Clinical and Pathophysiological Aspects of Intrahepatic Cholestasis of Pregnancy

Elisabeth A Wikström Shemer
Clinical and Pathophysiological Aspects of Intrahepatic Cholestasis of Pregnancy

Elisabeth A Wikström Shemer
ABSTRACT

Objective: The pathogenesis of intrahepatic cholestasis of pregnancy (ICP) involves impaired bile acid and estrogen/progesterone metabolism and excretion based on genetic and environmental factors. In this thesis we evaluated different pathophysiological and clinical aspects of ICP, i.e., serum levels of vitamin D, the morphology of ICP placentas, maternal and fetal outcomes in ICP at a time of active management, and ICP-associated pregnancy conditions.

Methods: In Paper I, we performed an observational study and compared the levels of active vitamin D (1,25-dihydroxy vitamin D3) in women with ICP and normal pregnancies. In Paper II we examined in a prospective case-control study morphological differences of placentas from untreated and ursodeoxycholic acid (UDCA) treated ICP, respectively, and normal pregnancies, by using stereology and systematic random sampling. In paper III, we estimated in a nationwide cohort study of more than 1.2 million singleton births in Sweden between 1997 and 2009 the actual prevalence of ICP and its association with adverse pregnancy and fetal outcomes, using data of the Swedish Medical Birth Registry (MBR). In Paper IV, we assessed in a hospital based retrospective cohort study the risk of emergency cesarean section (CS) and fetal asphyxia in ICP women with spontaneous and induced onset of labor at gestational weeks 37-39, by linkage of the MBR and a local obstetrical database.

Results: We report for the first time that women with ICP have lower levels of active vitamin D. We also show that ICP substantially affects the morphology of the placenta, with increased surface capillary area and syncytial knots. These changes were not observed in UDCA-treated ICP. In our nationwide population based study, we found a previously unknown strong association of ICP with gestational diabetes, preeclampsia and large for gestational age, and that ICP bears an increased risk of moderate prematurity but not of stillbirth at a time of active management. We found that induction of labor in women with ICP in gestational weeks 37-39 in a tertiary Swedish hospital did not increase the risks of emergency CS or fetal asphyxia.

Conclusions: Decreased levels of active vitamin D may contribute to the pathogenesis of ICP. ICP causes morphological changes in the placenta that might be improved by treatment with UDCA. Induction of labor in ICP does not increase the rate of emergency CS. The low risk of stillbirths at a time of modern management of ICP is reassuring but the strong association of ICP with gestational diabetes and preeclampsia needs consideration, e.g., by oral glucose tolerance testing and proper management of possibly coexisting conditions.

Keys words: Intrahepatic cholestasis of pregnancy, bile acids, vitamin D, placenta, stereology, labor induction, cesarean section, preeclampsia, gestational diabetes
LIST OF PUBLICATIONS

I. Wikström Shemer E, Marschall HU.
   Decreased 1,25-dihydroxy vitamin D levels in women with intrahepatic cholestasis of pregnancy

II. Wikström Shemer E, Thorsell M, Östlund E, Blomgren B, Marschall HU.
    Stereological assessment of placental morphology in intrahepatic cholestasis of pregnancy

III. Wikström Shemer E, Marschall HU, Ludvigsson JF, Stephansson O.
     Intrahepatic cholestasis of pregnancy and associated adverse pregnancy and fetal outcomes: a 12-year population based cohort study
     Submitted for publication, under review

IV. Wikström Shemer E, Thorsell M, Marschall HU, Kaijser M.
    Risks of emergency caesarean section and fetal asphyxia caused by induction of delivery in intrahepatic cholestasis of pregnancy: A hospital-based retrospective cohort study.
    Sexual and Reproductive Health, accepted for publication
# TABLE OF CONTENTS

1 Introduction ........................................................................................................................................ 1

2 Background ...................................................................................................................................... 1
   2.1 Epidemiology ................................................................................................................................. 2
   2.2 Cholestasis and bile acid physiology .............................................................................................. 2
   2.3 Genetics ........................................................................................................................................ 3
   2.4 Symptoms ..................................................................................................................................... 3
   2.5 Biochemical disparities .................................................................................................................. 3
   2.6 Co-existence of ICP and other liver and pregnancy related disorders ......................................... 4
   2.7 Placenta and ICP ........................................................................................................................... 5
   2.8 Fetal risks in ICP ........................................................................................................................... 5
   2.9 Modern management of ICP ......................................................................................................... 6

3 Aims ................................................................................................................................................ 8

4 Material and methods ..................................................................................................................... 9
   4.1 Paper I .......................................................................................................................................... 9
      4.1.1 Observational study ................................................................................................................ 9
   4.2 Paper II ....................................................................................................................................... 9
      4.2.1 Prospective case control-study .............................................................................................. 10
      4.2.2 Preparation and evaluation of placentas .............................................................................. 10
   4.3 Paper III ..................................................................................................................................... 14
      4.3.1 Retrospective cohort study ................................................................................................... 14
   4.4 Paper IV ..................................................................................................................................... 15
      4.4.1 Retrospective cohort study ................................................................................................... 15

5 Results .............................................................................................................................................. 18
   7.1 Paper I ....................................................................................................................................... 18
   7.2 Paper II ....................................................................................................................................... 19
   7.3 Paper III ..................................................................................................................................... 21
   7.4 Paper IV ..................................................................................................................................... 27

6 Discussion ........................................................................................................................................ 33

7 Conclusion ....................................................................................................................................... 37

8 Acknowledgements .......................................................................................................................... 38

9 References ........................................................................................................................................ 40
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>BSEP</td>
<td>Bile salt export pump</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CS</td>
<td>Cesarean section</td>
</tr>
<tr>
<td>CTG</td>
<td>Cardiotocography</td>
</tr>
<tr>
<td>FXR</td>
<td>Farnesoid X receptor</td>
</tr>
<tr>
<td>HE</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>ICP</td>
<td>Intrahepatic cholestasis of pregnancy</td>
</tr>
<tr>
<td>IUFD</td>
<td>Intrauterine fetal death</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>MBR</td>
<td>Swedish medical birth registry</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio (aOR, adjusted odds ratio)</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PBC</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SR</td>
<td>Sirius red</td>
</tr>
<tr>
<td>UDCA</td>
<td>Ursodeoxycholic acid</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a common liver disease during pregnancy\(^1\) with reported incidence rates between 0.4 and 15% in different countries and populations\(^2,3\). ICP is characterized by pruritus with elevated bile acids and/or transaminases in late second and third trimester of pregnancy. Pruritus spontaneously resolves and deranged liver function tests typically normalize within a few weeks after delivery.

ICP has been found to be associated with increased risks of preterm delivery, meconium staining of amniotic fluid, fetal bradycardia, asphyxial events, fetal distress, and fetal demise\(^3\). The underlying mechanisms associated with impaired fetal outcome are mainly unknown but have been associated with elevated maternal total serum bile acids, in particular, when exceeding 40 $\mu$mol/L at any time in pregnancy\(^4\). However, specific predictors of pregnancy outcomes have not been consistently identified\(^4,5\). Inconsistency of data on fetal risk can in part be attributed to different diagnostic criteria of ICP (increased bile acids and/or liver transaminases) and lack of serum bile acid data in some studies\(^1,2\).

Older studies on ICP reported stillbirth rates of up to 15%, decreasing to 3.5% or less in more recent studies\(^3\). Varying findings may also be attributed to changes in the management of ICP in the last two decades, by administration of ursodeoxycholic acid (UDCA) for relief from pruritus and biochemically reduction of maternal bile acids\(^6,7\) and induction of delivery in gestational weeks 37-38\(^3\), aiming to avoid stillbirth\(^3,8\). However, there are currently no specific biochemical tests or modalities of fetal monitoring for the prediction of stillbirth recommended.

The higher incidence in twin pregnancies and the induction of cholestasis by oral contraceptives or progesterone treatment indicate a major role of sex hormones, and specific alterations in progesterone and bile acid metabolism\(^9-15\) have been defined. The familial occurrence in some cases suggests genetic susceptibility\(^15-17\) in ICP.

The present thesis was designed to explore whether an environmental factor such as vitamin D deficiency could be associated with ICP and whether the morphology of ICP placentas might be altered. In addition, we wanted to study the epidemiology of ICP in Sweden with current management and finally, whether the risk of emergency cesarean section (CS) is increased when delivery is induced in ICP.

