Neonatal Outcome Based on Triple Serum Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18: Is Amniocentesis Always Necessary?

Loralee Hope Davison  
*Grand Valley State University*

Molly Rachel Kerr  
*Grand Valley State University*

Samantha Ann Mullin  
*Grand Valley State University*

Follow this and additional works at: [http://scholarworks.gvsu.edu/theses](http://scholarworks.gvsu.edu/theses)

**Recommended Citation**  
[http://scholarworks.gvsu.edu/theses/502](http://scholarworks.gvsu.edu/theses/502)
Neonatal Outcome Based on Triple Serum Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18: Is Amniocentesis Always Necessary?

By

Loralee Hope Davison
Molly Rachel Kerr
Samantha Ann Mullin

THESIS

Submitted to the Physician Assistant Program at Grand Valley State University Allendale, Michigan in partial fulfillment of the requirements for the degree of

MASTER OF PHYSICIAN ASSISTANT STUDIES

2000
Neonatal Outcome Based on Triple Serum Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18: Is Amniocentesis Always Necessary?

ABSTRACT

OBJECTIVE: Our goal was to evaluate whether a normal ultrasound following a positive triple marker serum screen reduces the risk of having a trisomy 18 fetus to a level that is less than the procedure-related risk for amniocentesis. STUDY DESIGN: A nonrandomized, non-concurrent cohort chart review was performed of all women who screened positive (risk > 1:250) for trisomy 18 by triple marker serum testing through Spectrum Health Downtown Genetic Screening Program from June 1996 through September 1999. Of the 13,618 serum samples screened, 158 were identified as being at an increased risk for trisomy 18. Pediatric outcome was obtained through office documentation, review of birth records, or phone contact with the mother. RESULTS: Four cases of trisomy 18 were identified and all had abnormalities on ultrasound. CONCLUSION: The presence of normal ultrasound was not associated with significant increased risk for trisomy 18. In this population, amniocentesis is not justified.
ACKNOWLEDGEMENTS

We would like to extend our appreciation to the following individuals for graciously giving their time and assistance: Dr. Thomas Marks, Justine Ritchie, and Dr. Dawn DeWitt. We also wish to extend a special thank you to Dr. Curtis Cook, who gave us the opportunity to get involved with this project. His long hours of assistance in data collection and revision of the paper are greatly appreciated. Another influential person we wish to express our gratitude to is Dr. Johnine Callahan, our committee chairman. She joined us half-way through and has dedicated many hours in guidance and revision of our project. We would also like to thank Dr. William Bell whose leadership was essential during the proposal process. The support of all those involved helped provide a valuable learning experience.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>CHAPTER.</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Background to the Problem</td>
<td>1</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>3</td>
</tr>
<tr>
<td>Purpose</td>
<td>4</td>
</tr>
<tr>
<td>Significance of the Problem</td>
<td>5</td>
</tr>
<tr>
<td>2. REVIEW OF LITERATURE</td>
<td>7</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>7</td>
</tr>
<tr>
<td>Prenatal Screening</td>
<td>10</td>
</tr>
<tr>
<td>Triple Marker Screening</td>
<td>12</td>
</tr>
<tr>
<td>Ultrasonographic Findings</td>
<td>19</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>26</td>
</tr>
<tr>
<td>Summary</td>
<td>29</td>
</tr>
<tr>
<td>3. METHODOLOGY</td>
<td>31</td>
</tr>
<tr>
<td>Study Design</td>
<td>31</td>
</tr>
<tr>
<td>Study Site</td>
<td>32</td>
</tr>
<tr>
<td>Subjects</td>
<td>33</td>
</tr>
<tr>
<td>Equipment and Instruments</td>
<td>34</td>
</tr>
<tr>
<td>Validity and Reliability of Amniocentesis</td>
<td>35</td>
</tr>
<tr>
<td>Procedure</td>
<td>35</td>
</tr>
<tr>
<td>4. DATA ANALYSIS</td>
<td>37</td>
</tr>
<tr>
<td>Study Population</td>
<td>37</td>
</tr>
<tr>
<td>Techniques of data analysis</td>
<td>37</td>
</tr>
<tr>
<td>Characteristics of subjects</td>
<td>38</td>
</tr>
<tr>
<td>5. DISCUSSION AND IMPLICATIONS</td>
<td>39</td>
</tr>
<tr>
<td>Discussion of findings</td>
<td>39</td>
</tr>
<tr>
<td>Application of practice</td>
<td>40</td>
</tr>
<tr>
<td>Limitations and Recommendations</td>
<td>40</td>
</tr>
<tr>
<td>Conclusions</td>
<td>41</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>42</td>
</tr>
</tbody>
</table>

iii
APPENDIX A – Data Tables

Table 1 – Maternal Demographic Data: Comparison of patients with positive triple marker serum screen for trisomy 18

Table 2 – Neonatal Data: Comparison of fetal data from pregnant women with a positive triple marker serum screen for trisomy 18

APPENDIX B – Data Collection Forms

APPENDIX C – Proposal and Project Approval Forms
CHAPTER ONE
INTRODUCTION

Background to Problem

Prenatal screening has become an important part of the care of pregnant women in our society. The standard of care today includes routine testing for fetal anomalies in conjunction with regularly scheduled obstetric visits. Routine testing means that the available screening is offered to the patient by her practitioner as part of antenatal care. The patient then has the option to either accept or decline such testing after being informed of its risks and benefits.¹ Prenatal screening allows parents to make informed decisions regarding the care of their baby.²

One specific use of prenatal screening is the detection of certain chromosomal abnormalities such as trisomy 21 and trisomy 18. The current methods used for determining the fetal anomalies associated with trisomy 21 and trisomy 18 are: the maternal triple marker serum screen; ultrasonography; and amniocentesis. The triple marker screen is offered to the pregnant woman as a first option in screening for chromosomal aberrations. This screen is typically offered between 15 and 22 weeks gestation. The second trimester triple marker serum screen consists of: maternal β-human chorionic gonadotropin (β-hCG); unconjugated estriol; and alpha fetoprotein (AFP). These markers have been shown to be of value in evaluating pregnancies at risk for various anomalies and
aneuploidies. Second trimester maternal levels of AFP, unconjugated estriol, and \( \beta \)-hCG are all at lower than normal levels in the presence of trisomy 18, with estriol being the most sensitive. Triple marker serum screen testing has allowed earlier detection of potential defects than routine sonographic screening alone; however, the triple screen test has also led to the identification of a population of patients that require genetic counseling and may require additional testing.

Ultrasound is the other non-invasive test used to detect chromosomal abnormalities. This method of surveying the fetus allows for information to be obtained about the fetus and its environment. Specific anomalies can be viewed on ultrasound that correlate highly with trisomy 21 and trisomy 18. The incorporation of ultrasound as a prenatal screening tool may be related to improved perinatal and maternal outcome while avoiding the use of invasive procedures.

Amniocentesis is the “gold standard” test in that it provides the definitive diagnosis of chromosomal aberrations. Women who screen positive on a triple test are typically offered chromosomal analysis with amniocentesis; however, this test is an invasive procedure involving needle aspiration of amniotic fluid and cells. Amniocentesis is a procedure which is associated with complications for the mother and her unborn fetus. Although the risk of procedure-related pregnancy loss is small, it is important for women undergoing this invasive procedure to be informed of such risks.
Trisomy 18 is a chromosomal abnormality that results in an extra chromosome 18, causing a condition that is eventually lethal. The second trimester incidence of trisomy 18 is approximately 1:2400 with a birth incidence of approximately 1:8000. The difference in the incidence rates is due to a 70% fetal loss in the third trimester. Even after successful delivery, the prognosis remains poor with at most a 10% survival rate up to one year. This poor prognosis makes diagnosis of trisomy 18 important. Prenatal diagnosis would provide the patient with a variety of options including termination of the pregnancy, avoidance of cesarean section, delivery locally with conservative management, or emotional preparation for the delivery of an abnormal neonate.

Data suggests that ultrasound following a positive triple marker screen may provide guidance regarding the decision for definitive diagnosis with amniocentesis in trisomy 21. Very recent data suggests that this may also be possible for trisomy 18; however, studies published thus far concerning abnormal triple screen have indicated the need for further evaluation with ultrasound or ultrasound in combination with amniocentesis. This project will focus on the utilization of non-invasive prenatal screening to guide the decision for amniocentesis for the purpose of trisomy 18 detection.

