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## **Diagnostic evaluation of fetal death with special reference to intrauterine infections**

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Det är gott att leva.  
Det är gott att finnas till, trots allt.  
(Ulf Lundell)

När det närvarande väger tyngre än det  
som aldrig blev, då är man en lycklig  
människa.  
(Vibeke Olsson, ”Koltrasten i  
Tegnérslunden”)



## **Abstract**

The incidence of intrauterine fetal death ( $\geq 28$  gestational weeks) is approximately 4/1000 births in Sweden. In order to arrive at a potential cause of death, a number of investigations have been recommended in the diagnostic evaluation of intrauterine fetal death (IUFD). One of the issues in this thesis was to review the causes of stillbirth and to evaluate a certain arsenal of diagnostic procedures in cases of IUFD.

We examined 188 cases of stillbirth occurring in Stockholm in 1998-99 according to an extensive investigational protocol. A presumptive explanation of the stillbirth was established in 91% of the cases. The most common factors associated with intrauterine fetal death could be identified as infections (24%), placental insufficiency/intrauterine growth restriction (22%), placental abruption (19%), intercurrent maternal conditions (12%), congenital malformations (10%), and umbilical cord complications (9%). Based on our studies, placental pathological examination and autopsy, together with some additional tests, are suggested to be included in the routine investigation of stillbirth to arrive at a minimum of unexplained cases of fetal death.

The association between some infectious disorders and fetal morbidity and mortality is well documented. This thesis focuses mainly on two agents: *Toxoplasma gondii* and parvovirus B19. When a primary toxoplasma infection occurs during pregnancy, *T. gondii* may be transmitted from the mother to the fetus. Fetal infection can result in inflammatory lesions in the brain, retina and choroid, which may lead to permanent neurological damage and visual impairment. Disseminated toxoplasma infection may cause fetal death. Many European countries have screening programs for the detection of congenital toxoplasmosis.

Anti-toxoplasma IgG and IgM antibodies were measured in eluates from PKU cards from 40,978 newborns in Stockholm and Skåne in 1997-98. The seroprevalence in pregnant women was 14.0% in Stockholm and 25.7% in Skåne. On comparing the seroprevalence measured between 1969 and 1987 in pregnant women in Stockholm with our data, it was noted that today the majority of seropositive pregnant women have seroconverted before entering the childbearing period. The incidence of primary toxoplasma infection in our study was 0.51/1000 susceptible pregnancies (9 months). The prevalence of congenital toxoplasmosis in live born children was 0.73/10000. We conclude that the incidence of toxoplasmosis during pregnancy is low in Sweden and introduction of a screening program cannot be recommended before the effectiveness of treatment has been evaluated. Health education directed at pregnant women may be sufficient in a country with so low an incidence of toxoplasma infection. Maternal primary infection with parvovirus B19 may be transmitted across the placenta. Several reports have shown that parvovirus B19 can cause fetal death in the second trimester, mainly in combination with hydrops fetalis. Some authors have reported that the infection might also be an important cause of stillbirth in late pregnancy in non-hydropic cases.

Placental and/or fetal tissues from 47 cases of IUFD were examined for the presence of parvovirus B19 DNA by the polymerase chain reaction (PCR). We found a significantly higher frequency of parvovirus B19 in fetal death (15%) compared to live-borns (0%). The majority of parvovirus B19 DNA-positive cases did not exhibit hydrops fetalis. We conclude that most cases of late fetal death due to infection with parvovirus B19 are non-hydropic and conventional diagnostic procedures for diagnosing parvovirus B19-associated fetal death can be greatly improved by adding B19 PCR.

INFPREG, an Internet-based database covering infectious disorders and pregnancy was introduced on the Web in 1999. In our evaluation of INFPREG, we sent an anonymous, self-administered questionnaire to all antenatal clinics in Sweden. The questionnaire consisted of sections covering use of computers, Internet access, and use of INFPREG in patient care.

Based on our results, we conclude that the majority of midwives at antenatal clinics in Sweden have access to the Internet and are confident in using an Internet-based knowledge center for infectious disorders in pregnancy. The significance of the Web as a medical information resource in antenatal care is likely to increase.

Key words: fetal death, stillbirth, investigational protocol, congenital toxoplasmosis, parvovirus B19, Internet, INFPREG

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## **List of original papers**

This thesis is based on the following papers, referred to by their Roman numerals:

- I. Petersson K, Stray-Pedersen B, Malm G, Forsgren M, Evengard B. Seroprevalence of *Toxoplasma gondii* among pregnant women in Sweden. *Acta Obstet Gynecol Scand.* 2000;79:824-9.
- II. Evengard B, Petersson K, Engman ML, Wiklund S, Ivarsson SA, Tear-Fahnehjelm K, Forsgren M, Gilbert R, Malm G. Low incidence of toxoplasma infection during pregnancy and in newborns in Sweden. *Epidemiol Infect.* 2001;127:121-7.
- III. Tolfvenstam T, Papadogiannakis N, Norbeck O, Petersson K, Broliden K. Frequency of human parvovirus B19 infection in intrauterine fetal death. *Lancet* 2001;357:1494-7.
- IV. Petersson K, Bremme K, Bottinga R, Hofsjö A, Hulthén-Varli I, Kublickas M, Norman M, Papadogiannakis N, Wångren K, Wolff K. Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998-99. *Acta Obstet Gynecol Scand.* 2002;81:284-92.
- V. Petersson K, Norbeck O, Westgren M and Broliden K. Detection of parvovirus B19, cytomegalovirus and enterovirus infections in intrauterine fetal death. Submitted.
- VI. Petersson K, Forsgren M, Sjödin M, Kublickas M and Westgren M. Dissemination of information in antenatal care on a national basis by an Internet-based system - INFPREG. Submitted.

## **Abbreviations**

B19	parvovirus B19
BMI	body mass index
CI	confidence interval
CMV	cytomegalovirus
CPM	confined placental mosaicism
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EMSCOT	European Research Network on Congenital Toxoplasmosis
FMH	fetomaternal hemorrhage
HELLP	hemolysis, elevated liver enzymes, low platelets
HIV	human immunodeficiency virus
ICD 10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICP	intrahepatic cholestasis of pregnancy
Ig	immunoglobulin
IT	information technology
IH	immunohistochemistry
IUFD	intrauterine fetal death
IUGR	intrauterine growth restriction
mAb	monoclonal antibody
NSP	non-structural protein
OR	odds ratio
PCR	polymerase chain reaction
PKU	phenylketonuria
Q/A	question/answer
RNA	ribonucleic acid
SGA	small for gestational age
SLE	systemic lupus erythematosus
TORCH	toxoplasma, rubella, cytomegalovirus, herpes simplex
WHO	World Health Organization



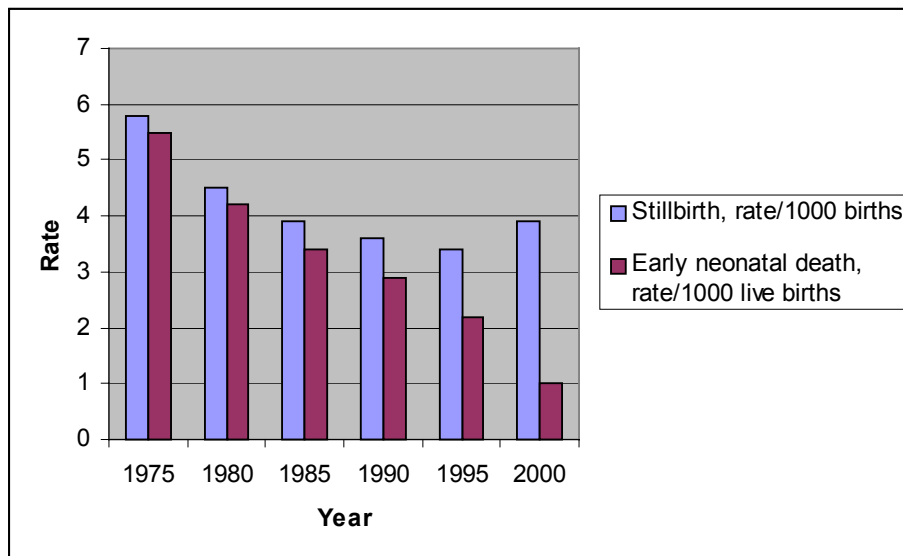
## **Definitions**

Intrauterine fetal death	Fetal death at gestational age of $\geq 22$ completed weeks (ICD 10-International Statistical Classification of Diseases and Related Health Problems, Tenth Revision) Fetal death at gestational age of $\geq 28$ completed weeks (The Swedish Medical Birth Register)
Early neonatal death	Death before 7 days of life among live-born infants
Perinatal death	Stillbirth and early neonatal death
Small for gestational age	Birth weight of less than 2 standard deviations below the mean birth weight for the gestational age