2 BACKGROUND

ICP was originally described in 1883 by Ahlfeld as recurrent jaundice in pregnancy that resolved following delivery\(^18\). During the last century, pruritus gravidarum, recurrent jaundice of pregnancy, hepatosis gravidarum, cholestasis gravidarum and obstetric cholestasis have all been used as synonyms. The Tenth International Classification of Diseases (ICD-10) uses since 1997 the term intrahepatic cholestasis of pregnancy\(^3\).
2.1 Epidemiology

The incidence of ICP shows large variation between different countries and populations\(^2\). It is most common in South America with the highest incidence (14%) reported from Chile\(^19\), although a lower incidence (1.5–4%) was later reported\(^20\).

In Northern Europe, ICP occurs in approximately 1.0–1.5% of pregnancies\(^4,21,22\), while in other countries the reported incidences have been lower (e.g. in France and Italy 0.4–1%\(^23,24\)). Also, ICP has seasonal variation, with more women affected during winter months in Sweden, Finland and Turkey\(^22,25,26\). In the United Kingdom, ICP affects only 0.6% of pregnancies in white Caucasians, but 1.4% of pregnancies of women with Indian or Pakistani origin\(^27\). The varying incidences may be explained by differences in diagnostic criteria, environmental or genetic background\(^3\).

Multiple pregnancy increases the risk of ICP 5-fold\(^4,28\) and the recurrence of ICP has been noted in 40–60% of patients\(^19\). The higher incidence in twin pregnancies and that most women present with symptoms of ICP in the third trimester when estrogen and progesterone levels are highest, suggest a hormonal component in ICP\(^2\). However, there are reports of ICP already presenting in the first trimester\(^25\). In one study, women over the age of 35 years were at increased risk of ICP\(^29\). Other risk factors include gallstones\(^4,30,31\) and hepatitis C seropositivity, the latter possibly being associated with early onset of the condition\(^23,32\). Increased drug sensitivities have been reported\(^33\). In the majority of women, ICP recurs in subsequent pregnancies, but disease severity cannot be predicted by the course in previous pregnancies\(^3\).

2.2 Cholestasis and bile acid physiology

Cholestasis is defined as impairment of bile flow due to intrahepatic or extrahepatic causes, leading to retention of hepatotoxic compounds, specifically bile acids\(^34\). Intrahepatic cholestasis indicates functional impairment of bile secretion whereas extrahepatic cholestasis is caused by obstruction of the bile duct, in particular by gallstones or pancreatic tumors.

Bile acids are physiological detergents that generate bile flow and facilitate intestinal absorption and transport of lipids, nutrients, and vitamins\(^35\). During the enterohepatic circulation, bile acids excreted from the liver into the duodenum are reabsorbed in the terminal ileum and via the portal vein recycled to the liver\(^36\). Specific transporters located in the basolateral membrane of the hepatocyte actively secrete bile acids (BSEP=ABCB11, gene symbol\(ABCB11\)), cholesterol (ABCG5/8,\(ABCG5/8\)) and phospholipids (MDR2=ABCB4,\(ABCB4\)) into the bile\(^35,37\). Bile acids also are signaling molecules that activate nuclear receptors such as the bile acid sensor farnesoid X receptor (FXR,\(FXR\)) and cell signaling pathways not only regulating bile acid turnover but also lipid, glucose, and energy metabolism\(^38,39\). Bile acids consist of a 24-carbon steroid core and a side chain carrying a carboxyl group. They are synthesized from cholesterol in the liver and conjugated with glycine or taurine before secretion into the bile\(^40\). Promoted by the ingestion of a meal, bile flow and plasma bile acid concentrations are highest postprandially\(^41\).
2.3 Genetics

Pedigrees from women with a history of ICP suggest a genetic background \(^{42}\); sisters of affected women may have a 12-fold increased risk \(^{43,44}\). Variants or mutations of genes encoding BSEP, MDR2 and FXR that may directly or indirectly impair excretion of bile acids and gestational hormones have so far been the main focus in research reports on genetic loci \(^{43,45-49}\). In particular, mutations of the phospholipid flippase MDR2 were found in about 10-15% of all cases with ICP, which is in agreement with the close association of ICP with gallstone disease \(^{4,17}\).

2.4 Symptoms

Diagnosis of ICP is based on otherwise unexplained pruritus during pregnancy and elevated bile acids (>10 \(\mu\)mol/L) and/or liver transaminases in absence of other liver disease \(^{2,3,8}\). Delayed onset of abnormal liver function tests has been reported, which is why serial liver function tests should be performed in these patients \(^{50}\).

Cholestatic pruritus presents as an irresistible desire to scratch with no skin changes do be seen except for excoriations. There are reports of ICP with debut of pruritus already in the first trimester \(^{22,51,52}\), although 80% of the patients present with itching in gestational weeks 30-32 \(^{4,23,30,31}\). The itching is worse at nighttime and most intense in the palms of the hands and soles, but it can also be generalized affecting other parts of the body. Insomnia is common with subsequent tiredness \(^3\). Pruritus typically subsides almost immediately after delivery and the serum bile acid and liver enzyme levels normalize within a few weeks \(^{53}\). The etiology of itching in ICP is unknown, but it has been speculated that bile salts deposited on nerve endings of the skin cause this sensation \(^{54,55}\). Another theory suggested that bile salts accumulate in the hepatocytes, inducing the release of pruritogenic compounds \(^{8,56}\). However, skin concentrations of bile acids do not correlate well with the sensation of itch \(^{54,55}\), and pruritus may start before the onset of biochemical abnormalities \(^{50}\).

Pruritus is most distressing and often the only symptom for women with ICP since jaundice is a rare finding \(^{4,57-60}\). Patients do not experience abdominal pain or encephalopathy as in other liver diseases of pregnancy \(^{1,61}\). Ultrasound examination reveals no dilatation of the biliary tract, but may show gallstones. As in other cholestatic conditions \(^{19,62}\), the patient might also experience nausea, anorexia, malaise, and on rare occasions, dark urine and pale stools indicating risk for impaired vitamin K absorption, and thus risk for postpartum hemorrhage \(^{19}\).

2.5 Biochemical disparities

Levels of bile acids and liver transaminases do not change significantly during pregnancy; however, bilirubin is lower in the third trimester due to hemodilution whereas alkaline phosphatase is elevated by the placental isoenzyme \(^{63}\). ICP is a diagnosis given for pregnant women with pruritus and elevated bile acids after exclusion of prevalent or incident liver disease \(^{8,62}\). There is as yet no consensus on whether a rise in serum bile
acids precedes the onset of symptoms, and there is no agreement on whether serum bile acids should be measured in the fasted (normal, <10 μmol/L) or post-prandial (normal, <14 μmol/L) state. Increased serum bile acid concentrations may be the first or only laboratory abnormality and they may go up to 100 times above the normal range. There are typical ICP-induced changes in serum profiles of bile acids, with a marked increase in the proportion of cholic acid and a shift towards a higher proportion of taurine-conjugated species. A Finnish study first reported a relationship between increased maternal serum bile acid levels and fetal distress. A large prospective study performed in the west of Sweden 1999-2002 showed that 80% of ICP women had bile acids below 40 μmol/L. Adverse pregnancy outcome (i.e., spontaneous preterm labour, asphyxial events, meconium-staining of amniotic fluid, placenta and membranes) did not occur until maternal serum bile acids exceeded 40 μmol/L, why the 96 cases found with this condition were considered to suffer from a severe form of ICP. Although this was the largest prospective study ever performed in ICP, it still lacked sufficient power to address the question whether any or only the severe form of ICP is associated with stillbirth and how medical intervention might improve fetal outcome. A significant increase of fetal complications rates at bile acid levels exceeding 40 μmol/L at any time of pregnancy was also found in most but not all other, albeit substantially smaller studies.

Little or no correlation has been found between serum total bile acid concentrations and other liver test values. Nevertheless, liver transaminase elevations, in ICP usually 2–10-fold, may be diagnostic for ICP in the absence of elevated serum bile acids, provided the exclusion of viral or autoimmune hepatitis by appropriate serological testing. Liver histology is unnecessary for the diagnosis of ICP. In those cases where it was incidentally performed, it revealed normal findings. As in other cholestatic conditions, total and LDL-cholesterol concentrations are higher in ICP than in normal pregnancy. All liver function test elevations typically return to normal within 6-8 weeks after delivery. However, there are case reports of persisting biochemical abnormalities for years without evidence for any other hepatobiliary disease.

