assessing the gestational age. In normal pregnancies, the anomalies were either suspected or diagnosed during the 18-week routine fetal examination. In women with hereditary or clinical risk factors or clinically abnormal development of the pregnancy, the anomalies were sometimes discovered by an ultrasound examination before or after the routine examination. If any abnormality was detected, a thorough scan was performed by obstetricians working at the center. A pediatric cardiologist was usually consulted during the first part of the study (1985–89) and regularly during the second part (1990–94). Karyotyping and biochemical analysis were performed in most cases. All data were prospectively stored in a computer database and the comparisons were based on the recorded findings in the ultrasound report.

When the autopsies were performed at other hospitals, the report was obtained with the permission from the relevant pathology department. At the Department of Pathology, Trondheim University Hospital, the autopsies during the years 1985–90 were performed by doctors in training, supervised by a consultant pathologist. When the NCFM was established in 1990, a pathologist with experience in perinatal pathology was included in the team. The autopsies were performed after joint meetings with the obstetricians, the videotapes being reviewed with the sonographic findings. The results of the sonographic examinations were available to the pathologist prior to the postmortem examination. From 1991, the autopsy protocol was standardized to include whole-body radiography and photography, to document visible external and internal abnormalities. Biometric measurements were recorded in order to evaluate the gestational age of the fetus. The heart was examined *in situ* before the arterial connections were cut. If more than one cardiac defect was found, the anomalies were classified according to the most serious defect; the clinical outcome was taken into consideration if the pregnancy was allowed to continue.

The heart defect and other non-cardiac abnormalities were evaluated for severity in order to determine the principal diagnosis. When possible, these were evaluated according to viability. Serious central nervous system (CNS) anomalies were considered to be more important than congenital heart defects; complex heart defects, with sinister prognosis or requiring immediate surgery, were considered more important than anomalies not immediately lethal.

The ultrasound and autopsy findings were categorized as follows[29]:

(1) Full agreement between the ultrasound and autopsy findings;

(2) Minor autopsy findings not found or not recorded at the ultrasound examination;

(3) Major autopsy findings not detected at the ultrasound examination, although other ultrasound findings indicated termination of pregnancy;

(4) No autopsy findings suspected at the ultrasound examination. In these cases the fetus/infant died naturally *in utero* or shortly after birth;

(5) Minor ultrasound findings not confirmed at autopsy. These unverified ultrasound findings did not precipitate unjustified management, as they were supplementary to other detected anomalies confirmed at autopsy.

(6) Major ultrasound findings not confirmed at autopsy. This category included false positives as well as cases in which the ultrasound findings were not verified at autopsy because of technical difficulties (traumatization/maceration of the fetus) at the postmortem, making a morphological diagnosis difficult.

## RESULTS

Twenty-seven (25%) of the 106 cases with congenital heart defects were examined during the years 1985–89 and 79 (75%) cases were examined in 1990–94. Of these 79

**Table 1** Survey of 106 cases with congenital heart defects (CHD)

| | *Major finding* | | *Minor finding* | | *Total* | |
|---|---|---|---|---|---|---|
| *Years* | *n* | % | *n* | % | *n* | % |
| 1985–89 | 16 | 59 | 11 | 41 | 27 | 25 |
| 1990–94 | 47 | 59 | 32 | 41 | 79 | 75 |
| 1985–94 | 63 | 59 | 43 | 41 | 106 | 100 |

Major finding, CHD was the most important diagnosis; minor finding, CHD was secondary to other anomalies regarded as more important

**Table 2** Organ system abnormalities as indication for induced abortion in 106 cases with congenital heart defects

| *Organ involved/abnormality* | *n* | % |
|---|---|---|
| Heart | 63 | 59 |
| Central nervous system | 15 | 14 |
| Diaphragmatic/abdominal wall defect | 10 | 9.5 |
| Fetal hydrops/cystic hygroma | 10 | 9.5 |
| Kidney | 3 | 3 |
| Miscellaneous | 5 | 5 |
| Total | 106 | 100 |

**Table 3** Classification of heart defects in 106 cases with congenital heart defects

| *Diagnosis* | *Total* | % |
|---|---|---|
| Ventricular septal defect | 48 | 28.6 |
| Atrial septal defect | 17 | 10.1 |
| Hypoplastic left ventricle | 15 | 8.9 |
| Atrioventricular septal defect | 14 | 8.3 |
| Coarctation of the aorta | 11 | 6.5 |
| Atrial and ventricular septal defect | 9 | 5.3 |
| Pulmonary hypoplasia/atresia | 9 | 5.3 |
| Systemic anomalous venous return | 6 | 3.6 |
| Hypoplastic right ventricle | 6 | 3.6 |
| Overriding aorta | 6 | 3.6 |
| Truncus arteriosus | 6 | 3.6 |
| Tetralogy of Fallot | 4 | 2.4 |
| Ebstein's anomaly | 4 | 2.4 |
| Double outlet right ventricle | 4 | 2.4 |
| Transposition of great arteries | 3 | 1.8 |
| Situs inversus | 2 | 1.2 |
| Ectopia cordis/pericardial defect | 2 | 1.2 |
| Rhabdomyoma | 1 | 0.6 |
| Double inlet right ventricle | 1 | 0.6 |
| Total | 168 | 100.0 |

examined during the latter period, i.e. after the establishment of the NCFM, 55 (70%) were referred from all over the country; 24 (30%) were from Trondheim and neighboring communities. There were 44% males and 56% females. The mean age of the mother at delivery was 29 years (range 18–43). Thirty-two of the women (30%) had experienced previous pregnancy loss; 14 of these (44%) had had more than one abortion. These were either spontaneous or induced for social reasons. In two of these cases there was a positive family history of congenital heart defects. During the first time period, the average gestational age at abortion or birth was 26 weeks (range 18–40 weeks) and during the second time period 23 weeks (range 13–41 weeks). The average gestational age at which the ultrasound diagnosis was made was 21 weeks (range 12–39 weeks). In 36 (34%) of the 106 cases, the diagnoses were made in connection with the routine ultrasound examination at the NCFM; the rest (66%) were referred cases, because of either suspected anomalies or otherwise abnormal development of the pregnancy.

In 66 (62%) of the 106 women, the pregnancy was terminated. One had a spontaneous abortion, 16 women (15%) experienced an intrauterine death, and in 23 cases (22%) the infant was born spontaneously or birth was induced. The heart defect was the principal diagnosis in 63 cases (59%). In 43 cases (41%), a developmental anomaly of another organ was regarded as the main diagnosis (Table 1). Of these, anomalies of the central nervous system were the most prevalent (Table 2).

A total of 168 heart defects were diagnosed in the 106 cases (Table 3). Ventricular septal defect (VSD) was the most common anomaly (48/168; 29%), with equal frequency in the two time periods. Atrioventricular septal defect (AVSD) occurred more frequently during the first time period (19% (5/27) versus 11% (9/79)), whereas hypoplastic left ventricle and atrial septal defect (ASD)

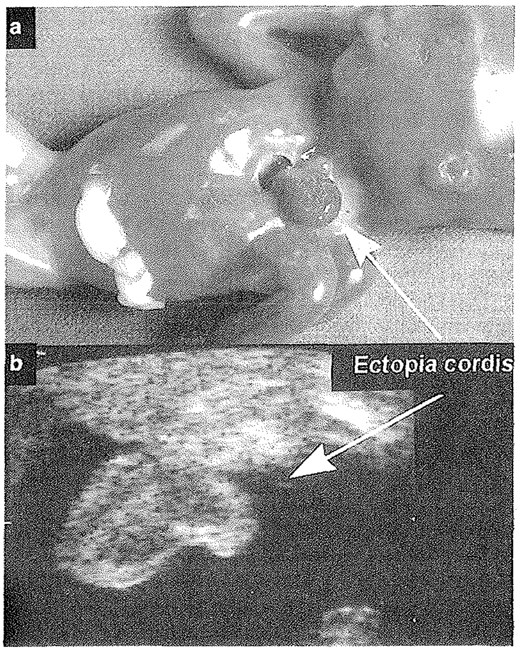


**Figure 1** Fetus, 20 weeks old, with ectopia cordis (arrows). (a) Autopsy photograph; (b) ultrasound scan

were more frequent during the second time period (both 18% (14/79) versus 4% (1/27) and 11% (3/27)). When the heart defect was the main diagnosis, AVSD and VSD were the lesions most frequently encountered (14/63; 22% and 13/63; 21%, respectively). Two rare cases, ectopia cordis and cardiac rhabdomyomas, were also found; they are shown in Figures 1 and 2.

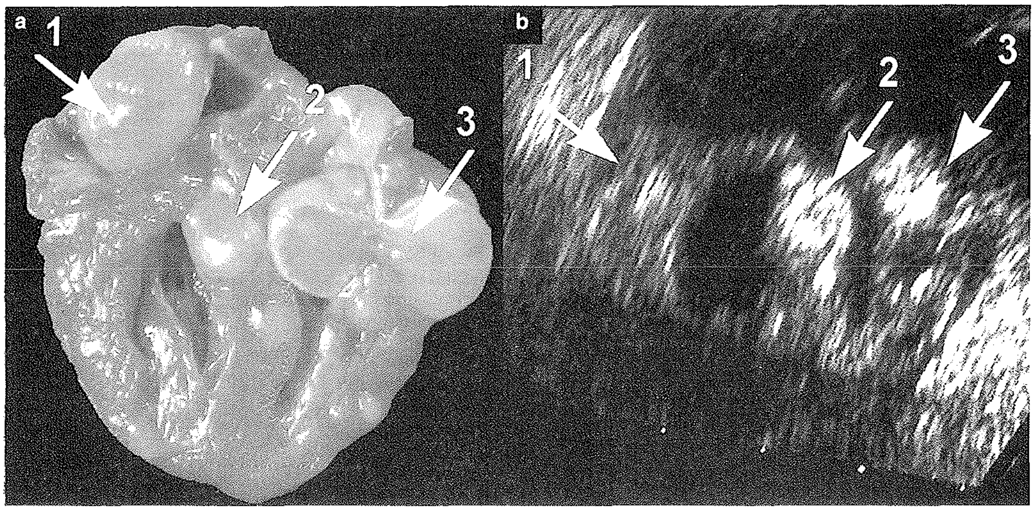


**Figure 2** Fetus, 34 weeks old. Congenital cardiac rhabdomyomas in a case with tuberous sclerosis (arrows point at different tumors). (a) Autopsy photograph; (b) ultrasound scan

The heart defects and the extracardiac anomalies are listed in Table 4. The congenital heart defects were isolated in 39 (37%) of the 106 cases. Of these, 15 were associated with fetal hydrops and/or cystic hygroma. Of the congenital heart defects occurring alone or in combination with fetal hydrops and/or cystic hygroma, AVSD and VSD were the most frequent diagnoses (10/39; 26%), followed by hypoplastic left ventricle (7/39; 18%). Other organ abnormalities were associated with the congenital heart defects in the remaining cases (67/106; 63%), a total of 118 anomalies being present (Table 5).

Fifteen of the heart defects were associated with major CNS anomalies; in these cases the CNS anomaly was the main indication for termination of pregnancy or the main cause of death (Table 6). In 12 (44%) of the 27 cases of congenital heart defects associated with CNS anomalies, the CNS anomalies were less severe. In 23 cases of congenital heart defects associated with an omphalocele or a diaphragmatic defect, a CNS anomaly was present in nine cases. Of all the combinations of congenital heart defects and CNS anomalies, 19/27 (70%) were VSDs, either isolated or in combination with other cardiac defects.

Table 7 demonstrates the concordance between the prenatal ultrasound and postmortem findings. Five (5%) of the 106 cases with congenital heart defects had an isolated secundum ASD not registered in the ultrasound report; these were excluded from this analysis. Of the remaining 101 cases, 74 (73%) were correctly diagnosed by ultrasound.

In 27 cases, discrepancies were found (Table 8). Cases 1–14 were from the first time period, and 15–27 from the last time period. Of all cases, 18 were classified into category 2 (minor autopsy findings not found on ultrasound). Nine were isolated VSDs, one was a primum ASD and six were coarctation of the aorta; one of these also had a VSD. In one case, the aortic and pulmonary outlets were initially normal and a fusion in the ascending part of the aorta was not detected. In one case, an overriding aorta was missed while a VSD was detected (Table 8).

Four cases were placed into category 3 (major autopsy findings not found on ultrasound) including a tetralogy of Fallot, a VSD with a truncus arteriosus, an AVSD with a truncus arteriosus and a VSD with an overriding aorta (Table 8; Cases 9–12). In these four cases, other anomalies had warranted a termination of pregnancy (one Dandy–Walker malformation, two renal dysplasia and one trisomy 18 with omphalocele).

Three congenital heart defects were overlooked at the routine ultrasound examination (category 4). Two of the three cases were live births; the third was a spontaneous abortion. The diagnoses were VSD with a truncus arteriosus, a hypoplastic left heart syndrome and a pulmonary stenosis, respectively (Table 8; Cases 13, 14, 25). Case 13 had VSD and truncus arteriosus. This infant, who presented facial dysmorphic features, was liveborn. A chromosomal abnormality was suspected, but karyotyping was not carried out. Case 14 died 1 day old because of a hypoplastic left ventricle.

In the two cases in categories 5 and 6 (Table 8; Cases 26 and 27) an AVSD was diagnosed by ultrasound but not verified at autopsy. The postmortem examination found a VSD in Case 26. In Case 27 the heart was macerated and therefore difficult to evaluate at autopsy. Trisomy 21 and trisomy 18 were present in these cases.

Overall, the most common anomaly not detected prenatally was VSD, which comprised 15 (56%) of the 27 overlooked anomalies. Coarctation of the aorta was not as frequent as VSD; six of 11 (55%) cases were not diagnosed at the ultrasound examination. Four of the six cases of truncus arteriosus were not found prenatally (Table 8; Cases 10, 11, 13, 15); three of these were associated with renal dysplasia. In five cases (Table 8; Cases 1, 10–13) the

**Table 4** Cases with congenital heart defects (CHD), extracardiac anomalies included ($n$ = 106)

| | |
|---|---|
| CHD as the sole manifestation | 24 |
| CHD with hydrops fetalis | 15 |
| CNS anomaly | 9 |
| CNS and kidney anomalies | 7 |
| CNS/kidney anomalies and diaphragmatic hernia | 1 |
| CNS/kidney anomalies and omphalocele | 1 |
| CNS anomaly and diaphragmatic hernia | 2 |
| CNS anomaly and omphalocele | 2 |
| CNS anomaly, omphalocele/diaphragmatic hernia and GI anomaly | 1 |
| CNS anomaly, omphalocele/diaphragmatic hernia and limb deformities | 1 |
| CNS anomaly, omphalocele and limb deformities | 1 |
| CNS anomaly and hydrops | 2 |
| Kidney anomaly | 7 |
| Kidney anomaly and diaphragmatic hernia | 2 |
| Kidney anomaly and omphalocele | 4 |
| Kidney and GI anomalies | 5 |
| Kidney anomaly and skeletal dysplasia (S-N) | 1 |
| Kidney/GI anomalies and skeletal dysplasia (S-N) | 1 |
| Kidney/GI anomalies and hydrops | 1 |
| Kidney anomaly and hydrops | 3 |
| GI anomaly and omphalocele | 2 |
| GI anomaly and limb deformities | 2 |
| GI anomaly and hydrops | 1 |
| Diaphragmatic hernia | 1 |
| Omphalocele | 1 |
| Omphalocele and limb deformities | 1 |
| Omphalocele and hydrops/cystic hygroma | 2 |
| Fetal hydrops and limb deformities | 1 |
| Pierre Robin syndrome | 1 |
| Cantrell's pentalogy | 1 |
| Arthrogryposis multiplex | 2 |
| Conjoined twins | 1 |
| Total | 106 |

CNS, central nervous system; GI, gastrointestinal; S-N, Saldino–Noonan syndrome

**Table 5** Extracardiac anomalies in 67 cases of congenital heart defects ($n$ = 118)

| *Organ involved/abnormality* | *n* | % |
|---|---|---|
| Kidney/urinary tract | 33 | 28 |
| Central nervous system | 27 | 23 |
| Fetal hydrops/cystic hygroma | 10 | 8 |
| Omphalocele | 16 | 14 |
| Gastrointestinal tract | 13 | 11 |
| Limb/skeleton | 11 | 9 |
| Diaphragmatic hernia | 7 | 6 |
| Miscellaneous | 1 | 1 |
| Total | 118 | 100 |

congenital heart defects consisted of two lesions that were missed at the ultrasound examination. Thus, the number of discrepant lesions was 32 (19%), and 136 (81%) of 168 different defects were correctly diagnosed.