## **Introduction**

The incidence of intrauterine fetal death (from the 28th gestational week) is reported to be 3.6/1000 births in Sweden.<sup>1</sup> Continuous improvements in perinatal care have resulted in a dramatic decrease in early neonatal mortality during the last few decades, but no comparable reduction of antenatal mortality has been observed (Fig. 1).<sup>1, 2</sup> A better knowledge of the etiology of stillbirth is imperative to achieve a further decrease in the perinatal mortality rate. Furthermore, a better understanding of the causes of intrauterine fetal death (IUFD) is essential for adequate health planning and for the setting of research priorities in perinatal medicine. On an individual basis, the parents are entitled to an explanation as to why their baby died and a diagnosis can probably be helpful in their grieving process.<sup>3</sup> Last, but not least, the cause of fetal death may be relevant to later pregnancies and make it possible to improve the outcome of such pregnancies.

**Figure 1. Stillbirth and early neonatal death in Sweden 1950-2000.**



Source; The Medical Birth Register, The National Board of Health and Welfare, Stockholm, Sweden.

## **DEFINITION OF FETAL DEATH**

Many studies over the years have shown that there is substantial international variation in the definition of perinatal mortality despite attempts of the World Health Organization (WHO) to introduce common definitions. Differences between different countries make it difficult to

compare death rates on an international basis.<sup>4</sup> WHO states: “It is recommended that national perinatal statistics should include all fetuses and infants delivered weighing at least 500 g or, when birth weight is unavailable, the corresponding gestational age (22 weeks) or body length (25 cm crown-heel), whether dead or alive”.<sup>5</sup> In Sweden, all live-born babies, regardless of gestational age, and stillborn fetuses born after 28 completed gestational weeks are registered in the national perinatal statistics.<sup>1</sup> However, since 1997, the diagnosis “intrauterine fetal death” is used for all stillbirths delivered after 22 completed gestational weeks according to ICD 10<sup>6</sup> and this definition is used in Papers III-V in this thesis.

### **CLASSIFICATION OF CAUSES OF FETAL DEATH**

Correct classification of the cause of death is of primary importance and a large number of systems for classification of perinatal mortality have been proposed. The aim of such classification is to identify categories mainly responsible for the perinatal mortality in a population. Common use of a classification makes comparisons possible between both regions and countries with regard to the etiology of stillbirths and enhances the identification of changes over time. An increased knowledge of the causes of death may also increase the chances of preventing stillbirths.

The Aberdeen classification described by Baird et al. in 1954<sup>7</sup> was developed in order to identify the maternal condition that initiated the events that led to death. Hey et al. suggested a revision of this classification in order to identify the pathological processes occurring in the baby in every perinatal death.<sup>8</sup> The Nordic Baltic perinatal death classification was developed in order to identify suboptimal care<sup>9</sup> in cases of perinatal death. NICE - a cause of death classification for stillbirths and neonatal deaths<sup>10</sup>- is etiologically and hierarchically oriented and has been developed for epidemiological purposes. The classification used in this thesis is a revised form of the one proposed by Fretts et al.<sup>11</sup> In this classification, the factors associated with fetal death are listed as the fetal or maternal condition that was most likely to have initiated the “process” that resulted in death.

### **RISK FACTORS FOR FETAL DEATH**

Several studies have been conducted in recent years on risk factors for fetal death. Advanced maternal age increases the risk of fetal death. Women  $\geq 35$  years have a 40-50% higher risk of stillbirth compared to women at age 20-29 years.<sup>12, 13</sup> This age-associated risk

is more pronounced among primiparas than among multiparas. A possible reason that could partly explain this age-related risk may be the higher incidence of multiple pregnancies, gestational diabetes, hypertension, preeclampsia, and fetal malformation among older women.

Smoking during pregnancy has been associated in several publications with fetal death.<sup>14</sup>,<sup>15</sup> A possible causative relationship has been described; smoking increases the risk of intrauterine growth retardation and abruption of the placenta.<sup>16</sup> Smoking seems to be a causative factor in stillbirth, especially in preterm pregnancies.<sup>17</sup>

Maternal weight at the first visit for antenatal care influences the risk of fetal death. A connection between a high body mass index (BMI) and IUFD has been described earlier by Little<sup>15</sup> and Cnattingius.<sup>18</sup> Stephansson et al. have recently conducted a case-control study including 700 primiparas with IUFD and 700 controls. Compared to lean women (BMI  $\leq$  19.9), overweight primiparas (BMI 25.0-29.9) have a doubled risk of stillbirth and this risk is even higher among obese primiparas (BMI  $\geq$  30.0).<sup>19</sup> Weight gain during pregnancy does not seem to affect the risk of IUFD.<sup>19, 20</sup>

Social factors, such as level of education and socioeconomic status, affect the risk of stillbirth.<sup>21</sup> A recently published Swedish study reports that, after adjustment for confounders such as age, smoking habits, and BMI, workers with low-level education have a doubled risk for stillbirth compared to highly educated so-called “white collar workers”. These differences could not be explained by such factors as the number of visits for the antenatal care, the proportion of pregnancies complicated by intrauterine growth retardation, or intercurrent maternal disorders.<sup>22</sup>

Differences concerning the risk of perinatal mortality in relation to race or ethnicity have been reported by several authors.<sup>23, 24</sup> A Swedish study by Essén et al.<sup>25</sup> found that immigrant women, especially women from sub-Saharan Africa, were at increased risk for perinatal mortality compared to native Swedish women.

Several studies have found that a previous stillbirth increases the risk 6-10 times for a subsequent stillbirth in the next pregnancy.<sup>26, 27</sup> This recurrence tendency is more common among women with diabetes or hypertensive disorders.<sup>27</sup>

## **ETIOLOGY OF FETAL DEATH**

In population-based registers, the cause of death is seldom noted and even though many countries have a “death certification register”, the quality of this information is generally

poor.<sup>28</sup> It has been reported that the most adequate information on causes of stillbirth is obtained from perinatal audits.<sup>29</sup> Several studies have been published concerning specific causes of intrauterine fetal death.<sup>11, 30-32</sup> The diagnostic tools used and the classifications followed differ from study to study, thus making comparisons difficult.

