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PREGNANCIES COMPLICATED BY OBESITY – FOCUS ON STILLBIRTH AND INFANTS BORN LARGE FOR GESTATIONAL AGE

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PREGNANCIES COMPLICATED BY OBESITY – FOCUS ON STILLBIRTH AND INFANTS BORN LARGE FOR GESTATIONAL AGE
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To Arvid, Kasper, Rasmus and Hedda

ABSTRACT

Background

The prevalence of obesity has increased globally and reached pandemic levels. Obesity is associated with increased risks of complications during conception, pregnancy and delivery as well as during the postpartum period. Obesity is most commonly associated with increased risk of giving birth to an infant with high birth weight for gestational age. Among other complications, obese women have a higher risk of stillbirth. Better models to identify women at risk of having infants large for gestational age or stillbirth is needed to be able to take preventive action. In addition, increased knowledge about mechanisms behind the association between obesity and stillbirth is needed.

Methods

Study I and study II were based on the first trimester database, crosslinked to the Swedish Medical Birth register. Predictive models for risk of giving birth to an infant large for gestational age and risk of stillbirth were constructed, based on maternal characteristics and biochemical markers in maternal blood from early pregnancy.

Study III compared placentas and cord blood erythropoietin concentrations from pregnancies of obese and normal weight women, with the aim to investigate signs of chronic fetal hypoxia. Study IV compared placental analyses from pregnancies of obese and normal weight women with stillborn and live born infants, with the aim to examine placental factors potentially mediating the increased risk of stillbirth with increasing BMI.

Results

The predictive model for risk of large for gestational age infants, in early pregnancy, in parous women with obesity had an AUC for the receiver operating characteristic (ROC) curve of 0.81 (95% CI; 0.79 to 0.82), with a sensitivity of 48 % at a fixed specificity of 90%. The predictive model for risk of stillbirth had an AUC for the ROC curve of 0.69 (95% CI 0.64-0.74). The sensitivity was 28 % at a fixed specificity of 90%.

The adjusted linear regression analysis showed a significant, positive association between maternal body mass index (BMI) and cord blood erythropoietin concentration, (β 0.97 CI 0.27 - 1.68, P-value 0.01). However, no significant difference in placental lesions between obese and normal weight women with uneventful pregnancies were found.

The effect of obesity on the risk of stillbirth decreased with 38 % when umbilical cord abnormalities were included in the logistic regression model. The effect of obesity on the risk of stillbirth decreased with 15% when chorioamnionitis was included in the logistic regression model.

Conclusion

Predictive models for risk of large for gestational age infants in obese parous women were fairly good. Predictive models for risk of stillbirth in overweight and obese women were reasonable. However, the capacity of the predictive model increased if small for gestational age was included in the model, indicating a potential to improve the predictive capacity if estimated fetal weight could be included.

There are signs of an increased risk of chronic fetal hypoxia in pregnancies of obese women.

In term pregnancies, umbilical cord abnormalities could possibly explain approximately one third of the increased risk of stillbirth with increasing BMI. Chorioamnionitis could possibly explain approximately 15% of the increased risk of stillbirth with increasing BMI in term pregnancies.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original articles and manuscripts, hereafter referred to by their Roman numerals (I-IV)

I. **Åmark H,** Westgren M, Persson M

Prediction of large for gestational age infants in pregnancies complicated by obesity: A population-based cohort study.

Acta Obstet Gynecol Scand. 2019 Jun;98(6):769-776.

II. **Åmark H**, Westgren M, Persson M

Prediction of stillbirth in women with overweight or obesity – A register-based cohort study.

PLoS One. 2018 Nov 19;13(11):e0206940

III. **Åmark H,** Sirotkina M, Westgren M, Papadogiannakis N, Persson M

Is obesity in pregnancy associated with signs of chronic fetal hypoxia?

Acta Obstet Gynecol Scand. 2020 Jun 18. doi: 10.1111/aogs.13941. Online ahead of print.PMID: 32557543

IV. **Åmark H**, Westgren M, Sirotkina M, Persson M, Papadogiannakis N

Maternal obesity and stillbirth at term; placental pathology
A case control study

Submitted: Obstetrics and Gynecology

CONTENTS

LIST OF ABBREVIATIONS

AUC	Area Under Curve
BMI	Body Mass Index
CI	Confidence Interval
DAG	Direct Acyclic Graph
GDM	Gestational Diabetes Mellitus
IGF	Insulin Growth Factor
IOM	Institute Of Medicine
LGA	Large for Gestational Age
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PAPP-A	Pregnancy Associated Plasma Protein A
ROC	Receiver operating characteristic
SGA	Small for Gestational Age

1 INTRODUCTION

The proportion of women with overweight or obesity in early pregnancy is continuously increasing in the world, including Sweden. The problem with obesity has reached pandemic proportions [1, 2]. Increasing body mass index (BMI) is associated with increased risks of complications during fertilization, implantation, pregnancy, delivery and the post-partum period. The risks are increased for both mother and child in the short-term as well as in the long-term perspective [3-7].