When defects such as VSD primum ASD and coarctation of the aorta were excluded, the concordance rose to 91%. Comparing the periods 1985–89 and 1990–94, the observed difference in category 1 (full agreement) was 48% (13/27) versus 82% (61/74), respectively, which was statistically significant ($p < 0.01$) (Table 7). The detection rate of VSD improved from 38% in the first time period to 86% in the second period ($p < 0.05$). Except for one case with pulmonary stenosis, all cases in categories 3 and 4 were from the first time period. The autopsy reports of the two cases in categories 5 and 6 from the second time period were not conclusive, and the AVSDs diagnosed at the ultrasound examinations were not considered as false positives.

In 87 (82%) of the 106 cases, a chromosome analysis was performed. In four cases (4%), the karyotyping was not successful. Fifty-three (64%) of the 83 cases with known karyotype had a chromosomal abnormality (Table 9). A chromosomal aberration was found in 30 (63%) of the 48 with VSD, nine (60%) of the 15 cases with hypoplastic left ventricle, nine (64%) of the 14 with AVSD and six (67%) of the nine combinations of ASD and VSD. Of the cases karyotyped, this percentage was higher (Table 10).

## DISCUSSION

The frequency of congenital heart defects (26%) in this autopsy study of fetuses and infants with developmental anomalies was within the expected range[20]. The complexity of the cardiac anatomy and the diversity of different heart defects emphasize the necessity of a referral center with highly qualified ultrasonographers and experienced perinatal pathologists[5,27]. Since a pediatric cardiologist worked together with the obstetricians in a team setup, this study cannot evaluate the contribution of the cardiologist to the diagnoses. We believe this dialog was valuable and increased the quality of the examination.

Complete agreement of 73% between the ultrasound diagnoses and postmortem findings in fetuses and infants with congenital heart defects seems acceptable. With minor discrepancies disregarded, the correlation rises to 91%; similar results have been found in other studies[21,23,30], but these also included surviving infants and are therefore not comparable with our autopsy series.

Our results showed a significant difference between the detection rate of heart defects in autopsied fetuses and infants during the two time periods 1985–89 and 1990–94. It is reasonable to attribute this to improved experience, technical advances and the introduction of the four-chamber view to the routine ultrasound examination in 1988[3,5]. The discovery of one anomaly will alert the experienced sonographer and increase the awareness for another anomaly.

The mean gestational age of the fetus or infant at autopsy decreased by 3 weeks from the first study period to the second. This is most probably related to the introduction of the 18-week routine ultrasound examination resulting in detection of congenital anomalies at an earlier gestational age than before. Improvement of the quality of the examination has also made earlier diagnosis possible[29].

Certain defects, such as a small VSD, ASD, mild valvular stenosis, simple transposition and aortic coarctation, can be very difficult to detect by ultrasound[30]. When the detection rate was calculated, the secundum ASDs were

**Table 6** Congenital heart defects associated with central nervous system (CNS) anomalies ($n$ = 27)

| *Heart defect* | *CNS anomaly* | *Karyotype* |
|---|---|---|
| VSD | hydrocephaly | |
| VSD | craniorachischisis | trisomy 18 |
| VSD | anencephaly | |
| VSD | holoprosencephaly | triploidy |
| VSD | Arnold–Chiari malformation | trisomy 18 |
| VSD | CNS dysplasia | trisomy 18 |
| VSD/overriding aorta | Arnold–Chiari malformation | trisomy 18 |
| VSD/overriding aorta | holoprosencephaly | trisomy 13 |
| ASD/VSD/tricuspid atresia | Arnold–Chiari malformation | |
| ASD/VSD | hydrocephaly | partial trisomy 13 |
| ASD | dysmorphism/eye anomalies | |
| ASD/aortic coarctation | hydrocephaly | |
| Hypoplastic left ventricle | anencephaly | |
| Hypoplastic left ventricle | hydrocephaly | trisomy 13 |
| Transposition/pulmonary atresia | craniorachischisis | trisomy 18 |
| Hypoplastic left heart, situs inversus, aortic coarctation | Dandy–Walker malformation, partial agenesis of the vermis | |
| Hypoplastic left heart, mitral atresia, ASD/VSD | CNS dysplasia | trisomy 18 |
| Hypoplastic left heart, VSD, aortic coarctation | CNS dysplasia | trisomy 18 |
| Hypoplastic left heart | choroid plexus cyst | trisomy 18 |
| Tetralogy of Fallot | Dandy–Walker malformation, hypoplasia of the corpus callosum | trisomy 18 |
| Tetralogy of Fallot | agenesis of corpus callosum, agyria, trisomy 18? | |
| AVSD, anomalous venous return (vena cava superior left side) | Dandy–Walker malformation | partial trisomy 14 |
| AVSD, double outlet right ventricle | choroid plexus cyst | trisomy 18 |
| ASD/VSD, dextroposition of heart | Dandy–Walker malformation | trisomy 18 |
| VSD/aortic coarctation | agenesis of corpus callosum | |
| Rhabdomyoma | tuberous sclerosis | |
| VSD/overriding aorta | choroid plexus cyst | trisomy 18 |

VSD, ventricular septal defect; ASD, atrial septal defect; AVSD, atrioventricular septal defect

**Table 7** Congenital heart defects: correlation between prenatal and postnatal findings ($n$ = 101)

| | *1985–89* | | *1990–94* | | *1985–94* | |
|---|---|---|---|---|---|---|
| *Category* | *n* | % | *n* | % | *n* | % |
| (1) Full agreement | 13 | 48 | 61 | 82 | 74 | 73 |
| (2) Minor autopsy findings not found by ultrasound | 8 | 30 | 10 | 13.5 | 18 | 18 |
| (3) Major autopsy findings not found by ultrasound | 4 | 15 | 0 | 0 | 4 | 4 |
| (4) No autopsy findings suspected by ultrasound | 2 | 7 | 1 | 1.4 | 3 | 3 |
| (5) Minor ultrasound findings not confirmed at autopsy | 0 | 0 | 1 | 1.4 | 1 | 1 |
| (6) Major ultrasound findings not confirmed at autopsy | 0 | 0 | 1 | 1.4 | 1 | 1 |
| Total | 27 | 100 | 74 | 100 | 101 | 100 |

excluded, since defining a secundum ASD in a fetus is not possible; this was therefore not considered as a false negative[26]. As most small VSDs spontaneously close after birth[9,31,32], the importance of their detection is probably more as an indicator for associated disorders such as chromosomal aberrations. Large VSDs, AVSDs and conditions with hypoplastic heart chambers are among the congenital heart defects that are easier to detect prenatally. The introduction of the four-chamber view has increased the detection rate of congenital heart defects. After the outflow tracts were included in the examination, the rate improved further[11,33].

Isolated VSDs accounted for more than half of the autopsy findings not detected by ultrasound. VSD was also the most common defect encountered. In studies of cardiac defects in liveborn infants, VSD represented the most common anomaly[9,10,12,31]. The detection rate of the VSDs in the present study increased significantly from the first to the second period. When the heart defect was the main diagnosis, AVSD and VSD were the most frequent lesions. This has also been found in other series[12,23].

The detection of aortic coarctation was difficult. Six (55%) of the 11 cases were missed at the ultrasound examination. Such false-negative cases have been recorded by others[11,28,30,34]. However, in the six cases of missed diagnosis of aortic coarctation, other abnormalities prompted further investigation. Four cases with a truncus arteriosus and two with an overriding aorta were missed, which probably reflected difficulties in assessment of the outflow tract[33]. Three of these six cases had been referred from other centers; two had been missed at a routine ultrasound examination and one case had been given a targeted

**Table 8** Congenital heart defects: survey of discrepancies between ultrasound and autopsy findings ($n$ = 27)

| *Case number* | *Prenatal diagnosis* | *Sex* | *GA at US diagnosis* | *GA at autopsy* | *Mode of death/birth* | *Final diagnosis following autopsy* | *Category* |
|---|---|---|---|---|---|---|---|
| 1 | Omphalocele, IUGR | M | 39 | 40 | liveborn | trisomy 18? IUGR, VSD, aortic coarctation, omphalocele, horseshoe kidney, clubfoot | 2 |
| 2 | Hydrocephaly | F | 39 | 39 | TOP | hydrocephaly, left renal agenesis, VSD | 2 |
| 3 | IUGR | F | 30 | 30 | liveborn | trisomy 18? IUGR, VSD, dysmorphism, malrotation of gut, horseshoe kidney | 2 |
| 4 | Anencephaly | F | 22 | 22 | TOP | anencephaly, VSD | 2 |
| 5 | VSD, mild hydronephrosis | M | 18 | 37 | liveborn | tetralogy of Fallot, focal renal dysplasia, bile duct atresia, peritonitis/pneumonia | 2 |
| 6 | Trisomy 21, fetal hydrops | M | 18 | 23 | TOP | trisomy 21, fetal hydrops, aortic coarctation | 2 |
| 7 | Cystic hygroma, fetal hydrops, VSD possible, pulmonary atresia, unilateral dysplastic kidney, Turner's syndrome? | F | 20 | 20 | TOP | cystic hygroma, fetal hydrops, VSD, pulmonary atresia, aortic coarctation, left dysplastic kidney, dysplastic gonads, Turner's syndrome? | 2 |
| 8 | Trisomy 18, omphalocele, radial aplasia, choroid plexus cysts | M | 18 | 20 | TOP | trisomy 18, omphalocele, VSD, radial aplasia, choroid plexus cysts, horseshoe kidney | 2 |
| 9 | IUGR, Dandy–Walker malformation | M | 30 | 32 | TOP | trisomy 18, IUGR, tetralogy of Fallot, Dandy–Walker malformation, hypoplasia of corpus callosum | 3 |
| 10 | Anhydramnios, renal dysplasia, skeletal dysplasia | F | 22 | 23 | TOP | VSD, truncus arteriosus, dysmorphism, cleft palate, renal dysplasia, skeletal dysplasia | 3 |
| 11 | Oligohydramnios, micrognathia, dysplastic kidneys, skeletal dysplasia, polydactyly | F | 20 | 20 | TOP | AVSD, truncus arteriosus, dysmorphism, cleft palate, micrognathia, dysplastic kidneys, urogenital agenesis, skeletal dysplasia and polydactyly consistent with Saldino–Noonan syndrome | 3 |
| 12 | Trisomy 18, omphalocele and radial aplasia | M | 15 | 16 | TOP | trisomy 18, omphalocele, VSD, overriding aorta, radial aplasia | 3 |
| 13 | Polyhydramnios | F | 33 | 35 | liveborn | VSD, truncus arteriosus, facial dysmorphism, cleft palate | 4 |
| 14 | None | F | | 37 | liveborn | hypoplastic left heart, aortic and mitral atresia | 4 |
| 15 | Anhydramnios and dysplastic kidneys | M | 21 | 21 | TOP | dysplastic kidneys, urethral/anal atresia, truncus arteriosus, right-sided aortic arch | 2 |
| 16 | Dichorionic and diamniotic twins, twin I with fetal hydrops, nuchal edema, dilated cerebral ventricles and dilated renal pelvis right side | M | 29 | 32 | IUFD | twin I with IUGR, fetal hydrops, hypoplastic lungs, VSD | 2 |
| 17 | Hydrocephaly and bilateral clubfeet | F | 19 | 22 | IUFD | hydrocephaly, secundum ASD, aortic coarctation, bilateral clubfeet | 2 |
| 18 | Oligohydramnios, fetal hydrops with hydrothorax and ascites, cystic hygroma and bilateral hydronephrosis | F | 17 | 18 | TOP | fetal hydrops, cystic hygroma, ASD/VSD, dysplastic kidneys, bicornuate uterus, streak gonad left side | 2 |
| 19 | Trisomy 18, nuchal edema and pericardial effusion | F | 18 | 22 | TOP | trisomy 18, dysmorphism, nuchal edema, aortic coarctation, secundum ASD, mild ureteropelvic obstruction | 2 |
| 20 | Polyhydramnios, atresia of upper gastrointestinal tract, vertebral anomalies, deformed left ear, right ear with preauricular tags, Goldenhar syndrome? | F | 31 | 34 | IUFD | aortic coarctation, secundum ASD, hypoplastic lungs, duodenal atresia, multiple vertebral anomalies, deformed left ear and preauricular tags right ear; Goldenhar syndrome possible | 2 |
| 21 | Trisomy 21, nuchal edema, hydrothorax left side and choroid plexus cyst left lateral ventricle | F | 16 | 17 | TOP | trisomy 21, nuchal edema, VSD and bilateral peripheral lung infarcts | 2 |
| 22 | Oligohydramnios, IUGR, holoprosencephaly, lumbosacral bifid spine with myelomeningocele, dysplastic right kidney; triploidy? | F | 22 | 22 | TOP | triploidy, IUGR, holoprosencephaly, lumbosacral bifid spine with meningocele, omphalocele, renal dysplasia right side with ureteral atresia and hypoplastic bladder, left renal agenesis, VSD | 2 |
| 23 | Fetal hydrops, cystic hygroma, omphalocele, cleft lip/palate, clubfoot | M | 13 | 14 | TOP | trisomy 18, cystic hygroma, omphalocele, VSD, cleft lip/palate and clubfoot | 2 |
| 24 | Oligohydramnios, fetal hydrops and cystic hygroma; Turner's syndrome probable | F | 20 | 21 | IUFD | Turner's syndrome, fetal hydrops, cystic hygroma, primum ASD and horseshoe kidney | 2 |
| 25 | None | F | | 20 | SA | pulmonary stenosis 1 mm; early membrane rupture with chorioamnionitis | 4 |
| 26 | AVSD and ascites | M | 18 | 19 | TOP | trisomy 21 and VSD (AVSD on US not found at autopsy) | 5 |
| 27 | AVSD and nuchal edema | M | 12 | 13 | TOP | trisomy 18 and AVSD probable (macerated fetus) (AVSD on US not confirmed at autopsy) | 6 |

GA, gestational age in weeks; US, ultrasound; IUGR, intrauterine growth restriction; VSD, ventricular septal defect; AVSD, atrioventricular septal defect; IUFD, intrauterine fetal death; ASD, atrial septal defect; TOP, termination of pregnancy; SA, spontaneous abortion examination because two siblings had congenital heart defects. In these cases, the congenital heart defects was associated with other anomalies. The difficulty of detecting a congenital heart defects by routine ultrasound examination has been documented[4].