### **Intrauterine growth restriction (IUGR)**

The relationship between low birth weight and perinatal mortality and morbidity is well documented. Stillborn infants weigh less at delivery than live-born babies of equivalent gestational age. This is commonly attributed to a process of growth restriction preceding death and probably sharing the same underlying causes as placental insufficiency.<sup>33</sup>

IUGR is considered to be one of the most important causes of stillbirth. Intrauterine growth restriction is known to be associated with multiple pregnancy<sup>34</sup>, congenital malformations<sup>35</sup>, fetal chromosomal abnormalities<sup>36</sup>, and preeclampsia<sup>37</sup>, but it is also a well-recognized fact that, in most cases, none of these conditions are present.<sup>17</sup> In a study by Gardosi et al., 41% of all cases of stillborn infants were small-for-gestational age (SGA) and a higher proportion of preterm (<37 weeks) than term stillborns were SGA. The definition of SGA in that publication was derived from a population-based standard. It has been suggested that, in order to identify small babies at risk for an adverse perinatal outcome, a customized birth weight standard should be used, which includes adjustment for maternal height, booking weight, and ethnic origin in addition to conventional variables such as parity, gestational age, and fetal gender.<sup>17</sup> De Jong et al. showed that this customized birth weight standard significantly improves the identification of infants who have failed to reach the expected birth weight and who are at increased risk for adverse perinatal events.<sup>38</sup> Another issue when diagnosing growth restriction in stillborn babies is whether or not the birth weight remains unchanged after death. On comparing fetuses experiencing antenatal and intrapartal death, the antenatal dead fetuses weigh less than intrapartal dead babies, suggesting that the low birth weight of stillborn infants may be partly due to weight loss following death.<sup>39</sup> The risk of IUFD in postterm pregnancies is increased from gestational week 41 and this risk is dramatically increased if the fetus is small for its gestational age.<sup>40</sup>

### **Maternal medical disorders**

Both type 1 and type 2 diabetes is associated with an increased risk of IUFD.<sup>41, 42</sup> The risk of IUFD among type 1 diabetic women is reported to be 4-5 times higher than among the non-diabetic population in Sweden.<sup>1</sup> The majority of stillbirths related to diabetes are reported to occur in patients with poor glycemic control and complications of macrosomia, polyhydramnios, intrauterine fetal growth restriction, and preeclampsia. Fetal death in uncomplicated gestational diabetes is uncommon.<sup>43</sup>

Hypertensive disorders (gestational hypertension, preeclampsia, chronic hypertension and superimposed preeclampsia) are common medical complications of pregnancy and constitute a major cause of perinatal morbidity and mortality. In a recent meta-analyse, the authors concluded that chronic hypertension increases the risk of perinatal mortality and morbidity even in the absence of superimposed preeclampsia.<sup>44</sup> McCowan et al. reported that, in the absence of superimposed preeclampsia, women with chronic hypertension had perinatal outcomes similar to those in the general population.<sup>45</sup> Martin et al. reported a stillbirth rate of 2% in patients with severe preeclampsia without HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome and a stillbirth rate of 5% in patients with HELLP syndrome.<sup>46</sup>

An increased risk of IUFD has been reported in women with hereditary antithrombin deficiency, activated protein C resistance, and protein C and protein S deficiency.<sup>47, 48</sup> Phospholipid antibody syndrome, with acquired phospholipid antibodies, correlates with IUFD and miscarriages due to impaired implantation, thrombosis, and infarctions in the placenta.<sup>49-51</sup> Phospholipid syndrome may occur isolated or associated with other autoimmune diseases such as SLE.<sup>52</sup>

Both maternal hypothyroidism and hyperthyroidism have been reported to be causative in stillbirths.<sup>53, 54</sup>

Intrahepatic cholestasis of pregnancy (ICP) with pruritus and increased levels of bile acids is reported to be associated with increased rates of fetal mortality and morbidity.<sup>55</sup> It is under debate whether or not an adverse perinatal outcome can be improved by active intervention or treatment.<sup>56-58</sup>

### **Fetal chromosome abnormalities and congenital anomalies**

Chromosome aberration increases the risk of perinatal death. Kuleshov et al. reported that 14% of cases of IUFD showed abnormal karyotypes.<sup>59</sup> This large number of karyotype

abnormalities in fetal death could not be verified in a report from France.<sup>60</sup> The most common autosomal trisomies in stillborns are 21, 18, and 13, and the most common abnormal karyotype is 45x.<sup>61</sup>

An increased frequency of adverse pregnancy outcome, including pregnancy loss, intrauterine growth restriction, and premature labor, has been observed in association with confined placental mosaicism (CPM), which is characterized by a discrepancy between the karyotype of the fetus and placenta. Specific chromosomal trisomies have been observed in CPM more frequently than others, with trisomies 7, 16 and 18 being more prevalent.<sup>62, 63</sup> Although chromosome aberrations dominate, there is also a significant group of fetuses that die intrauterine due to malformations and syndromes of other etiologies.<sup>64</sup> However, the majority of babies with lethal malformations will die after birth, for example, of congenital heart defects, pulmonary hypoplasia, and lethal genetic disorders such as Potter's syndrome, anencephaly, and hernia of the diaphragm.<sup>65</sup>

Compared to singletons, twins are at increased risk of stillbirth. Severely discordant monochorionic and dichorionic twins have significantly worse perinatal mortality and morbidity than mildly discordant and concordant twins.<sup>66</sup> Twin transfusion syndrome, occurring only in monochorionic pregnancies, carries a high risk of stillbirth in one or both of the twins.<sup>67</sup> If one twin dies in utero, the prognosis for a surviving dichorionic twin is relatively good. By contrast, the surviving monochorionic twin has a poor prognosis with a high frequency of neurological damage.<sup>68</sup>

### **Placental and umbilical cord complications**

Many and varied pathological findings in the placenta associated with stillbirth have been described, for example, inflammation of the membranes and/or umbilical cord, lesions consistent with uteroplacental vascular insufficiency, mainly seen as infarction and decidual arteriopathy<sup>69</sup> and signs of abruption.<sup>70</sup>

Cord complications (knots, encirclement, prolapse) have been described as being directly causative in fetal death<sup>71</sup>, although several authors question whether umbilical cord encirclement can cause fetal death.<sup>72, 73</sup> Abnormal umbilical cord coiling has been related to adverse perinatal outcomes.<sup>74</sup>

Massive fetomaternal hemorrhage (FMH) has been associated with stillbirth and fetal anomalies. Trauma to the uterus and abruption of the placenta have been described as events

that may lead to fetomaternal transfusion, but in most cases of the condition, no underlying cause can be identified. Samadi et al. reported an incidence of 4% of massive FMH among unselected cases of fetal death.<sup>75</sup>

## **Infections**

The placenta and the fetus can be infected either by transplacental (hematogenous) transmission or by an ascending infection from the vagina. The proportion of stillbirths associated with infections has been reported to be 6-15% of all cases of stillbirth in different studies.<sup>11, 30, 31, 76</sup> Several agents are considered to be related to fetal death. Congenital viral infection with parvovirus B19<sup>77-79</sup> and cytomegalovirus (CMV)<sup>80</sup> has been reported to cause fetal death. Infection with some enteroviruses has been associated with stillbirth.<sup>81</sup> Maternal rubella early in pregnancy may be a cause of fetal death<sup>82</sup> and intrauterine infection with herpes simplex has, in some case reports, been associated with stillbirth, but it is considered to be very rare.<sup>83</sup> Maternal primary infection with *Toxoplasma gondii* can be transmitted to the fetus and cause congenital toxoplasmosis and fetal death.<sup>84</sup> Several bacterial agents have been associated with perinatal mortality: Group B *Streptococci*<sup>85</sup>, *Escherichia coli*<sup>86</sup>, *Listeria monocytogenes*<sup>83, 87</sup>, lues<sup>88</sup>, genital mycoplasma and *Ureaplasma urealyticum*<sup>83, 89</sup>. Chorioamnionitis due to candida infection and intrauterine devices has been observed in cases of stillbirth.<sup>90</sup> Malaria is a well-known cause of stillbirth.<sup>91</sup> Fetal death as a consequence of severe maternal sepsis with thrombosis in the placenta and stillbirths have been reported.<sup>92</sup>

### **Toxoplasmosis and infection with parvovirus B19 during pregnancy**

We have chosen to focus mainly on two agents in this thesis: infection with *Toxoplasma gondii* and parvovirus B19.

When a primary toxoplasma infection occurs during pregnancy, *T.gondii* may be transmitted from the mother to the fetus. Fetal infection can result in inflammatory lesions in the brain, retina and choroids, which may lead to permanent neurological damage and visual impairment. Disseminated infection may cause fetal death.<sup>93</sup> The incidence of congenital infection in live-borns and stillborns in Sweden has not been known before now.