One of the most common complications in pregnancies of obese women is giving birth to a large for gestational age (LGA) infant. LGA is defined as birthweight equal to or above the 97th percentile for gestational age and sex (>2 SD) [8]. Giving birth to an LGA infant is associated with increased risks for both mother and infant during delivery. There are increased risks of obesity, high blood pressure and diabetes in adolescence and adulthood for the LGA born infant [9, 10]. The knowledge of how to identify women at highest risk of giving birth to an LGA infant is a prerequisite for the preventive strategies aiming at reducing the incidence of LGA infants.

One of the most severe complications associated with obesity is stillbirth. The risk of stillbirth among obese women increase almost linearly with increasing maternal BMI in early pregnancy [3]. The rates of stillbirth have been stable during the past decades in high-resource European countries, while the incidence of neonatal death has continued to decrease. Consequently, stillbirth is the main contributor to perinatal death [11, 12]. The incidence of stillbirth differs between high-resource countries. There are interregional differences in incidence within Sweden indicating a possibility to decrease the incidence in some regions. In 2016 Flenady et al. wrote that "Future research must focus on stillbirth prediction, understanding placental pathways to stillbirth and causal pathways to unexplained stillbirth" [13]. In order to further decrease risks of stillbirth, increased knowledge on pathogenic mechanisms is needed. Also, early identification of women with high risk of stillbirth may open possibilities for prevention.

2 BACKGROUND

2.1 OBESITY

2.1.1 Definitions

BMI is defined as an individual's weight in kilograms divided by the square of the height in meters (kg/m^2). BMI is strongly correlated with, and therefore often used as a marker of, the amount of body fat [14]. Overweight and obesity are defined as BMI of 25.0 to 29.9 and BMI ≥ 30.0 respectively. Obesity is further sub-classified into obesity class I, class II and class III, please see table 1.

Table 1. Classification of BMI by the World Health Organization (WHO)

BMI (kg/m2)	**Classification**
>18.5	Underweight
18.5-24.9	Normal weight
25-29.9	Overweight
30-34.9	Obesity class I
35-39.9	Obesity class II
≥ 40	Obesity class III

2.1.2 Prevalence

The prevalence of obesity is increasing worldwide [2]. The most rapid increase has been observed in high-income, English-speaking countries [2]. In the United States the prevalence of obesity among women in reproductive age increased from 28% in 1999 to 34% in 2008 [15]. Most countries in Europe have a prevalence of overweight or obesity between 30-37% in women in reproductive age [16]. The highest prevalence of overweight or obesity in Europe, 48.4%, was reported in Scotland [16]. In Sweden more than one in three women in early pregnancy are overweight or obese compared to one in four 20 years ago, figure 1 [12].

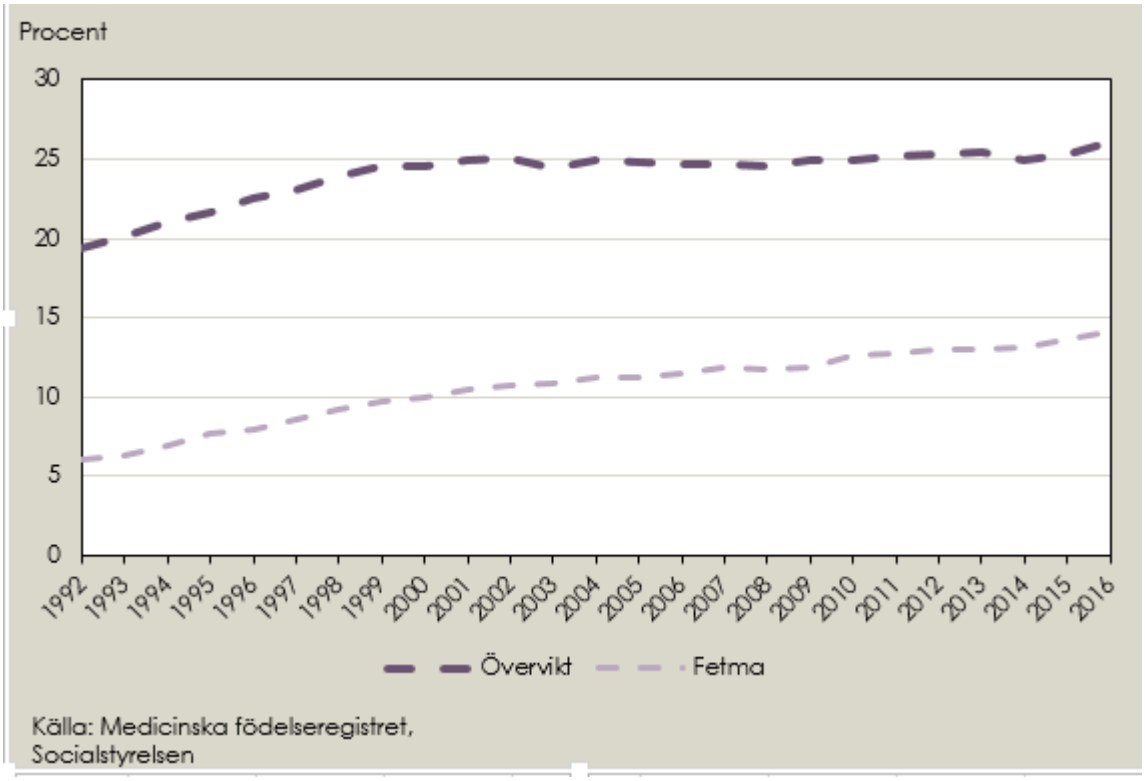

Figure 1. The increasing proportion of overweight and obesity in early pregnancy in Sweden between 1992 to 2016 [17].