Statement of Problem

Research involving the prenatal diagnosis of trisomy 18, without the use of amniocentesis, has been mostly inconclusive. Research exists on the sonographic findings of trisomy 18 and the parameters of triple marker serum screen, but little research has been conducted on the risk of trisomy 18 based on
the combination of these two antenatal tests. The combination of ultrasound with
the biochemical markers is thought to yield a more sensitive predictive risk of
fetal anomalies for women to base their prenatal decisions on. Many women in
institutions throughout West Michigan are currently being offered triple marker
screening as part of routine prenatal care. Women who are offered the option to
undergo maternal serum screening would be counseled on the significance of the
results so that they are able to make informed decisions on further testing. Those
women who screen positive for trisomy 18, which is defined as a risk greater than
1:250, typically undergo further evaluation by ultrasonography. The question
which arises is how to counsel women on the likelihood of trisomy 18 based on
ultrasound findings and abnormal triple screen. Specifically, does a normal
ultrasound further reduce the risk of trisomy 18 and possibly obviate the need for
invasive testing by amniocentesis?

**Purpose**

The purpose of our study is to reduce invasive prenatal testing with its
associated perinatal risks. Kellner et al. report a potential loss of 3 to 4 unaffected
fetuses out of a group of 700 patients as a result of amniocentesis. Older women
who have delayed childbearing and those that have had previous treatment for
infertility may not consider the risk of amniocentesis to be low enough to justify
detecting the chromosomal abnormality. We hope to reduce the amount of
invasive testing by determining whether a normal ultrasound following an
abnormal triple screen reduces the risk of trisomy 18 to the point where
amniocentesis would carry a greater risk than that of the chromosomal
abnormality. This information will contribute to a step-wise approach in evaluating pregnant women that have screened positive for trisomy 18.

**Significance of the Problem**

Prenatal diagnosis of chromosomal abnormalities is important in that it allows the practitioner to provide the patient with options. In this study, we are trying to determine the probability of the fetus having trisomy 18 with less invasive and less risky procedures. Unfortunately, the majority of genetic amniocenteses are performed on normal fetuses. The average pregnancy loss due to genetic amniocentesis is roughly 1 in 270. Therefore, it is important to determine techniques that define a more select group of candidates for invasive prenatal testing to decrease fetal losses due to amniocentesis. By reducing invasive procedures, there is a decreased risk to the fetus as well as a reduction in unnecessary health care expenditure. Therefore, we hope to decrease the size of the population that will need further invasive testing for definitive diagnosis of trisomy 18. As health care providers, education is a vital component in delivering quality patient care. This research will contribute information for providers to use in counseling patients who have an abnormal triple screen.

Further research is needed in detecting trisomy 18 with triple marker screening and ultrasound. Yankowitz et al. conducted a recent study to evaluate the efficacy of prenatal serum screening for trisomy 18. The investigators concluded that combining serum screening with detailed ultrasound might further define the population that would most benefit from invasive testing. We hope to extend the validity of the study with this objective in mind by investigating a new
sample population in a different geographical area. We will evaluate a population of patients at an increased risk for trisomy 18 based on mid-second trimester triple serum screening and review the patients' ultrasound findings. The expected outcome of this study is to determine how risk may be assessed on the basis of ultrasound findings.
CHAPTER TWO  
REVIEW OF LITERATURE  

Trisomy 18

Trisomy 18 is the second most common autosomal trisomy with a birth incidence between 1:7000 and 1:8000.\textsuperscript{16-18} The low birth incidence of trisomy 18 is directly related to the increased number (70\%) of spontaneous abortions in the third trimester of life.\textsuperscript{18} This relatively lethal condition is most often caused by a meiotic non-disjunction of chromosome 18.\textsuperscript{19} Similar to trisomy 21, the birth incidence of trisomy 18 increases with maternal age.\textsuperscript{18} Long term survival is rare in that approximately 50\% of trisomic 18 fetuses will die within 10 days to 2 months of birth and over 90\% by 100 days.\textsuperscript{3,17} An infant with trisomy 18 will rarely survive beyond one year since this condition is associated with multiple structural abnormalities and extreme growth retardation.\textsuperscript{16,18} These structural malformations can be seen and detected on ultrasonography.\textsuperscript{16,20}

Trisomy 18 remained an unrecognized entity until 1960 when the first full report of a child with trisomy 18 syndrome was described by Edwards, Harnden, Cameron, Crosse, and Wolff.\textsuperscript{21} At the same time, Patau, Smith, Therman, Inhorm, and Wagner reported the discovery of two infants with a similar syndrome; however, the researchers were only able to identify the extra chromosome as being from the E group which consists of chromosome numbers 16 to 18. In an addendum, they mentioned 4 more cases and were able to identify the
Subsequent reports of similarly affected infants soon followed. Patau, Therman, Smith, and DeMars compared the abnormalities of the two affected fetuses against the infant Edwards et al. had described and concluded that all of these cases represented the same disorder caused by an extra chromosome number 18. The most prominent clinical abnormalities noted in these trisomy 18 infants were: mental retardation with moderate hypertonicity; low-set malformed ears; small mandible; flexion of the fingers with the index finger overlapping the third; and severe failure to thrive. The flexion of the fingers with the index overlapping the third digit was significant for diagnosis of the syndrome because it is such an unusual anomaly. The phenotypic expression of trisomy 18 was found to vary with a wide range of anomalies. Each one, however, was not present in every patient.

Another early study in 1962 reported on 6 definite cases and 1 possible case of trisomy 18. These cases were confirmed by cytological preparation and analysis. The most frequent anomalies noted of these seven trisomy 18 cases were: apparent mental retardation; failure to thrive; flexion and deviation of the fingers; low-set and malformed ears; micrognathia; defectively ossified and short sternum; diaphragmatic hernia or eventration; hypertonia; limited hip abduction; dorsiflexed and short great toe; malformation of the heart; malformation of the kidney; prominent occiput; diastasis recti abdominus or peri-umbilical hernia; rocker-bottom feet or equinovarus; and limpness and hypotonia at birth. The investigators concluded that the trisomy 18 syndrome was a well-defined entity.
While there are many published descriptions of trisomy 18, there is little information about the cause of death and the length of survival. Embleton et al. explored the cause of death because many clinicians blamed the rapid demise of the trisomic fetus on congenital heart disease.\(^4\) The researchers conducted a retrospective study for all cases of trisomy 18 between 1986 and 1992. Of the 282,583 births during that time, 66 fetuses at 18 weeks gestation with trisomy 18 were identified. Of the 66 trisomic pregnancies, 23 were terminated, 6 spontaneously aborted, 3 were stillborn, and 34 were born living. The observed prevalence was 1 in 4274 at 18 weeks gestation. The prevalence decreases to 1 in 8333 live births at the time of delivery.\(^4\) Trisomy 18 was diagnosed antenatally in 28 cases. Of these 28 cases, only two were carried to term. The mode of delivery in 64% of the cases was by cesarean section. In this group, only one case had been diagnosed antenatally and cesarean section was performed to save the unaffected twin of the trisomic baby. Thirteen of these cesarean sections were performed due to fetal complications such as fetal distress and cord prolapse. Prior diagnosis might have allowed for vaginal delivery. Twenty-one infants were determined to have cardiac malformations. Nine babies died within a few hours following birth. Only three cases were presumed to expire from cardiac abnormalities. None of the infants who lived beyond two days died of cardiac complications; therefore, cardiac surgery would not have affected fetal outcome. The most common mode of death was found to be central apnea, and this was the cause of death in 10 cases.\(^4\)
Prenatal Screening

Screening programs for pregnant women have caused much debate since the advent of tests such as amniocentesis and chorionic villus sampling. More specifically, there has been continued debate about which populations should undergo such testing because of health care cost and the risks associated with them. Previously, prenatal screening was only offered to women over 35 because of the invasive nature of the tests. Without a non-invasive screening test, fetal loss due to testing and the cost of the procedure limited the efficacy of prenatal diagnosis. Use of such screening programs as ultrasound and triple marker screening have raised many questions about counseling and testing protocol.

Screening for chromosomal abnormalities has been traditionally offered to women considered to be at risk because of advanced age or other risk factors. Almost all women presently are offered some form of prenatal screening which may include ultrasound, triple marker screen, and amniocentesis. The prenatal screening tools available to women during pregnancy have continued to improve and multiply throughout the past ten years. With new technology and availability of prenatal screening, some researchers have become concerned about the specificity and sensitivity of the tests. For example, Dornan et al. expressed concern about the extension of these tests into populations which have not been researched and whether this would result in increasing numbers of false positive results and more unnecessary invasive testing. Dornan et al. felt prenatal diagnosis through early screening would allow parents to make informed
decisions. They foresaw a need for uniformity in the way the tests are offered and interpreted in order to decrease false positive results.²

Increased availability of procedures to detect fetal abnormalities prenatally has caused some researchers to question the impact caused by the technology. A study done by Forrester et al. examined the effect of prenatal diagnosis and elective termination on the prevalence of certain birth defects.³⁵ They included electively terminated cases into calculations of birth prevalence rates and found that this increased the rates by more than 50% for 5 of the 10 birth defects studied. Elective terminations had the most effect on the rates of neural tube defects (NTD) and chromosomal abnormalities. By taking into consideration the number of elected terminations, there was a 58% increase in trisomy 13 rates and 100% increase in trisomy 18 rates.³⁵ Birth defects such as anencephaly, trisomy 18, and trisomy 21 that result in a poor prognosis or substantial disability were more likely to end in elective terminations after prenatal diagnosis.