2.6 Co-existence of ICP and other liver and pregnancy disorders

There have been some reports of the co-existence of ICP with other pregnancy-related disorders including preeclampsia, acute fatty liver of pregnancy, gestational diabetes, or otherwise impaired glucose tolerance. A longitudinal retrospective population-based cohort study from Finland found an association of ICP with numerous liver diseases, in particular significant increased risks of hepatitis C and gallstone-related morbidity (cholecystitis, cholangitis, nonalcoholic pancreatitis), but also nonalcoholic liver cirrhosis and primary biliary cirrhosis (PBC). Thus, ICP may be the initial manifestation of a chronic liver disease, which may question the diagnosis of ICP in these patients and in turn point to the importance of testing hepatitis C serology and controlling serum liver tests after delivery in women with ICP.
2.7 Placenta and ICP

It has been suggested that the risk of adverse fetal outcomes relates to the toxic effects of bile acids, possibly via vasoconstrictive effects as shown on isolated human placental chorionic veins. Human data concerning the morphology of the placenta in ICP are few, and most reports of stillbirth placentas did not show gross abnormalities or signs of chronic placental insufficiency. However, Costoya et al. described already some 30 years ago reduced intervillous spaces, cytotrophoblastic proliferation and increased syncytial sprout formation in ICP placentas. Decreased intervillous spaces and in addition, crowded villi in ICP placentas were also found in a recent study by Geenes et al. Also, in placentas from UDCA-treated ICP a reduced number of syncytial knots was found. Overall, the changes observed are suggested to impair fetal oxygenation due to decreased transport through trophoblasts and diminished blood flow through intervillous spaces. In order to facilitate oxygen uptake and to compensate for fetal hypoxia, vascular hyperplasia or angiogenesis in the terminal chorionic villi may develop as a mechanism to increase the surface of the vascular bed. Importantly, this process was associated with perinatal morbidity and mortality.

2.8 Fetal risks in ICP

Fetal morbidity and likely, also mortality in ICP is higher than in pregnancies without this condition. However, there is controversy about the extent of fetal risks. The main complication of ICP is prematurity, possibly by increased sensitive of ICP myometrium to oxytocine. However, many preterm deliveries are due to the common practice of induction of labor in ICP, aiming to avoid stillbirth. As yet, there are no studies with sufficient power to allow accurate estimations of the occurrence of adverse outcomes including stillbirth.

Several studies suggest that bile acids accumulate in the fetal compartment and thus are likely to exacerbate fetal risk. The Swedish observational study that found 693 cases of ICP among 45,485 pregnancies reported a 1%-2% increase in risk of spontaneous preterm labour, asphyxial events (defined as operative delivery due to asphyxia, Apgar score <7 at 5 min or arterial cord pH <7.05) or meconium staining of the amniotic fluid and/or placenta and membranes for every additional μmol/L of maternal serum bile acids. However, this study did not find an increase in adverse outcomes compared to women without ICP when maternal serum bile acids were below 40 μmol/L. Thus the authors suggested no increased risk for the fetus with mild ICP according to this definition.

Smaller studies from Finland and American Latina population showed similar results, although the effect of bile acid levels on adverse events varied. Interestingly, one recent retrospective study reported no correlation of maternal bile acids and fetal complications in an American Latina population.

Stillbirth in ICP rarely occurs before the last month of pregnancy. The risk is difficult to estimate as the etiology of stillbirth is unknown and fetal autopsy usually is normal. Rates of 3.5% or less were reported in more recent studies whereas the Swedish ICP trial did not find increased risk of stillbirth.
Stillbirth in ICP is thought to occur suddenly, without preceding warning signs such as growth restriction or uteroplacental insufficiency, which makes fetal surveillance difficult. There are conflicting reports on Doppler ultrasound findings. Guerra et al. concluded that there were no significant changes in any of the blood flow velocity indices determined by umbilical Doppler blood flow analysis in ICP, while a recent study found significantly increased Doppler flow velocities in ICP despite normal cardiotocography (CTG). In agreement with this finding, a case of stillbirth in ICP has been observed within 8 hours following a normal CTG.

2.9 Modern management of ICP

Obstetric treatment
Induction of labor at gestational weeks 37-38 has been advocated to prevent obstetric complications by prolonged pregnancy and to possibly reduce the risk of stillbirth, since stillbirth in ICP in previous studies tended to cluster at this age of gestation and is unpredictable by conventional fetal surveillance. Since this kind of active management of ICP is already common practice in many countries, it is very difficult to perform sufficiently powered randomized prospective trials to evaluate this concept, in particular, as the overall incidence of stillbirth is very low. In Stockholm, after 28 weeks of gestation, 3-4 stillbirths/1,000 births were recorded in 2002-2005, but only 0.04 stillbirths/1,000 births were attributed to ICP. Interestingly, as to more common fetal complications, a recent randomized trial of induced labor vs. expectant management reported no significant difference in maternal and fetal outcomes, including emergency CS rates, between ICP women randomized to early term delivery and ICP women who obtained expectant management.

Medical treatment
Pruritus is commonly treated with UDCA. The patient may be instructed to estimate the intensity of her pruritus on a 100-mm long visual analogue scale with the endpoints “no pruritus at all” and “worst possible pruritus”. This scale is in particular useful for monitoring and the evaluation of possible medical treatment effects on this symptom. Beyond amelioration of pruritus, UDCA also improves liver function tests in many cases. Thus, many obstetricians worldwide treat ICP with UDCA, as presently no better choice of drug exists.

However, there is some controversy about the overall benefit of UDCA. A recent meta-analysis showed significant improvement of pruritus and also a decrease of total prematurity, respiratory distress syndrome in the newborn and fetal distress, although no statistical difference was found for the rate of Apgar scores <7 at 5 minutes. This meta-analysis unfortunately did not evaluate the latest blinded, placebo-controlled clinical trial that actually include the largest number of women with ICP. This particular trial concluded that although UDCA significantly improved pruritus, the size of the benefit might be too small for most doctors to recommend it or for most women to want to take it. Interestingly, UDCA treatment induced a significant reduction in serum levels of alanine transaminase, γ-glutamyltransferase, and bilirubin, but not of bile acids. There were no differences in mode of delivery, birth weight, estimated blood loss, or variables of neonatal morbidity.
UDCA is a tertiary, naturally occurring hydrophilic bile acid that constitutes 3-5% of the physiological bile acid pool in humans. It has been used in various cholestatic disorders and is recommended as first-line treatment of PBC. Interestingly, UDCA prevented the deposition of perisinusoidal collagen and reduced the apoptotic activity in PBC patients after 2 years of therapy. UDCA induces changes in the expression of metabolizing enzymes and transporters that reduce bile acid cytotoxicity and improve biliary and renal excretion. It was also shown that UDCA increases expression of placental bile acid transporters and their regulating nuclear factors, which may enhance maternal-fetal bile acid transfer.

Other medications have been used, e.g. cholestyramine, S-adenosyl-L-methionine, and dexamethasone, but have shown to be much less effective in relieving pruritus and reducing bile acids and liver functions tests. Antihistamines are frequently used to alleviate pruritus and they may be beneficial for patients experiencing nocturnal itch. Vitamin K should be supplemented at long-standing cholestasis with possible malabsorption.

Although estrogen has been linked to ICP, it is possible for women with a prior history of ICP to use combined oral contraceptives. However, these women should be advised of the risk of pruritus and elevated liver enzymes when using combined pills. They can commence oral contraceptives with low-dose estrogen or progesterone-only products once the liver tests have normalized following delivery.
The aim of this thesis was to explore possible causes of adverse fetal outcome in ICP and to provide information about the pathophysiology, epidemiology and clinical management of ICP.

The hypotheses and questions explored were:

- Do levels of an environmental factor such as vitamin D differ in women with ICP, as the disease has seasonal variation?
- Could levels of vitamin D correlate with signs of fetal distress?
- Does the ICP placenta differ from placentas of normal pregnancies as ICP pregnancies are at increased risk of adverse events?
- What happens to placentas where the mother was treated with UDCA?
- What has happened on a nationwide basis regarding diagnosis, management and outcome of ICP since the introduction of ICD 10 in 1997?
- Can we see on a nationwide basis if women who were prescribed UDCA during pregnancy differed in maternal and fetal delivery outcomes?
- Do women with ICP carry a higher risk for other pregnancy related diseases?
- What are the risks of emergency CS in induced early term delivery in ICP?
- Are induced ICP women and fetuses at increased risk of adverse outcomes?
4  MATERIALS AND METHODS

This thesis includes observational (paper I) and prospective (paper II) and retrospective (papers III, IV), respectively, case-control studies with methodology from clinical chemistry (paper I), pathology (paper II), and epidemiology (paper III and IV). These methods are described in separate sections below.