2.1.3 Characteristics of overweight and obese women

The prevalence of overweight and obesity is not equally distributed in society. There is an association between pre-pregnancy obesity and country of birth. Women born in Africa, Middle East and South America have an increased risk of overweight or obesity compared to women born in Sweden. However, women born in Europe (except the Nordic countries), North America, Australia, former USSR and Asia have lower risk of overweight or obesity as compared to women born in Sweden [18]. Besides country of birth, socioeconomic factors are important risk factors for obesity, with level of education being one of the most important. Women with lower level of education have an increased risk of obesity [18]. In addition, women with lower level of education had a more prominent increase in BMI between pregnancies than higher educated women, with the largest difference observed in women with excessive gestational weight gain during the first pregnancy [19]. Since women tend to gain weight between pregnancies, women with obesity are more often parous women [20].

2.1.4 Gestational weight gain

There are risks associated with both higher and lower gestational weight gain than recommended during pregnancy [21], table 2

Table 2. Institute of Medicine (IOM) [22] recommendations for gestational weight gain

Pre-pregnancy BMI (kg/m2)	Recommended gestational weight gain
>18.5	12.5-18 kg
18.5-24.9	11.5-16 kg
25-29.9	7-11.5 kg
>30	5-9 kg

Women with lower gestational weight gain than recommended have a reduced risk of giving birth to a large for gestational age (LGA) infant and an increased risk of giving birth to a small for gestational age (SGA) infant, with the highest risk in women with BMI < 18.5 before pregnancy [21]. In addition, lower gestational weight gain than recommended is associated with an increased risk of preterm birth [21].

Women with excessive gestational weight gain have a decreased risk of giving birth to an SGA infant and an increased risk of giving birth to an LGA infant. In addition, there is an increased risk of cesarean section, gestational hypertension, gestational diabetes (GDM), preeclampsia, induction of labor and blood transfusion [21, 23]. Obese women with weight loss during pregnancy or lower gestational weight gain than recommended have an increased risk of preterm birth and an increased risk to give birth to an SGA infant [21]. However, weight loss between pregnancies in overweight or obese women decreases the risk of giving birth to an LGA infant and is not associated with increased risk of preterm birth or SGA [24, 25].

2.1.5 Obesity and pregnancy complications

Obesity is a major risk factor for several complications during pregnancy, delivery and puerperium [7]. Obesity is the most important modifiable risk factor for pregnancy complications, including stillbirth, large for gestational age infants, preeclampsia and gestational diabetes [3, 26]. It has been estimated that, if 10% of women with pre-pregnancy obesity achieved a normal weight before pregnancy, 700 fetal deaths per year could be prevented in the USA [27].

2.1.5.1 Obesity and gestational diabetes

Obesity increases the risk of GDM [28], which is an independent risk factor for preeclampsia and LGA [29, 30].

Normal pregnancy includes a more insulin resistant state which in some cases reaches a level defined as GDM. However, the HAPO study [31], which was an international multicenter study, including over 25,000 women without pre-gestational diabetes or GDM, has shown increased risks of adverse maternal and neonatal outcomes even at levels of hyperglycemia beneath the diagnostic threshold for GDM [29, 31, 32]. These findings have led to stricter criteria for the diagnosis of GDM. No international or national consensus on screening and diagnosis of GDM existed before 2016. However, in 2016 new, stricter criteria for the diagnosis of GDM were adopted by the American Diabetes Association and WHO. Since 2018, The Swedish National Board of Health recommend the same criteria [33]. According to the new criteria, a diagnosis of GDM should be given in case of a fasting glucose level ≥ 5.1 mmol/L, and/or a glucose level ≥ 10.0 mmol/L at 1 hour after oral glucose tolerance test (OGTT, 75 g glucose) and/or a glucose level ≥ 8.5 mmol/L at 2 hours after OGTT [33].

Risks associated with obesity and risks associated with GDM are partly the same. The risks of preeclampsia and hypertension seem to be higher in obese women than in women with GDM alone [34]. The risk of giving birth to an LGA infant is increased both in women with obesity and in women with GDM, with the highest risk in pregnancies with both GDM and obesity [29, 30]. The impact of obesity and maternal hyperglycemia seems to be independent and additive with respect to the risk of giving birth to an LGA infant and to the risk of hypertensive disorders in pregnancy, including preeclampsia [29, 34]. The stricter criteria for the diagnosis of GDM will lead to an increasing number of women being diagnosed. These women will be treated with the aim of keeping the levels of glucose in the blood as close to normal as possible, to reduce the risks associated with GDM. The benefits of employing stricter criteria remain to be investigated.