Maternal primary infection with parvovirus B19 may be transmitted across the placenta. Several reports have shown that parvovirus B19 can cause fetal death in the second trimester,



mainly in combination with hydrops fetalis.<sup>77</sup> Some authors have reported that the infection might also be an important cause of stillbirth in late pregnancy in non-hydrotic cases.<sup>78, 94</sup> The frequency of parvovirus B19, especially in non-hydrotic cases of intrauterine fetal death, needs to be further investigated.

### **Unexplained stillbirth**

The proportion of stillbirths in which no identifiable cause can be determined ranges from 12-50% in the literature.<sup>11, 30-32, 95, 96</sup> It has been suggested that the risk factors for unexplained deaths may differ from the risk factors among women delivered of fetuses with a specified cause of death. Froen et al. reported that the risk of sudden intrauterine unexplained death increased with gestational age, advanced maternal age, high cigarette use, low education, and overweight or obesity. Primiparity and previous stillbirths or spontaneous abortions were not associated with sudden intrauterine unexplained death in that study.<sup>97</sup> Huang reported from a study of 196 unexplained fetal deaths occurring during 1961-74 and 1978-96 that the following factors were independently associated with unexplained fetal death: maternal prepregnancy weight greater than 68 kg, birth weight ratio (defined as ratio of birth weight to mean weight for gestational age) between 0.75 and 0.85 or over 1.15, fewer than four antenatal visits by women whose fetuses died at 37 weeks or later, primiparity, parity of three or more, low socioeconomic status, cord loops and, for the 1978-96 period only, maternal age of 40 years or more.<sup>95</sup> The scope of investigative procedures used in the studies differs, however, making the groups of unexplained stillbirths difficult to compare.

### **DIAGNOSTICS IN FETAL DEATH**

In order to arrive at a potential cause, a number of investigations have been recommended in the diagnostic evaluation of IUFD.<sup>30, 98-101</sup> With an extensive and relevant test protocol, several authors have reported a reduction of unexplained deaths.<sup>30, 102, 103</sup> Incerpi et al. stated that certain laboratory tests can be eliminated in the fetal death workup, and that a complete systematic method that incorporates placental pathological conditions, as well as autopsy findings, should prove to be beneficial in the evaluation of stillbirths.<sup>100</sup> In conclusion, there is no generally accepted “gold standard protocol” in cases of fetal death. One of the issues in this thesis is to review the causes of stillbirth and to evaluate a certain arsenal of diagnostic procedures in cases of intrauterine fetal death.

## **THE INTERNET AS A RESOURCE FOR DISSEMINATION OF INFORMATION ON INFECTIOUS DISORDERS IN PREGNANCY**

Intrauterine infection and its relation to perinatal morbidity and mortality have been highlighted in recent years. Rapid developments in such fields as microbiological diagnostics and pharmacotherapy have made it difficult for clinicians to keep their knowledge up to date. Practice may easily become outdated and there is an obvious risk of suboptimal care. Medical information on the Internet offers great advantages due to its ready availability and the possibility of current updating. Updated knowledge concerning the management of infectious disorders in pregnancy may improve the quality of care of these patients, and consequently, decrease the perinatal mortality and morbidity associated with infections.

## **Aims of the study**

- To evaluate the seroprevalence of toxoplasmosis in pregnant women of different ages and origins in Sweden.
- To estimate the incidence of seroconversion and the rate of transmission of Toxoplasma infection during pregnancy and the incidence of congenital toxoplasmosis in Sweden.
- To review the causes of stillbirth in a well-defined population and to evaluate a certain arsenal of diagnostic procedures in cases of intrauterine fetal death.
- To estimate the frequency of infectious diseases in fetal death with a focus on viral and toxoplasma infections.
- To study the frequency of infections with parvovirus B19, CMV and enterovirus in unselected cases of fetal death and healthy controls.
- To compare different diagnostic procedures (polymerase chain reaction (PCR), immunohistochemistry (IH), and histopathological examination) in diagnosing parvovirus B19 infection in cases of fetal death.
- To evaluate INFPREG, an IT-based database for the dissemination of knowledge concerning infectious diseases during pregnancy.

## **Material and methods**

### **Papers I and II**

All mothers of newborns born in the Stockholm area between April 1, 1997, and July 31, 1998, and in the Skåne area (in the southern part of Sweden) between May 1, 1997, and July 31, 1998, were invited to participate. The mothers of 40,978 newborns (n=26,885 in Stockholm County and n=14,093 in Skåne County) gave their consent to have their newborns tested, representing 97,1% of all newborns during the study period.

Eluates from PKU cards from the participating children were analyzed for the presence of anti-toxoplasma IgG and IgM antibodies by the enzyme-linked immunosorbent assay (ELISA). If specific IgG and/or IgM antibodies were detected, a stored maternal serum sample drawn during early pregnancy for HIV and rubella IgG testing was requested and analyzed for specific IgG and IgM antibodies. If toxoplasma IgM and/or high levels of IgG were demonstrable in this maternal sample, the avidity of IgG was measured to further elucidate the time of onset of infection. All newborns to women with a primary infection during pregnancy were followed clinically and serologically for one year. The diagnosis of congenital toxoplasmosis was based on specific IgM and IgA antibodies in confirmatory neonatal serum and persistence of IgG at the age of 12 months.

### **Paper III**

Placental and fetal tissues were prospectively collected from cases of fetal death (n=47) occurring between January, 1998, and May, 1999, at Huddinge University Hospital, Stockholm Söder Hospital, and Södertälje Hospital. Tissues were analyzed by B19 NSP PCR after DNA extraction. Formalin-fixed paraffin-embedded tissues were examined by IH staining using a mAb directed to capsid antigen. B19 PCR-positive fetal tissues and placenta were thoroughly examined for the presence of histopathological indicators of B19 infection by a senior perinatal pathologist. For comparison, we included 37 referred cases of spontaneous abortion (<22 weeks of gestation) of unknown etiology and 29 referred cases of fetal loss (<22 weeks of gestation) of known etiology derived from legal abortions. Consecutively collected placental tissues from 53 normal term pregnancies were included in order to estimate the frequency of asymptomatic carriers of parvovirus B19. All samples were processed in the same manner and coded until the end of the study.

#### **Paper IV**

All cases of intrauterine fetal death (n=188) occurring in any of the five hospitals in Stockholm County (Danderyd Hospital, Huddinge University Hospital, Karolinska University Hospital, Södertälje Hospital, and Stockholm Söder Hospital) during the period January 1, 1998 – December 31, 1999, were included. The inclusion criteria were a gestational age of  $\geq$  22 completed weeks and no signs of life when born (i.e. an Apgar score of 0,0,0). A detailed examination of the mother, placenta, and fetus was performed according to an extensive test protocol. An Internet-based database was developed for the purpose of collecting study results. Test results from the examinations of the mother, placenta and fetus, together with data from the prenatal maternity and delivery records, were entered in this database. All cases of fetal death were reviewed by a group of obstetricians representing all of the five hospitals and a perinatal pathologist. Test results from the examinations of the mother, placenta, and fetus, together with data from the prenatal maternity and delivery records, were scrutinized and, following the classification, the group was assigned a probable cause of death. Maternal characteristics, including smoking, parity, occupation, age, assisted reproduction, and simplex/multiple pregnancy, were compared between women giving birth to stillborn and live-born babies during the study period employing logistic regression analysis.