4.1  PAPER I

4.1.1  Observational study

Study participants
Twenty-two women with ICP at presentation, in comparison with 11 healthy women at delivery at the Department of Obstetrics and Gynecology at Danderyd Hospital, Stockholm, Sweden. Diagnosis of ICP was made based on elevated bile acids (>10 \( \mu \text{mol/L} \)) and pruritus that spontaneously disappeared after delivery. All women were Caucasians. After obtaining informed and written consent, we collected data regarding health and obstetric history, medications and nutritional supplements. Exclusion criteria were multiple pregnancies, maternal disease other than ICP and smoking. Serum levels of 1,25-D\( _3 \) and PTH were analyzed by \(^{125}\text{I}-\text{radioimmunoassay} \) (Nichols Institute Diagnostics, San Clemente, CA, USA).

Statistical analysis
After confirming equal distributions within groups, serum parameters (mean \( \pm \) SD) were tested for significant differences using an unpaired, two-tailed t-test. A \( P \)-value <0.05 was considered statistically significant.

4.2  PAPER II

4.2.1  Prospective case control-study

Study participants
Twenty women with ICP and 8 women with normal pregnancies as controls who delivered during the period 2008-2009 at the Department of Obstetrics and Gynecology at Danderyd Hospital in Stockholm, Sweden. Diagnosis of ICP was based on otherwise unexplained pruritus and elevated bile acids (>10 \( \mu \text{mol/L} \)) and/or liver transaminases. Exclusion criteria were multiple pregnancies, maternal disease other than ICP (gestational or essential hypertension, preeclampsia, gestational diabetes and hypothyroidism), and smoking. All women studied were Caucasians. After informed written and oral consent, data was obtained regarding health and obstetric history, medications and nutritional supplements. Information was also obtained for gestational week at diagnosis and delivery, bile acids and liver transaminases at time of diagnosis and delivery, mode of delivery, treatment with UDCA, umbilical pH, placental weight, birth weight and obstetric outcome.
Management
Fetal monitoring was performed by weekly non-stress test CTG. Ten women with ICP were treated with UDCA (10-15 mg/kg/day). The other ten were not since they were given the diagnosis at gestational week 37 or thereafter and considered for induction of delivery within the next 1-14 days, if bile acids exceeded 30 μmol/L or pruritus became unbearable.

4.2.2. Preparation and evaluation of placentas
Placentas were collected immediately after delivery, and following trimming and stripping of the cord, weighed and immersed in 4% neutral buffered formaldehyde solution.

Stereology
Stereology is a methodology that permits the objective, quantitative description of morphology of the placenta by efficient and design-based methods. Applied to placentas in normal and abnormal pregnancies, this methodology has proved of great value for challenging earlier misconceptions. Stereology improves the interpretation of processes of growth, morphogenesis and the understanding of adaptation and functioning at the whole-organ level.

Systematic random sampling of the placentas
Systematic random sampling of tissue from the placentas was performed at each stage of preparation in order to achieve a representative selection of explants for stereological assessment. For this purpose, the placentas were placed on a cutting-board with 1 cm slits. A start number was obtained by using a random number table. The placentas were cut into 1 cm slabs (fractionation number 1).

Slabs from the random start number and every subsequent third slab were collected. Random start number in this example is 3.
These were then put on another cutting-board with 0.5 cm slits (fractionation number two).

As before, the random number table provided the start number for the second fractionation where every fourth slab was collected. Here, the random start number is 2.
The collected specimens were put in cassettes and immersed in 4% neutral buffered formaldehyde solution for a minimum of 24 h, then dehydrated according to a standard protocol and finally embedded in paraffin $^{105,106}$.

**Volume estimation of the placenta (reference volume)**
Placental volume estimation was performed according to the Cavalieri formula $^{108}$. The slabs generated at the first fractionation were placed with the cut surface facing upwards. An overhead film with a point grid was thrown on the slab and the points hitting the surface were counted. The points counted on all slabs were summed together, and multiplied with the area per point and the slab thickness. This gave the estimated volume of the placenta.

![Diagram of placental volume estimation](image)

**Slide preparation**
From each placenta, 6 paraffin-embedded specimens were chosen by using a random number table and cut into sections of 5 $\mu$m for staining with hematoxylin and eosin (HE) for visualizing blood vessels and Sirius red (SR) for collagen. They were also cut in 20 $\mu$m sections, stained with HE to estimate the number of syncytial knots. The total number of specimens from the placentas was 168. The examining investigators were blinded to ICP and control groups, as specimens were given random numbers of 1-168.

The selected placenta slabs were put in cassettes, processed and embedded in paraffin, sectioned at selected thickness, mounted on slides and stained.
A low Apgar score was defined as less than 7 at five minutes. Neonatal death was defined as death of the infant from 0 to 27 days after birth. The presence of meconium aspiration was obtained by diagnosis at discharge from delivery or neonatal care hospital (ICD-10: P24). We also linked data from the Swedish MBR with the Education and Prescribed Drug Registries (the latter established 1st of July 2005) by using the unique personal identity number assigned to each citizen at birth or immigration. Through the Swedish Education Register we obtained information on the number of years of formal education completed as of 1 January 2010, categorized as ≤11 vs. ≥12 years, and through the Prescribed Drug Register we obtained filled prescriptions for UDCA during pregnancy in the years 2006-2009.

**Statistical analysis**

The main outcome measure was stillbirth. Secondary outcomes were gestational diabetes, preeclampsia, preterm birth (classified into spontaneous or iatrogenic), neonatal death, low Apgar score, meconium aspiration, large for gestational age, macrosomia, small for gestational age and mode of onset of labor. Using logistic regression we estimated the risk of adverse pregnancy outcomes in relation to ICP by crude and adjusted odds ratios with 95% confidence intervals (CI). Data were analyzed using the SAS software version 9.2.

We compared women with a diagnosis of ICP with women with no such diagnosis, taking into account possible confounders (maternal age, body mass index, parity, years of formal education, cigarette smoking, and calendar year of delivery). Because observations are not independent in women who delivered more than once during the study period, we calculated estimates using clustered data in the generalized estimation equation method (PROC GENMOD). Trends for rates of ICP-diagnosis and stillbirth by calendar year of delivery were analyzed in Chi-2 tests that were also used for differences in maternal characteristics for women with and without ICP.

### 4.4 PAPER IV

#### 4.4.1 Retrospective cohort study

**Study participants**

25,870 women with singleton pregnancies who delivered during the period 2002-2006 at Danderyd Hospital, Stockholm, Sweden. Of these, 333 (1.3%) had a diagnosis of ICP and 25,537 and 98.7% did not (controls). Of women with ICP, 39 (12%) had an elective CS and the corresponding figure for controls was 2,727 (9%). The indications for elective CS in women with ICP included previous CS (n=9), psychosocial indication (n=11), breech presentation (n=10), back pain (n=3), anal sphincter injury in previous pregnancy (n=3), in vitro fertilization (n=2), and macrosomia (n=1). The diagnosis of ICP was made in the presence of otherwise unexplained pruritus and elevated serum bile acids (>10 μmol/L) and/or aminotranferases (ALT, upper limit of normal, ULN, <45 U/L; AST, <36 U/L) in at least one of serial evaluations.

**Management**

Fetal monitoring by weekly non-stress CTG test and oral administration of UDCA (10-20 mg/kg/day) if pruritus was intolerable or if maternal bile acids exceeded 30 μmol/L. ICP
women were considered for induction of labor at 37-38 weeks of gestation or without delay if diagnosed with ICP later than gestational week 37. In cases of severe maternal disease unresponsive to therapy, obstetric complications or non-reassuring fetal testing, immediate induction of delivery was considered. However, according to the patients’ preference, spontaneous onset of labor could be awaited in uncomplicated cases.

Entries analyzed
Obstetric outcome in ICP was assessed through linkage of the Swedish Medical Birth Registry and a local hospital based obstetrical database based on the patient’s medical files. CS, postpartum hemorrhage and fetal asphyxia, corresponding to ICD-codes P21, O68, O72, and O82, respectively. Induction of labor was also defined according to ICD-10 (O61.0). Preterm labor was defined as labor at gestational age ≤37 weeks. Data on body mass index (BMI) was registered in 60% of the patients. All ICP patients’ obstetrical files were manually reviewed to verify the indications for emergency CS and to retrieve data on maternal bile acids, liver transaminases, bilirubin and UDCA treatment. For controls, due to the large cohort, data were solely obtained through the obstetrical database.