2.1.5.2 Obesity and hypertensive disorders

Essential hypertension is defined as blood pressure ≥ 140/90 mmHg, measured on two separate occasions. Pregnancy induced hypertension is defined as blood pressure ≥ 140/90 after the 20th gestational week, measured on two occasions, in a woman who was normotensive prior to pregnancy. Preeclampsia is defined as pregnancy induced hypertension with significant proteinuria and/or additional symptoms from other organs, increased creatine level, twice normal level of liver transaminases, pulmonary edema or cerebral/visceral symptoms [35].

There are numerous studies reporting increased risk of preeclampsia with increasing maternal BMI [4, 36-38]. The risks of both mild and severe preeclampsia increase with increasing BMI, as

well as the risk of pregnancy induced hypertension [38, 39]. The pathophysiology of preeclampsia is not fully understood. However, dysfunctional trophoblast invasion and migration in the endometrial lining of the uterus resulting in a disrupted remodeling of the spiral arterioles, hence reducing placental perfusion, will likely contribute [37].

Obesity is accompanied by a state of subclinical inflammation, with increased levels of pro-inflammatory cytokines [40]. The inflammatory state of obese women is likely to contribute to the increased risk of preeclampsia. The state of vascular inflammation is associated with both BMI and hypertension [40].

It has been shown that hyperinsulinemia is associated with a reduced trophoblast invasion and poor placentation [41-43]. In addition, hyperinsulinemia inhibits the physiological decrease in blood pressure during mid-gestation and increases the risk of hypertension later in pregnancy [42]. Nitric oxide is crucial for keeping normotension during pregnancy [44] and hyperinsulinemia leads to decreased nitric oxide production [44]. Additionally, the higher levels of pro-inflammatory cytokines will also contribute to decreased levels of nitric oxide and increased levels of vasoconstrictors in maternal blood [37]. These mechanisms are likely to contribute to the increased risk of hypertension in pregnancies complicated by obesity.

There is also evidence of a maternal cardiovascular component in the etiology of preeclampsia [45]. During normal pregnancy, the heart is remodeled with increased chamber dimensions, wall thickness and increased mass [45]. Pregnancy is a cardiovascular burden with increasing blood volume, increasing heart minute volume and a slight increase in heart rate. Obese women have less margins than normal weight women to handle the extra cardiovascular burden associated with pregnancy [45].

2.1.5.3 Obesity, time to delivery and mode of delivery

Duration of first stage of labor increases with increasing maternal BMI as well as if the fetus is large [46, 47]. Particularly the early phase of the first stage of labor is prolonged in obese women [48]. However, the second stage of labor is not prolonged in obese women [47]. Obese women have an increased risk of post term pregnancy [49], resulting in an increased risk of induction of labor. It has been shown that obese women needed more doses of misoprostol during induction of labor and a longer duration of oxytocin administration at increased concentration and they still ran an increased risk of cesarean section [50]. Several reviews show that obese women have an increased risk of instrumental vaginal birth as well as cesarean section [51, 52]. It has been hypothesized that the increased risk of cesarean section and a prolonged first stage of labor are due to factors like cholesterol incorporated in the

myometrium affecting contractility, increase of pelvic soft tissue narrowing the birth canal, increased frequency of macrosomic fetuses and a poorer response to oxytocin [53]. However, there is evidence that obese women have the same strength of contractions as normal weight women [54].

2.1.6 Minimizing risks associated with obesity during pregnancy- what can be done?

Obesity is associated with risks of adverse outcome for both mother and child. Intervention programs including diet and exercise during pregnancy have demonstrated a slight reduction in gestational weight gain, however, not reduced BMI and have failed to show decreased risks except for small differences in birthweight and a small decrease in the incidence of GDM [55]. After bariatric surgery the risk of GDM, LGA, hypertension and post-partum hemorrhage decreases; however, the risk of SGA increases [56]. Weight-loss between pregnancies leads to decreased risks of GDM and LGA without increased risks of SGA or preterm birth [24]. Observational studies that have reported decreased risks of GDM, LGA and hypertension with weight loss between pregnancies have compared women who really did lose weight with women who did not. Supposing BMI is the harmful exposure, decreased risks cannot be expected if the exposure is not changed. In interventional studies during pregnancy most women will not lose weight. Hence, the different results may be explained by different degree of exposure.

Breastfeeding is less frequent and of shorter duration in obese women than normal weight women [53]. However, the effect of breastfeeding on post-pregnancy weight loss is conflicting [57, 58].