#### **Paper V**

The study was conducted prospectively on January 1, 1998- February 28, 2001, at Huddinge University Hospital in Stockholm, Sweden. Samples were obtained from 52 of the 60 cases of IUFD during the study period. Placental biopsy specimens were collected from 46 of the cases and fetal blood and/or amniotic fluid from 43 of the cases. Placental biopsy specimens from normal term pregnancies (n=53) were used as controls. The tissues were examined for parvovirus B19 DNA, CMV DNA, and enterovirus RNA using the PCR technique. Maternal virus and toxoplasma serology was performed on maternal blood (n=46) and virus isolation was performed in maternal stool samples (n=31). All cases of fetal death were examined according to an extensive investigational protocol. The same senior pathologist performed the histopathological investigation in all cases of IUFD. Clinical data were extracted from the patients' obstetric medical records.

#### **Paper VI**

INFPREG (infectious diseases during pregnancy) is an interactive Internet-based database containing updated information concerning infectious diseases during pregnancy. It was

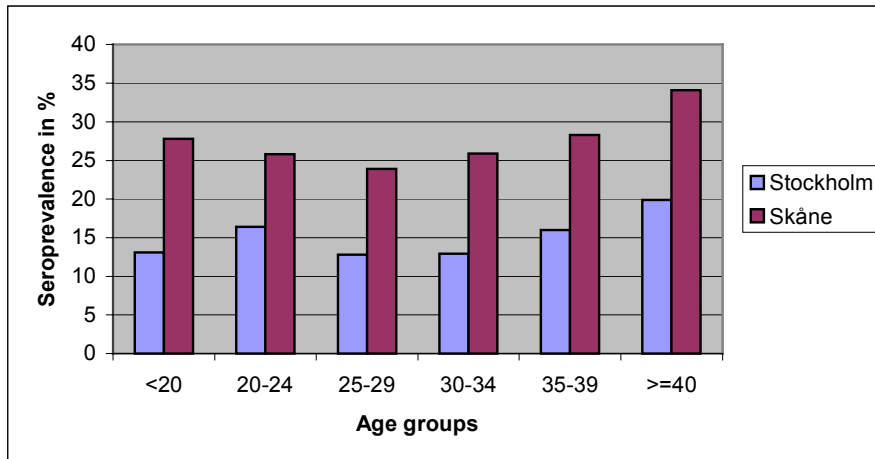
introduced on the Internet in 1999. At the present moment, it consists of 32 different chapters on various infectious diseases in pregnancy. All chapters are structured in the same way and are presented in two versions, one for medical personnel and one for the general public. In addition, there is an interactive Q/A facility for medical personnel, which is password-protected. All chapters are updated routinely every year. Information about INFPREG has only been directed toward medical personnel and not to the general public. The information has been made available through pamphlets, lectures, and papers in national medical and midwifery journals. In our evaluation of INFPREG, we sent an anonymous, self-administered questionnaire to all antenatal clinics in Sweden in 2000 (n=515) and 2002 (n=503). The questionnaire consisted of sections covering the use of computers, Internet access and the use of INFPREG in patient care.

## Results and discussion

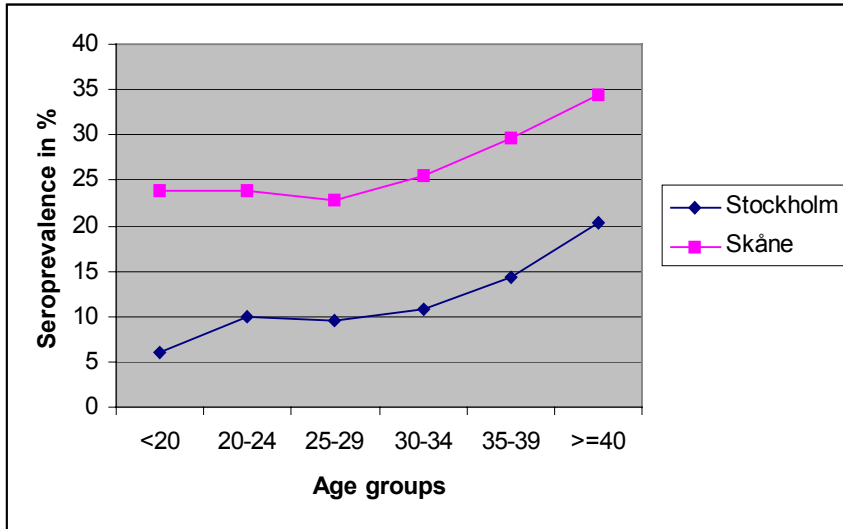
### Papers I and II

Specific IgG was found in 3772 cards in Stockholm and 3618 cards in Skåne, giving a seroprevalence among pregnant women of 14.0% in Stockholm area and 25.7% in Skåne. Clear differences were found between age groups and between women born in the Nordic countries and women born outside the Nordic countries (Figs. 2,3,4).

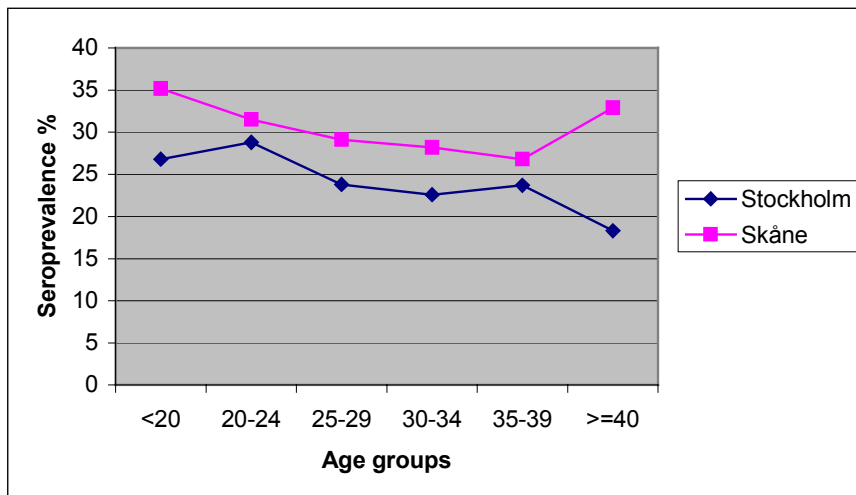
**Fig. 2 Prevalence of *Toxoplasma gondii*-specific IgG antibodies in pregnant women in different age groups in Stockholm and Skåne in 1997-98.**



**Fig.3 Seroprevalence of *Toxoplasma gondii* in different age groups among women born in the Nordic countries and living in Stockholm and Skåne in 1997-98.**



**Fig. 4 Seroprevalence of *Toxoplasma gondii* in different age groups among women born outside the Nordic countries and living in Stockholm and Skåne in 1997-98.**

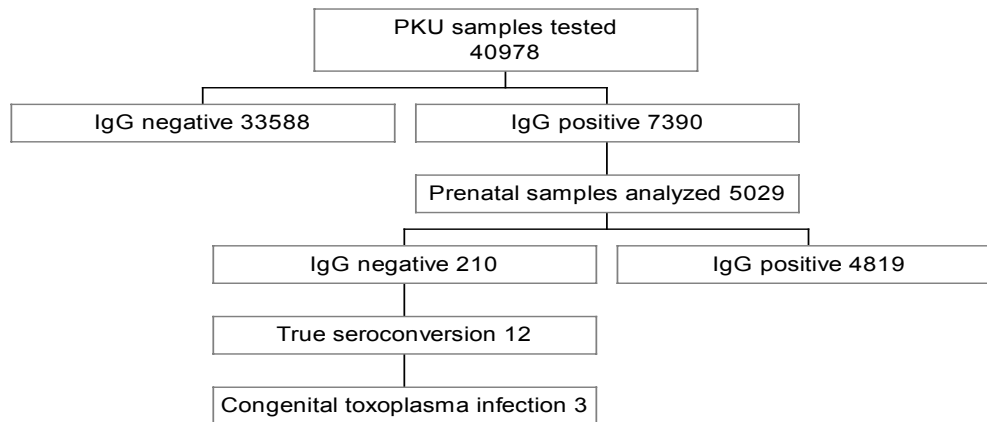




On examining different age groups, the seroprevalence was found to increase with increasing age in the subgroups of women born in the Nordic countries. This tendency was not observed in women born outside the Nordic countries. Data from a previous study by Forsgren et al.<sup>104</sup> were available for a comparison between birth cohorts among Nordic women in Stockholm. On comparing our results from Stockholm for 1997-98 with the seroprevalence measured in pregnant women in Stockholm in 1969, 1979, and 1987, it became obvious that the seroprevalence in different birth cohorts has remained at the same level throughout a 20-year period. We conclude that the majority of seropositive pregnant women in Sweden today have seroconverted before entering the childbearing period and that the percentage of women in Sweden contracting toxoplasmosis during the childbearing period is low.