Statistical analysis
First, we analyzed the association between ICP and risk of emergency CS in ICP with spontaneous onset of labor, compared to controls with spontaneous onset of labor. We then estimated the risk of emergency CS in ICP with induction of labor, compared to controls with induction of labor. We performed a subgroup analysis where we exclude women with the diagnoses preeclampsia, gestational hypertension, or diabetes. Risk for emergency CS after induction of labor was analyzed with emergency CS as dependent and ICP as independent variables using the logistic procedure in SAS (SAS Institute Inc., Cary, NC, USA). Since other studies have found BMI, age, use of epidural analgesia and parity to be factors affecting labor outcome, we adjusted for these potential confounders by successively adding these variables to the strata statement. Tests of heterogeneity were done by the chi-square test. Patient characteristics, fetal and maternal outcome were analyzed by Chi-2 tests and Wilcoxon signed-rank test. A P-value <0.05 was considered statistically significant.
Population based registers used for PAPERS III-IV

The Swedish Medical Birth Registry
Data from the Swedish MBR include information on about 98% of births in Sweden since 1973 and contain information on the mother as well as the pregnancy, delivery, and neonatal period. Data is prospectively collected, starting at the woman’s first antenatal visit.\(^{116}\)

The Swedish Educational Registry
The Swedish Educational Registry was established in 1985 and includes the population between 16 to 74 years that are registered as Swedish residents at the 1\(^{st}\) of January each year. The registry is yearly updated with graduation and examination data from regular educational institutions in Sweden, and the highest completed educational level is registered.

The Swedish Drug Registry
The Swedish Prescribed Drug Register contains information about age, sex and unique identifier of the patient as well as the prescriber's profession and practice. The register provides data on exposure to drugs and is useful to study patterns of drug utilization. The possibilities for record linkage to other health registers gives, from an international perspective, good opportunities to explore drug and disease associations and the risks, benefits, effectiveness and health economic effects of drug use.\(^{117}\)

The Swedish Identity Number
The Swedish personal identity number was introduced in 1947. Originally, it consisted of a 9-digit number representing the year, month and day of birth together with a 3-digit number to make the personal identity number unique. In 1967, a fourth digit was introduced, that verifies that the birth data and the 3-digit number are correct.\(^ {118}\) Using the personal identity number, data from different registers can be linked together.

Ethical considerations for PAPERS I-IV
Ethical approvals from the regional ethical board of Stockholm were obtained before commencing the studies. In papers I (Dnr 2009/926-32, Dnr 2007/1221-32 and Dnr 2005/708-31) and II (Dnr 2007/1221-32 and Dnr 2005/708-31), informed consent was obtained from all the participating women. In papers III (Dnr 2011/1860-32 and Dnr 2008/1182-31/4) and IV (Dnr 2011/1586-31 and Dnr 2006/1129-31/4), individual consent was not required due to the large number of women, which was approved by the ethical board.
5 RESULTS

5.1 PAPER I

ICP diagnosis was made at mean gestational age 34.3 ± 3.2 weeks. These women delivered at 37.5 ± 1.6 weeks, whereas controls delivered at term (39.7 ± 0.5 weeks). Of the 22 ICP patients, six (30%) had spontaneous vaginal delivery; 14 received induction of labor, resulting in 11 vaginal deliveries and three caesarean sections; and two had planned caesarean section: one preterm pregnancy and substantially elevated bile acids (122 μmol/L), and one patient due to breach. All babies, both in ICP and control groups had normal umbilical cord pH and APGAR >9 at 5 minutes. Thirteen ICP patients (59%) were treated with UDCA, 1 g/day until delivery, with no treatment-related differences in outcome. Seventeen ICP patients (77%) and 7 controls (64%) had oral multivitamin supplement, resulting in a maximum daily dose of 5 mg of vitamin D. Of note, in Sweden, milk is supplemented with vitamin D (4 mg/L).

ICP women had significantly lower serum 1,25-D3 compared to normal healthy pregnant women (controls). Serum 1,25-D3 levels were 76.4 ± 23.1 ng/L in ICP women, without seasonal variation, and 112.0 ± 40 ng/L in controls, which is significantly different (p = 0.0041). 1,25-D3 levels were inversely associated (p < 0.01) with the incidence of meconium staining of amniotic fluid (Fig. 1) but not to bile acids (Fig. 2).

![Figure 1](image-url)  
**Figure 1.** Lower 1,25-dihydroxy vitamin D3 (1,25-D3) levels (p < 0.01) in women with ICP (55.1 ± 17.9 ng/L) with meconium staining than in healthy controls (83.3 ± 18.3 ng/L; mean ± SD)

There was no statistically significant association (p = 0.32) between meconium staining and serum bile acids (35.0 ± 24.7 μmol/L) (Fig. 2). PTH levels were 26.9 ± 12.6 ng/L in ICP and 25.8 ± 6 ng/L in controls (no statistical significant difference). There was no statistically significant correlation in ICP patients between serum 1,25-D3 and PTH, bile acid or ALT (180 ± 123 U/L) levels. Bilirubin (<14 μmol/L) and International Normalized Ratio (INR) (<0.9) were normal in all cases.
### Table 6. Risks of adverse gestational and fetal outcomes with and without treatment with UDCA.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>UDCA</th>
<th>No UDCA</th>
<th>No ICP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Rate</td>
<td>OR*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>746</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gestational diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>4.0</td>
<td>4.18</td>
</tr>
<tr>
<td>No</td>
<td>716</td>
<td>96.0</td>
<td>1</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37</td>
<td>5.0</td>
<td>1.92</td>
</tr>
<tr>
<td>No</td>
<td>709</td>
<td>95.0</td>
<td>1</td>
</tr>
<tr>
<td>Caesarean section a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>164</td>
<td>22.0</td>
<td>1.33</td>
</tr>
<tr>
<td>No</td>
<td>582</td>
<td>78.0</td>
<td>1</td>
</tr>
<tr>
<td>Moderately preterm birth (32+0-36+6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>139</td>
<td>18.8</td>
<td>5.46</td>
</tr>
<tr>
<td>No</td>
<td>601</td>
<td>81.2</td>
<td>1</td>
</tr>
<tr>
<td>Induction of labor a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>382</td>
<td>51.3</td>
<td>1.33</td>
</tr>
<tr>
<td>No</td>
<td>362</td>
<td>48.7</td>
<td>1</td>
</tr>
<tr>
<td>Apgar &lt;7 at 5 min of age a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>1.5</td>
<td>1.44</td>
</tr>
<tr>
<td>No</td>
<td>734</td>
<td>98.5</td>
<td>1</td>
</tr>
<tr>
<td>Large for gestational age a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57</td>
<td>7.6</td>
<td>2.38</td>
</tr>
<tr>
<td>No</td>
<td>689</td>
<td>92.4</td>
<td>1</td>
</tr>
</tbody>
</table>

a, b as Table 4

#### 5.4 PAPER IV

ICP was diagnosed in 333 (1.3%) of 25,870 singleton pregnancies. Maternal or gestational age, BMI, use of epidural analgesia, and parity did not differ between women with ICP and controls (Table 3). When comparing ICP with spontaneous vs. induced onset of labor, no differences were found in duration of ICP diagnosis, serum levels of bile acids, ALT/AST, bilirubin, or proportion treated with UDCA (Table 7).

In ICP with spontaneous onset of labor, the proportion of emergency CS was 12.5% and not different from controls with spontaneous onset of labor (9.3%) (OR 1.40, 95% CI 0.63-3.10). Excluding women with preeclampsia, gestational hypertension or diabetes mellitus did not change this result (Table 8).

Among induced women, the proportions delivered by emergency caesarean section were 9% in ICP and 16.5% in controls, which is significantly different (OR 0.45; 95% CI 0.25-0.82). Excluding ICP women with preeclampsia, gestational hypertension or diabetes mellitus again did not change this result (Table 8).
### Table 7. Maternal characteristics and mode of labor onset of women with and without ICP who delivered in gestational weeks 37 – 39.