In conclusion, actions taken during pregnancy have failed to substantially affect the risks of LGA, birthweight, GDM and hypertension. The most effective action is to achieve a BMI as close to normal as possible before pregnancy [24]. Dietary advice with limited gestational weight gain, awareness of increasing blood pressure, estimated fetal weight and knowledge about a normal labor in obese women, may help reduce the negative effects of obesity during pregnancy. Women with overweight or obesity have a higher frequency of substantial inter-pregnancy weight gain [59], which will further increase their risks due to obesity. A low gestational weight gain during pregnancy is associated with a decreased risk of inter-pregnancy weight gain [58, 60].

2.2 STILLBIRTH

In Sweden, stillbirth is defined as a fetus born dead at gestational week ≥ 22+0 [61]. The incidence of stillbirth is highest in low-resource regions of the world but remains a problem in high-resource regions where stillbirth accounts for the majority of all perinatal deaths. The average stillbirth rate is around 5/1000 births in high-income regions and in Sweden 3-4/1000 births [62, 63]. During the past decades the incidence of stillbirth has declined slowly or remain stable over time in most high resource countries [12, 63, 64]. However, during the same time period the incidence of neonatal mortality has declined faster and consequently the proportion of perinatal deaths due to stillbirth has increased, figure 2 [11, 12].

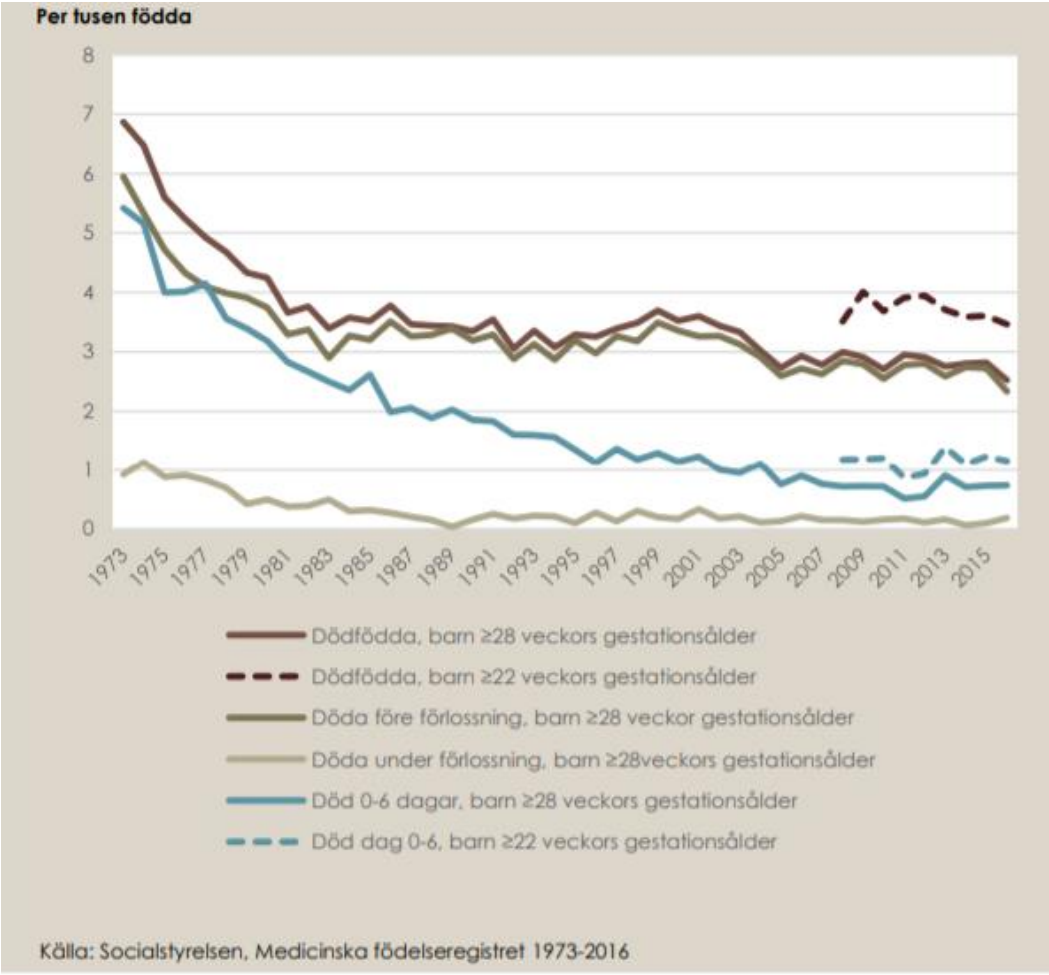

Figure 2. The proportion of stillbirth and neonatal death (day 0-6) for each year per 1000 born infants in Sweden [65].

Several risk factors for stillbirth have been identified, including, low and high maternal age, nulliparity, previous stillbirth and maternal medical conditions such as diabetes, preeclampsia, systemic lupus erythematous (SLE) and antiphospholipid syndrome [26, 66]. The two main modifiable risk factors are obesity and smoking [26]. Several of the risk factors are

associated with placental dysfunction and fetal growth restriction, which is found in a large proportion of stillbirths [26, 62, 64, 67]. In addition, fetal anomalies, infections, umbilical cord complications and placental abruption might cause stillbirth. However, for a large proportion of stillbirths the cause is unknown [64, 68].