There is often controversy over the need to have a screening program for trisomy 18 due to its low birth prevalence and the high mortality rate for affected infants. Prenatal diagnosis of trisomy 18 allows the parents to prepare themselves for the birth of abnormal child with a poor chance for survival. On a psychosocial level this information can prove to be valuable to parents to allow for adequate grieving and decision making. Medical concerns such as a high rate of cesarean section in association with trisomy 18, support the need for antenatal identification of these infants as well. When trisomy 18 remains undiagnosed prenatally, there is a high rate of cesarean section in these mothers due to the
frequent association between trisomy 18 and intrauterine growth retardation (IUGR). Diagnosis of fetal distress is twice as common in infants with growth retardation. Cases in which primary cesarean section was performed for fetal distress resulted in an unjustified risk to the mother when ultimate fetal outcome is unfavorable either way. 

**Triple Marker Screening**

As stated previously, invasive prenatal tests cannot be offered to every pregnant woman because of the cost of such procedures and the associated complications. A number of non-invasive screening tests aimed at selecting higher risk pregnancies have been developed but are continuously in need of improvement. Screening for chromosomal abnormalities includes serum marker screening, advanced maternal age, and ultrasound findings. The maternal triple marker serum screen is the most widely used prenatal screen for chromosomal abnormalities. This screen consists of three biochemical markers: β-human chorionic gonadotropin (β-hCG); unconjugated estriol (uE3); and alpha fetoprotein (AFP). Each of these biochemical markers is produced by the fetus or the placenta and passes into maternal circulation. Human chorionic gonadotropin is produced by the placenta and is a glycoprotein similar to luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). The hCG consists of an alpha-subunit and a beta-subunit. The levels of β-hCG follow a specific pattern throughout the gestation of a fetus. This pattern begins with implantation to 8 weeks gestation when the levels begin rise. The
concentration plateaus from 8 to 12 weeks gestation, decreases from 12 to 18 weeks, and plateaus again until term. Unconjugated estriol is formed from dehydroepiandrosterone sulfate (DHEAS) which is converted in the fetal liver to 16-alpha-OH-DHEAS and then becomes unconjugated estriol in the placenta. AFP is produced primarily in the fetal liver as well as the yolk sac and the gastrointestinal tract. Fetal plasma levels of AFP peak between 10 and 13 weeks and then decrease until term.

Maternal serum screening is a blood test that is typically offered between 16 and 21 weeks gestation. The biochemical markers are calculated and reported in Multiples of the Median (MoM). These serum markers are initially measured in mass units and then converted to MoMs based upon proven normal pregnancies at the same gestational age. By reporting the values in this way, the values can be adjusted for biological variables that are known to affect the maternal serum levels such as maternal weight, insulin-dependent diabetes, and race. In screening specifically for trisomy 18, the levels for all three biochemical markers are lower than normal values for gestation age.

Merkatz et al. were the first to observe a decreased maternal biochemical marker in a trisomic 18 fetus. Their research was prompted by an index case of "undetectable" maternal serum AFP in a 28 year-old primigravid women at 16 weeks that resulted in the birth of a trisomy 18 infant. During the pregnancy the mother had inquired about amniocentesis but was told the procedure was not recommended due to her young age; however, she did undergo routine AFP screening and had two results that were described as "below sensitivity" of the
test. At the time, the literature revealed that the significance of low midtrimester maternal AFP levels was unclear but seemed to be correlated with higher than estimated rates of spontaneous abortion. This observation incited Merkatz et al. to further research this relationship.  

Merkatz et al. used three different populations in a retrospective study of prenatally diagnosed chromosomal abnormalities. The first group was evaluated for maternal age distribution. The researchers found that among all trisomy 18 and trisomy 21 births, 68% occurred in women who were 34 years of age or younger. Due to the fact that almost three-fourths of trisomic affected births occur in women who are younger than the standard cutoff of age 35 and due to the expense of prenatal diagnosis, Merkatz et al. suggested the need for a screening tool to identify a high-risk subset of pregnant women. The second and third populations evaluated the use of AFP as a screening tool. They concluded that using low AFP values as a criterion to do further prenatal testing for diagnosis would reduce the number of amniocenteses to approximately one chromosomal abnormalities for every 35 to 40 performed. This ratio is compared to 1 to 2 chromosomal abnormalities for every 100 amniocenteses performed when maternal age is used as the criterion.  

The earliest screens measuring biochemical analytes were first performed using AFP exclusively to screen for Down’s syndrome. With continuing research, β-hCG and uE3 were used in addition to AFP because of an increased detection rate of aneuploidy. Kellner et al. found using all three markers together in
correlation with maternal age increased the detection rate of chromosomal
abnormalities versus AFP and hCG without estriol. The results of the study also
indicate a higher detection rate by using the three serum markers with other
chromosomal abnormalities without a change in the false positive rate.

With progression in research on the accuracy of the triple marker
screening test for detecting chromosomal abnormalities, Kellner et al. raised the
question whether amniocentesis needs to be offered to a more defined
population. Using the triple screen in women age 35 and greater in the study
group, a total of 700 women were spared from undergoing amniocentesis, while 4
out of 5 cases of trisomy 21 were detected by offering amniocentesis to only 186
women. Statistically, the loss of 3 to 4 unaffected fetuses would be spared as
well. On the other hand, offering amniocentesis to women less than 35 years of
age resulted in an increase detection of abnormalities. The researchers also noted
that the ultrasound was able to detect an additional 2 cases of trisomy 21 and
suggested that it may be a useful additional screening tool. In conclusion, the
researchers found the triple screen in correlation with maternal age has increased
detection of chromosomal abnormalities, allowing for another option for older
women who are usually candidates for amniocentesis.

Maternal serum screening for trisomy 18 was initiated as an extension of
second-trimester maternal blood screening for Down's syndrome. In 1992
Kellner et al. began screening for trisomy 18 in addition to their trisomy 21
screening study. The study population included 8,649 women of the original
10,605 who were initially screened for trisomy 21. A test was considered screen
positive for trisomy 18 if the three biochemical markers were low. The screen positive test was determined with the following values: \( AFP \leq 0.75 \text{ MoM} \), \( uE3 \leq 0.60 \text{ MoM} \), and \( \beta\text{-hCG} \leq 0.55 \text{ MoM} \). Of the 8649 serum samples tested, 23 women screened positive for trisomy 18. Twenty of these women elected to undergo amniocentesis. Two cases were detected by amniocentesis resulting in 1 case per 11.5 amniocenteses. There was 1 case that was missed by the screening and one fetal death in which trisomy 18 could not be ruled out.\textsuperscript{14}

Leporrier et al. examined 33 cases of known trisomy 18 pregnancies in order to evaluate two maternal serum biochemical markers, specifically unconjugated estriol and human chorionic gonadotropin.\textsuperscript{16} The levels for both \( uE3 \) and \( hCG \) were found to be significantly lower as compared to unaffected pregnancies. The researchers point out the advantages to using these biochemical markers as routine screening are a reduction in the number of amniocenteses performed, as well as earlier detection of the disease.\textsuperscript{16}

With continuing research on the detection of trisomy 18, the triple serum screen was found to be a better screen than AFP alone or AFP and \( \beta\text{-hCG} \) in combination. With the addition of estriol, 60% of pregnancies with trisomy 18 would be detected at the same false positive rate of 0.2%. The detection rate doubles in comparison to using only AFP and \( \beta\text{-hCG} \).\textsuperscript{3} In 1993, Barkai et al. concurred with previous studies that the triple marker screen could be modified to detect trisomy 18 as well as trisomy 21.\textsuperscript{20} The difference between the two syndromes is a decreased level of \( \beta\text{-hCG} \) in trisomy 18 in contrast with an
increased level in trisomy 21. Depending on the risk cut-off rate of either 1:10 or 1:100, multiple marker screening could potentially detect 67-80% of trisomy 18 cases, with a false positive rate of 0.3-0.6% respectively.\textsuperscript{20}

As prenatal screening programs routinely began using the triple marker screen for the detection of trisomy 18, research was directed towards evaluating the efficacy of the triple marker screen. A prospective intervention trial to evaluate the performance of a screening protocol for trisomy 18 in routine practice was carried out by Palomaki et al. in 1991.\textsuperscript{31} A total of 92 of the 19,491 singleton pregnancies studied were identified as high risk, meaning they were screen positive for trisomy 18. The following serum biochemical marker levels were defined as a positive screen or high risk pregnancy: AFP \(\leq 0.75\) MoM, uE3 \(\leq 0.60\) MoM, and hCG \(\leq 0.55\) MoM. Ninety-six percent of the women elected further karyotyping by amniocentesis. Six of the 92 screen positive women actually had fetuses with trisomy 18, making the odds of having an affected fetus 1:14. The results indicate an estimated detection rate of 85% using this screening protocol. Thus, the method utilizing the three serum markers compared to the alternative method of using \(\beta\)-hCG alone as a screening tool shows an improved detection rate and a decreased false positive rate.\textsuperscript{31}