All four cases with major autopsy findings not seen at the ultrasound examination were from the first time period. In these four cases, other anomalies were the reason for terminating the pregnancy. With regard to the three cases in which none of the autopsy findings were seen at the routine ultrasound examination, two were from the first time period (VSD with truncus arteriosus, and hypoplastic left heart syndrome). These infants were liveborn and no undue action was taken. All these cases would probably have been detected with today's experience. The last case (a spontaneous abortion in week 20) had a pulmonary stenosis not detected. In another series studied elsewhere, only half of the cases with pulmonary stenosis were detected prenatally[33].

Constellations of anomalies in different organ systems, regardless of karyotype, are well documented[9,21,22,35–38], although the proportional incidence of multiple anomalies does not seem to have been considered in depth. In a combined clinical and autopsy study, gastrointestinal, skeletal, urogenital, CNS and respiratory anomalies were the non-cardiac anomalies involved in the mentioned order of frequency[39]. In the present study, a diaphragmatic defect or an omphalocele was found in more than one-third of the cases with non-cardiac anomalies. The combination of congenital heart defects and CNS anomalies was even higher, although according to other studies this association is relatively infrequent[36,37]. These studies, however, included congenital heart defects in a setting of both clinical findings and autopsy findings. CNS anomalies detected at ultrasound examination are often serious and probably account for the high incidence in this autopsy series. Cases with the combination of congenital heart defects and CNS anomalies had a high degree of chromosomal aberrations. An abnormal karyotype is seen almost twice as frequently in cases with combined anomalies than in cases with isolated anomalies[17]. Sixty-three per cent (54/86) of the lesions with VSD, AVSD, hypoplastic left ventricle and the combination of ASD and VSD were associated with a chromosomal aberration. This was higher than in other studies[27] and was probably related to the frequency of amniocenteses in cases with congenital heart defects.

**Table 9** Frequency of chromosomal abnormalities ($n$ = 106)

| *Time period* | *Cases karyotyped* | *Normal karyotype* $n$ | % | *Abnormal karyotype* $n$ | % |
|---|---|---|---|---|---|
| 1985–89 | 13 | 4 | 31 | 9 | 69 |
| 1990–94 | 70 | 26 | 37 | 44 | 63 |
| 1985–94 | 83 | 30 | 36 | 53 | 64 |

It is generally more difficult to detect congenital heart defects by ultrasound than it is to detect CNS anomalies[2,19,20]. The nature of the cardiac anatomy and the wide spectrum of defects make the heart a difficult organ to examine. If the fetus is small, the heart can be difficult to examine at autopsy as well; on the other hand, ultrasound imaging including Doppler examination can give a very good indication of morphological alterations. In high-risk pregnancies, an ultrasound scan can be performed at the end of the first trimester. Because of the steadily decreasing gestational age at which fetuses with congenital heart defects are aborted, a new approach to the perinatal autopsy is important. Since cardiac defects, especially small VSDs, can be extremely difficult to visualize by simple dissection when the fetus is only 12–13 weeks old, dissection of the heart with the help of a stereomicroscope is necessary for such an examination. Opening the heart *in situ* is important, and has become an established routine that should be followed in order to obtain a correct view of both the intracardiac connections and the great arteries.

Knowing the sonographic diagnoses before autopsy may introduce a bias. A pathologist is trained to describe only what is seen and should not be influenced by ultrasound findings. We do not believe such knowledge has introduced false-positive diagnoses. Information about ultrasound findings will probably reduce the possibility of missing subtle diagnoses at autopsy and make the final diagnoses more complete. The collaboration between pathologists and ultrasonographers is thus beneficial for the development of both prenatal diagnostic ultrasound and perinatal pathology. The examination of embryonic and fetal lethal anomalies is complex and autopsy should be considered as a complementary examination necessary to confirm the final diagnosis. A conscientious autopsy is a prerequisite for correct diagnosis, genetic counselling and epidemiological studies[40–50]. Regardless of how accurate the ultrasound examination becomes, the autopsy plays an important role in the quality control of antenatal diagnostic methods[19,20,42,43,45,46,51].

**Table 10** Percentage of chromosomal abnormalities in the most common congenital heart defects, 1985–94

| | *Total* | *Known karyotype* | *Normal karyotype* | *Abnormal karyotype* $n$ | *% karyotyped* |
|---|---|---|---|---|---|
| VSD | 48 | 39 | 9 | 30 | 77 |
| Hypoplastic left ventricle | 15 | 13 | 4 | 9 | 69 |
| AVSD | 14 | 13 | 4 | 9 | 69 |
| ASD/VSD | 9 | 7 | 1 | 6 | 86 |
| Total | 86 | 72 | 18 | 54 | 75 |

VSD, ventricular septal defect; AVSD, atrioventricular septal defect; ASD, atrial septal defect

## CONCLUSION

This study from a tertiary center has shown that the prenatal diagnosis of major congenital heart defects was correct in 91% of the cases, with no false positives. In a comparison of the two time periods 1985–89 and 1990–94, the improvement in diagnosis was significant. Close co-operation between ultrasonographers and pathologists was mutually stimulating for the development of prenatal diagnosis and perinatal pathology.

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# PAPER III

# Fetuses and infants with congenital urinary system anomalies: correlation between prenatal ultrasound and postmortem findings


Isaksen, Christina V. (1,2) M.D. Consultant Pathologist

Eik-Nes, Sturla H. (2) M.D. Ph.D. Professor of Obstetrics and Gynecology

Blaas, Harm-Gerd (2) M.D. Consultant Gynecologist

Torp, Sverre H (1) M.D. Ph.D. Consultant Pathologist, Associate Professor

1) Institute of Morphology and Department of Pathology

2) National Center for Fetal Medicine, Department of Obstetrics and Gynecology

Trondheim University Hospital, Norwegian University of Science and Technology, Trondheim, Norway


Short title: Comparison of prenatal ultrasound and postmortem findings of urinary system anomalies


Key words: ultrasonography, autopsy, kidney, urinary system, anomalies



Mailing address: Dr. Christina Vogt Isaksen

Department of Pathology

Trondheim University Hospital

7006 Trondheim, Norway

tel. +47 73868653

fax +47 73868654

e-mail: Christina.Isaksen@medisin.ntnu.no

## Abstract

**Objective** Detection of congenital urinary system anomalies is an important part of prenatal ultrasound examination. The present study compares prenatal ultrasonographic findings and postmortem examinations of fetuses and infants with renal and urinary tract anomalies.

**Design** Criteria for inclusion were an ultrasound examination at the National Center for Fetal Medicine (NCFM) and autopsy performed during the period 1985 to 1994. Results from the ultrasound examination and autopsy regarding urinary system anomalies were categorized according to the degree of concordance.

**Results** Urinary system anomalies were found in 112 (27%) of 408 fetuses with congenital anomalies. The renal and/or urinary tract anomaly was the principal reason for induced abortion or cause of death in 50 cases (45%). In 97 (87%) of the 112 cases there was full agreement between the ultrasound observations and the autopsy findings. In 5 cases the autopsy revealed minor findings not mentioned in the ultrasound report. The main diagnosis was thus correct in 102 cases (91%). In 4 cases major autopsy findings had not been found by ultrasound examination; in another 4, none of the autopsy findings were suspected by ultrasound, and in 2, minor ultrasound findings were not confirmed at autopsy.

**Conclusion** The accordance between ultrasound diagnoses and postmortem examinations proved to be satisfactory. The close cooperation between ultrasonographers and perinatal pathologists is mutually beneficial. In addition to serving as a complement to the prenatal diagnosis, postmortem examination is of vital importance for the quality control of ultrasonography in fetal diagnostics and plays an important role for genetic counseling.

## Introduction

Detection of fetal anomalies is an important part of prenatal care. The introduction of routine fetal ultrasonographic examination has improved the detection rate of fetal anomalies and recent technical developments of ultrasound equipment have taken the diagnostic accuracy to a higher level. As a consequence, congenital anomalies are diagnosed earlier than previously. In cases where abortion is elected this may lessen the physical and psychological trauma these women are exposed to[1]. The decreasing fetal age at termination of pregnancy (TOP) with the small size of the fetus represents a challenge in perinatal pathology and requires a high level of experience.

The overall prevalence of congenital urinary system anomalies has been 2 per 1000 live births[2]. Urinary system anomalies represent a large group of fetal anomalies detected by sonography[3]. Ultrasonographic screening has disclosed an overall frequency of fetal uropathy of 0.28% and about 2/3 of these had urinary tract dilatation[4,5]. In two different antenatal studies the most frequent cause of significant urinary tract dilatation was obstruction of the ureteropelvic junction[6,7]. Upper urinary tract dilatation together with renal dysplasia are the largest group of urinary system anomalies[2,4,8,9]. The incidence of bilateral renal agenesis is low, 0.1- 0.3/1000 births[10,12].

Autopsy studies comparing the prenatal ultrasound diagnosis with postmortem examination have shown that the detection of renal anomalies varies from 60% to over 90%. In these studies the urinary system anomaly was part of a general analysis of all types of anomalies and did not focus specifically on particular organ systems[13,14,15-19]. A few studies comparing prenatal and postnatal urinary system anomalies have focused on both clinical follow-ups and postmortem examinations; the detection rate has varied between 65% and 100%[4,20-22]. Except for one study[23], they all comprised a limited number of cases, and in one, only minor renal anomalies were studied[22]. To our knowledge, a comprehensive focus on prenatal diagnoses of urinary system anomalies comparing them with autopsy results has not been done.

This study was designed to prospectively evaluate the correlation between prenatal ultrasound and autopsy examination, and at the same time record urinary system anomalies in a mixed low-risk and high-risk population.

**Material and Methods**

Autopsied fetuses and infants who during the pregnancies had been examined with ultrasound at the ultrasound laboratory, Department of Obstetrics and Gynecology, Trondheim University Hospital and later proved to have a urinary system abnormality are included in this study. The hospital serves the city of Trondheim with surrounding areas with a total of 250 000 inhabitants. The National Center for Fetal Medicine (NCFM) was established in 1990 and is the Norwegian referral center for pregnant women with suspected or verified fetal anomalies.

Four hundred and eight autopsies of fetuses and infants with developmental anomalies were performed from 1985 through 1994, 365 of these at the Department of Pathology, Trondheim University Hospital, and the rest at other hospitals cooperating with the center. In 112 (27%) of the 408 cases, urinary system anomalies were diagnosed and included in this study.

The anomalies were suspected or diagnosed during the routine fetal ultrasound examination offered to all pregnant women at 18 weeks or at a selective ultrasound scan performed because of hereditary risk factors or an abnormally developing pregnancy. A thorough description of the ultrasound findings was recorded including information about supplementary examinations. All data were prospectively stored in a computer data base and the comparisons were based on the recorded findings in the ultrasound and autopsy report. The ultrasound examinations were performed by obstetricians working at the center using Hitachi EUB 565, Dornier AI 3200 and Vingmed Sound CFM 750 machines. The machines were equipped with transducers with frequencies ranging from 3.5 to 7.5 MHz.

At the Department of Pathology, Trondheim University Hospital, a standardized autopsy was carried out. From 1985 to 1989 the autopsies were performed by doctors in training, and from 1990 by a consultant pathologist with experience in perinatal pathology who became part of the team at the NCFM. The ultrasound report was available for the pathologist prior to the autopsy. Postnatal radiography of the fetus or infant and photographic documentation of dysmorphic features and anomalies were regularly performed after 1990. At autopsy, all organs were examined. In some cases where special photographic documentation was desirable, the kidneys were left in situ after removal of other organs (Figure 1 and 2). When the autopsies were performed at other hospitals, permission to use the report was obtained.

The ultrasound and autopsy findings were categorized as follows[24,25].

1) Full agreement between the ultrasound and autopsy findings.

2) Minor autopsy findings not found or not recorded at the ultrasound examination.

3) Major autopsy findings not detected at the ultrasound examination, though other ultrasound findings indicated termination of pregnancy.

4) No autopsy findings suspected at the ultrasound examination. In these cases the fetus or infant deceased naturally in utero or shortly after birth.

5) Minor ultrasound findings not confirmed at autopsy. This category includes findings supplementary to other detected anomalies which were confirmed at autopsy.

6) Major ultrasound findings not confirmed at autopsy. In addition to false positives this category includes cases where postmortem changes interfered with making a proper morphological diagnosis.

**Results**

Eighty-eight (79%) of the 112 cases were referred from all over the country, the rest came from the city of Trondheim. The sex distribution was 47% female and 53% male. The mean age of the mothers at the time of abortion or birth was 28 years (range 18-42). A previous pregnancy loss had been experienced by 32% of the women. The average gestational age of the fetus or infant was 28 weeks in 1985 as opposed to 25 weeks in 1994 (range 16-40). In 75 (67%) of the 112 cases termination of pregnancy (TOP) was effectuated, 2 (2%) were spontaneous abortions, 11 (10%) were intrauterine deaths, and 24 (21%) were liveborn.

A urinary system anomaly was the main diagnosis in 50 of the 112 cases (Table 1). The renal or urinary tract anomaly was isolated in 38 (76%) of the 50 cases. Thirty-five were either renal agenesis or various forms of cystic renal disease, two cases were intrauterine fetal deaths with urethral obstruction and dilated bladder, and one case was a fetus with hydroureter and hydronephrosis. The latter was spontaneously aborted after drainage for polyhydramnion. In the other 12 cases where the main diagnosis of urinary system anomaly was associated with abnormalities of other organs, anal atresia was the most frequent associated anomaly (5 of 12 cases).

In 62 cases, the renal or urinary tract anomaly was considered less important than an anomaly in another organ system (Table 2). Considering all organ manifestations associated with urinary system anomalies, congenital heart defects were the most common.

More than one urinary system anomaly was registered in 49 (44%) of the 112 cases. The total number of urinary system anomalies found was 171, 44 (26%) of these were multicystic renal dysplasias, 18 of which were associated with ureteral hypoplasia, posterior urethral valves or urethral atresia. In 15 (13%) of 112 cases a horseshoe kidney was found. In 12 of these 15 cases the karyotype was known; 9 were associated with a chromosomal aberration (3 Turner syndrome, 6 trisomy 18). The classification of the urinary system anomalies is shown in Table 3.

In 97 (87%) of the 112 cases there was full agreement between the ultrasound observations and the autopsy findings (Table 4; category 1). Discrepancies between the sonographic observations and the autopsy findings were found in the remaining 15 cases. Different degrees of accordance were registered and are listed in Table 5, cases 1 to 7 from the period 1985-89 and cases 8 to 15 from the period 1990-94.

In five cases the autopsy revealed minor findings not mentioned in the ultrasound report (Table 5; category 2) and in 4 cases major urinary system anomalies registered at autopsy were not mentioned in the ultrasound report (Table 5; category 3). Four other fetuses and infants had autopsy findings not observed at the routine ultrasound examination (Table 5; category 4). These died in utero between gestational week 23 and 38.