<table>
<thead>
<tr>
<th></th>
<th>Women without ICP induced to labor</th>
<th>Women without ICP spontaneous labor</th>
<th>Women with ICP induced to labor</th>
<th>Women with ICP spontaneous labor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>968</td>
<td>8218</td>
<td>144</td>
<td>56</td>
</tr>
<tr>
<td><strong>Maternal age (years)</strong></td>
<td>33 ± 4</td>
<td>33 ± 5</td>
<td>33 ± 4</td>
<td>33 ± 4</td>
</tr>
<tr>
<td><strong>Nulliparous</strong></td>
<td>562 (58%)</td>
<td>4405 (54%)</td>
<td>73 (51%)</td>
<td>34 (61%)</td>
</tr>
<tr>
<td><strong>Multiparous</strong></td>
<td>406 (42%)</td>
<td>3813 (46%)</td>
<td>71 (49%)</td>
<td>22 (39%)</td>
</tr>
<tr>
<td><strong>Median gestational age (weeks)</strong></td>
<td>38.3 ± 0.7</td>
<td>38.5 ± 0.7</td>
<td>38.5 ± 0.7</td>
<td>38.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Mean duration of ICP (days)</strong></td>
<td>19 ± 18</td>
<td></td>
<td>17 ± 15</td>
<td>9 (16%)</td>
</tr>
<tr>
<td><strong>Treatment with UDCA</strong></td>
<td>29 (20%)</td>
<td>9 (16%)</td>
<td>22.5 ± 3.6</td>
<td>22.6 ± 3.0</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.6 ± 4.3</td>
<td>22.8 ± 3.8</td>
<td>22.5 ± 3.6</td>
<td>22.6 ± 3.0</td>
</tr>
<tr>
<td><strong>Birth weight (grams)</strong></td>
<td>3476 ± 428</td>
<td>3487 ± 452</td>
<td>29.7 ± 27.9</td>
<td>11.4 ± 5.4</td>
</tr>
<tr>
<td><strong>Serum bile acids at diagnosis of ICP</strong></td>
<td>23.6 ± 22.5</td>
<td>14.1 ± 10</td>
<td>11.5 ± 5.6</td>
<td>11.2 ± 5.8</td>
</tr>
<tr>
<td><strong>Serum bile acids at delivery</strong></td>
<td>29.7 ± 27.9</td>
<td>21.8 ± 18.9</td>
<td>120 ± 10</td>
<td>114 ± 84</td>
</tr>
<tr>
<td><strong>Bilirubin at time of ICP diagnosis</strong></td>
<td>11.4 ± 5.4</td>
<td>11.2 ± 5.8</td>
<td>114 ± 96</td>
<td>114 ± 114</td>
</tr>
<tr>
<td><strong>Bilirubin at delivery</strong></td>
<td>11.5 ± 5.6</td>
<td>11.7 ± 5.8</td>
<td>180 ± 138</td>
<td>168 ± 126</td>
</tr>
<tr>
<td><strong>AST at time of ICP diagnosis</strong></td>
<td>120 ± 10</td>
<td>114 ± 84</td>
<td>180 ± 156</td>
<td>156 ± 126</td>
</tr>
<tr>
<td><strong>AST at delivery</strong></td>
<td>114 ± 96</td>
<td>114 ± 114</td>
<td>297 ± 186</td>
<td>297 ± 186</td>
</tr>
<tr>
<td><strong>ALT at time of ICP diagnosis</strong></td>
<td>180 ± 138</td>
<td>168 ± 126</td>
<td>180 ± 156</td>
<td>156 ± 126</td>
</tr>
<tr>
<td><strong>ALT at delivery</strong></td>
<td>180 ± 156</td>
<td>156 ± 126</td>
<td>180 ± 156</td>
<td>156 ± 126</td>
</tr>
</tbody>
</table>

*Elective caesarean sections excluded.

a. medians with standard deviations; b. absolute numbers and proportions. None of the differences was statistically significant. Bile acids, bilirubin [µmol/L]; ALT, AST [U/L], normal, ALT<45, AST<36

ICP with spontaneous onset of labor comprised a statistically significant increased risk for fetal asphyxia (OR 2.48; 95% CI 0.59-10.28) as compared controls and spontaneous onset of labor. Also here, exclusion of women with preeclampsia, gestational hypertension or diabetes mellitus had no influence on the results. Risk of fetal asphyxia in induced labor did not differ in ICP and controls (Table 9).

The proportion of hemorrhage was significantly different in ICP with spontaneous onset of labor (12.5%) compared to controls with spontaneous onset of labor (5.8%) (OR 2.31; 95% CI 1.04-5.13). There were no differences in blood loss in induced ICP compared to induced controls, even after excluding women with preeclampsia, gestational hypertension or diabetes mellitus (Table 10). Furthermore, excluding pregnancies with missing data on BMI did not alter the results (Data not shown).

Dysfunctional labor was the most common indication for emergency CS in ICP (n=9; 69%) as well as controls (n=94; 54%). The proportion of women who had emergency CS because of fetal asphyxia did not differ between the two groups (n=4, 30.7% vs. n=60, 34.5%). Among women who had induction of labor, 4 with ICP (2.8%) and 60 (34.5%) of controls had emergency CS because of suspicion of fetal asphyxia (Table 11).
Table 8. Risk of acute CS by mode of onset of labor among women with ICP and intended vaginal delivery in gestational weeks 37 – 39.

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>Acute caesarean section after spontaneous onset of labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP</td>
<td>8218</td>
<td>761 (9.3%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>56</td>
<td>7 (12.5%)</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>P for independence</strong></td>
<td></td>
<td></td>
<td><strong>0.41</strong></td>
</tr>
</tbody>
</table>

No ICP without preeclampsia, hypertension, or diabetes

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td></td>
<td>7880</td>
<td>687 (8.7%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td>50</td>
<td>6 (12%)</td>
<td>1.43</td>
</tr>
<tr>
<td><strong>P for independence</strong></td>
<td></td>
<td></td>
<td><strong>0.45</strong></td>
</tr>
</tbody>
</table>

Acute caesarean section after induced labor

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>No ICP</td>
<td>968</td>
<td>160 (16.5%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>144</td>
<td>13 (9.0%)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>P for independence</strong></td>
<td></td>
<td></td>
<td><strong>0.0074</strong></td>
</tr>
</tbody>
</table>

No ICP without preeclampsia, hypertension, or diabetes

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td></td>
<td>702</td>
<td>112 (15.9%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td>129</td>
<td>10 (7.8%)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>P for independence</strong></td>
<td></td>
<td></td>
<td><strong>0.01</strong></td>
</tr>
</tbody>
</table>

* Adjusted for maternal age, parity, BMI, and intrapartal epidural anesthesia.
Table 9. Risk of fetal asphyxia by mode of onset of labor among women with ICP and intended vaginal delivery in gestational weeks 37 – 39.

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Asphyxia after spontaneous onset of labor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP</td>
<td>8218</td>
<td>121 (1.5%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>56</td>
<td>2 (3.5%)</td>
<td>2.48</td>
</tr>
<tr>
<td><em>P for independence</em></td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP without preeclampsia, hypertension, or diabetes</td>
<td>7880</td>
<td>111 (1.4%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td>50</td>
<td>2 (4.0%)</td>
<td>2.92</td>
</tr>
<tr>
<td><em>P for independence</em></td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Asphyxia after induced labor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP</td>
<td>968</td>
<td>14 (1.5%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>144</td>
<td>1 (0.7%)</td>
<td>0.48</td>
</tr>
<tr>
<td><em>P for independence</em></td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP without preeclampsia, hypertension, or diabetes</td>
<td>702</td>
<td>8 (1.1%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td>129</td>
<td>1 (0.8%)</td>
<td>0.68</td>
</tr>
<tr>
<td><em>P for independence</em></td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, parity, BMI, and intrapartum epidural anesthesia.
Table 10. Risk of blood loss of >1000 ml by mode of onset of labor among women with ICP and intended vaginal delivery in gestational weeks 37 – 39.

<table>
<thead>
<tr>
<th>Deliveries</th>
<th>Events</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n (%)</td>
<td>Odds Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Blood loss &gt; 1000 ml spontaneous onset of labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP</td>
<td>8218</td>
<td>479 (5.8%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>56</td>
<td>7 (12.5%)</td>
<td>2.31</td>
</tr>
<tr>
<td>P for independence</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP without preeclampsia, hypertension, or diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7880</td>
<td>454 (5.8%)</td>
<td>ref</td>
<td>1</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>7 (14%)</td>
<td>2.66</td>
<td>1.19 - 5.95</td>
</tr>
<tr>
<td>P for independence</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood loss &gt; 1000 ml when induced labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP</td>
<td>968</td>
<td>86 (8.9%)</td>
<td>ref</td>
</tr>
<tr>
<td>ICP</td>
<td>144</td>
<td>10 (6.9%)</td>
<td>0.77</td>
</tr>
<tr>
<td>P for independence</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ICP without preeclampsia, hypertension, or diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>702</td>
<td>53 (7.6%)</td>
<td>ref</td>
<td>1</td>
</tr>
<tr>
<td>ICP without preeclampsia, hypertension, or diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>129</td>
<td>9 (6.9%)</td>
<td>0.92</td>
<td>0.44 - 1.91</td>
</tr>
<tr>
<td>P for independence</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, parity, BMI, and intrapartal epidural anesthesia
Table 11. Suspected fetal asphyxia, dysfunctional labor and indications for emergency cesarean section in women with and without ICP and induction of labor.