True seroconversion was detected in 12 women (Fig. 5). At follow-up, three of the 12 children born to women showing evidence of seroconversion during pregnancy were found to have congenital toxoplasma infections. The incidence of primary toxoplasma infection (defined as seroconversion during pregnancy in women giving birth to live children) was 0.51/1000 susceptible pregnancies (9 months). The mother-to-child transmission rate was 25% (3/12). The prevalence of congenital toxoplasmosis in live-born children was 0.73/10,000 (3/40,978). The three infected children received antiparasitic treatment for 12 months. Two of these children showed clinical signs of infection; one had hydrocephalus, intracranial calcifications, a small corneal diameter and chorioretinitis in the right eye and the other had cranial calcifications. All three children are developing normally today.

**Figure 5. Toxoplasma IgG and IgM in pregnant women and newborns.**



Another study on the diagnostic evaluation of fetal death was conducted in Stockholm in 1998-99 (Paper IV). Maternal toxoplasma serology was included in the investigation protocol and was measured in blood retrieved at the first visit for antenatal care and at the time of stillbirth. None of the 147 women investigated showed definitive serological evidence of seroconversion for toxoplasma during pregnancy. These results make us confident that we did not miss any case of congenital toxoplasmosis due to fetal death in Stockholm during the study.

The best approach to prevention and control of congenital toxoplasmosis is not clear. Routine testing for the detection of maternal infection with *T. gondii* during pregnancy is offered in some European countries. Primary infection is defined as seroconversion (appearance of specific Toxoplasma immunoglobulin IgG and IgM) between two blood collections. When seroconversion is established, the pregnant woman is treated with medication to prevent transmission and an amniocentesis is performed. The diagnosis of congenital infection is clinched by findings of toxoplasma DNA by PCR and/or by isolation of *T. gondii* by mouse inoculation in amniotic fluid.<sup>105</sup> The evidence of effectiveness of

antenatal treatment with antiparasitic drugs regarding the risk of transmission and development of clinical sequelae in the infected fetus is under debate. A systematic review by Wallon et al.<sup>106</sup> showed that no controlled trials have been conducted regarding antiparasitic treatment and the risk of transmission. In a Cochrane review published in 2000, the authors concluded, “Despite a large number of studies performed over the last three decades, we still do not know whether antenatal treatment in women with presumed congenital toxoplasmosis reduces the transmission of *Toxoplasma gondii*”.<sup>107</sup> A recent study by Foulon et al. found no effect of any prenatal treatment on transmission after adjustment for gestation at maternal infection. However, they reported an odds ratio of 0.3 (95% confidence interval 0.1-0.9) for clinical signs at one year of age in children whose mothers were treated antenatally compared to children with untreated mothers.<sup>108</sup> Gilbert et al. reported that on comparing the relative risk of mother-to-child transmission and clinical manifestations between centers with intensive prenatal treatment, short-term treatment, and no treatment, they found no clear evidence that the risk of transmission or clinical manifestations was the lowest in centers with the most intensive treatment.<sup>109</sup>

After birth, a definite diagnosis of congenital toxoplasmosis can be made on detection of parasites (mouse inoculation or PCR) or on detection of specific antibodies in blood from the newborn.<sup>110</sup> Toxoplasma-specific IgM in neonatal blood has been routinely analyzed in New England for ten years and was introduced on a routine basis in Denmark in 1999. It is estimated that 70-80% of all children with congenital toxoplasmosis will be identified by this screening procedure.<sup>111</sup>

Postnatal treatment of congenitally infected infants is considered to be effective in those with symptoms.<sup>112</sup> However, most congenitally infected infants are asymptomatic at birth.<sup>113</sup> Whether antiparasitic treatment of this group is beneficial is uncertain although Guerina et al. reported in 1994 that early treatment even in subclinical cases might reduce the severe long-term sequelae.<sup>114</sup> Today, most centers treat infected children, even if they show no clinical signs of congenital toxoplasmosis.

Health education on how to avoid maternal toxoplasma infection during pregnancy is an important aspect of any program for prevention of congenital toxoplasmosis. Foulon et al. reported that the incidence of maternal toxoplasma infection could be reduced by 63% when health education was introduced.<sup>115</sup> EMSCOT (European Research Network on Congenital Toxoplasmosis) reported from a multicenter study comprising 252 infected women and 858

controls that the risk factors most strongly predictive of acute toxoplasma infection in pregnant women were eating undercooked or cured meat products, contact with soil, and travel outside Europe and the United States and Canada. Contact with cats was not a risk factor.<sup>116</sup>

The incidence of toxoplasma infection during pregnancy and congenital toxoplasmosis is low in Sweden. Introduction of a screening program during pregnancy or in the neonatal period cannot be recommended before the effectiveness of treatment has been evaluated. Health education for pregnant women may be sufficient in a country with so low an incidence of toxoplasma infection.

### **Paper III**

Seven of the 47 cases (15%) of fetal death were positive for parvovirus B19 DNA in fetal and/or placental tissue. Fetal tissue from two of 37 (5%) miscarriages was positive for parvovirus B19 DNA. Fetal and placental tissues from legal abortions (n=29) and placental tissues from normal term pregnancies (n=53) were all found to be negative.

Thus, the occurrence of parvovirus B19 DNA in the group of stillborn babies was significantly higher than in normal pregnancies at term ( $P < 0.05$ , chi-square test).

Histopathological examination revealed the presence of amphiphilic inclusions in nucleated fetal erythrocytes characteristic of parvovirus B19 in two cases of PCR-positive fetal death and in one case of PCR-positive miscarriage. The same three cases were positive by IH. All PCR-negative cases from all study groups were negative by IH. All but one of the DNA-positive cases of intrauterine death were non-hydropic. We conclude from these findings that most cases of late fetal death due to infection with parvovirus B19 are non-hydropic, and that conventional diagnostic procedures for diagnosing parvovirus B19-associated fetal death can be greatly improved by the addition of B19 PCR. PCR analysis for the detection of parvovirus B19 in placental and fetal tissues may be included in the routine investigation protocol in cases of fetal death.

Parvovirus B19 infects the fetus across the placenta and the rate of transplacental transmission is estimated to be approximately 24-51%.<sup>117, 118</sup> A fetal infection may cause an inhibition of the erythropoiesis leading to fetal anemia, fetal hydrops, and death.<sup>78, 119</sup> Parvoviral DNA has been found in fetal myocardial cells, suggesting that the infection may cause clinical complications, such as myocarditis or cardiac arrest, in the fetus.<sup>94, 120</sup> The estimated excess risk for fetal loss in women with parvovirus B19 infection during pregnancy

is estimated to be approximately 10%.<sup>118, 121</sup> Fetal B19 infection may also be asymptomatic, and there have been several observations of infants born healthy despite evidence of intrauterine infection.<sup>121</sup>

The seroprevalence is reported to be approximately 60% in women of childbearing age<sup>122, 123</sup> and the annual seroconversion rate has been estimated to be 1.5% and 13% during respectively non-epidemic and epidemic years.<sup>123</sup> In this Danish study, the risk during epidemics, of contracting the infection in pregnant women was reported to be correlated with the level of contact with children. Nursery school teachers were reported to have the highest occupational risk. Some authors have suggested that exclusion from work during endemic periods should be considered for pregnant women with an occupational risk of contracting B19 infection (e.g. nursery school teachers).<sup>124, 125</sup> The effectiveness of this prevention strategy is debatable since most infections are acquired through exposure to the woman's own children.<sup>123, 126</sup>