Two cases were classified in category 5; dysplastic kidneys were suspected at the ultrasound examination in case 7, but autopsy revealed bilateral renal agenesis. A horseshoe kidney in case 15 was interpreted at ultrasound as agenesis of right kidney and dysplastic left kidney. In this case, other anomalies led to TOP. None of the 15 horseshoe kidneys were diagnosed at the ultrasound examination; they are listed separately in Table 6.

The main diagnosis was correct in 102 cases (91%). This includes cases with full agreement and cases with minor autopsy findings not detected prenatally. If we compare the two time periods 1985-89 and 1990-94, the overall accordance between ultrasound and autopsy diagnoses was approximately the same during the two periods; 85% and 88%. As for the types of anomalies not detected, there seem to be no major differences.

Of the 112 cases with urinary system anomalies, an amniocentesis or fetal blood sampling for chromosomal analysis was performed in 82 cases. Karyotyping was not successful in 7 of them due to cell culture problems. A chromosomal abnormality was detected in 28 (37%) of the 75 cases that were successfully karyotyped (Table 7). The karyotype was known in 23 (50%) of all 46 cases during the first time period; during the last time period it was known in 52 (79%) of the 66 cases.

**Discussion**

The aim of this study was to register renal and urinary tract anomalies and to compare the findings of the ultrasonographic examination with the postmortem findings in 112 fetuses and infants with urinary system anomalies. The frequent occurrence of serious urinary system anomalies such as multicystic renal dysplasia reflects that this is a selected population with almost 80% referred patients. The possibility of bias will always be present. Some anomalies may be missed when other more important anomalies are sufficient for further management, or, the finding of one anomaly may trigger the attention to look for other anomalies. This may influence the diagnosis of subtle lesions.

In our study, full agreement between the ultrasound examination and the autopsy report was found in 87%. In 91% the main diagnosis was correct (Table 4). Other follow-up studies of ultrasonography of urinary system anomalies include, for the most part, a combination of clinical and postmortem cases. Postnatal confirmation of the prenatal diagnoses is described in 50% to 78% of cases from the late eighties[20,21,23]. In a recent study of 55 cases, there was an agreement of 81% between antenatal and postnatal diagnoses[4]. The major discrepancies consisted of difficulties in discriminating dysplastic kidneys from hydronephrosis. In an early second-trimester sonographic screening, 21 anomalies consisting of unilateral renal agenesis, pelvic kidney and double collecting system were all confirmed postnatally or at postmortem examination[22].

Oligo- or anhydramnios will usually trigger the attention towards a thorough examination of the urinary system. The presence of oligo- or anhydramnios makes the interpretation of the ultrasound findings more difficult and can therefore be responsible for inaccuracies[11,21,26].

In our series, a better agreement was found between the prenatal and postnatal diagnoses in cases with central nervous system anomalies[24] than with urinary tract anomalies. The opposite was true for congenital heart defects; in this study the correlation between the two methods was better for urinary system anomalies than for congenital heart defects[25]. This has also been found by others[16,27]. Unlike small VSDs and aortic coarctations which are difficult or impossible to detect ultrasonographically, even moderate obstructions of the urinary tract can be detected as they may lead to dilatation of the renal pelvis. While early obstruction is more likely to cause renal dysplasia, late obstruction may lead to hydronephrosis, which therefore is more likely to be observed at a later stage in pregnancy[28,29].

Isolated unilateral kidney lesions such as agenesis, hypoplasia or dysplasia escape detection more often than bilateral lesions, probably because they will not cause amniotic fluid alterations and thus not trigger the awareness for a renal anomaly. Focal dysplastic lesions, especially if subtle, can also be difficult to detect. The importance of detecting minor renal abnormalities has been emphasized, and of these unilateral agenesis is the most common[22].

Bilateral dysplastic lesions do not pose any great diagnostic problems whereas bilateral renal agenesis can be difficult to discern both because of reduced amniotic fluid and because the adrenals in these cases may mimic kidneys[2,30]. Later in pregnancy, the kidneys become more hypoechogenic which makes them even more difficult to differentiate from the adrenal glands[22,30]. In one case from the first time period, bilateral renal agenesis was falsely interpreted as dysplastic kidneys by ultrasound. This did not have any consequences for the management.

The level and severity of urinary tract obstruction and the time of its onset influences the morphological changes[29, 31, 32]. Of 5 discrepant cases with urinary tract obstruction, 2 had hydronephrosis and hydroureter, one had ureteropelvic junction atresia, and 2 had obstructive lesions of the urethra (Table 5). In the above mentioned cases, the fetus or infant was either live born or died in utero, but the obstructive lesions were not considered as the cause of death. These urinary tract obstructions may have occurred later in pregnancy thus accounting for the diagnosis being missed at the 18th week routine ultrasound examination.

Horseshoe kidneys are difficult to detect because the connection may be missed on the two-dimentional plane[2]. They occur in 1 of 600 individuals and represent one of the more frequent renal anomalies[2]. The horseshoe kidneys in our material were all associated with other anomalies; none of them were detected by ultrasound examination. During the later years the ability to diagnose horseshoe kidneys has improved considerably.

The percentage of overlooked minor ultrasound findings was reduced from the first (1985-89) to the second (1990-94) time period (Table 4). The numbers are small, but we believe this is an expression of improved ultrasound expertise. The downward shift in gestational age at abortion or birth from 28 weeks during the first year of registration to 25 weeks during the last year acknowledges both the introduction of the routine ultrasound examination at 17-18 gestational weeks and the technical improvements of ultrasound equipment[16].

In our study, uni- or bilateral multicystic renal dysplasia was the most common anomaly and occurred in 39% of the fetuses. Except for one intrauterine fetal death with unilateral dysplastic and hypoplastic kidney and one fetus with holoprosencephaly and cystic renal dysplasia, the renal dysplasias were either suspected or correctly diagnosed prenatally giving a detection rate of 95%. Ureteropelvic obstruction and duplication anomalies were less common, which is as expected taking into account that we are dealing with postmortem examinations. The detection rate for hydronephrosis and ureteropelvic obstruction was 87%. Obstructive lesions were most often found in connection with other more serious anomalies.

CNS anomalies and CHDs were the most frequent associated conditions, followed by gastro-intestinal anomalies, diaphragmatic hernia and abdominal wall defects. When the urinary system anomaly was associated with a CNS anomaly, the latter was usually the reason for TOP or cause of death. Multiple organ anomalies with combinations of renal anomalies, CNS anomalies and CHD have been described[15-17, 31, 33-35]. An abnormal karyotype is often present in such cases and was demonstrated in 25% of all the urinary system anomalies in this study. According to other authors, from 2 to 33% of renal anomalies are associated with chromosomal aberrations[11, 36] and approximately 50% are associated with other anomalies[10,11,37]. The combination of urinary system anomalies and anal atresia is understandable considering that during embryonic development the distal urinary tract and the distal gut have a common origin in the cloaca[38,39].

At autopsy, renal anomalies, if not subtle like some medullary cystic disorders, are usually easy to diagnose. Distinguishing between polycystic disease of the kidneys and multicystic renal dysplasia may be difficult on gross examination. The examination by light microscopy usually renders the correct diagnosis. Dilatations of the urinary tract are easy to discern, though it may be difficult to determine the level of obstruction. The diagnosis of urethral obstruction, specifically posterior urethral valves, may be missed at gross inspection, even with careful dissection.

Apart from serving as a complement to the prenatal diagnosis, postmortem examination is considered important for the quality control of ultrasonography in fetal diagnostics. Considering the variety of etiological factors governing renal cystic lesions, the morphological diagnosis classifying them into nonhereditary and hereditary forms is important not only for epidemiologic studies but also for the genetic guidance of the parents[40].

**Conclusion**

In our project consisting of 408 fetuses and infants with developmental anomalies, renal and urinary tract anomalies occurred with a frequency of 27%, next after CNS anomalies (34%). The prenatal ultrasound findings from this tertiary center were compared with the results of postmortem examination. The main diagnosis in the 112 cases with urinary system anomalies was correct in 91%, thus showing a good correlation between the prenatal and postmortem examination. This comparison of ultrasound and autopsy diagnoses does not differ greatly from the results observed in cases with central nervous system anomalies and congenital heart defects[24, 25].

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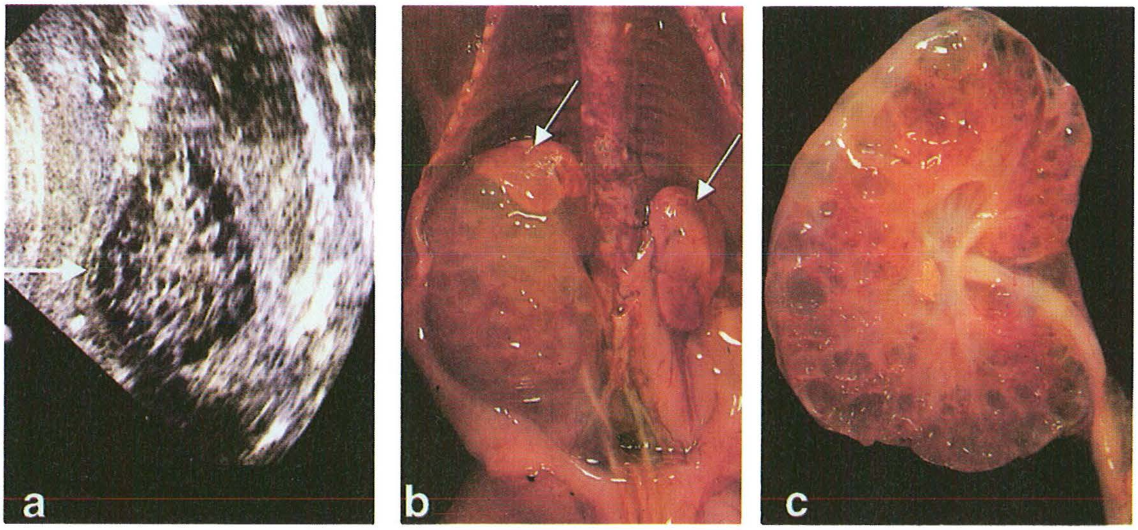


Figure 1 Fetus, 18 weeks old. Cystic dysplastic right kidney and agenesis left kidney. (a) Ultrasound scan of right kidney (arrow); (b) in situ photograph at autopsy including right kidney and both suprarenal glands (arrows on suprarenal glands); (c) cut surface of right kidney

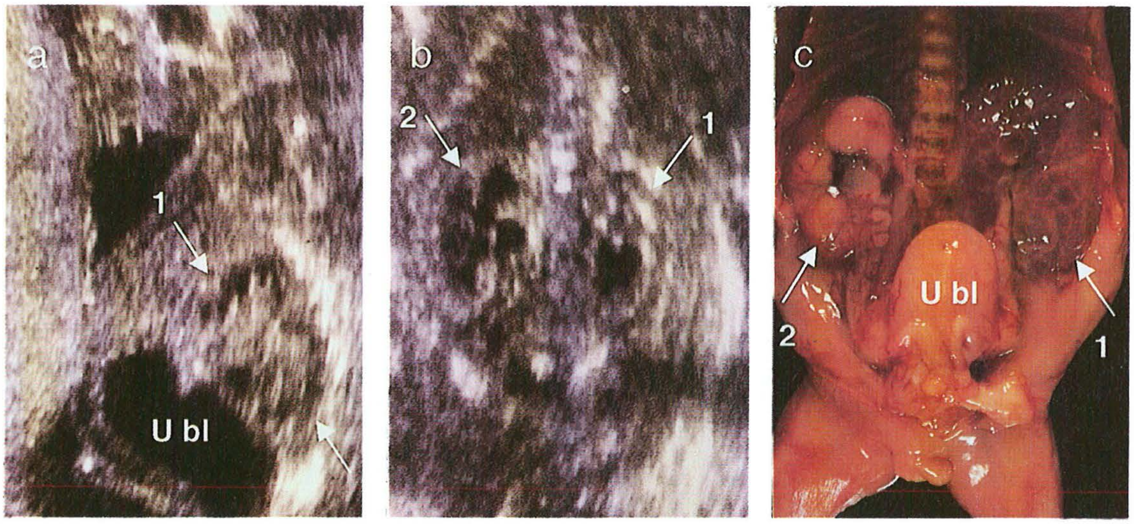


Figure 2 Fetus, 20 weeks old. Urethral obstruction with dilated urinary bladder, hydronephrosis right kidney and cystic dysplastic left kidney. (a) Ultrasound scan of urinary bladder (U bl) and left kidney (arrow); (b) ultrasound scan of kidneys (arrows; 1 left, 2 right); (c) in situ photograph at autopsy including bladder (U bl) and both kidneys (arrows)

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**Table 1 Main diagnosis classified according to organ system in 112 cases with urinary system anomalies**

| Organ system | Total n | Percent of total |
|---|---|---|
| Urinary system | 50 | 45 |
| Central nervous system | 20 | 18 |
| Cardiovascular system | 19 | 17 |
| Diaphragm/abdominal wall defects | 12 | 11 |
| Fetal hydrops /cystic hygroma | 7 | 6 |
| Miscellaneous | 4 | 3 |
| Total | 112 | 100 |

**Table 2 Cases including urinary system anomalies: main diagnoses and additional findings (n = 112)**

| | Main finding n | Additional finding n | Total n |
|---|---|---|---|
| Urinary system | 50 | 62 | 112 |
| Cardiovascular system | 19 | 14 | 33 |
| Central nervous system | 20 | 4 | 24 |
| Gastro-intestinal system | 2 | 21 | 23 |
| Skeletal system | 2 | 21 | 23 |
| Diaphragm/abdominal wall defects | 12 | 7 | 19 |
| Fetal hydrops | 7 | 6 | 13 |
| Genital system | | 9 | 9 |
| Lungs | | 1 | 1 |
| Total | 112 | | |

**Table 3 Classification of urinary system anomalies (n=112)**

| **Diagnosis** | **n** | **%** |
|---|---|---|
| Renal agenesis: | | |
| -bilateral | 11 | 6.4 |
| -unilateral | 17 | 9.9 |
| Renal hypoplasia | 2 | 1.2 |
| Duplication of renal pelvis and ureter | 2 | 1.2 |
| Horseshoe kidney | 15 | 8.8 |
| Multicystic renal dysplasia: | | |
| -bilateral | 28 | 16.4 |
| -unilateral | 16 | 9.3 |
| Multicystic renal dysplasia associated with: -ureteral hypoplasia | 10 | 5.8 |
| -posterior urethral valves | 1 | 0.6 |
| -urethral atresia | 7 | 4.1 |
| Congenital hydronephrosis: | | |
| -bilateral | 5 | 2.9 |
| -unilateral | 9 | 5.2 |
| -ureteropelvic obstruction | 9 | 5.2 |
| Anomaly of the ureter: | | |
| -duplication | 1 | 0.6 |
| -dilatation | 2 | 1.2 |
| -atresia | 3 | 1.8 |
| Polycystic disease | 6 | 3.5 |
| Meckel-Gruber syndrome | 2 | 1.2 |
| Renal disease uncertain classification | 1 | 0.6 |
| Medullary cystic disorders | 2 | 1.2 |
| Anomalies of the bladder: | | |
| -dilatation | 6 | 3.5 |
| -agenesis | 9 | 5.2 |
| -duplication | 2 | 1.2 |
| Anomalies of the urethra: | | |
| -posterior valves | 2 | 1.2 |
| -atresia | 3 | 1.8 |
| Total | 171 | 100 |