A study by Yankowitz et al. was done to determine the efficacy of the prenatal serum screen for trisomy 18.\textsuperscript{6} Maternal serum samples were drawn between 15 and 20 weeks gestation. Those screens considered positive for trisomy 18 had maternal serum AFP \(\leq 0.75\) MOM, estriol \(\leq 0.60\) MOM, and \(\beta\)-
hCG ≤ 0.55 MOM. Serum AFP values were corrected for maternal weight, race, and Type 1 diabetes mellitus. Estriol and β-hCG were corrected for maternal weight. Fetal ultrasound evaluation included biometric parameters, assessment of fetal structure, and amniotic fluid. Patient follow-up was obtained through evaluation of karyotype if amniocentesis was performed, birth certificate data, or telephone contact with the patient or physician. During the study 40,762 women were screened with 175 being screen positive for trisomy 18. The results indicated that eight fetuses out of the 175 that had a positive triple screen were actually diagnosed with trisomy 18. One hundred twenty-one women of the 175 that had a positive screen elected to undergo amniocentesis, which resulted in the identification of only one trisomic 18 fetus. Of the 8 fetuses who were afflicted with trisomy 18, only 3 mothers elected to undergo ultrasound after having screened positive for the triple marker serum screen. Remarkably, all three of the fetuses showed abnormalities on ultrasound.

Yankowitz et al. argued that combining serum screening with genetic ultrasound may improve the predictive value of those who screen positive for trisomy 18. This would be accomplished by targeting a more precise group at a higher risk of trisomy 18 for amniocentesis. By combining ultrasound with the triple marker screen, the researchers theorized that the number of women undergoing amniocentesis could have been reduced from 121 to 27. Yankowitz et al. go on to suggest that all women who screen positive should first undergo ultrasound and then be counseled for further genetic testing based on the
calculated risk of trisomy 18. Normal ultrasound results would potentially eliminate 80% of the risk of trisomy 18.6

**Ultrasonographic Findings**

Ultrasound is a diagnostic tool that is used by physicians to assist them in detection of multiple gestations and fetal abnormalities. In women with unreliable dates of last menses, an estimation of gestational age can also be determined through the use of ultrasound. Real time ultrasound using high frequency sound waves to produce a two-dimensional image gives the physician the capability to survey both structural and functional characteristics of the fetus, as well as the location and morphology of the placenta. A study performed by Magriples et al. evaluated the accuracy of routine ultrasound screening for fetal anatomy and anomalies.32 The investigators found routine ultrasound screening to be extremely sensitive in the detection of fetal anomalies in women screened before 26 weeks gestation. Sensitivity and specificity of ultrasound screening in this study were found to be 71.4% and 99.4% respectively.32 Findings are significant due to the fact that a majority of fetal malformations occur in pregnant women having no known risk factors.

Ultrasound has made it possible to visualize structural malformations associated with chromosomal abnormalities and neural tube defects. There has been ongoing research on the use of ultrasound as a routine prenatal screening for specific chromosomal abnormalities such as Down's syndrome and trisomy 18. The motivation to incorporate ultrasound as a routine prenatal screening is to improve perinatal and maternal outcome while decreasing the use of invasive
testing. Recent research has been able to describe sonographically detectable abnormalities associated with specific chromosomal anomalies.

Benacerraf et al. conducted a series of studies focusing on the accuracy and limitations of ultrasound in the detection of trisomy 18. This resulted in their development of a scoring system for sonographic features. In one of their early studies, Benacerraf et al. examined the accuracy and limitations of prenatal ultrasound in identifying fetuses with trisomies 13 and 18. Patients scanned between January 1, 1984 and February 1, 1987 who had cytogenetic diagnoses of trisomy 13 or 18 were included in the study. All ultrasounds were done between 15 and 40 weeks gestation without any knowledge of karyotype. There were 15 cases of trisomy 18. Twelve of the 15 cases had abnormalities detected by ultrasound. Eleven of these fetuses had abnormalities of the hands or feet, three had diaphragmatic hernia, and one had micrognathia. Of the twelve cases with abnormalities detected by ultrasound, six were scanned between 15 and 22 weeks, one at 25 weeks and 5 between 31 and 40 weeks. All three fetuses who did not have sonographic abnormalities were seen between 16 and 17 weeks. Sonographic identification for trisomy 18 in this study was approximately 80%. With this study Benacerraf et al. were unable to conclude whether ultrasound is predictive in diagnosing trisomies due to the small number of cases.

In a following study, Benacerraf et al. further examined abnormalities seen on ultrasound and derived a scoring system to be used to detect fetuses at increased risk for aneuploidy. The system was scored as follows: nuchal fold 2, major defect 2, short femur 1, short humerus 1, and pyelectasis 1. A score of 2
would mean increased risk of aneuploidy. Using this system there is a potential for identifying as many as 80% of trisomy 21 fetuses, 100% of trisomy 13 and 18 fetuses while keeping a low false-positive rate of 4.4%.^4

In a 1994 follow-up study, Benacerraf et al. evaluated the ability to determine autosomal trisomy fetuses by using their previously determined scoring system for sonographic features.^^ They selected a new population that was scanned before karyotyping to avoid overlap with their earlier study. The two new criteria added to the scoring system were hyperechoic bowel = 1 and choroid plexus cysts = 1. The amniocentesis findings identified 60 trisomic fetuses with 13 trisomy 18 fetuses. These were compared to 106 control fetuses that underwent scanning and amniocentesis at approximately the same time. A score of two was used as an indication of increased risk of aneuploidy warranting karyotyping. This score of 2 identified 11 of the 13 trisomy 18 fetuses or 85%. The 2 fetuses with trisomy 18 that were not identified were scanned at 15 weeks or earlier which limits the structural survey. According to Benacerraf et al., the optimal time to perform sonographic evaluation is 18 weeks since the fetal heart can be evaluated better. If only fetuses that were scanned at 18 weeks or older were included then 11 of 11 or 100% of trisomy 18 fetuses would have been detected. Benacerraf et al. suggest that the scoring system with triple marker screening may allow for a refined prediction of risk of aneuploidy and thereby performing fewer amniocentesis that have a higher sensitivity and positive predictive value in detecting aneuploidy.^35
A case review of the ultrasonographic findings in 47 fetuses with trisomy 18 was carried out by Nyberg et al. in 1992. Twenty-nine of the 47 total fetuses were examined between 14 and 24 weeks. The other 18 fetuses were scanned after 24 weeks. Abnormalities excluding choroid plexus cysts were identified in 83% of the fetuses. The abnormalities detected in this study included the following: intrauterine growth retardation (51%); cardiac defects (38%); cystic hygromas, lymphedema or nuchal thickening (19%); prominent or enlarged cisterna magna (19%); meningomyelocele (17%); omphalocele (21%); renal abnormalities (15%); single umbilical artery (13%); clubbed or rocker bottom feet (21%); and clenched hands (19%). Cardiac defects, enlarged cisterna magna, and IUGR were more likely to be detected in the third trimester in contrast with cystic hygromas. Clubbed feet or persistently clenched hands were the most common abnormalities of the extremities. This study demonstrates the use of ultrasound in detecting trisomy 18 in reporting ultrasonographic abnormalities associated with the syndrome. This further points to the efficacy of using ultrasound to evaluate patients with an increased risk for trisomy 18 based on serum biochemical markers.