Causes of stillbirth differ between preterm and term pregnancies. Infections and umbilical cord complications are more common in term stillbirths, whereas preeclampsia and abruptio placental are more common in pre-term stillbirths [69]. Fetuses small for gestational age have an increased risk of stillbirth both in term and pre-term pregnancies. However, the proportion of SGA fetuses in term stillbirths is much lower than the proportion of SGA among pre-term stillbirths [69].

2.2.1 Obesity and the risk of stillbirth

The risk of stillbirth increases with increasing maternal BMI [3]. Obese women have an increased risk of hypertension, preeclampsia and impaired placental function [67], which are possible contributing factors to the association between obesity and stillbirth [70]. However, the risk of stillbirth remains increased in obese women also after taking obesity-related conditions and obesity associated factors into account, such as preeclampsia, diabetes, hypertension, ethnicity, maternal age, parity, smoking and socioeconomic status [5, 26, 71, 72]. These findings indicate that obesity per se has an independent impact on the risk of stillbirth [70]. There are probably several mechanisms contributing to this effect; placental dysfunction and inflammation as well as metabolic and hormonal changes associated with obesity have been suggested [70].

Fetuses of obese women have an increased risk of hyperinsulinemia, measured as level of C-peptid in cord blood [32, 73]. Fetal hyperinsulinemia may lead to chronic fetal hypoxia [74]. Experimental studies on fetal rhesus monkeys and lambs demonstrate that induced hyperinsulinemia increases risk of hypoxia [75]. Hyperglycemia during pregnancy in women with type 1 diabetes mellitus increases the risk of stillbirth [76].

In addition to other functions, fat tissue plays a role in the immune system, which enhances the inflammatory state in obese women via increased number of macrophages and increased adipokines [77]. The low-grade inflammation is supposed to partly explain the increased risks of cardiovascular disease among obese persons as well as increased risk of cancer associated with obesity in non-pregnant individuals [77]. During pregnancy the low-grade inflammation is thought to contribute to some of the pregnancy complications associated with obesity such as

preeclampsia, gestational diabetes and hypertension [40]. Chorioamnionitis has been suggested to account for approximately 10% of the increased risk of stillbirth due to obesity [78].

It has been shown that both SGA and LGA fetuses have an increased risk of stillbirth, however, in particular SGA fetuses [79]. Obese women have a reduced risk of giving birth to an SGA infant [80, 81]. However, SGA fetuses with obese mothers are at higher risk of stillbirth compared to SGA fetuses with normal weight mothers [82]. Both SGA and LGA fetuses are at increased risk of chronic fetal hypoxia [80]. SGA fetuses have a higher incidence of low oxygen levels in both the umbilical vein and umbilical artery [80]. The umbilical vein transports oxygenated blood from the woman to the fetus and the umbilical artery contains de-oxygenated blood from the fetus to the woman. SGA fetuses may have decreased levels of oxygen due to impaired placental function, possibly also influencing fetal growth [80]. LGA fetuses have a higher prevalence of low oxygen levels in the umbilical artery, which may reflect an increased oxygen demand [80].

2.2.2 Reducing the number of stillbirths – what can be done?

To be able to further decrease the incidence of stillbirth, more knowledge about the causes of stillbirth is needed. One way to collect more structured information is to use audit protocols to collect detailed characteristics of the stillbirths, such as the fetal and maternal associated conditions, the placenta pathology and the autopsy [64]. A project with this aim has been going on in Stockholm since 1998 by the Stockholm stillbirth group. Audit protocols can also be used to try to find avoidable cases and possible preventive strategies.

A large proportion of stillborn infants are born SGA [83]. It has been advocated that repeated estimations of fetal weight would detect fetuses at risk to allow interventions that could potentially decrease stillbirth rates [84]. A screening ultrasound in gestational week 35-36 would potentially identify a larger proportion of SGA fetuses; however, the predictive capacity for negative perinatal outcome among the found SGA fetuses is poor [85, 86]. In addition, an ultrasound in gestational week 35-36 will miss most of SGA stillborn fetuses since the SGA fetuses who will suffer from stillbirth, to a large extent, die in earlier gestational weeks [69]. The incidence of SGA among stillborn infants in the Stockholm Stillbirth database is around 10% for term infants and around 40% for pre-term infants, figure 3 [8]. Hence, a third trimester ultrasound in a low-risk population has been shown to be able to detect a larger proportion of SGA fetuses, although not affect the outcome for the infant [87-89]. However, ultrasound examinations aiming to find SGA fetuses may be

beneficial for certain groups of high-risk women. Predictive models can possibly be used to be able to identify groups where increased surveillance in pregnancy is most needed [13].