**Table 4 Correlation of prenatal ultrasound findings and autopsy; Renal and urinary system anomalies (n=112)**

| Category | 1985-89 | | 1990-94 | | 1985-94 | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| 1) Full agreement | 39 | 85 | 58 | 88 | 97 | 87 |
| 2) Minor autopsy findings not found by ultrasound | 3 | 7 | 2 | 3 | 5 | 4 |
| 3) Major autopsy findings not found by ultrasound | 2 | 4 | 2 | 3 | 4 | 3.5 |
| 4) None of the autopsy findings suspected by ultrasound | 1 | 2 | 3 | 4 | 4 | 3.5 |
| 5) Minor ultrasound findings not confirmed at autopsy | 1 | 2 | 1 | 2 | 2 | 2 |
| 6) Major ultrasound findings not confirmed at autopsy | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 46 | 100 | 66 | 100 | 112 | 100 |

**Table 5 Kidney and urinary tract anomalies: survey of discrepancies between ultrasound and autopsy findings (n=15).**

| Case | Prenatal diagnosis | Sex | GA weeks | Mode of death/birth | Final diagnosis following autopsy | Category |
|---|---|---|---|---|---|---|
| 1 | Omphalocele, skeletal deformities in spinal column and lower extremities | F | 33 | TOP | LBWC with hypoplastic right kidney, aplasia of right ovary and fallopian tube, skeletal deformities in spinal column, pelvis and lower limbs | 2 |
| 2 | Right kidney and urinary bladder not localized, possibly a kidney on the left side; lung hypoplasia and Potter's syndrome | M | 36 | TOP (LB) | Multicystic renal dysplasia with hypoplasia (0.8 g) of right kidney (birth induced on suspicion of Potter type II, lived 2 h) | 2 |
| 3 | Myelomeningocele, omphalocele, skeletal deformities in spinal column and amelia one lower extremity, ARS? | F | 37 | IUFD (wanted to continue pregnancy) | LBWC, anal atresia, spinal deformities, amelia right lower extremity, urethral atresia with dilated bladder, hydroureter and hydronephrosis in a horseshoe kidney | 2 |
| 4 | Hydrocephaly and oligohydramnios | F | 40 | TOP | Hydrocephaly, right renal agenesis | 3 |
| 5 | Hydrocephaly | 2 | 22 | TOP | Hydrocephaly, bilateral radial aplasia, left renal agenesis, two ureters right kidney | 3 |
| 6 | None | M | 29 | IUFD | IUGR, skeletal deformities upper limbs, right renal agenesis | 4 |
| 7 | Oligohydramnios and suspected dysplastic kidneys | F | 18 | TOP | Bilateral renal agenesis | 5 |
| 8 | Polyhydramnios | M | 24 | LB | Bilateral hydronephrosis/hydroureter; polyhydramnios, spontaneous birth after amniotic fluid drainage, hyaline membrane disease, | 2 |
| 9 | Polyhydramnios, hypoplastic right ventricle, VSD, oligodactyly right foot | F | 33 | LB | Trisomy 18, hypoplastic right ventricle, VSD, esophageal atresia and tracheo-esophageal fistula, anal atresia, ureteropelvic junction atresia, syndactyly right foot (cesarean section, lived 1 day) | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10 | Holoprosencephaly, abdominal wall defect, dilated rectum with anal atresia and fistula | M | 18 | TOP | Holoprosencephaly, cleft lip/palate, cystic renal dysplasia, urethral atresia, anal atresia with fistula to bladder | 3 |
| 11 | Holoprosencephaly, lumbosacral meningocele, dysplastic right kidney, | F | 22 | TOP | Triploidy, IUGR, holoprosencephaly, lumbosacral meningocele, omphalocele, VSD, left renal agenesis, ureteropelvic atresia right side with cystic renal dysplasia, syndactyly left hand | 3 |
| 12 | None | M | 23 | IUFD | Urethral atresia with megacystis | 4 |
| 13 | None | M | 38 | IUFD | Dysplastic and hypoplastic left kidney, incomplete duplication of bladder, IUGR | 4 |
| 14 | None | F | 36 | IUFD | Posterior urethral valves with dilated bladder; intrauterine asphyxia (placental infarction and retroplacental hemorrhage) | 4 |
| 15 | Trisomy 18, Arnold-Chiari malformation, VSD, clenched fingers, bilateral clubfeet, right renal agenesis and dysplastic left kidney | M | 29 | TOP | Trisomy 18, IUGR, Arnold-Chiari malformation, VSD, clenched fingers, bilateral clubfeet, horseshoe kidney | 5 |

TOP=termination of pregnancy
IUFD=intrauterine fetal death
LB=liveborn
IUGR=intrauterine growth retardation
LBWC=limb-body-wall-complex
ARS=Amniotic rupture syndrome
VSD=ventricular septal defect

**Table 6 Urinary system anomalies: survey of cases with horseshoe kidneys (n=15)**

| Case | Prenatal diagnosis | Sex | GA weeks | Mode of death/birth | Final diagnosis following autopsy |
|---|---|---|---|---|---|
| 1 | Polyhydramnios, omphalocele | M | 40 | LB | Trisomy 18?, IUGR, omphalocele, VSD, aortic coarctation, clubfeet, horseshoe kidney |
| 2 | Omphalocele, hypoplastic right ventricle with tricuspidal atresia, VSD, choroid plexus cysts, low set ears, rockerbottom feet | M | 18 | TOP | Trisomy 18? omphalocele, hypoplastic right ventricle with tricuspidal atresia, VSD, low set ears, horseshoe kidney |
| 3 | Polyhydramnios, IUGR | F | 30 | LB | Trisomy 18?, IUGR, VSD, dysmorphic facial features, malrotation of gut, horseshoe kidney |
| 4 | Anhydramnios, cystic hygroma | F | 21 | IUFD | Turner syndrome (mosaic) cystic hygroma, horseshoe kidney |
| 5 | Trisomy 18, Arnold-Chiari malformation, cystic hygroma, fetal hydrops, clenched fingers | F | 19 | TOP | Trismoy 18, Arnold-Chiari malformation, cystic hygroma, fetal hydrops, clubfeet, syndactyly, horseshoe kidney |
| 6 | Trisomy 18, choroid plexus cysts, omphalocele, bilateral radial aplasia, | M | 20 | TOP | Trisomy 18, omphalocele, VSD, bilateral radial aplasia, horseshoe kidney |
| 7 | Fetal hydrops | F | 28 | | Fetal hydrops, horseshoe kidney |
| 8 | Myelomeningocele, omphalocele, skeletal deformities in spinal column and amelia one lower extremity, ABS? | F | 37 | IUFD | LBWC, anal atresia, spinal deformities, amelia right lower extremity, urethral atresia with dilated bladder, hydroureter and hydronephrosis in a horseshoe kidney |
| 9 | Trisomy 18, omphalocele, VSD, deformities right hand | F | 24 | TOP | Trisomy 18, omphalocele, VSD, dysmorphic facial features, deformities right hand, horseshoe kidney |
| 10 | Trisomy 18, Arnold-Chiari malformation, VSD, clenched fingers, bilateral clubfeet, right renal agenesis and dysplastic left kidney | M | 29 | TOP | Trisomy 18, IUGR, Arnold-Chiari malformation, VSD, clenched fingers, clubfeet, horseshoe kidney |

| | | | | | |
|---|---|---|---|---|---|
| 11 | LBWC witth deformities in spinal column and lower extremities | M | 34 | IUFD | LBWC with extensive skeletal deformities, ASD, horseshoe kidney |
| 12 | IUGR, cystic hygroma and fetal hydrops | F | 21 | IUFD | Turner syndrome, IUGR, cystic hygroma, hydrops, ASD, horseshoe kidney |
| 13 | IUGR, VSD, dysmorphic features, clenched finger, rockerbottom feet | F | 36 | LB | Trisomy 18, IUGR, VSD, dysmorphic facial features, clenched fingers, rockerbottom feet, Meckel's diverticula, horseshoe kidney |
| 14 | Trisomy 18, IUGR, hypoplastic left ventricle, VSD, clenched fingers | F | 41 | IUFD | Trisomy 18, IUGR, hypoplastic left ventricle, VSD, aortic coarctation, CNS dysplasia, clenched fingers, horseshoe kidney |
| 15 | Fetal hydrops, cystic hygroma | F | 20 | TOP | Turner syndrome, cystic hygroma, fetal hydrops, horseshoe kidney |

TOP=termination of pregnancy
IUFD=intrauterine fetal death
LB=liveborn
IUGR=intrauterine growth retardation
LBWC=limb-body-wall-complex
VSD=ventricular septal defect

**Table 7 Frequency of chromosomal abnormalities**

| Years | Cases karyotyped | Normal | | Abnormal | |
|---|---|---|---|---|---|
| | N | N | % | N | % |
| 1985-89 | 23 | 15 | 65 | 8 | 35 |
| 1990-94 | 52 | 32 | 62 | 20 | 38 |
| 1985-94 | 75 | 47 | 63 | 28 | 37 |

# PAPER IV

# A CORRELATIVE STUDY OF PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS IN FETUSES AND INFANTS WITH AN ABNORMAL KARYOTYPE


Christina V. Isaksen (1,2) M.D.

Sturla H. Eik-Nes (2) M.D. Ph.D.

Harm-Gerd Blaas (2) M.D. Ph.D.

Sverre H. Torp, (1) M.D. Ph.D.

Carl B. van der Hagen (3) M.D.

Eli Ormerod (3) B.Sc.

1) Department of Laboratory Medicine and

2) National Center for Fetal Medicine, Department of Obstetrics and Gynecology

Trondheim University Hospital, Norwegian University of Science and Technology, Trondheim, Norway

3) Department of Medical Genetics, Oslo University Hospital, Oslo, Norway


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Mailing address: Dr. Christina Vogt Isaksen
Department of Pathology
Trondheim University Hospital
N-7006 Trondheim, Norway
Tel. no. +47 73868653
Fax no. +47 73868654
e-mail: Christina.Isaksen@medisin.ntnu.no

## ABSTRACT

**Objective** To compare ultrasound and postmortem findings in 98 fetuses and infants with an abnormal karyotype.

**Design** Criteria for inclusion were an ultrasound examination at the National Center for Fetal Medicine (NCFM), an abnormal karyotype, and an autopsy performed during the period 1985 to 1994.

**Results** Trisomy 18 and 21 were the two most common abnormal karyotypes. The highest number of congenital anomalies was observed in cases with trisomy 13 and 18; congenital heart defects (CHD) were most prevalent among fetuses with trisomy 18. In 80% there was full agreement between the ultrasound and autopsy findings, in another 8 % there was nearly complete concordance. Thus, in 88% of the cases, the main prenatal ultrasonographic diagnosis was correct. In 6% major autopsy findings were not detected by ultrasound examination, in 1% none of the autopsy findings were detected by routine ultrasound and in 5% ultrasound findings were not verified at autopsy. When the correlation was related to individual autosomal trisomies, structural anomalies were most often correctly diagnosed in fetuses with trisomy 13, with the main diagnosis correct in all cases; second in accuracy were the ultrasound diagnoses in fetuses with trisomy 21 with main diagnosis correct in 96%; for trisomy 18 the concordance was not as good, with the main diagnosis correct in 71%.

**Conclusion** The present comparison of ultrasonographic diagnoses with postmortem findings demonstrates good accordance between the two methods. It also demonstrates the importance of being aware of anomalies known to occur in the different aneuploidies.

**INTRODUCTION**

Approximately 10% of all clinically recognizable conceptions in the human species have been estimated to be chromosomally abnormal[1]. Chromosome aberrations are involved in over half of all spontaneous abortions or miscarriages and 5% of stillborns. This indicates that the major loss of chromosomally abnormal zygotes appears early in gestation[2-5]. Still, chromosome abnormalities affect approximately 0.5% of newborns[2,5,6]. The sinister prognosis associated with such conditions has been an incitement for maternal serum screening and prenatal ultrasound, followed by amniocentesis and chorionic villi biopsy in selected cases.

The fact that various chromosome aberrations are associated with fetal structural anomalies, detectable by ultrasound examination[7], is illustrated by studies from tertiary referral centers where all of the fetuses with trisomy 13, over three fourths of those with trisomy 18, and almost half of those with trisomy 21, have significant structural anomalies that are possible to detect by a second trimester ultrasonography scan[8-11]. Chromosome aberrations are also common when multiple anomalies are present[12] with a risk of chromosomal abnormality as high as 35%[13].

The use of improved technical equipment in ultrasonography with high frequency transducers has made the detection of discrete anomalies possible. Minor structural abnormalities, so-called "chromosomal markers", have a potential for screening in connection with the 18th week routine ultrasound scan[7] as well as earlier scans[14].

The aim of this study was to look at the spectrum of structural anomalies found in fetuses with various abnormal karyotypes and compare the prenatal ultrasound findings with the results of a subsequent postmortem investigation.

**MATERIAL AND METHODS**

From January 1985 through December 1994, a total of 408 autopsies of fetuses and infants with developmental anomalies were performed; 365 at the Department of Pathology, Trondheim University Hospital, and the rest at other cooperating hospitals. In all cases, an ultrasound examination during pregnancy had been performed at the ultrasound laboratory of the Department of Obstetrics and Gynecology, Trondheim University Hospital. In 1990, this unit was established as the National Center for Fetal Medicine (NCFM) and acts as a referral center for pregnant women from all over Norway.

Obstetricians working at the NCFM performed the targeted ultrasound examinations. The scan included a survey of fetal anatomy, biometric measurements of the fetus, and placental location. Fetal biparietal diameter, measured at the routine ultrasound examination, was the basis for evaluation of gestational age.

The prenatally collected samples were derived from amniocentesis, cordocentesis, cardiocentesis and chorionic villus sampling (CVS). In some cases of intrauterine fetal death, achilles tendon biopsy specimens were karyotyped. When liveborn, blood for karyotyping was collected after birth. Procedures for karyotyping were effectuated in 312 of the 408 cases. In 13 cases karyotyping was not successful.

Anamnestic information, sonographic findings, and the results of chromosome/biochemical analyses were all registered and prospectively stored in a computer data base. The ultrasound machines employed were Hitachi EUB 565, Dornier AI 3200 and Vingmed Sound CFM 750. They were equipped with transducers with frequencies ranging from 3.5-7.5 MHz. The comparisons were based on the recorded findings in the ultrasound and autopsy reports.

Postmortem, the fetuses/infants were examined according to a standardized autopsy protocol[15]. Until 1990, the autopsies were done at the Department of

Pathology, Trondheim University Hospital by junior pathologists in training, supervised by a senior consultant pathologist. When the NCFM was established in 1990, a senior pathologist, specialising in perinatal pathology, performed the postmortem examinations. The pathologist participated at regular meetings with the obstetricians working at the NCFM, reviewing the videotapes with the sonographic findings prior to autopsy,. Whole-body radiography and photographic documentation of the findings were routinely performed at the postmortem examination. All organs were examined, the heart in situ, and the brain removed under water, in order to avoid traumatizing actions at its removal. Except for 12 cases, the placenta was examined both grossly and microscopically.