While previous studies have reported an 80 to 85% ability to detect abnormalities specific to trisomy 18, the ultrasounds have inconsistently been performed at various gestational ages between 12 and 40 weeks. Most pregnant women undergo a screening ultrasonogram early in the second trimester. A study by Shields et al. focused on the prenatal detection of ultrasonographic abnormalities in fetuses with trisomy 18 during the early second trimester.
patient population included all patients that were referred to the University of Washington Prenatal Diagnosis Center between January 1, 1987 to June 30, 1996. The fetuses of these women were between 14 and 22 weeks gestation with a fetal karotype indicating trisomy 18. Thirty-five cases of trisomy 18 were identified. The mean time of evaluation for the fetuses was 17.2 ± 2.0 weeks. The ultrasound evaluation included standard biometry measurements of biparietal diameter, head circumference, abdominal circumference, and femur length as well as fetal anatomic evaluation of the cerebral ventricles, posterior fossa, spine, four-chamber view of the heart, stomach, kidneys, and bladder. Of the 35 fetuses studied, 30 (86%) had at least one sonographic abnormality detected. The most common abnormality detected was persistent abnormal positioning of fetal fingers or a clenched fist. Abnormalities in regard to fetal hands were found in 18 cases with 16 having positional abnormalities of the fingers. Abnormal positioning of the hand was seen in almost 90% of the fetuses whose hands were evaluated. Shields et al. comment that while careful documentation of hands would not identify all abnormal fetuses, it would take little additional time to obtain during a routine scan. This finding seems to have a high predictive value in detecting chromosomally abnormal fetuses. Of the 5 fetuses that had anatomically normal ultrasounds, there was only one case in which the hands were noted to be sonographically normal. The next most common finding was choroid plexus cyst. Fifteen patients or 43% were noted to have this abnormality. In 5 of these 15 cases, a choroid plexus cyst was the only abnormality noted. Other anomalies found included: abnormal cranial shape such as the lemon-shaped and the classic
strawberry-shaped heads; two-vessel umbilical cord; cardiac defects; intrauterine growth restriction; omphalocele; neural tube defects; and cystic hygroma or lymphangiectasia. This study notes that a majority of fetuses with trisomy 18 will have ultrasonographic abnormalities that can be detected between 14 and 22 weeks during the screening ultrasonographic examination.37

In a previous study by Shields, 274 fetuses had isolated choroid plexus cysts on ultrasound with only 7 fetuses having abnormal karotypes.38 The risk of trisomy 18 in fetuses with an isolated choroid cyst noted on second trimester ultrasound was determined to be 1.9%. At this time, Shields et al. suggested that an isolated choroid cyst in a fetus with a normal maternal serum screen may not need invasive testing.38

Vintzileos et al. and Bahado-Singh et al. both conducted studies that focused on determining the importance of ultrasound and triple marker screen used in combination to detect trisomy 21 risk. Bahado-Singh et al. used ultrasonographic biometry and anatomic survey to obtain the best combination of biometry parameters for the detection of Down’s syndrome and other chromosomal anomalies.39 This study established values for nuchal thickness, humerus length, femur length, and fetal anatomy which would elicit a statistically significant percentage of chromosome abnormality in a positive triple screen population. With the use of a combination of ultrasonographic parameters in the present study, fetuses at greatest risk for trisomy 21 and other significant chromosomal abnormalities such as trisomy 18 among positive triple screen pregnancies were identified. The researchers thus concluded that a normal
ultrasonography result significantly reduces the risk of any clinically significant chromosomal defect. A previous study by Bahado-Singh et al. showed that the chance of finding Down's syndrome in a triple-screen-positive pregnancy is significantly reduced with a normal nuchal thickness and no fetal structural abnormalities on ultrasonography. They also established that the risk from amniocentesis significantly exceeds the risk of finding Down syndrome in these same fetuses with normal nuchal thickness and no fetal structural abnormalities. Thus, a major benefit of defining those triple-screen-positive pregnancies at low risk for chromosomal abnormalities as noted in the present study is a potential reduction of performing genetic amniocentesis on such a population.

In response to conflicting results of prenatal ultrasonographic findings in trisomy 21, Vintzileos et al. conducted a study to establish the average sensitivity and specificity of ultrasonographic markers in the second trimester of pregnancy. Based on these findings, the researchers were able to derive an adjusted risk for trisomy 21 for both high- and low-risk populations. The significance of this data is that second-trimester ultrasonography is an important key in adjusting the need for genetic amniocentesis in women with abnormal triple serum biochemistry results. These results allow for better selection of candidates for genetic amniocentesis and thus minimize amniocentesis-related fetal loss. Both studies by Vintzileos et al. and Bahado-Singh et al. validate the significance of ultrasonographic biometry in combination with a positive triple serum screen in detecting trisomy 21 prenatally.
Amniocentesis

Amniocentesis was first known to be performed for genetic studies in the 1950's; however, the first report that a fetal karyotype could be obtained from cultured amniocytes for the purpose of prenatal diagnosis was made by Steel and Breg in 1966. Then, in 1972, Hsu et al. reported one of the first cases of trisomy 18 diagnosed prenatally with the use of transabdominal amniocentesis. This procedure was performed at 16.5 weeks gestation on a 40 year old woman secondary to her advanced maternal age. Chromosomal analysis, performed on the amniotic fluid culture, indicated trisomy 18. Using prenatal amniocentesis and pathologic examination, the researchers were able to conclude that trisomy 18 could be detected as early as twenty weeks gestation.

With the heightened use of amniocentesis, safety, as well as its effects on the fetus and maternal outcome, was under debate. In 1971, the National Institute of Child Health and Human Development (NICHD) initiated the National Registry for Amniocentesis to conduct a prospective study to investigate the safety and accuracy of mid-trimester amniocentesis. The study involved 1,040 subjects and 982 controls. The registry data focused on immediate complications of amniocentesis such as fetal loss at the time of delivery, birth difficulties, congenital abnormalities, and follow-up at one year of age in order to note any possible developmental problems. The overall fetal loss by the women undergoing amniocentesis was 3.5% compared to 3.2% for the controls. There were no significant findings for the infants in death rates, medical history, or physical growth between the two groups at the one-year follow-up. Overall, the NICHD
study group concluded that amniocentesis was a safe procedure; however, they
did not discount the potential risks that accompany the procedure even though the
data indicated the actual occurrence of these risks to be small. The researchers
felt the results of the study should help to advocate for the use of amniocentesis in
older women with an increased risk for Down’s syndrome.\textsuperscript{42}

Since the time of the report by the NICHD, several studies have followed
and have shown a small but significant procedure-related loss associated with
amniocentesis.\textsuperscript{43-46} According to the bulletin constructed by the ACOG study
group on the safety and efficacy of amniocentesis, the risk of fetal loss associated
with amniocentesis was quoted as 1:200 procedures.\textsuperscript{47} The procedure involves the
introduction of an ultrasound guided 20- to 22-guage spinal needle through the
abdominal wall. The needle is then passed through the uterus and into the
amniotic sac. Approximately 20 to 30 ml of amniotic fluid is extracted. The fluid
contains amniotic cells which are then cultured and karyotyped.\textsuperscript{11} Amniocentesis
is considered the gold standard for invasive prenatal diagnostic procedures;
however, it is essential that patients be informed of the possible risks of
amniocentesis including a procedural related loss of 0.5 to 1.0%.\textsuperscript{10,11,45}

Second trimester amniocentesis is currently offered on a routine basis to
women at risk for Down’s syndrome and other fetal aneuploidies.\textsuperscript{10,40,41,47} The
population of women at increased risk for fetal aneuploidies includes all women
age 35 years and older since the risk of having a fetus with an autosomal trisomy
increases significantly after this age.\textsuperscript{12,40} Other indications for prenatal diagnosis
by amniocentesis include a positive maternal serum screen, history of a child with
a chromosomal abnormality, family history of neural tube defects, and abnormal results on fetal ultrasonography.\textsuperscript{45} Although less than 25\% of trisomy 21 infants are born to mothers over the age 35, the risk of having an affected fetus in the second trimester increases to 1 in 200.\textsuperscript{12,48} The risk of procedure-related fetal loss in this age group is considered to be the same or less than the risk of having a child with trisomy 21.\textsuperscript{40} Because the birth incidence of trisomy 18 is much lower than that of trisomy 21, the risk of fetal loss secondary to amniocentesis may actually be higher than the risk of having a trisomy 18 infant.

The study by Spencer and colleagues demonstrates the need for further research on performing routine amniocenteses on pregnant women who screen positive for trisomy 18.\textsuperscript{18} In this particular study, the birth incidence of trisomy 21 was found to be 12.6/10,000 as compared to an incidence of 1.3/10,000 for trisomy 18. With the use of maternal serum AFP and $\beta$-hCG for the prenatal diagnosis of trisomy 18 and trisomy 21, a detection rate of 50\% with a false positive rate of 1\% and a detection rate of 70\% with a false positive rate of 5\% were obtained, respectively. Therefore, 8.8 cases of trisomy 21 would be detected for every 500 amniocenteses performed giving one case per 57 amniocenteses; however, the detection rate for trisomy 18 would result in 154 women undergoing amniocentesis to detect one case of trisomy 18. This is significant in that this would result in a loss of just as many normal fetuses as abnormal fetuses detected. Thus, it was concluded that the risk caused by follow-up amniocentesis was too great for the number of trisomy 18 fetuses detected at that time.\textsuperscript{18}
Routine second-trimester amniocentesis for women at an increased risk of trisomy 18 has not been found justifiable from a cost/benefit standpoint either. This aspect of routine amniocentesis was explored in a 1998 study by Vintileos et al. The researchers performed a cost/benefit analysis and cost effectiveness determination of three prenatal strategies for the detection of trisomy 18. The strategy of universal amniocentesis for all women at an increased risk of trisomy 18 was found to be much more costly than the strategy of no prenatal diagnostic work-up of at-risk patients, and the strategy of genetic amniocentesis reserved for at-risk patients with abnormal ultrasound results. The universal amniocentesis approach would generate an annual cost of approximately $12 million dollars and 40 fetal losses whereas the targeted genetic ultrasound approach would cost only $5 million dollars annually with a fetal loss of 8 as the result of amniocentesis. This study demonstrates that from an economic standpoint universal amniocentesis is not a cost effective option for mothers at an increased risk for trisomy 18.