<table>
<thead>
<tr>
<th></th>
<th>Pregnancies with induction of labor as denominator</th>
<th>Pregnancies with emergency cesarean section as denominator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP (n=144)</td>
<td>ICP (n=13)</td>
</tr>
<tr>
<td></td>
<td>No ICP (n=968)</td>
<td>No ICP (n=174)</td>
</tr>
<tr>
<td>Suspected fetal asphyxia</td>
<td>4 (2.8 %)</td>
<td>4 (30.7%)</td>
</tr>
<tr>
<td></td>
<td>60 (6.3%)</td>
<td>60 (34.5%)</td>
</tr>
<tr>
<td>Dysfunctional labor</td>
<td>9 (6.3%)</td>
<td>9 (69%)</td>
</tr>
<tr>
<td></td>
<td>94 (9.7%)</td>
<td>94 (54%)</td>
</tr>
</tbody>
</table>

**Indications for emergency CS**

<table>
<thead>
<tr>
<th></th>
<th>Pregnancies with induction of labor as denominator</th>
<th>Pregnancies with emergency cesarean section as denominator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP (n=144)</td>
<td>ICP (n=13)</td>
</tr>
<tr>
<td></td>
<td>No ICP (n=968)</td>
<td>No ICP (n=174)</td>
</tr>
<tr>
<td>Fetal malpresentation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 (0.5%)</td>
<td>5 (3.0%)</td>
</tr>
<tr>
<td>Psychosocial</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15 (1.5%)</td>
<td>15 (8.5%)</td>
</tr>
</tbody>
</table>
Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-specific liver disease affecting up to 1.5% of all pregnancies in Sweden. Etiology and pathogenesis are still unknown and risks and appropriate management under intensive debate. This Thesis provides important information to either aspect.

Family clustering and ethical differences in the prevalence of ICP clearly indicate a genetic factor that in part has been substantiated by finding significant associations between variants of hepatobiliary transport proteins and their regulating nuclear factors, and ICP. However, variants or mutations are found in not more than 15% of all women affected with ICP. Thus, beyond sex hormones environmental factors may play an important additional role. Among these, trace elements and vitamins are likely candidates, in particular, as their availability varies during different seasons, which is also reflected by the observation both from the northern and the southern hemisphere that the incidence of ICP peaks in winter months. This led us to test the hypothesis that serum vitamin D levels might differ between ICP and normal pregnancies. Indeed, we found significantly lower levels of the active form of vitamin D (1,25-dihydroxy vitamin D; 1,25-D3) in women with ICP. In addition, lower levels of 1,25-D3 were associated with a higher incidence of meconium staining, which commonly is considered as an indicator of fetal distress. These findings were new. Previous studies of vitamin D levels in ICP probably missed significant associations since these focused on levels of the much easier measurable precursor of 1,25-D3, i.e., 25-D3. We are confident that our data are more relevant since they also reflect more specifically the metabolism of vitamin D during pregnancy. In particular, the high level of 1,25-D3 in normal pregnancy is thought to be due to an increased placental 1,25-D3, which is substrate-dependently catalyzed by 1-alpha-hydroxylase, and disconnected from the tight regulation of calcium homeostasis in the renal tubuli. In contrast, in a non–pregnant state, plasma concentrations of 1,25-D3 are controlled via feedback regulation of renal 1-alpha-hydroxylase.

ICP is characterized by otherwise unexplained pruritus and elevated serum bile acids that however, might not be causative for the itching. Other compounds such as lysophosphatidic acid via decreased autotaxin activity may cholestatic pruritus, in particular in ICP. Also impaired metabolism and excretion of steroid hormones are probably more relevant for the pathogenesis and the pruritus of ICP then bile acid disturbances. Nevertheless, elevated bile acids indicate cholestasis, which is generally impaired bile flow that ultimately may lead to decreased absorption of fat-soluble vitamins, including vitamin D. We do not believe that this was the reason for lowered serum 1,25-D3 levels in our ICP patients since they had a short history of pruritus and all normal INR values, indicating unaffected intestinal uptake of fat-soluble vitamin K.

However, lower levels of active vitamin D may affect bile acid metabolism and excretion in liver, intestine and placenta since a number of these processes in part are regulated by vitamin D via its nuclear receptor, VDR. For example, administration of pharmacological doses of vitamin D was found to enhance bile acid metabolism in mice. On the other hand, the cholestatic bile acid lithocholic acid has a high affinity ligand to VDR. Thus, one may hypothesize a direct link between increased toxic bile acids
and decreased 1,25-D3 in susceptible individuals, either by directly competing at VDR or by impaired signaling, e.g., for the expression of bile acid metabolizing/excreting proteins or placental 1-alpha-hydroxylase, which converts vitamin D to the active hormone.

Taken together, our finding of decreased levels of active vitamin D in ICP (Paper I) is certainly only of circumstantial evidence but in agreement with concepts on the etiology and pathogenesis of ICP and needs further substantiation. Indeed, we are aware of ongoing laboratory experimental animal studies of vitamin D in pregnancy as well as systematic testing of vitamin D levels in different larger ICP populations. However, it is questionable whether also controlled interventional studies of vitamin D in ICP will ever be performed as already now oral supplements of vitamin D are given.

ICP is a pregnancy complication with substantial risk for the fetus, in particular prematurity. In this respect it is somewhat surprising that the pathology of the placenta previously was paid only very little attention. When this Thesis was planned, we only found one single study from 1980 that reported reduced intervillous spaces, cytotrophoblastic proliferation and increased syncytiotrophoblastic sprout formation in placentas ICP when compared to normal pregnancies of the same gestational age. We therefore performed a systematic evaluation of placental pathology in ICP using a highly advanced morphological approach, i.e. stereology with computerized random sampling. This methodology was chosen as it permits the objective, quantitative description of morphology by efficient and design-based methods. Stereology improves the interpretation of processes of growth, morphogenesis and the understanding of adaptation and functioning at the whole-organ level. Indeed, this methodology has proved of great value for challenging earlier misconceptions when applied to placentas of abnormal pregnancies, such as preeclampsia and intrauterine growth retardation.

Our findings of increased surface areas of terminal villi and capillaries, and a higher number of syncytiotrophoblastic knots in ICP placentas (Paper II) are in agreement with a condition of long-standing placental hypoperfusion or low-grade tissue hypoxemia. Increased placental capillary growth might suggest a response to low-grade hypoxia induced by higher levels of maternal bile acids since in extracted isolated chorionic veins of ICP placentas, bile acids were found to induce vasoconstriction. Our finding of an increased number of syncytiotrophoblastic knots in ICP placentas, which in part confirms the data of 1980, further strengthens the hypothesis of bile acid-induced hypoxia. Syncytiotrophoblastic knots consisting of aggregated old syncytiotrophoblastic nuclei have been described as a sign of hypoxia and are commonly seen in placentas of gestational diabetes and preeclampsia. Interestingly, UDCA-treated ICP had a significantly lower number of syncytiotrophoblastic knots compared to untreated ICP, and a reduction of the collagen area fraction as compared to placentas of both controls and untreated ICP. Both changes may be seen as a beneficial effect of UDCA on morphology ultimately improving fetal blood supply, but the design of the study and the numbers of investigated placentas were too small to draw more robust conclusions. However, at the same time, Catherine Williamson’s group at Imperial College, London, provided data obtained by substantially different methodology that is in striking agreement with our results. They reported several morphological abnormalities of the placenta, including an increase in the number of syncytiotrophoblastic knots. These findings could be reproduced in an in vitro (explant) model when exposed to primary bile acids, which then allowed defining a placental phenotype of ICP consisting
with our data. Furthermore, they also demonstrated that UDCA had a protective effect on placental tissue both in vivo and in vitro.

Taken together, our stereology data (Paper II) substantially add to the knowledge of the placental phenotype of ICP and in particular, to the concept that treatment with UDCA might have beneficial effects on placental blood supply.

Oral treatment with UDCA is part of modern management of ICP aiming firstly to relieve the mother from pruritus and is as such recommended by a number of obstetric societies. A recent meta-analysis concluded that UDCA is effective in reducing pruritus and improving liver test results in patients with ICP. However, as the number of randomized trials with UDCA vs. placebo is very limited, the meta-analysis also included data of studies where placebo was given to other medications than UDCA. Furthermore, the as yet largest double-blinded randomized placebo-controlled trial of UDCA, the PITCH trial in the UK, was not evaluated. This particular study including 125 women with ICP showed that although UDCA significantly reduces pruritus, the size of the benefit might be too small for most doctors to recommend it, or for most women to want to take it. This finding is in line with the previously largest controlled trial comparing UDCA vs. placebo in 94 women with ICP that found significant improvement of pruritus only in the small group of patients with severe ICP, defined as serum bile acids exceeding 40 μmol/L at any time of pregnancy. Treatment with UDCA is also performed with the aim to prevent fetal complications, although data to support this concept are lacking. This is not surprising since studies of fetal complications also addressing the prevention of the most feared complication of ICP, i.e. stillbirth, would have to include more than 4000 women with ICP in each arm.