The time that elapsed between abortion/death and autopsy was approximately 1 day, with a range of a few hours up to 5 days. Until autopsy was performed, the corpses were stored at low temperatures (4-8$^{o}$ C). Whole organs or specimens of them were fixed in 10% formalin; slices from the organs, including the placenta, were processed routinely with paraffin embedding, and the cut sections (4-5μm thick) stained with hematoxylin-erythrosin-safranin.

The recorded ultrasound and autopsy findings were categorized and correlated as reported previously[16,17]:

1) Full agreement between the ultrasound and autopsy findings.

2) Minor autopsy findings not detected or not recorded at the ultrasound examination.

3) Major autopsy findings not detected at the ultrasound examination.

4) None of the autopsy findings suspected at the ultrasound examination.

5) Minor ultrasound findings not confirmed at autopsy. This category includes findings supplementary to other detected anomalies which were confirmed at autopsy.

6) Major ultrasound findings not confirmed at autopsy. This category includes false positives, as well as cases where postmortem changes interfered with making a proper morphological diagnosis.

**RESULTS**

**Clinical data**

During the ten-year period, 299 (73%) of the 408 fetuses/infants with congenital anomalies were successfully karyotyped. An abnormal karyotype was found in 98 (33%) of the 299 cases. They form the patient material in the present report. In this group amniocentesis was performed in 71 cases (72%), cordocentesis in 19 (20%), cardiocentesis in 4 (4%), CVS in 3 (3%) and in one case blood was collected from a liveborn infant after birth.

After the NCFM was established in 1990, a significant number of the women (72%) were referred from other parts of the country. The mean age of the 98 women at the time of abortion or birth, was 30 years (range 18 to 43). Thirty-four (35%) of them had experienced a previous abortion, 14 more than once. In 81 women (83%) the pregnancy was terminated, in 11 (11%) intrauterine death occurred. Six women (6%) delivered liveborn infants. They were all older than 36 gestational weeks and lived only for a few hours. The sex distribution was 60 girls and 38 boys. The mean gestational age at the time of abortion/birth was 20 weeks (range 12-41), while the mean gestional age at which the diagnostic ultrasound was performed was 19 weeks (range 12-34). The placenta was examined in 86 of the cases, it was normal in 66 (77%); in 2 cases a partial hydatiform mole was present, whereas in 18 cases (21%), degenerative changes, fibrinous or hemorraghic infarcts or hemorrhages were recorded. A single umbilical artery was found in 26 placentas (30%).

**General observations**

The incidence of abnormal karyotypes is shown in Table 1. The most common chromosome abnormality was trisomy 18, which was observed in 31 (32%) of the cases, followed by trisomy 21 which occurred in 23 (24%) cases.

In 5 of those with trisomy 21, in 2 cases with 47,XXY (Klinefelter syndrome), in 2 cases with 47,XXX anomaly and in one case with a deletion on chromosome 9, the postmortem examination did not reveal any structural anomaly. In 8 of these cases maternal age was the indication for karyotyping, in one case karyotyping was performed because of a sibling with trisomy 18, and in one case hydrothorax prompted karyotyping. In the remaining 88 fetuses and infants, 196 abnormalities, including fetal hydrops, were found. Congenital heart defect (CHD) was the most prevalent organ anomaly, followed by urinary system and central nervous system (CNS) anomalies. Nuchal edema, cystic hygroma, and/or generalised fetal hydrops, was found in 39 of all 98 cases (40%) (Table 2).

Table 2 shows the distribution of anomalies related to the various karyotypes. Trisomy 13 and 18 had most single anomalies per case. The highest incidence of CHD, (27/31; 87%), occurred in fetuses and infants with trisomy 18.

***Trisomy 13***: The average gestational age of the fetuses was 19 weeks. The distribution of anomalies is shown in Table 2. Chromosomal markers and combinations of anomalies detected at ultrasound and postmortem examination are listed in Table 3.

***Trisomy 18***: The average gestational age was 23 weeks. Anomalies of the heart, CNS and kidneys, together with omphalocele and various skeletal deformities, were the predominating anomalies (Table 2). Twenty-three (85%) of the 27 cases with a congenital heart defect had a ventricular septal defect (VSD), either isolated or combined with other cardiac defects, most commonly a hypoplastic ventricle or an atrioventricular septal defect (AVSD). Four of the VSDs were associated with an overriding aorta.

A CNS anomaly (choroid plexus cysts excluded) was found in 10 (32%) of the 31 cases. All but one were associated with a CHD. The CNS anomalies included 3 cases with the Arnold-Chiari malformation, 2 with anencephaly and rachischisis, 2 with the Dandy-Walker malformation and 3 with CNS dysplasias. An omphalocele or a diaphragmatic hernia did not occur without the presence of a CHD. The most frequent combination of organ anomalies was CHD and omphalocele; altogether 12 (39%) of the trisomy 18 cases had this combination. Six of 9 horseshoe kidneys observed were found in fetuses with trisomy 18. The distribution of chromosomal markers detected by ultrasound and autopsy are listed in Table 4. Not all ultrasound detected choroid plexus cysts nor ultrasound findings such as nuchal edema were confirmed at autopsy.

***Trisomy 21***: The average gestational age was 19 weeks. Five cases were without gross anomalies, 7 had combined CHD and hydrops. Nuchal edema or cystic hygroma was present in 15 (65%) of the 23 fetuses and infants with trisomy 21 (Table 2). In 12 of these, the findings were remarked both at the ultrasound and postmortem examination, in 2 cases only at the ultrasound examination and in one case a cystic hygroma was not diagnosed by ultrasound. Three discrepancies were found in fetuses with trisomy 21, an aortic coarctation and a VSD were missed in two cases, and in one case an ASVD suspected at the prenatal ultrasound examination was not confirmed at autopsy (Table 5; cases 1,9 and 16).

***Turner syndrome, 45,X***: The average gestational age of the 14 fetuses with Turner syndrome was 19 weeks. All had hydrops and/or cystic hygroma; 3 had horseshoe kidneys and 2 a CHD (Table 2). None of the 3 horseshoe kidneys were detected at the ultrasound examination and in one case an ASD primum was not detected (Table 5; case 11).

***Triploidy, 69,XXX/69,XXY***: The average gestational age, 24 weeks, was higher in comparison with the main aneuploidies. The most common anomalies were CHD, CNS and renal anomalies (Table 2). No special combination predominated. The renal anomalies were either dysplasia or agenesis. Syndactyly was present in 4 cases. None of the fetuses or infants had hydrops or cystic hygroma. In one case dysmorphic features and bifid spine were not detected at the ultrasound examination and in another case a VSD, unilateral renal agenesis, omphalocele and syndactyly were not detected (Table 5; cases 7 and 13).

**General survey of sonographic and autopsy findings**

As shown in Table 6, the prenatal ultrasound diagnoses were in agreement with those made at autopsy in 78 (80%) of the 98 cases. The 20 discrepancies are listed chronologically in Table 5; cases 1-7 from the first time period 1985-89, and cases 8-20 from the second time period, 1990-94. Almost half of the inconsistencies concerned minor discrepancies (category 2). These were VSD and aortic coarctation, as well as syndactyly and ureteropelvic junction stenosis (Table 5; cases 1-4 and 8-11). None of the horseshoe kidneys were detected at the ultrasound examination. Disregarding the minor autopsy findings overlooked (category 2), the detection rate by means of ultrasonography was 88%.

In 6 cases major autopsy findings were not detected at the ultrasound examination (category 3). Among these were tetralogy of Fallot, VSD and overriding aorta, omphalocele, diaphragmatic hernia and oesophageal atresia with tracheo-oesophageal fistula. In all these cases (Table 5; cases 5,6,12 -15), the chromosome abnormality and/or other concomitant anomalies were the indication for termination of pregnancy.

In one patient (Table 5; case 7), none of the autopsy findings were suspected at the routine ultrasound examination (category 4). The infant was delivered by cesarean section in the 34th week because of serious intrauterine growth retardation. Dysmorphic features at birth prompted a chromosome analysis which revealed triploidy.

Five fetuses (Table 5; cases 16-20) in category 5 and 6 with ultrasound findings not confirmed at autopsy, were from the second time period. In one of them (case 17), a horseshoe kidney was interpreted as agenesis of the right kidney and dysplasia of the left. In a case of trisomy 18 (case 18), ultrasound revealed a slight dilatation of the renal pelvis and an increased echogenicity of the kidneys. This was interpreted as an expression of dysplasia but could not be confirmed at the postmortem examination. In two fetuses an AVSD at ultrasound examination was not verified at autopsy; a VSD was confirmed at the postmortem examination in one (case 16), and in the other (case 19), the fetus was macerated and the AVSD could not be confirmed. Finally, in case 20, the autopsy failed to confirm the prenatal findings as the fetus was fragmented by the abortion procedure. Choroid plexus cysts were not included in this table. Only 2 of 9 plexus cysts in fetuses with trisomy 18 were confirmed at postmortem examination (Table 4).

An overview of discrepancies in the various chromosome aberrations is shown in Table 7. Most discrepancies were found in fetuses and infants with trisomy 18, the overall detection rate for anomalies in trisomy 18 was only 58%, whereas for the fetuses and infants with other abnormal karyotypes, the detection rate varied between 75 and 100%.

## DISCUSSION

Most fetuses with major chromosomal abnormalities have associated structural anomalies that can be recognized by ultrasound examination[12.18.19]. The association of anomalies like CHD, omphalocele and cystic hygroma with aneuploidy, is well known[18,20-24]. Fetuses with ultrasonographic findings, like nuchal edema, choroid plexus cysts and mild hydronephrosis, have increased risk of chromosome aberrations[23, 25-27]. The rapidly accumulating experience with fetal ultrasonography has led to the recognition of a whole spectrum of chromosomal markers, including dysmorphic facial features and limb deformities, as well as specific anomalies and combinations of anomalies. Intrauterine growth retardation and altered biometric measurements are also used as markers for the prediction of chromosome aberrations[22].

Up to 50% of early spontaneous pregnancy losses in the first trimester are associated with chromosome aberrations[2-5]. An early sonographic examination will increase the chance to detect anomalies in fetuses with chromosome aberrations[3, 22, 28]. We may expect to discover additional markers for aneuploidy in the future. Our observation that the gestational age of the fetuses with Turner syndrome was lower than for some of the other with chromosome aberrations is in good accordance with the results of other studies[19] and is related to the early diagnosis of hydrops and cystic hygroma[12,29].

The incidence of known chromosome aberrations in our study was 24%. Since 27% of cases with congenital anomalies were not karyotyped, the true incidence of abnormal karyotypes might be higher. Our study compares favorably with the results in a study by Gagnon et al. where chromosome abnormalities were found in 22% of cases with congenital anomalies[30]. Furthermore, the distribution of the different categories of numerical chromosome abnormalities and the incidence of structural abnormalities are comparable with those of a recent, comprehensive report[19]. Thirty-three percent of the karyotyped fetuses had a chromosome aberration, a prevalence we consider high. Ultrasonographers and sonologists are becoming increasingly aware of anomalies associated with specific chromosome abnormalities, which can guide them in their search for additional markers[12,19,31].

Nuchal translucency as a sign of nuchal edema or cystic hygroma is the most consistent ultrasonographic marker for chromosome aberrations[20,23,25,32,33]. Fetal aneuploidy in cystic hygromas varies with incidences from 22-90%[12,25,27,34,35]. In the present study 39 (40%) of the 98 fetuses and infants had cystic hygroma and/or fetal hydrops; this figure corresponds with those of other studies[19,25]. Such findings, combined with a maternal age risk, may identify more than 80% of fetuses with trisomy 21[26,34]. Prevalences for chromosome abnormalities based on maternal age have been estimated and found to be helpful in determining the significance of ultrasonographic markers[26,34,36]. Nuchal cystic hygroma is associated with Turner syndrome and nuchal edema with trisomies in addition to various other conditions[25,26,37]. In our study, cystic hygroma occurred in all patients with Turner syndrome. In addition, almost two thirds of the patients with trisomy 21 had nuchal edema or cystic hygroma.

CHD was the most frequent disorder found in fetuses with chromosome abnormalities. The high frequency (almost 90%) of CHD in fetuses with trisomy 18, and in trisomy 21 fetuses (almost 50%), is in good accordance with other series[38-42]. The most common cardiac lesion seen in trisomy 18 is VSD[40,43], which in our study comprised the majority (85%) of CHD. Likewise, AVSD or VSD occurred in 82% of the heart defects in the cases with trisomy 21. Obviously, AVSD is a marker at the routine ultrasound examination which leads to karyotyping and detection of trisomy 21.

After Down syndrome, trisomy 18 is the second most common autosomal trisomy in liveborns[42,44,45]. There were more fetuses with trisomy 18 than trisomy 21 in this series which can be explained by the higher detection rate of trisomy 18 than trisomy 21 at the routine ultrasound examination.

As we have shown, almost half of the discrepancies between the prenatal diagnoses and postmortem examination concerned minor anomalies, among these small VSDs and syndactylies[19,46]. In 1988 the four-chamber view was introduced to the routine ultrasound examination, facilitating the detection of septal defects[47]. Structural anomalies like horseshoe kidneys may be extremely difficult to detect by ultrasound examination[21,48], and was misinterpreted in one of the cases. Some anomalies such as choroid plexus cysts and nuchal edema may have no clinical implications, but their presence will trigger an observant ultrasonographer to look for other possible chromosomal markers. They can both be transitory, and thus not present at autopsy[36,49]. With improved techniques and skills in examinating the outflow tract, aortic coarctation has become easier to find ultrasonographically[50]. Oesophageal atresia with tracheo-oesophageal fistula is difficult to detect but may be diagnosed in utero[19,51,52]. All the cases with preaxial upper limb reduction were diagnosed prenatally. Among chromosome aberrations in humans, radial aplasia is almost unique to trisomy 18 except for cases with deletion of the long arm of chromosome 4[53]. In 6 cases major pathological autopsy findings were not detected sonographically, but in all of them other anomalies prompted karyotyping, and the management was based on the abnormal karyotype.

In one case of triploidy where dysmorphic features and a sacral bifid spine were overlooked at the routine ultrasound examination, a caesarean section could have been avoided if the fetal karyotype had been known, otherwise no action was taken altering the pregnancy management. It is still important to map all anomalies as beeing aware of even small deviations can trigger attention towards a chromosome aberration.

The list of sonographically detectable anomalies associated with autosomal trisomies is long, particularly for trisomy 18[44]. Thus, it can become rather laborious and time consuming to carefully scrutinize all the possibilities for structural alterations. Having found several anomalies indicating a chromosome aberration, there may be a risk of putting less emphasis into finding all accessory anomalies. In our study, minor anomalies in fetuses with trisomy 18 seemed to be more frequently overlooked.

Anomalies can also be overlooked at autopsy because the pathologist is not able to identify them or because the fetus has become seriously traumatized or macerated. Four of the 5 patients with ultrasound findings not confirmed at autopsy, were fetuses with trisomy 18, in two of these cases the fetus was macerated and the anomaly not possible to verify. In these cases the accordance between the ultrasound and postmortem examination must be interpreted with caution. Trisomy 13, trisomy 21 and Turner syndrome were in most cases correctly diagnosed. Two cases with Klinefelter syndrome were without structural anomalies, the karyotyping was performed because of advanced maternal age.