Management of B19-infected pregnant women may be problematic since fetal death can occur as early as a few days post infection up to several months later.<sup>118, 127</sup> Maternal infections often go unrecognized due to non-existent or diffuse symptoms.<sup>128</sup> Fetal affection can be suspected during ultrasonographic examination if the fetus exhibits a hydropic appearance. Intrauterine transfusion has been used to treat fetal anemia.<sup>129</sup> So far, no large studies have been conducted to determine if intrauterine blood transfusion is associated with an increased survival rate in fetal B19 infection. However, several series of cases have been reported suggesting that it may enhance the rate of fetal survival.<sup>130, 131</sup> The most comprehensive investigation made retrospectively studied 38 fetuses with intrauterine B19 infection and hydrops. Nine of 12 fetuses treated with blood transfusion survived compared to 13 of 26 non-treated fetuses.<sup>132</sup> Maternal intravenous immunoglobulin treatment has been suggested in cases of fetal B19 infection, since these passively transferred antibodies may neutralize the virus infection. This regimen has been employed in some isolated cases, but the positive benefit of this treatment has yet to be evaluated.<sup>133</sup>

A prophylactic vaccine against parvovirus B19 is being tested in the USA<sup>134</sup> and, hopefully, will be available in Sweden soon. Non-immune individuals with hemolytic anemia and immunocompromised children will probably be given high priority when a vaccination program is introduced, but immunization of non-immune fertile women will certainly be discussed.

#### **Paper IV**

A presumptive explanation of the stillbirth was established in 91% of the cases. The most common factors associated with IUFD could be identified as infections (24%), placental insufficiency/intrauterine growth restriction (22%), placental abruption (19%), intercurrent maternal conditions (12%), congenital malformations (10%), and umbilical cord complications (9%). Infection was considered to be the only factor or one of two contributing factors to intrauterine fetal death in 46 cases (24%). The most common bacterial infections were Group B *streptococci*, *enterococci*, and *Escherichia Coli*. There was one case of syphilis. Parvovirus B19 infection was demonstrated as the cause of death in four cases, enterovirus in two, and CMV and candida in one case each. No case of congenital toxoplasmosis was observed.

On comparing the cohort of women in the same area giving birth to live babies (n=34,648) with women experiencing IUFD (n=183), some risk factors for IUFD were observed. Significant independent variables for intrauterine fetal death were: maternal age  $\geq 40$  years (OR 2.66, 95% CI 1.53-4.62,  $P < 0.001$ ), smoking (OR 1.69, 95% CI 1.13-2.52,  $P < 0.01$ ), multiple pregnancy (OR 3.17, 95% CI 1.86-5.41,  $P < 0.0001$ ), and unemployment (OR 1.64, 95% CI 1.22-2.21,  $P < 0.001$ ).

We conclude that a relevant test protocol in cases of IUFD reduces the number of unexplained cases to a minimum. Infections were identified as one of the major contributors to fetal death in the present study. The risk factors for stillbirth were thus considered to be advanced maternal age, smoking, multiple pregnancy, and unemployment, all being risk factors that have been observed in several other studies.<sup>135</sup>

A number of tests have been advocated to arrive at potential causes in cases of fetal death. Placental examination and autopsy have proven to be the most valuable examinations in determining the cause of death.<sup>100</sup> Rayburn et al. reported from a study comprising 89 cases of fetal death that histological placental abnormalities were supportive of prior impressions in 77% of the cases, contradictory to prior impressions in 11%, or the sole contributors in explaining the cause of death in 11%.<sup>136</sup> Saller et al. found that in 94 cases with conclusive autopsies, the pathological diagnosis confirmed the clinical diagnosis in 55%, and it changed or significantly added to the clinical diagnosis in 45%.<sup>137</sup> In conformity with previous findings, we were able to demonstrate that placental pathological examinations and autopsies are valuable examinations in determining the factors associated with IUFD. These

investigations contributed to the diagnosis in 54% and 36% of the cases, respectively. However, in contrast to Incerpi et al.<sup>100</sup> we do not agree that placental examination and autopsy are sufficient in the effort to arrive at an explanation in cases of fetal death. Regarding the spectrum of underlying causes of stillbirth, we consider that some additional tests should be performed. According to the results presented in Papers III-V, together with data from other studies referred to in the Introduction of this thesis, the IUFD collaboration group in Stockholm has proposed the investigational protocol presented in Table 2.

**Table 2. Investigational protocol in cases of fetal death.**

<p><b>Routine investigations performed in all patients with stillbirth</b></p> <ul style="list-style-type: none"> <li>• Anti-cardiolipin antibodies, Lupus anticoagulant, Factor V mutation (in maternal blood)</li> <li>• PCR in placental and/or fetal tissue/amnion for parvovirus B19 and CMV (PCR enterovirus can be considered)</li> <li>• Bacterial culture in fetal cardiac blood, amnion, cervix</li> <li>• Karyotype (amnion and placenta)</li> <li>• Hemoglobin, alanine aminotransferase (ALAT), bile acids, platelets, antithrombin, prothrombin complex, activated partial thromboplastin time (APTT) and fetal hemoglobin/Kleihauer Betke (in maternal blood)</li> <li>• TORCH serology and serology for parvovirus B19 (maternal blood)</li> <li>• Placental examination</li> <li>• Autopsy</li> </ul> <p><b>Extended investigation in cases with intrauterine growth restriction, infarctions and/or thrombosis in the placenta, preeclampsia, abruption of the placenta, maternal history of thromboembolic disease</b></p> <ul style="list-style-type: none"> <li>• Protein S</li> <li>• Protein C</li> <li>• Prothrombin polymorphism</li> <li>• Homocysteine</li> </ul> <p><b>Investigations under special indications</b></p> <ul style="list-style-type: none"> <li>• Thyrotropin, free thyroxine and thyroid-stimulating immunoglobulin in cases of suspected maternal thyroid disease</li> <li>• X-ray if fetal skeletal malformation is suspected</li> <li>• Platelet antibodies in maternal blood if internal bleeding is noted in the fetus</li> </ul>
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Source; IUFD collaboration group in Stockholm 2002.

### **Paper V**

Samples were obtained from 52 cases of fetal death and analyzed for the presence of parvovirus B19 DNA, CMV DNA, and enterovirus RNA. Viral nucleic acid was found in one

or more tissue samples from a total of six cases: parvovirus B19 in two, CMV in three, and enterovirus in one. Virus isolation for enterovirus in maternal stool samples was performed in 31 cases, all of which were found to be negative. The serological investigation in 46 of the mothers showed seroconversion for parvovirus B19 only in the two parvovirus B19 DNA-positive cases but no signs of primary infection with the other agents tested: CMV, herpes simplex, rubella, or toxoplasma in any mother. In the three CMV DNA-positive cases, maternal IgG but no IgM or rise in IgG activity was found. According to their medical records, none of the women suffering from IUFD associated with viral infection had shown clinical signs of infection during pregnancy. In the six PCR-positive cases of fetal death, no other obvious cause of death was established. The placental biopsy specimens from 53 normal term pregnancies were all found to be PCR-negative for parvovirus B19, CMV, and enterovirus.