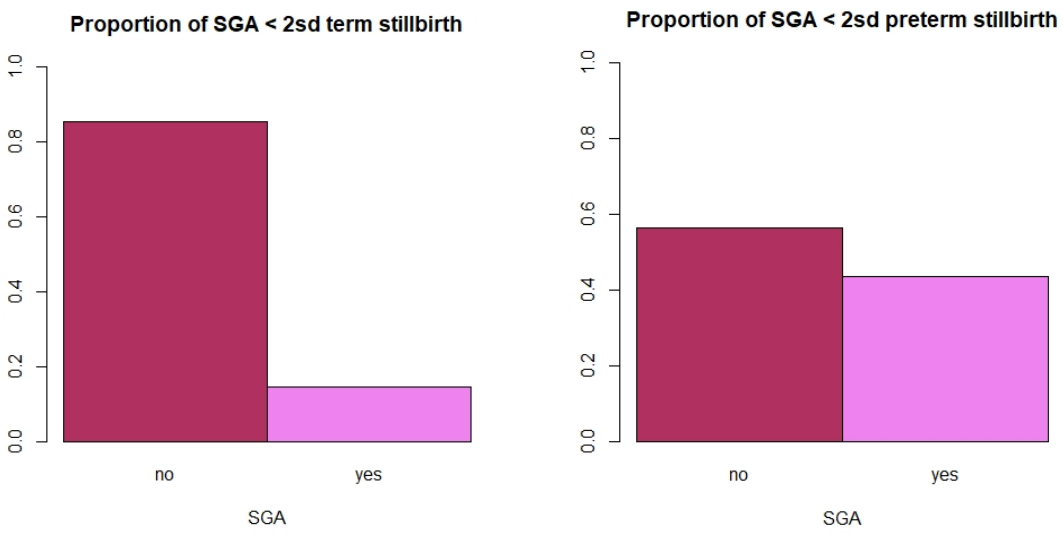

Figure 3: Proportion of stillborn term infants SGA no/yes and stillborn preterm infants SGA no/yes.

Low-dose aspirin has been widely evaluated as a method for prevention of placental-related complications and in particular prevention of pre-eclampsia. It has also been shown to decrease the risk of stillbirth with fourteen percent in women with risk factors for stillbirth [90].

Sleeping position and sleep disordered breathing could be associated with the risk of stillbirth. There are evidence that a supine sleeping position could potentially increase the risk of stillbirth [91]. The evidence of sleeping disordered breathing as a risk factor for stillbirth is conflicting, possibly due to the rare outcome and difficulties to reach enough power in studies [92].

It is known that the risk of stillbirth at term increases for each extra gestational week [93]. There is an on-going debate about the optimal time for induction of labor due to prolonged pregnancy, with the risk of stillbirth and negative neonatal outcome weighted against the risk of other adverse effects associated with induction of labor [94]. The risk of stillbirth in obese women has a more pronounced increase from gestational week 39, compared to normal weight women [95]. Indicating that induction of labor would have a greater effect in preventing stillbirth among obese women compared to normal weight women.

In areas where the stillbirth incidence has decreased rates have decreased over the whole continuum of gestational ages. This is likely a reflection of an overall improvement in perinatal care [96].

2.2.3 Erythropoietin

Erythropoietin is a glycoprotein hormone, stimulating red blood cell production [97]. Erythropoietin is mainly produced in the fetal liver in late pregnancy, and in the infants kidneys after birth [98]. The fetus has several mechanisms to decrease the risk and the effects of hypoxia and respond to reduced oxygen tension [99]. Fetal hemoglobin has a higher affinity for oxygen compared to maternal hemoglobin [99]. The fetal circulation may adapt to maintain adequate blood supply to the brain and heart [99]. The fetus will stop moving to decrease the oxygen demand [99]. In addition, the erythropoiesis will be stimulated in response to elevated levels of erythropoietin [97]. The levels of erythropoietin seem to be stable during pre-term and slightly rise in later gestational weeks [100]. The level of erythropoietin starts to rise two to three hours after the onset of a moderate to severe hypoxia [74, 75, 101]. There are several different receptors with affinity to erythropoietin [97]. It has been argued that a fast increase and high levels of erythropoietin will mainly affect receptors with a low affinity of erythropoietin, mediating short-term effects of erythropoietin including anti-inflammation, anti-apoptotic and antioxidative effects [97]. These effects seem to be neuroprotective [97]. It has been shown that neonates treated with therapeutic cooling combined with high doses of erythropoietin had decreased severity of brain injury than neonates who got therapeutic cooling only [97]. Chronic hypoxia, lasting for days, leads to a smaller, prolonged increase in erythropoietin concentration which will lead to enhanced erythropoiesis, neurogenesis and angiogenesis [97]. Fetal blood erythropoietin level has a strong correlation to erythropoietin level in amniotic fluid [102]. The level of erythropoietin in amniotic fluid can possibly differentiate between acute and chronic causes of stillbirth with high levels of erythropoietin in amniotic fluid when the hypoxic situation has lasted for a long time and normal levels of erythropoietin in amniotic fluid when the hypoxia/asphyxia was acute [103].