## CONCLUSION

Most chromosome aberrations, except trisomy 21, occurring in connection with structural anomalies are lethal. Learning to suspect or recognize a chromosome abnormality when dealing with anomalous fetuses has implications for further management. In particular, chromosomal markers are important in order to trigger our attention towards the possibility of an abnormal karyotype. The present comparison of ultrasonographic diagnoses with postmortem findings shows an overall good accordance between the two methods and confirms the importance of awareness of the variety of anomalies encountered in fetuses with an abnormal karyotype.

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**Table 1 Abnormal karyotypes (n=98)**

| Chromosome abnormality | 1985-89 n | 1990-94 n | 1985-94 n |
|---|---|---|---|
| | Time period | | |
| Trisomy 13 | 2 | 7 | 9 |
| Trisomy 18* | 5 | 26 | 31 |
| Trisomy 21 | 6 | 17 | 23 |
| 69, XXX/XXY | 4 | 4 | 8 |
| 45, X | 6 | 8 | 14 |
| 47, XXX | 1 | 1 | 2 |
| 47, XXY | | 2 | 2 |
| Other karyotypes** | 2 | 7 | 9 |
| Total | 26 | 72 | 98 |

* Includes 2 cases with partial trisomy 18 ( 46 XY,18p+ and 46 XX, 18p+)

** Other karyotypes:

46 XX/46 XX, -13, + mar? (13)
46 XX, der (14), p (2;14) (p13;q32)
46 XX, der (2) t (2;14) (q33;q24)
46 XY, der (4) t (4;5) (q34;q33)
47 XY, der (13) t (3,13) (q29;q21.2)
46 XX, del (9) (q35)
46 XY, inv (4) (q21; q27)
46 XX, 11p+
46 XY, der (11) t (8;11) (q24.11;q25)

**Table 2 Distribution of findings in relation to abnormal karyotype**

| | Trisomy 13 n=9 | Trisomy 18 n=31 | Trisomy 21 n=23 | Triploidy n=8 | 45,X n=14 | Other karyotypes n=13 | Total n=98 |
|---|---|---|---|---|---|---|---|
| No structural anomaly | | | 5 | | | 5 | 10 |
| Nuchal edema/cystic hygroma/ fetal hydrops | 3 | 5 | 15 | | 14 | 2 | 39 |
| Congenital heart defects | 5 | 27 | 11 | 4 | 2 | 4 | 53 |
| Urinary system anomalies | 4 | 11 | 2 | 4 | 4 | 1 | 26 |
| Central nervous system anomalies | 5 | 10 | 1 | 4 | | 2 | 22 |
| Skeletal deformities | 1 | 12 | 1 | 3 | | 1 | 18 |
| Omphalocele | 2 | 10 | | 2 | | | 14 |
| Gastro-intestinal anomalies | 1 | 6 | | 1 | | | 8 |
| Diaphragmatic hernia | 2 | 3 | | | | 1 | 6 |
| Omphalocele and diaphragmatic hernia | 1 | 2 | | | | | 3 |
| Genital anomalies | 2 | 1 | | | | | 3 |
| Miscellaneous | 1 | 1 | | 1 | | 1 | 4 |
| **Total** | **27** | **88** | **35** | **19** | **20** | **17** | **206** |

**Table 3 Trisomy 13: distribution of chromosomal markers and combinations of anomalies detected by ultrasound (US) and autopsy**

**Trisomy 13 (n=9)**

| **Chromosomal markers** | US | Autopsy | Missed at US |
|---|---|---|---|
| Low-set ears | 1 | 1 | |
| Small chin | 4 | 4 | |
| Cleft lip/palate | 3 | 3 | |
| Syndactyly | 0 | 1 | 1 |
| Polydactyly | 3 | 4 | 1 |
| Club feet | 2 | 2 | |
| Fetal hydrops | 3 | 3 | |
| Single umbilical artery | 3 | 3 | |
| **Other anomalies** | | | |
| CNS anomalies and CHD | 3 | 3 | |
| CHD/omphalocele | 2 | 2 | |
| CHD/ diaphragmatic hernia | 1 | 1 | |

CNS: central nervous system
CHD: congenital heart defect

**Table 4 Trisomy 18: distribution of chromosomal markers detected by ultrasound (US) and autopsy**

**Trisomy 18 (n=31)**

| Chromosomal markers | US | Autopsy | Missed at US | Not confirmed at autopsy | Full agreement |
|---|---|---|---|---|---|
| Abnormal head shape | 10 | 5 | | 5 | |
| Small mouth and nose | 3 | 4 | 1 | | |
| Low-set ears | 10 | 17 | 7 | | |
| Small chin | 9 | 13 | 4 | | |
| Cleft lip/palate | 2 | 2 | | | 2 |
| Syndactyly | | 2 | 2 | | |
| Polydactyly | | 1 | 1 | | |
| Clenched fists | 10 | 11 | 1 | | |
| Rocker bottom feet | 3 | 3 | | | 3 |
| Club feet | 8 | 7 | | 1 | |
| Choroid plexus cyst | 9 | 2 | | 7 | |
| Nuchal edema | 8 | 4 | | 4 | |
| Single umbilical artery | 5 | 7 | 2 | | |

**Table 5 Chromosomal abnormalities and discrepancies between ultrasound and autopsy findings**

| Case nr. | Final diagnosis after autopsy | Sex | GA (weeks) at autopsy | Mode of death/ delivery | Autopsy findings not recognized at ultrasound examination (cat.2,3,4) Ultrasound findings not confirmed at autopsy (cat.5,6) | Category |
|---|---|---|---|---|---|---|
| 1 | Trisomy 21, fetal hydrops, aortic coarctation | M | 25 | TOP | Aortic coarctation | 2 |
| 2 | Trisomy 18, Arnold-Chiari malformation, cystic hygroma, syndactyly right 3rd and 4th finger, bilateral clubfeet, horseshoe kidney | F | 19 | TOP | Syndactyly right 3rd and 4th finger, clubfeet, horseshoe kidney | 2 |
| 3 | Trisomy 18, omphalocele, VSD, bilateral radial aplasia, low set ears, horseshoe kidney | M | 19 | TOP | VSD 2mm, horseshoe kidney | 2 |
| 4 | Trisomy 13, cleft lip/palate, bilateral polydactyly lower extremities | F | 26 | TOP | Polydactyly | 2 |
| 5 | Trisomy 18, IUGR, Dandy-Walker malformation, hypoplasia of corpus callosum, tetralogy of Fallot | M | 32 | TOP | Tetralogy of Fallot with pulmonary stenosis | 3 |
| 6 | Trisomy 18, omphalocele, radial aplasia, VSD, overriding aorta | M | 16 | TOP | VSD and overriding aorta | 3 |
| 7 | Triploidy, IUGR, dysmorphism, sacral bifid spine, club feet, placental insufficiency | F | 34 | LB | Dysmorphism and sacral bifid spine not registered at the US examination | 4 |
| 8 | Partial trisomy 18 (46, XX, 8p+), dysmorphism, nuchal edema, aortic coarctation, ureteropelvic stenosis | F | 22 | TOP | Aortic coarctation, ureteropelvic stenosis | 2 |
| 9 | Trisomy 21, nuchal oedema, VSD, bilateral peripheral lung infarcts with pleural effusion | F | 17 | TOP | VSD | 2 |
| 10 | Trisomy 18, cystic hygroma, omphalocele, VSD, cleft lip/palate, club foot right side | M | 14 | TOP | VSD 1.5 mm | 2 |
| 11 | Turner syndrome, IUGR, cystic hygroma, fetal hydrops, ASD primum, horseshoe kidney | F | 17 | IUFD | ASD primum, horseshoe kidney | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | Trisomy 18, VSD, hypoplastic right ventricle, tricuspid and pulmonary atresia, oesophageal atresia with tracheoesophageal fistula, anal atresia, ureteropelvic atresia, syndactyly right 3.and 4. toe | F | 35 | LB | Oesophageal atresia with tracheo-oesophageal fistula, ureteropelvic atresia, syndactyly right 3.and 4. toe | 3 |
| 13 | Triploidy, IUGR, holoprosencephaly, lumbosacral myelomeningocele, omphalocele, left renal agenesis, renal dysplasia right side with ureteral atresia and hypoplastic bladder, VSD, syndactyly right 3rd and 4th finger | F | 22 | TOP | VSD, omphalocele, left renal agenesis, syndactyly | 3 |
| 14 | Trisomy 18, Arnold-Chiari malformation, omphalocele, left diaphragmatic hernia, oesophageal atresia with fistula, VSD, overriding aorta, right radial aplasia, bilateral club feet | M | 18 | TOP | Oesophageal atresia with fistula, diaphragmatic hernia and omphalocele | 3 |
| 15 | Trisomy 18, IUGR, Dandy-Walker malformation, omphalocele, left diaphragmatic hernia, ASD secundum, VSD, bilateral radial aplasia, rocker bottom feet | M | 18 | TOP | Left diaphragmatic hernia | 3 |
| 16 | Trisomy 21, VSD | M | 19 | TOP | AVSD at US not verified at autopsy | 5 |
| 17 | Trisomy 18, IUGR, Arnold-Chiari malformation, VSD, clenched fingers, bilateral club feet, horseshoe kidney | M | 29 | TOP | Horseshoe kidney interpreted as right renal agenesis and left renal dysplasia | 5 |
| 18 | Trisomy 18, cystic hygroma, fetal hydrops, VSD, bilateral radial aplasia, bilateral club feet | M | 18 | TOP | Bilateral hydronephrosis with possible dysplastic kidney changes not confirmed at autopsy | 5 |
| 19 | Trisomy 18, AVSD probable (macerated fetus) | M | 13 | TOP | AVSD on US not confirmed at autopsy | 6 |
| 20 | Trisomy 18, fetal hydrops | F | 12 | TOP | Fragmented fetus not possible to evaluate | 6 |

GA: gestational age
US: ultrasound
TOP: termination of pregnancy
LB: liveborn
IUFD: intrauterine fetal death
IUGR: intrauterine growth retardation
ASD: atrial septal defect
VSD: ventricular septal defect
ASVD: atrioventricular septal defect

**Table 6 Chromosome abnormalities: correlation prenatal and postnatal findings (n=98)**

| Category | 1985-89 | | 1990-94 | | 1985-94 | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| 1) Full agreement | 19 | 73 | 59 | 82 | 78 | 80 |
| 2) Minor autopsy findings not detected by ultrasound | 4 | 15 | 4 | 5,5 | 8 | 8 |
| 3) Major autopsy findings not detected by ultrasound | 2 | 8 | 4 | 5,5 | 6 | 6 |
| 4) No autopsy findings suspected by ultrasound | 1 | 4 | 0 | 0 | 1 | 1 |
| 5) Minor ultrasound findings not confirmed at autopsy | 0 | 0 | 3 | 4 | 3 | 3 |
| 6) Major ultrasound findings not confirmed at autopsy | 0 | 0 | 2 | 3 | 2 | 2 |
| Total | 26 | 100 | 72 | 100 | 98 | 100 |

**Table 7 Correlation prenatal and postnatal findings**

| Karyotype | TOTAL | Full agreement | | Minor autopsy findings not detected by ultrasound | | Major autopsy findings not detected by ultrasound | | None of the autopsy findings suspected by ultrasound | | Minor ultrasound findings not confirmed at autopsy | | Major ultrasound findings not confirmed at autopsy | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | n | % | n | % | n | % | n | % | n | % | n | % |
| Trisomy 13 | 9 | 8 | 89 | 1 | 11 | | | | | | | | |
| Trisomy 18 | 31 | 18 | 58 | 4 | 13 | 5 | 16 | | | 2 | 7 | 2 | 6 |
| Trisomy 21 | 23 | 20 | 87 | 2 | 9 | | | | | 1 | 4 | | |
| 69,XXX | 8 | 6 | 75 | | | 1 | 12. 5 | 1 | 12.5 | | | | |
| 45,X | 14 | 13 | 93 | 1 | 7 | | | | | | | | |
| Other karyotypes* | 13 | 13 | 100 | | | | | | | | | | |
| Total | 98 | 78 | 80 | 8 | 8 | 6 | 6 | 1 | 1 | 3 | 3 | 2 | 2 |

*47, XXX; 47, XXY; se footnote** Table 1

# PAPER V

**Detection of Trisomy 18 on Formalin-Fixed and Paraffin-Embedded Material by Fluorescence in situ Hybridization (FISH)**

Christina Vogt Isaksen*, Borgny Ytterhus, Sølvi Skarsvåg

Department of Laboratory Medicine and Department of Pathology, Norwegian University of Science and Technology, Trondheim University Hospital, Trondheim, Norway

*Corresponding author: Christina Vogt Isaksen
Department of Pathology
Regionsykehuset i Trondheim
7006 Trondheim, Norway
tel. +47 73868653
fax +47 73868654
e-mail: Christina.Isaksen@medisin.ntnu.no

Short title: Detection of trisomy 18 by FISH

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**Abstract**

Formalin-fixed and paraffin-embedded autopsy material from 10 fetuses and infants with unknown karyotype and anomalies suggestive of trisomy 18 were subjected to fluoresence in situ hybridization (FISH). Nuclei were extracted from the tissues and hybridized with a chromosome-18-specific centromere probe. The hybridization was successful in 9 of 10 cases. Two cases showed 3 hybridization signals in the majority of the nuclei (74% and 85%). These had anomalies frequent for trisomy 18 (congenital heart defect, omphalocele and horseshoe kidney). Two cases showed a mixture of 2 and 3 signals (47/49% and 59/36%), suggesting the possibility of mosaicism. One of these cases had anomalies consistent with a trisomy 18 phenotype. In the other case intrauterine growth retardation and syndactylies suggested triploidy. Hybridization with a chromosome-8-specific probe gave a distribution of 2 and 3 signals (34% and 62%, respectively). This result strengthened the suspicion of a possible triploid mosaicism. In 5 of the cases the majority of the nuclei showed 2 signals (85% to 88%). However, as only one type of tissue was examined for enumeration of chromosome 18, the possibility of organ mosaicism or other chromosomal aberrations cannot be excluded. The FISH technique is applicable on macerated and autolysed formalin-fixed tissue, making it possible to retrospectively analyse autopsy material from aborted and stillborn fetuses and infants. This contributes to a better quality of perinatal autopsies and is helpful in further parental counselling.

Key words: fluorescence in situ hybridization, trisomy 18

**Introduction**

Most chromosomal aberrations in the fetus are associated with anomalies [1-4]. The likelihood of a chromosomal abnormality is increased when multiple anomalies are present [5,6]. After Down syndrome, trisomy 18 is the most common autosomal trisomy in liveborns with an estimated incidence between 1 in 3000 and 1 in 11000 [7,8-11]. Since Edwards et al. described trisomy 18 [12], more than 130 single abnormalities have been reported in conjunction with this condition [8,13]. The most common are dysmorphic facial features, overlapping fingers, congenital heart defects, omphalocele and horseshoe kidney [14].