Summary

The research and development of screening programs are focused on reducing the number of women undergoing invasive procedures for definitive diagnosis of chromosomal abnormalities. Performing amniocenteses on all women who screen positive on triple marker serum screen for chromosomal abnormalities is both costly and carries the risk for fetal loss. This lends support to further define a population at higher risk of trisomy 18 in order to reduce the number of amniocenteses performed. When the risk of the test is greater than the
risk of the abnormality, the patient needs guidelines to make informed decisions regarding further evaluation.

Several studies have indicated the efficacy of triple serum marker screen in identifying trisomy 18.\textsuperscript{6,31} Many studies have also described abnormalities associated with trisomy 18.\textsuperscript{21-23} Furthermore, studies have demonstrated the ability of these abnormalities to be detected on ultrasound.\textsuperscript{33-38} While there were a few ultrasounds that had no visible abnormalities but resulted in fetuses with trisomy 18, most of those ultrasounds were performed before 17 weeks gestation.\textsuperscript{33,35} Most research published thus far has centered on altering the risk of having a fetus affected with trisomy 21 using non-invasive methods. These studies demonstrate the significance of using ultrasound and triple marker screen in combination to determine trisomy 21 risk.\textsuperscript{15,39} The parameters and framework from these studies can be extended to research involving trisomy 18. More specifically, the use of ultrasound and triple screen in combination to identify a population at higher risk for trisomy 18 must be researched.
CHAPTER THREE
METHODOLOGY

Study Design

This study examined the risk of trisomy 18 based upon triple marker serum screen results and ultrasound findings. The project was a correlational study of the relationship between an abnormal triple marker screen, a normal ultrasound, and the risk of trisomy 18. A non-experimental design, specifically, a non-concurrent cohort study, was conducted to obtain data. This was accomplished through a retrospective and prospective chart review of a cohort of pregnant women who had screened positive for trisomy 18. The retrospective aspect was conducted between June 1, 1996 through July 31, 1998. The remaining cases were followed from August 1, 1998 until September 30, 1999. The starting date for the study was chosen because June 1996 was when trisomy 18 risk was first reported to the physician population. September 1999 was chosen as a cut off date in order to allow adequate time to obtain pediatric outcome prior to presentation of this project in March of 2000. The advantage of using this particular study design was that it permitted a large population to be studied within a specified amount of time. Also, due to the low incidence of trisomy 18, access to the largest population possible was needed to obtain a sufficient number of trisomy 18 cases. The use of a retrospective design does
have the possibility of missing data, loss to follow-up, and lack of uniformity in the recorded information.

The focus of the study centered around the results of the triple marker screen and ultrasound as well as the outcome in terms of presence or absence of trisomy 18. The triple marker serum screen results and ultrasound findings constitute the independent variables. The triple marker screen consists of testing serum levels of AFP, β-hCG, and unconjugated estriol. In a positive test for trisomy 18, all of the serum levels are lower than normal in the second trimester. Ultrasound was the other screening tool used to detect the presence of trisomy 18. Research has shown that there are specific abnormalities seen on ultrasound commonly associated with trisomy 18. These sonographic findings include: intrauterine growth retardation; congenital heart disease; single umbilical artery; cystic hygromas; choroid plexus cysts; omphalocele; clenched hands; rocker bottom feet; meningomyelocele; renal anomaly; and enlarged cisterna magna. The dependent variable in the study was the presence or absence of trisomy 18.

**Study Site and Subjects**

**Study Site**

The central site of the study was conducted through Spectrum Health Downtown campus in conjunction with West Michigan Perinatology and Spectrum Health Downtown Genetic and Cytogenetic departments. These facilities serve the population of West Michigan along with surrounding areas.
The demographic profile of this population ranges from urban to rural encompassing various ethnic and socioeconomic backgrounds.

Subjects

Subjects were initially recruited by referral through their primary physician for a triple marker serum screen based on their gestational age. All pregnant women with fetuses between the gestational age of 15 to 22 weeks are offered the triple marker serum screen. The study population included women who were screened through Spectrum Health Downtown Campus Genetic Screening Program. The cut-off levels at Spectrum Health Genetics lab for the biochemical analytes in the serum marker screen consist of the following: AFP 0.70 MoM, estriol 0.55 MoM, and β-hCG 0.50. Using these levels, there is a 50% detection rate with a 2% false positive rate. Based on the levels of β-hCG, AFP, and estriol, a risk for trisomy 18 was generated. The study sample included all women with a calculated serum screen risk of greater than 1:250 for trisomy 18. These women then underwent ultrasound or ultrasound in combination with amniocentesis. A computer-generated list of all positive results for trisomy 18 was compiled. The risk calculation for trisomy 18 was instituted June 1, 1996. The women included in this study had screened positive between this date and September 30, 1999. Prior to June 1, 1996, the triple marker screen was being done but was not evaluated for trisomy 18 risk calculation. Excluded from the study were those with a normal risk based on recalculation with change in
estimated date of confinement (EDC), fetal demise at less than 20 weeks, and termination at less than 20 weeks without amniocentesis.

**Equipment and Instruments**

This study was a chart review in which data were collected concerning ultrasound findings and fetal outcome. No direct instrumentation was used during the study. Ultrasound and amniocentesis were done prior to the chart review.

The technique of ultrasound is widely used as a general, non-invasive screening tool and it is considered to be a safe method for obtaining information about the fetus and its environment. Ultrasound biometry is now the gold standard for assessing fetal growth. A number of second-trimester ultrasound findings have been found to be frequently associated with fetal aneuploidy including: smaller biparietal diameter; shorter femur length and humerus length; thickened nuchal fold; pyelectasis; increased bowel echogenicity; abnormal heart anatomy and abnormal flexion of the hands. In trisomy 18, the most common structural abnormalities present on ultrasound consist of: cardiac or abdominal wall defects; central nervous system abnormalities; renal abnormalities; single umbilical artery; structural anomalies of the hands and feet; and intrauterine growth retardation. The specificity and sensitivity of the ultrasound depends on a number of factors including the quality of screening equipment, the expertise of the ultrasonographer, and the specific abnormality being evaluated.

Amniocentesis for prenatal diagnosis is the most common invasive procedure offered to pregnant women who have an increased risk of chromosomal aberration. The procedure involves the introduction of an ultrasound-guided
needle through the uterus into the amniotic sac. A small amount of amniotic fluid is aspirated with the needle and then sent to a lab for genetic evaluation and karyotyping.47

**Validity and Reliability of Amniocentesis**

In 1976, the diagnostic accuracy of mid-trimester amniocentesis was found to be 99.4% by the NICHD National Registry for Amniocentesis Study Group (NICHD). Since the early 1970’s, the accuracy and reliability of amniocentesis has been well established and is considered the “gold standard” for the diagnosis of chromosomal abnormalities.10,11

**Procedure**

The data was primarily collected through chart review of those women who screened positive for trisomy 18 using triple marker screen through Spectrum Health Downtown Campus Genetic Screening Program. A list of the women with abnormal triple screens was generated. The data needed for the study were retrieved from individual patient charts. A majority of the charts were available from the West Michigan Perinatology Office. The remaining charts were obtained through mailings and telephone calls to the woman’s primary care provider at the time of pregnancy. Review consisted of maternal demographic data, triple screen results, ultrasound evaluation, amniocentesis rates and results, and outcome assessment based on amniocentesis results, office documentation, review of birth records, or telephone contact with mother.

The data was collected by the three authors of the study, all of whom are physician assistant students, and also Dr. Dawn DeWitt. All data collectors were
consistent in finding and recording the information from the individual charts. In cases in which charts could not be reviewed directly, a standard form was sent to the practitioners office requesting the specific information needed. Upon receiving the completed form, the information was transcribed in a manner consistent with the data directly obtained. Data collection began September 1998 and continued through November 1999.

Because of the design of the study, there exist missing data and incomplete data. An exhaustive search for data was performed and all attempts to locate missing data was carried out. For charts outside of the West Michigan Perinatology office, a mailing was sent requesting information from practitioners on subjects in the study. Follow-up phone calls were made to those offices which had not responded to the initial request by mail. Attempts to further complete pediatric follow-up was carried out through phone calls to the mother and review of Spectrum Health’s labor and delivery log book.

There were no direct risks to the individual patients since this study was a chart review. Maintaining patient confidentiality was the only concern for this type of study. Confidentiality was preserved by sequential assignment of numbers to all patients so that names and identifying information were protected. Throughout the study, patients were only identified and referred to by their assigned number. Individual informed consent was not necessary due to preserved confidentiality and absence of direct patient contact.