Perinatal mortality including stillbirths has in older studies been reported with an incidence of 10-15%. This was reduced to 3.5% or less with more recent active management, which besides treatment with UDCA includes intensified fetal monitoring and planned deliveries at gestation weeks 37-38. The latter is based on the clustering of stillbirths in ICP after gestational week 36. In Paper III we present data supporting this practice since we did not find an increased risk for stillbirth in women with ICP as compared with non-ICP women under a period where induction of labor was performed in more than half of women with ICP. Actually, there was a numeric decline in the incidence of stillbirth from 3 per year in 1997 to 0 per year in 2009 but this was not statistically significant. Nevertheless, fetal complications rates still are higher in ICP then in normal pregnancies. Infants in ICP are delivered more prematurely, either spontaneously or by induction, are more likely to have a low (<7) 5-min Apgar score. Thus, ICP remains a serious condition. In particular, as our large population-based cohort study revealed a strong association of ICP with two other serious complication of pregnancy, i.e. gestational diabetes and preeclampsia that previously only occasionally was observed.

Interestingly, preeclampsia and gestational diabetes seem to share common pathogenetic pathways. Pregnancy exaggerates atherogenic-like responses, including insulin resistance and dyslipidemia, manifesting as preeclampsia and gestational diabetes. Thus, gestational diabetes and preeclampsia are more common in women with overweight and obesity. However, in our cohort (Paper III), women with ICP were less likely to have...
a high BMI. Consequently overweight and obesity could not explain the association between ICP and these pregnancy related diseases. On the other hand, we found an increased risk of large for gestational age (LGA) in ICP, which further strengthens the link of ICP and gestational diabetes, as LGA is a well-known complication to gestational diabetes\textsuperscript{137,138}. Of note, the association between ICP and LGA remained after excluding women with gestational diabetes. Since elevated bile acids are the hallmark of ICP they might play a pathogenetic role in preeclampsia and gestational diabetes as well\textsuperscript{139}. This speculation is supported by animal studies that have shown that bile acids in addition to their well-established roles in dietary lipid absorption and cholesterol homeostasis, also act as signaling molecules with systemic endocrine functions affecting glucose and lipid turn-over and energy expenditure\textsuperscript{38,39}. However, related humans studies still are lacking. Hopefully, our findings of a strong association of ICP with gestational diabetes and preeclampsia stimulate studies on possibly shared pathomechanism(s) of ICP, preeclampsia and gestational diabetes. Since this approach might result in optimized treatment strategies for either disease.

Although the epidemiologic data of ICP are very reassuring in term of stillbirth risks, the common practice of induction of delivery in gestational weeks 37-39 may generate other complications, in particular increased numbers of emergency caesarean sections (CS). Our single hospital based retrospective cohort study did not show this to be the case (\textbf{Paper IV}). The risk of emergency CS, which was around 10\% both for women with and without ICP, with spontaneous onset of labor in gestational weeks 37-39 is around 10\%, did not increase, when labor was induced. On the contrary, the risk of emergency CS was lower among women with ICP than among controls, even after exclusion of women with pregnancy complications such as preeclampsia, gestational hypertension or diabetes mellitus from study and control groups. It is intriguing to speculate whether increased bile acid levels might have enhanced uterine contractility through activation of the oxytocin receptor pathway\textsuperscript{90}, which is also reflected by the decreased amount of oxytocin that is required to stimulate contractions in women with ICP\textsuperscript{90}.

Taken together, we have shown that ICP during the last 15 years was more often diagnosed, up to 0.58\% of all pregnancies, which may indicate increased awareness of this condition (\textbf{Paper III}). We found that a (small) majority of women with ICP were induced for delivery, which possibly relates to the low number of stillbirths that do not differ from normal pregnancies (\textbf{Paper III}). We have also found highly significant associations with ICP and two other serious complications of pregnancy, gestational diabetes and preeclampsia that we believe need consideration in the management of either condition. Finally, we have shown that ICP is not associated with an increased number of emergency CS, neither for spontaneous or induced deliveries (\textbf{Paper IV}).
CONCLUSION

This thesis reports that women with ICP have lower levels of active vitamin D and that decreased levels correlate to meconium staining, which is considered as a sign of fetal distress. We show that ICP affects the placental morphology, in part resembling changes observed in other complications of pregnancy, and that treatment with UDCA may result in a reduction of collagen in ICP placentas. We found a strong association of ICP with gestational diabetes and preeclampsia, which needs to be considered in future management of either condition, but no increased risk of stillbirth in ICP pregnancies. We demonstrate that the common practice of induction of labor in ICP of gestational weeks 37-39 did not increase the risks of emergency CS or fetal asphyxia.

FUTURE RESEARCH AREAS

Further studies of ICP-related morbidity should focus on pathomechanism(s) leading to ICP, preeclampsia and/or gestational diabetes. This approach may result in optimized treatment strategies for either disease.
First of all I wish to thank the women who participated in the studies.

**Professor Hanns-Ulrich Marschall.** My main supervisor, who believed in a doctor’s ideas and visions. I have the deepest respect for your scientific skills and never ending energy and enthusiasm. I am so thankful that you shared with me your profound knowledge of intrahepatic cholestasis of pregnancy and taught me how to do science. I deeply appreciate all the time we spent discussing science together and your commitment to support my projects.

**Isaac Shemer.** My lifetime love, science partner and dearest of friends. Your support and encouragement helped me to fulfil my visions and to dare to pursue my ideas into a doctoral thesis.

**Associate professor Bo Blomgren.** My co-supervisor. Thank you so much for teaching me stereology and help in preparation and evaluation of the huge amounts of placenta explants. And I really appreciate your sense of humour and all the funny sayings. It is fun to do research with you.

**Associate professor Olof Stephansson.** My co-supervisor, who made it possible to explore the epidemiological aspects of ICP and showed me how true excellence work in academia.

**Eva Östlund.** My co-supervisor and former boss. I so much appreciate your efforts in facilitating for me to enroll patients and collecting placenta in the Department of Obstetrics and Gynecology at Danderyd Hospital. I am grateful for all the efforts in enabling research time despite the everyday demands of the clinic.

**Professor Catherine Williamson, London, UK.** My external mentor. Thank you so much for guidance and help in understanding ICP.

**Malin Thorsell.** What would I have done without you? Excellent research partner, talented statistician as well as the greatest of friends. Always available for a “food for thoughts chat” and a true clinician who loves science.

**Associate professor Magnus Kaijser.** I am so thankful for your skilled knowledge in statistics and help in paper IV.

**Associate professor Jonas Ludvigsson.** Co-author on paper III. It was really fun to work with you and to experience true excellence and cooperation. Thanks!

**Professor Gabriel Fried in postum.** You believed in my ideas from the beginning and paid the first application to the ethical committee. I really appreciate your openness and I am so very sorry that you are not with us anymore.

**Gabriel Edström and Karin Bartoff Edström.** You are amazing friends and I love spending time over long dinners with you, kids playing discussing other things in life than science.

**Associate professor Bo Anzen.** My former mentor during specialization in Obstetrics and Gynecology. I very much appreciate that you shared your experience and believe in new ideas. Thanks!

**Charlotta Wistrand.** You are amazing. Wise, warm hearted and smart. Who could ask for a better research partner and friend?

**Janne Rapp.** I really appreciate to be your friend and share our common interest of bringing ideas that can make a change.
Helena Kopp Kallner, Maria Persson, Karin Wickström, Ulrika Heddini, Caroline Elmer, thanks for great research dinners and sharing of thoughts and experiences of our PhD’s process.

Gunilla Zettermark and Hilde Larsson. You give great study support and are invaluable to a struggling PhD student.


Åsa Regner. It is really fun to discuss new ideas with you. And it was great travelling together in Bangladesh.

ALL my former friends and colleagues at the Department of OB & GYN, Danderyd Hospital. You are great, and I really appreciated working with you.

ALL my former friends and colleagues at BB Stockholm. It was great not only working with you but also to share thoughts about life in general.

Matthew Volsky. My dear brother-in-law, friend and business partner. I learned so much from “the American way”. You have no idea how much I appreciate your hard work and support.

Lena Wickman Wikström. My mother and my best friend. Without your love and support I would never have finished this thesis. Thank you so much for all the help and love you give to me and my family.

Dag Wikström. My father whom always imprinted in me that with education come obligation to use your knowledge to improve the conditions of humanity.

My sister Catharina and brothers Jonathan, Jakob and Andreas and sister in law Dana. I am so happy to have you as my family. You warm my heart.

My children Daniel, Ella and Jonathan. You are the joy of my life. Thank you for being patient with me and accepting having an absent-minded mother. And, let’s enjoy our family’s next step in New Zealand!