Prenatal karyotyping is traditionally performed on cultured cells from amniotic fluid, cord blood or chorionic villi. The main indications for doing a cytogenetic investigation are high maternal age, family history of inheritable genetic disease, and anomalies detected at a prenatal ultrasound examination. Ultrasound findings such as nuchal edema, hydrops and dysmorphic features as well as CHD together with an omphalocele are common indications for karyotyping [3,15,16].

Some spontaneously aborted fetuses and infants suffering an intrauterine or perinatal death may have anomalies suggestive of a chromosomal aberration. In such cases classical chromosomal studies are difficult due to lack of viable fetal cells. From an epidemiological and counselling viewpoint the presence of a genetic defect in such cases is important to verify.

Detection of chromosomal aberrations in archival formalin-fixed material is possible by fluorescence *in situ* hybridization (FISH) [17,18-21]. FISH is a molecular cytogenetic technique which allows detection of specific numerical and structural chromosomal aberrations in interphase nuclei [18,19,22,23]. The clinical utility of this method in perinatal pathology is primarily aneuploidies (trisomies, monosomies and polyploidies) [24]. In fetuses and infants with multiple anomalies and unknown karyotype, the use of FISH on archival material in order to screen for of the most common chromosomal aneuploidies is important in view of the consequences for the parents (low recurrence risk, prenatal diagnosis in subsequent pregnancies) [17].

The aim of this study was to enumerate chromosome 18 in an autopsy series of fetuses and infants with congenital anomalies suggestive of trisomy 18, but not previously karyotyped. Extracted nuclei from the autopsy material were hybridized with a chromosome-18-specific centromere probe. Fetuses and infants prenatally karyotyped by traditional cytogenetics were included as controls.

## Material and Method

Paraffin-embedded tissue from autopsies of 17 fetuses and infants with congenital anomalies were subjected to chromosome enumeration by FISH. Termination of pregnancy was performed in 7 cases, in 2 cases an intrauterine fetal death occurred and in 8 cases the infant was liveborn. The gestational age varied from 17-40 weeks, with an average of 26.4 weeks. The time elapsed from abortion/death to autopsy, varied between 1 and 5 days.

Study group:

Ten cases with unknown karyotype having anomalies suspicious of trisomy 18, were selected for FISH. Details of clinical information with dysmorphic features and anomalies are given in Table 1. The time elapsed from autopsy to the present investigation varied between 5 and 13 years.

Control group:

Paraffin-embedded material from seven fetuses and infants prenatally karyotyped by traditional cytogenetic methods were used as controls. Four had trisomy 18, one partial trisomy 18 and 2 had a normal karyotype. Three were examined blindly.

FISH:

All slides from the autopsies were reviewed and the degree of tissue maceration and autolysis evaluated by light microscopy. The thymus was usually better preserved than other organs and was therefore chosen for FISH. When thymus was not available, material from spleen, kidney or testis was used. The FISH technique was performed as described by Köpf et al. [21] using a chromosome-18-specific probe (CEP 18 Alpha-Satellite Green, Vysis, Cat.no. 32-132018, Vysis Inc., Downers Grove, IL, U.S.). The signals were simultaneously counted by two persons in a double fluorescence microscope (Zeiss Axiophot) at 400x magnification, following the recommendations of the producer. Two signals of the same size in close proximity not connected by a link, were counted as two signals. A diffuse signal was regarded as a signal if it was contiguous and within an acceptable boundary. Two small signals connected by a visible link were counted as one signal. Nuclei with zero signals were counted only if the other nuclei in the field of view had signals. Overlapping nuclei and nuclei with uncertain signals were not counted. Two hundred nuclei were counted in each case. The hybridization was regarded as a failure if more than 5% of the enumerated nuclei lacked signals.

**Results**

The hybridization results are summarized in Table 1. In cases 1 and 3, three signals were present in the majority of the nuclei (74% and 85% respectively) indicating trisomy 18 (Fig. 1a). Case 4 and 6 showed a mixture of two and three signals suggesting the possibility of mosaicism of trisomy 18. The distribution between nuclei with two and three signals was 47/49% and 59/36% respectively. Five cases demonstrated two signals in the majority of the nuclei (86 to 89%) (Fig.1b). In case 9 successful hybridization was not obtained, even after several post treatments with protease as recommended by Köpf et al. [21]. Three of the cases gave signals when rehybridized once and 3 cases were treated with protease and rehybridized twice before they showed acceptable signals.

Of the 7 cases with known karyotype, 4 were correctly identified as trisomy 18, and 3 were correctly identified as disomy, including one with partial trisomy 18 (46XX, 18p+) not detectable with the centromere probe used. Seventy-six to 88% of the nuclei from the cases with trisomy 18 showed 3 signals. The corresponding figures in the other samples ranged from 88 to 90%.

The degree of tissue maceration and autolysis varied from slight to marked (Table 1). There was no correlation between the observed degradation of the material and the FISH results. The only case where hybridization was not achived was on material from a liveborn term infant who lived for 45 minutes. The autopsy was performed 2 days after death and there was little autolysis. The storage time of the paraffin blocks did not influence the hybridization results. We do not know the length of formalin-fixation before paraffin embedding was performed.

**Discussion**

The ten fetuses and infants with unknown karyotype having anomalies suggestive of trisomy 18, were selected for FISH on the basis of type of anomaly or combinations of anomalies. Previous studies have documented the use of FISH on archival autopsy material [17-21,25-27] which was confirmed in the present study.

The hybridization results were compatible with the dysmorphic features and anomalies described in the different cases. In cases 1 and 3 where the majority of the nuclei showed 3 signals indicating trisomy 18, there were dysmorphic facial features such as low-set ears, small mouth and micrognathia, in addition to omphalocele, VSD and horseshoe kidneys, anomalies characteristic of trisomy 18. Dysmorphic facial features, omphalocele, and horseshoe kidney occur in almost one third of cases with trisomy 18 [14,28-30] and CHD in over 90% [14,29,31-33]. The combination of omphalocele and VSD is frequent, and is observed to occur in over one third of trisomy 18 cases [34,35].

In case 4 and 6 the nuclei showed a combination of 2 and 3 signals which may suggest trisomy 18 mosaicism. These results were verified by repeated hybridizations. Case 4 presented with clenched fingers, VSD and horseshoe kidney, but did not have an omphalocele. Case 6 had neither CHD, horseshoe kidney nor an omphalocele. Extreme growth retardation, as well as syndactylies, are findings characteristic of triploidy. In order to investigate this possibility, hybridization with a chromosome-8- specific probe (CEP 8 Spectrum Orange, Vysis Cat.no. 30-160008, Vysis Inc., Downers Grove, IL, U.S.) was later performed. Thirty-four percent of the nuclei showed 2 signals and 62% showed 3 signals strengthening the clinical suspicion of a possible triploidy or triploid mosaicism.

Ten percent of fetuses with an additional chromosome 18, are reported to be mosaics [10,36]. The anomalies present in complete and mosaic trisomy 18 have been described as similar [37] though neonates with mosaic trisomy 18 are said to be less severly affected with increased viability [38, 39]. Some individuals may be tissue-specific mosaics [40]. In our study only one type of tissue from each case was examined and organ mosaicism can therefore not be excluded.

Cases 2, 5, 7, 8 and 10 had one or more anomalies that can be found in trisomy 18, though neither the findings nor the constellation of anomalies were as characteristic of this aneuploidy as in cases 1 and 3. Thus the observed disomy 18 was not found contradictory to the clinical findings. However, in this study only enumeration of chromosome 18 was performed, and the possibility of organ mosaicism, structural abnormalities of chromosome 18 or other chromosomal aberrations cannot be excluded. The advantage of FISH in screening for the most common aneuploidies (trisomy 13, 18, 21, triploidy and Turner syndrome) is limited by the fact that these constitute 60-70% of all chromosomal aberrations [41-43].

Other groups have excluded the use of macerated or autolysed cases [44]. In this study, we experienced that the simplified protocol published by Köpf et al. [21] worked on different tissues with different degrees of autolysis, observed by light microscopy. A successful FISH result is consistent with clear signals in the majority of the nuclei (over 95%). The efficiency of detection is dependent on the material subjected for FISH. Acceptable results are more easily obtained on fresh material compared to paraffin-embedded and formalin-fixed tissues [20,45]. Autopsy specimens that are macerated and autolysed before fixation, are less suitable for FISH than material fixed freshly.

Signal registration and counting are two important factors when interpreting the results. In this study we have strictly followed the producers recommendations when enumerating signals in each nucleus. Some autopsy specimens demonstrated split or scattered signals which were difficult to interpret. Therefore, signals were counted simultaneously by two persons in a double microscope.

Of the 7 cases with known karoytype, 76-90% of the nuclei showed the expected number of signals. When analysing the cases with unknown karyotype, at least 70% of the nuclei had to show 2 or 3 signals in order to be classified as disomy or trisomy, respectively. In other studies performed on formalin-fixed and paraffin-embedded material these limits have varied between 33-98% [18,20,27]. One case with 3 signals in 11% of the nuclei and 2 signals in 65% was considered a mosaic [18]. Shashi et al. have in a case study reported that 17% of the cells from a liver biopsy with unknown karyotype demonstrated 3 signals, a result interpreted as organ mosaicism [27]. Mosaic cases can therefore represent a problem of interpretation. In our study, two cases showed a mixture of 2 and 3 signals (47/49% and 59/36%, respectively). These results were reproduced and the two cases regarded as possible mosaics. Recently, pilot studies for proficiency testing using FISH with chromosome-specific probes have shown high concordance between laboratories [46]. Improved protocols and probes have increased the detection efficacy, leading to expanded diagnostic applications of FISH.

In spontaneous abortions, stillbirths and perinatal deaths, the fetus or infant can have features or anomalies strongly suggestive of a chromosomal aberration. The application of FISH to interphase cells screening for the most common aneuploidies, trisomy 13, 18 and 21, triploidy and Turner syndrome has a large potential on fresh, frozen and formalin-fixed material [26,43,45]. Performing FISH on autopsy material is valuable in cases where karyotyping on viable cells has not previously been performed. The recurrence risk of an anomalous fetus is lower in cases with numerical chromosomal aberrations than in some of the other conditions associated with congenital anomalies [17]. This is of importance in counselling the parents, reassuring them of the sporadic nature of the detected chromosomal aberration. Another advantage of FISH is the use on uncultured amniotic cells [24,42,47-49]. In cases with fetal anomalies and advanced gestational age, a rapid preliminary result can aid in better management [24,50,51]. However, standard chromosomal analysis should always serve as the primary and confirmatory evaluation [50,52].

**Conclusion**

In this study we have performed FISH on autopsy material in order to detect trisomy 18 retrospectively in cases where karyotyping had not previously been performed. A previously described protocol was used, giving acceptable signals in 16 out of 17 study and control cases. The FISH technique can be a supplementary tool in the diagnosis of certain chromosomal aberrations, contributing to a better quality of perinatal autopsies and also be of help in further parental counselling.

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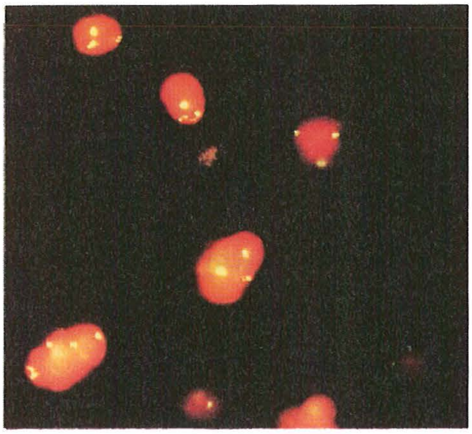
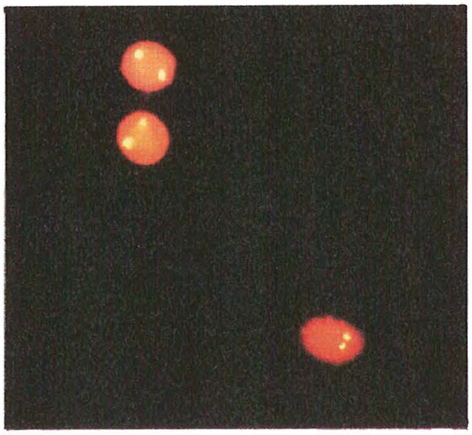
Figure 1 FISH using chromosome-18-specific probe on formalin-fixed autopsy material



a) Nuclei showing 3 signals consistent with trisomy 18



b) Nuclei showing 2 signals consistent with disomy for chromosome 18

**Table 1 Fetuses and infants with congenital anomalies suspect of trisomy 18**

| Case no | GA | Clinical data | Suspected clinical diagnosis | Organ examined and degree of autolysis* | | FISH result | |
|---|---|---|---|---|---|---|---|
| | | | | | | Number of signals | Percentage (200 nuclei counted) |
| 1 | 40 w | LB. IUGR, dysmorphic facial features, VSD, coarctation of aorta, omphalocele, skeletal anomalies, clubfeet, horseshoe kidney, single umbilical artery. Died 1 hour after delivery | Trisomy 18 (suspected at birth) | Kidney | ++ | 3 | 74 |
| 2 | 39 w | IUFD. Hydrocephalus, aqueductal stenosis? agenesis left kidney, VSD | | Thymus | ++ | 2 | 86 |
| 3 | 18 w | TOP. Low-set ears, micrognathia, rocker-bottom feet, choroid plexus cysts, ASD, VSD, hypoplastic right ventricle with tricuspidal atresia, omphalocele, horseshoe kidney | Trisomy 18 (suspected at US examination) | Spleen | + | 3 | 85 |
| 4 | 30 w | IUGR. Dysmorphic facial features (low-set ears, small mouth), clenched fingers, VSD, horseshoe kidney. Died with HMD 3 days old | Trisomy 18 (suspected at autopsy) | Thymus | +++ | 2+3 | 47/49 |
| 5 | 23 w | TOP. Cleft lip/palate, low-set ears, micrognathia, large VSD and pulmonary atresia, bilateral renal dysplasia with hydroureter and hydronephrosis left side, skeletal dysplasia, Saldino-Noonan type? | | Thymus | +++ | 2 | 85 |
| 6 | 30w | LB. IUGR, dysmorphic facial features (low-set ears, micrognathia), narrow chest and hypoplastic lungs without lobular division, syndactyly upper extremities and right lower extremity. Died within 24 hours | Trisomy 18 (suspected at birth) | Thymus | + | 2+3 | 59/36 (18)<br>34/62 (8) |
| 7 | 37 w | LB, hypoplastic left ventricle with mitral atresia. Lived 1 day | | Thymus | + | 2 | 85 |
| 8 | 22 w | TOP. Hydrocephalus, bilateral radial aplasia, left renal agenesis, double ureter right side | | Thymus | + | 2 | 88 |
| 9 | 40 w | LB. VSD, hypoplastic left ventricle and double inlet right ventricle. Lived for 45 minutes | | thymus, spleen | + | 0 | not successful |
| 10 | 17 w | IUFD. Deformed ears and nose, small mouth and cleft lip/palate, anal atresia, hydronephrosis right kidney | | testis | +++ | 2 | 88 |

**Abbreviations:** GA: gestational age
LB: liveborn
IUGR: intrauterine growth retardation,
VSD: ventricular septal defect
IUFD: intrauterine fetal death
TOP: termination of pregnancy
ASD: atrial septal defect
US: ultrasound
HMD: hyaline membrane disease
* Degree of autolysis: + slight; ++ moderate; +++ marked

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