This study was done in conjunction with Dawn DeWitt, MD, chief Obstetrics and Gynecology resident.
CHAPTER FOUR
RESULTS/DATA ANALYSIS

Study Population

A total of 13,618 maternal subjects were screened for fetal trisomy 18 from June 1996 to September 1999. Of the total number screened, 158 (1.2%) had a positive result. Ten were excluded from the study due to fetal demise or termination at less than 20 weeks gestation. Another 8 were excluded with recalculation of estimated date of confinement (EDC) and 1 subject was removed due to her non-pregnant status. Ultrasound data was not available on 19 of the 139 identified making the total number in our study population 120 (86.3%).

Techniques of Data Analysis

The study population was divided into two groups: normal ultrasound and abnormal ultrasound. The two groups were compared demographically to initially rule out any biases since the study was non-randomized. A comparison analysis of the collected data was performed using Pearson Chi-Square, Mann Whitney U, or Fischer Exact where appropriate. Significance was defined as p< 0.05. Comparison of normal ultrasound to abnormal ultrasound was based on the following maternal demographic components: age, advanced maternal age (age > 35 years), diabetes, weight, estimated gestational age (EGA) at ultrasound, EGA at triple screen, and triple screen risk. Assessment of neonatal data included the percentage undergoing amniocentesis, the percentage of aneuploidies, and the
percentage of trisomy 18. Results of this data are shown in Table 1 and Table 2, respectively (See Appendix A). Data analysis was provided by Justine Ritchie, Assistant Professor of Mathematics and Statistics at Grand Valley State University.

**Characteristics of Subjects**

Differences in the maternal data were not significant in any of the categories except weight distribution. The mean weight for the normal ultrasound group was 158.1 pounds as compared to 143.1 pound for the abnormal ultrasound group \( (p < 0.03) \). The results of the neonatal data demonstrate statistically significant differences between the normal and abnormal ultrasound groups. The total number of aneuploidies detected in the study was 10. The percentage of aneuploidy was 38.1% in the abnormal group and 2.5% in the normal group. Among the 8 aneuploidies with abnormal ultrasounds, there were 4 that were found to be trisomy 18. The other aneuploidies visualized on ultrasound consisted of 1 case of trisomy 16, 46 XX 9ph, 46 XX 4p-, and 1 triploidy. The two aneuploidies with normal ultrasounds were karyotyped as 46XX with an inversion of 11q and 47XXY. No cases of trisomy 18 were found in the normal ultrasound group, whereas all 4 cases of trisomy 18 were found in the abnormal ultrasound group \( (p < 0.001) \).
CHAPTER FIVE
DISCUSSION AND IMPLICATIONS

Discussion of Findings

Analysis of maternal data demonstrates that the only characteristic significantly different between the two identified groups is that of weight distribution. One may argue that, in theory, ultrasound is more difficult to perform and evaluate on larger women due to poor visualization of fetal anatomy. In our study, however, no cases of trisomy 18 were missed on ultrasound. According to the results of the neonatal data, a higher percentage of amniocentesis procedures were performed in the abnormal compared to the normal set. This is an expected outcome in that many of those having an abnormal test typically pursue further evaluation. A majority of the aneuploidies had detectable abnormalities visualized on ultrasound. All of the trisomy 18 cases had abnormalities that were detected on ultrasound. Four other aneuploidies were identified on ultrasound. These consisted of 1 case of trisomy 16, 1 case of female with 46 chromosomes with an abnormality of the heterochromatin on the short arm of chromosome 9, 1 case of a female with 46 chromosomes with a deletion on the short arm of chromosome 4, and 1 case of triploidy. Two aneuploidies, 46XX with an inversion of chromosome 11q and 47XYY, did not present with abnormalities on ultrasound. Both of these are considered minor chromosome abnormalities and are not expected to be detected on ultrasound.
Application of Practice

The results of our study potentially will affect the genetic counseling of women at high risk for trisomy 18 as defined by triple marker screen. We hope to provide those women who screen positive for trisomy 18, yet have normal ultrasound results, the ability to make a more informed decision about amniocentesis based on their adjusted risk. Although amniocentesis is a definitive test for the determination of chromosomal abnormalities, it is not without risk. In theory, the application of our data in a clinical setting would lead to a reduction in the number of amniocenteses performed on women with normal fetal ultrasounds.

Limitations and Recommendations

Limitations of this study include the size of our sample population and a limited number of trisomy 18 cases within this population. Complete data was unable to be recovered as this information was contained in patient charts located in various offices throughout Michigan. Due to the fact that this study was non-randomized, we are unable to extend our findings to the general population.

Before the results of this study can be implemented into clinical practice, further studies targeting a larger population will lend itself to the reliability of this study. Furthermore, a prospective study following a population at increased risk for trisomy 18 based on triple marker screen will minimize amount of incomplete data. Additional studies are needed that focus on the detection of trisomy 18 using non-invasive procedures.
Conclusions

The purpose of this study was to determine whether the risk of trisomy 18 in women who screened positive for trisomy 18 yet had normal ultrasound results was less than the procedure-related risk of amniocentesis. In other words, is the risk of amniocentesis-related loss greater than the risk of having a trisomy 18 fetus? We found in our study population that the presence of normal ultrasound findings is not associated with an increased risk of trisomy 18. The analyzed data showed that women with a positive triple screen but a normal ultrasound were at significantly less risk of actually having a trisomy 18 fetus than the women with abnormal ultrasounds. Furthermore, abnormal ultrasound findings are significantly associated with an increased risk of trisomy 18 and other aneuploidies. All cases of trisomy 18 in this study population had an abnormal ultrasound. Therefore, amniocentesis for all positive triple screens in this study population is not justified for the identification of affected infants.
REFERENCES


APPENDIX A

Data Tables
Table 1
Maternal Demographic Data: Comparison of patients with positive triple marker serum screen for trisomy 18

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Normal Ultrasound Group</th>
<th>Abnormal Ultrasound Group</th>
<th>Statistical Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(^1) (years)</td>
<td>31.0</td>
<td>29.7</td>
<td>NS</td>
</tr>
<tr>
<td>AMA(^2) (%)</td>
<td>34.7</td>
<td>22.7</td>
<td>NS</td>
</tr>
<tr>
<td>Weight(^3) (lbs)</td>
<td>158.1</td>
<td>143.1</td>
<td>p &lt;0.03</td>
</tr>
<tr>
<td>DM(^4) (%)</td>
<td>2.0</td>
<td>4.5</td>
<td>NS</td>
</tr>
<tr>
<td>EGA at TS(^5) (wks)</td>
<td>17.6</td>
<td>17.9</td>
<td>NS</td>
</tr>
<tr>
<td>EGA at US(^6) (wks)</td>
<td>20.6</td>
<td>19.8</td>
<td>NS</td>
</tr>
<tr>
<td>TS Risk(^7)</td>
<td>1:130</td>
<td>1:103</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Statistical significance defined as p <0.05

1. Mean age of subjects in years
2. % of subjects with advanced maternal age (age ≥35)
3. Mean weight of subjects in pounds
4. % of subjects with diabetes mellitus
5. Mean estimated gestational age at triple screen
6. Mean estimated gestational age at ultrasound
7. Mean calculated triple screen risk ratio
Table 2
Neonatal Data: Comparison of fetal data from pregnant women with a positive triple marker serum screen for trisomy 18

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Normal Ultrasound Group</th>
<th>Abnormal Ultrasound Group</th>
<th>Statistical Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniocentesis↑ (%)</td>
<td>31.9</td>
<td>68.2</td>
<td>P &lt;0.002</td>
</tr>
<tr>
<td>Trisomy 18↑ (%)</td>
<td>0</td>
<td>19</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>Aneuploidy↑ (%)</td>
<td>2.5**</td>
<td>38.1</td>
<td>P &lt;0.001</td>
</tr>
</tbody>
</table>

*Statistical significance is defined as p <0.05
**2.5%(n=2) of the fetuses in the normal ultrasound group had some type of aneuploidy. However, these aneuploidies were not significant as fetal outcome is expected to be good. Furthermore, no abnormalities from these two aneuploidies are expected to be detected on ultrasound.