Viral infection is one of several causes of stillbirth. In most cases, it is not possible to diagnose the infection based on symptoms in the pregnant woman. Maternal “TORCH” (toxoplasma, rubella, cytomegalovirus, herpes simplex) serology has been carried out routinely in cases of fetal death at many centers. Serological signs of infection alone are not sufficient to explain fetal death, but it can, in combination with the results of other investigations, contribute to an explanation. Several authors have questioned the effect of this investigation in stillbirths.<sup>100, 101</sup>

Maternal serology is considered to be the “gold standard” for diagnosing toxoplasma infection and seroconversion indicates acquisition of infection during pregnancy.<sup>93</sup> The incidence of congenital toxoplasmosis is low in Sweden and the frequency of fetal death due to toxoplasma infection must be considered to be low. Desmonts and Couvreur have reported that stillbirth or neonatal death occurs in 5% of the pregnancies with maternal toxoplasma infection in the first trimester, in 2% in pregnancies where the women acquired the infection in the second trimester and the corresponding figure is 0% for the third trimester.<sup>138</sup>

Maternal rubella early in pregnancy may be a cause of fetal death, but it must be considered to be an extreme rarity in the Swedish population today due to the energetic immunization program. No case of congenital rubella has been registered in Sweden since 1985.<sup>82, 139, 140</sup> However, it has been reported in recent years that a higher percentage of Swedish parents than before refuse to have their children vaccinated due to worries about negative side effects.<sup>141</sup> If the vaccination coverage continues to decrease, rubella may again be regarded as a cause of fetal complications in Sweden.



CMV is the leading cause of intrauterine infection and, according to a prospective Swedish study in the 1980s by Ahlfors et al., 0.5 % of all children have a congenital CMV infection.<sup>142</sup> CMV can cause multiorgan disease and clinical manifestations include hepatomegaly, splenomegaly, thrombocytopenia, pneumonitis, growth retardation, and microcephaly.<sup>80</sup> Approximately 10-20% of the congenitally infected newborns will suffer from long-term sequelae such as hearing loss, mental retardation, and motor disability.<sup>143</sup>,<sup>144</sup> CMV has been reported to cause spontaneous abortion and fetal death, but its incidence is not clear.<sup>143</sup> Griffiths reported that, in 11 pregnancies with first trimester primary CMV infection, 2 resulted in late fetal loss (gestational weeks 24 and 29).<sup>145</sup> Both primary and recurrent maternal CMV infection can cause fetal damage, although the risk of adverse sequelae in secondary infections seems to be low.<sup>146</sup> The use of serology in the determination of secondary maternal infection during pregnancy can be difficult to evaluate since detection of IgM can be missed when the test is taken at the time when IUFD is diagnosed. The need for other investigations in diagnosing CMV associated with stillbirth is therefore obvious.

In some case reports, intrauterine infection with herpes simplex has been associated with stillbirth, but this is considered to be very rare.<sup>83</sup>

Serology for enterovirus and parvovirus B19 is not included in the “traditional TORCH serology”. The majority of maternal enterovirus infections do not affect the fetus but it has been reported that infection with coxsackievirus B2, B3 and B4,<sup>147</sup> and echovirus 11<sup>148</sup> has been associated with intrauterine fetal death. It has been assumed that enterovirus causes fetal myocarditis and CNS infection.<sup>147</sup>, <sup>149</sup> Maternal infection can be identified by means of serology or virus isolation in stool samples although these tests are known to have a low sensitivity and specificity in establishing the diagnosis of the acute phase of an enteroviral infection.<sup>150</sup>

It has been reported that even when placental and autopsy findings are consistent with infection with parvovirus B19 in cases of stillbirth, serology on maternal samples at birth may reveal a delayed or absent B19 IgG response.<sup>78</sup>

In conclusion, the use of “TORCH” serology is not sufficient for screening for viral infections in cases of stillbirth.

In some cases, the viral infection can be identified by typical morphological findings, such as the presence of typical inclusions of CMV or parvovirus B19, which can be confirmed by

specific immunohistochemistry. However, in many cases, it has not been possible to diagnose the infectious agent by autopsy or placental examination alone. PCR on placental and/or fetal tissue is a necessary complement in diagnosing these infections. Although findings of viral genomes are not proof of causality between the infection and the stillbirth, the combination of PCR technique, placental examination, and autopsy may increase the chances of diagnosing viral infections associated with fetal death. We therefore suggest that PCR on placental and/or fetal tissue for parvovirus B19, CMV, and enterovirus may be included in the routine investigation of stillbirths.

## **Paper VI**

The questionnaire was answered by 404 midwives in 2000 and by 501 in 2002. In 2000, 81% of the midwives had access to computers at their antenatal clinics and this number had increased to 93% in 2002. Sixty-eight percent in 2000 and 88% in 2002 had computers with access to the Internet.

Of the participating midwives, 74% in 2000 and 84% in 2002 had received information concerning INFPREG. In 2000, 29% of the midwives had used INFPREG. This figure had increased to 58% in 2002. The information obtained from INFPREG had been implemented in patient care by 67% of the midwives who had used INFPREG in 2000 and by 81% in 2002. Forty-five percent answered in 2000 that they needed more information on how to use INFPREG and this figure was almost the same in 2002 (43%).

Our results suggests that the majority of midwives at antenatal clinics in Sweden have access to the Internet and are confident in using an Internet-based knowledge center covering infectious disorders in pregnancy. INFPREG seems to being used increasingly in patient care.

## **Conclusions and future perspectives**

**Paper I:** The seroprevalence of toxoplasma in pregnant women in Stockholm was 14.0%: 11.1% in women born in the Nordic countries and 24.3% in women born outside the Nordic countries. The seroprevalence in Skåne was 25.7%: 24.9% in women born in the Nordic countries and 29.4% in women born outside the Nordic countries. On comparing the seroprevalence in Nordic women living in Stockholm determined in 1969, 1979, 1987, and 1997-98, it becomes obvious that the seroprevalence in different birth cohorts has remained at the same level throughout a 20-year period.

**Paper II:** We present here, for the first time, baseline data on the incidence of congenital toxoplasmosis in Sweden. The incidence of seroconversion for toxoplasma was 0.51/1000 susceptible pregnancies (9 months). The overall risk for mother-to-child transmission was 25% (3/12). The incidence of congenital toxoplasmosis was 0.73/10,000 (3/40,978). Introduction of a screening program cannot be recommended before the effectiveness of treatment has been evaluated. Health education for all pregnant women concerning risk factors for toxoplasma infection may be recommended.

**Paper III:** Most cases of late fetal death due to infection with parvovirus B19 are non-hydropic and conventional diagnostic procedures for diagnosing parvovirus B19-associated fetal death can be greatly improved by adding B19 PCR.

**Paper IV:** A presumptive explanation of the stillbirth could be established in 91% of the cases investigated in Paper IV. The most common factors associated with intrauterine fetal death could be identified as infections (24%), placental insufficiency/intrauterine growth restriction (22%), placental abruption (19%), intercurrent maternal conditions (12%), congenital malformations (10%), and umbilical cord complications (9%). The strength of abnormal factors associated with fetal death is difficult to estimate. The only way to address this problem properly is to carry out a case-control study. The present study will serve as a base for a case-control study in Sweden.

**Paper V:** Fetal infection with parvovirus B19, CMV, and enterovirus is associated with stillbirth. The use of "TORCH" serology is not sufficient for screening for viral infections in

cases of fetal death. PCR on placental and/or fetal tissue for parvovirus B19, CMV, and enterovirus may be included in the routine investigation of stillbirths.

**Paper VI:** The majority of midwives at antenatal clinics in Sweden are confident in using an Internet-based knowledge center covering infectious disorders in pregnancy, but many still want more information about how to use the Internet for retrieving medical information. The Internet creates new and exciting opportunities regarding the dissemination of information, education, patient involvement, clinical research, etc. The significance of the Web as a medical information resource in antenatal care is likely to increase. The impact on practice needs to be studied further.

**Future perspectives:** Evaluating and understanding the significance of various infectious disorders in pregnancy is a complex matter. Future improvements in this area will probably entail screening procedures, vaccinations, better diagnostic and therapeutic tools, and better education of the general and medical public. In this thesis, we have addressed some of these ingredients. Finally, the complexity of infectious disorders in pregnancy calls for collaboration between many different fields in medicine. A multidisciplinary approach, as used in our work, is probably a prerequisite if progress is to be made in this field.

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