1. % of subjects who had amniocentesis performed
2. % of fetuses actually having trisomy 18
3. % of fetuses with aneuploidy as determined by amniocentesis
APPENDIX B

Data Collection Forms
### Data Sheet

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Weight</th>
<th>Race</th>
<th>Parity</th>
<th>Diabetes</th>
<th>EGA at TS</th>
<th>Date TS</th>
<th>TS Risk</th>
<th>B-HCG</th>
<th>AFP</th>
<th>Estradiol</th>
<th>EGA at US</th>
<th>Date US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echogenic bowel</td>
<td>Abd. wall defect</td>
<td>Other abnl.</td>
<td>BPD</td>
<td>AC</td>
<td>FL</td>
<td>RL</td>
<td>Amniocentesis</td>
<td>Results</td>
<td>Pediatric follow up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>------------</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----------------</td>
<td>---------</td>
<td>---------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuchal thickness</td>
<td>Renal pyelectasis</td>
<td>Cardiac defect</td>
<td>Type cardiac defect</td>
<td>Limbs &lt; 5%</td>
<td>Choroid plexus cyst</td>
<td>SUA</td>
<td>Abnl hands</td>
<td>Abnl feet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>---------------------</td>
<td>------------</td>
<td>---------------------</td>
<td>-----</td>
<td>------------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6mm</td>
<td>&gt;4mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX C

Proposal and Project Approval Forms
BUTTERWORTH HOSPITAL RESEARCH AND HUMAN RIGHTS
ASSURANCE FORM

Instructions to Research Investigator: Complete Section I and II with signature. Complete either A, B, C or D as appropriate to your project. Investigators are referred to the Research Guidebook and the enclosed Butterworth Hospital policies. Consultants please refer to E. Please return this form to the Butterworth Hospital Research Office using the enclosed envelope.

SECTION I:

Investigator: Samantha A. Mullin
Address: 16324 92nd Ave. Kent #1
Allendale, MI 49401

Telephone: (616) 892-5605
Co-Investigator: Curtis R. Cook, M.D. Dawn D. DeWitt, M.D.
Title of Protocol: Ultrasound Findings and Neonatal Outcome in Pregnancies At Increased Risk of Trisomy 18 Based on Triple Serum Screening

Other institutions involved: √ Yes ___ No
(ex: hospital, school)
If yes, name of other institution: AVSC evw /enc

A. Research Presenting Possible Risk to Patients:
e.g. drug and medical device trials, surgical and other invasive procedures, studies involving randomization, placebo controls, including Phase IV, etc.
If you have not done so previously, PLEASE SUBMIT:
One copy of the complete protocol, including budget, data forms, consent form, bibliography. If not a Butterworth Hospital staff member, please include CV and institutional letter of approval.

B. Research Presenting Minimal Risk to Subjects:
In order for your study to be categorized as a "minimal risk" project it must fall into one or more of the following areas: PLEASE INDICATE THE CATEGORY.
1. Collection of hair and nail clippings, excreta and external secretions, uncannulated saliva, placenta removed at delivery, amniotic fluid at time of rupture of the membrane, deciduous teeth, and permanent teeth if patient care indicates a need for extraction. Collection of dental plaque and calculus done in a non-invasive manner performed according to standard prophylactic techniques.

2. Collection of blood sample by venipuncture, in amounts not exceeding 450 milliliters in an eight (8) week period, and no more often than twice a week, from subjects over 18 years of age, in good health, and not pregnant.

3. Recording of data from subjects 18 years or older using non-invasive procedures routinely employed in clinical practice (e.g., weighing, testing sensory acuity, electrocardiography, electroencephalography, thermography - not x-rays or microwaves).

4. Moderate exercise by healthy volunteers.

5. Voice recordings made for research purposes.

6. Research on Behavior: perception studies; cognition; game theory; test development, where the investigator does not manipulate subjects' behavior and the research will not involve stress to subjects.

C. Research Presenting No Risk to Subject:
In order for your research to be considered as a "no risk" study, it must fall into one or more of the following categories. PLEASE INDICATE WHICH AREA(S) APPLY:

1. Use of educational tests for which there is no subject identifying data.

2. Research involving collection or study of charts, specimens, or medical records for which there will be no subject identifying data.

3. Research involving questionnaire, surveys, and/or interviews. Subjects cannot be identified from data; subjects' response, if known, will not place
them at risk; research does not deal with sensitive aspects of subjects' behavior (e.g., illegal conduct, drug or alcohol use, sexual behavior). All conditions must be met.

D. Non-clinical studies, includes teaching and laboratory protocols and those listed in 21 CRF, subpart A, Sec. 58.3.

E. Consultant. Reason for consultation: ____________________________________________


SECTION II:

ALL INVESTIGATORS MUST SIGN THE FOLLOWING STATEMENT OF ASSURANCE:

My signature indicates my research project(s) / activity is prepared and will be conducted, in accordance with federal and institutional policies pertaining to research. Those federal policies are as outlined by the FDA, USDA and HHS. I have read and understand Butterworth Hospital’s policies concerning research, which include those on federal regulations, misconduct, conflict of interest and authorship and I agree:

1. To obtain informed consent for subjects who are to participate in this project.

2. To promptly report to the Research and Human Rights Committee, in writing, any changes made in the protocol to protect the life of subjects. This includes but is not limited to title and/or investigator changes.

3. To promptly report to the Research and Human Rights Committee, in writing, any unanticipated or adverse effects, including study patient deaths, which become apparent during the course or as a result of experimentation and the actions taken as a result.

4. To obtain prior approval from the committee before amending or altering the scope of the project or implementing changes in the approved consent form.
5. To cooperate with members of the committee charged with the continuing review of this project.

6. To maintain documentation of consent forms and progress reports as required by federal and institutional policy.

7. To maintain the confidentiality of this information at all times. Any unauthorized disclosure to a person or company is considered a breach of confidentiality, and as such a violation of hospital policies, including the misconduct policy. It may also be a reason for legal action to be taken against you.

8. If doing consulting work relative to any research activity, I agree to follow and adhere to the policies as cited.

Signature: _______________________________ Date: 07/08/98

SECTION III

INSTITUTIONAL REVIEW COMPLETION BY THE HUMAN RIGHTS COMMITTEE:

Disposition of the Protocol: approved Date of Committee Meeting: 8/7/97

In accordance with institutional policy, this protocol was approved via the procedures for expedited review.

______________________________ 14 Aug 98
Research Administrator Date

Assure2 (01/19/94) Revised 4/21/94
THESIS/PROJECT PROPOSAL APPROVAL
For the Degrees of:
Master of Science in Occupational Therapy
Master of Science in Physical Therapy
Master of Physician Assistant Studies

Student Name(s): Lora Davison, Holly Kerr, Samantha Mullin

Topic Area: Perinatology / Obstetrics

Title of Thesis/Project: Neonatal Outcome Based on Triple Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18

Anticipated Completion Date: March 1, 2000

Research Advisor/Committee Approval: The aforementioned student(s) has/have completed a satisfactory research proposal and may now submit project materials to HSRB. Following HSRB approval, student(s) may begin data collection.

Typed Name(s)                     Signature

[Redacted] Major Advisor

[Redacted] Committee Member

[Redacted] Committee Member

Date of Proposal Approval 4.9.99
GRAND VALLEY STATE UNIVERSITY
HUMAN RESEARCH REVIEW COMMITTEE

Principal Investigator: Lora Davison, Molly Kerr, Samantha Mullin

Department or School: School of Health Professions, Physician Assistant Studies

Address and Telephone: Contact person: Samantha Mullin 10318 42nd Ave, Allendale, MI 49401 892-7922

Title of the Project: Neoplastic Outcome Based on Triple Serum Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18.

Summary of the Project: See attached proposal.

In what capacity does this project involve human subjects? (E.g., surveys, interviews, clinical trial, use of medical records, etc.)

[ ] Medical Record chart review

Check one:

[ ] This is a report on research on human subjects which is exempted by 46.101 of the Federal Register 4616:8336, January 26, 1981. (Refer to instructions on the reverse of this form.)

[ ] This is a request for expedited review as described in 46.110 of the Federal Register 46(16):8336, January 26, 1981. (Refer to instructions on the reverse of this form.)

[ ] This is a request for full review. (Refer to instructions on the reverse of this form.)

Principal Investigator: 4/7/99

Department/Unit Chair and Advisor: 4/7/99

Date: 4/7/99

Date: 4/7/99

NOTE: Proposals which do not include a summary of the project and which fail to respond to the requirements stated in the instructions for applicants (on the back of this form) will not be considered and will be sent back to the authors.
April 14, 1999

Samantha Mullin, Loralee Davison, Molly Kerr
10318 42nd Ave. #12
Allendale, MI 49401

Dear Samantha:

Your proposed project entitled "Neonatal Outcome Based on Triple Serum Screening and Ultrasound Findings in Pregnancies with an Increased Risk of Trisomy 18" has been reviewed. It has been approved as a study which is exempt from the regulations by section 46.101 of the Federal Register 46(16):8336, January 26, 1981.

Sincerely,

Paul Huizenga, Chair
Human Research Review Committee
MEDICAL RECORDS CONFIDENTIALLY STATEMENT

Spectrum Health Downtown Hospital/Clinical Facility places great importance in the confidentiality of medical records. Use of the medical records for research or learning experience is permitted, provided the researcher or student realizes his/her role in responsibility in protecting the confidentiality of personally identifiable information. Misuse of information collected could result in personal liability and the implementation of punitive action.

I acknowledge that I have read the above statement and take the responsibility for proper and limited use of the confidential information in my research project or educational activity.

Signature

Date 3/23/00

Master's Project

Research Project/Educational Activity

Instructors Signature