2.2.3.1 Relevant difference in erythropoietin concentration

It is well known that fetal hypoxia is associated with increased levels of amniotic and cord blood erythropoietin [74, 97]. Erythropoietin levels increased rapidly with acute hypoxia, however, stabilized at a slightly increased level with chronic hypoxia [104]. In fetal lamb exposed to low grade hypoxia, erythropoietin levels increased from a mean of 23 mU/ml to 145 mU/ml during the first 24 hours, then stabilized at a mean level of 31 mU/L in 7 days [104]. In a comparison between term pregnancies before gestational week 40+6 and pregnancies

from gestational week 41+0 the median erythropoietin levels were 20 U/l and 34 U/L, respectively [100]. Among normal weight women compared to obese women the erythropoietin levels were 25 U/L and 64 U/L, respectively, however including an increased number of complicated deliveries [51].

Median cord blood erythropoietin levels in uncomplicated pregnancies of 20-35 U/L before the onset of labor and with an upper limit ranging between 50-60 U/L are considered normal[74].

2.2.4 Obesity and placental changes

Placental analyses can reveal important pathological pathways between mother and fetus. Placental analyses are crucial in the evaluation of stillbirth and in the attempt to find the cause of stillbirth [105, 106].

Studies on placental pathology from women with obesity are scarce and results are conflicting. A large cohort study showed an increased proportion of placental infarctions and signs of chorioamnionitis in term and pre-term placentas from pregnancies complicated by obesity [107]. It has also been shown that obesity is associated with increased number of placental vascular lesions, in uncomplicated pregnancies [108]. Obesity has also been associated with focal villous immaturity and increased number of capillaries per villous but without inflammatory changes [109]. However, another study showed increased levels of inflammatory mediators but no vascular lesions [110]. Several studies have small sample sizes [108-110]. Even in uncomplicated pregnancies with normal delivery, a healthy and normal-sized infant and a healthy woman, there are a substantial proportion of pathological placental lesions [107-109]. The high proportions of placental lesions in healthy pregnancies, probably reflect that the placenta is an organ with large capacity and with a short life-time.

It has been shown that obese mice have very early changes in the placental transcriptome, affecting a number of important genes for angiogenesis and placental development [111]. It is possible that similar placental changes also in human pregnancies of obese women contribute to the elevated risk of complications.

To our knowledge, there are so far no studies comparing placentas of normal weight and obese women with respect to placental lesions possibly leading to fetal chronic hypoxia or placental lesions possibly compensating for chronic fetal hypoxia.

2.2.5 Placental lesions associated with hypoxia

The placenta has a high capacity to deliver oxygen. There are several placental lesions associated with a hypoxic situation reflecting either, signs of mechanisms to secure the oxygen supply or signs of processes impairing the blood flow. These lesions are associated with several conditions such as preeclampsia, intrauterine fetal growth restriction, stillbirth and preterm birth [112]. However, lesions are also found in normal pregnancies and many fetuses will be well oxygenated although there are placental lesions associated with chronic fetal hypoxia [112]. Thus, interpretation of meaning of different placental lesions is not always straight forward [112].

2.2.5.1 Mechanisms behind placental lesions associated with chronic fetal hypoxia

Placental lesions associated with hypoxia is divided in three groups according to the underlying reason for hypoxia. The three groups are; pre-placental hypoxia, decreased utero-placental supply and post placental hypoxia [113]. Pre-placental hypoxia may be present in women with anemia and women living at high altitude, the volume of vessels in the villi will increase to increase the possibility for oxygen exchange [114]. These changes are usually fairly uniformly distributed in the placenta [112]. Decreased utero-placental supply is a decreased perfusion through the spiral arteries, for example when the spiral arteries have not undergone normal remodeling. That may result in the same kind of placental changes as pre-placental hypoxia, however with focal lesions [112]. In both conditions the placenta is often large and the ratio between placental weight and fetal weight is increased [113, 115]. These lesions are signs of compensatory mechanisms due to fetal hypoxia. Post-placental hypoxia occurs when the obstruction of the blood flow is distal to the placenta and may for example be due to a mechanical obstruction of the umbilical vessels [113]. If there is an obstruction in the umbilical cord the amount of oxygen in the placenta will increase and angiogenesis in the villi will decrease, the newly formed villi will be long and thin with limited branching, called distal villi hypoplasia [80].

Impaired placental oxygen supply leads to increased levels of angiogenic placental factors in maternal blood. Increased level of angiogenic placental factors leads to increased placental size to be able to support the fetus with adequate amount of oxygen [115]. Obese women may require an increased amount of oxygen herself, in some cases affecting the placental oxygen supply, pre-placental hypoxia, resulting in increased level of angiogenic placental factors [115]. An increased placental size may be a compensation of a hypoxic situation.

In addition, there are placental insults, seen as placental lesions, which impair the blood flow and could lead to fetal hypoxia. For example, placental infarctions or placental abruptio could lead to both acute and chronic fetal hypoxia.

2.2.5.2 Placental lesions associated with chronic fetal hypoxia

There have been different definitions of placental lesions in the obstetric field which have made it hard to compare scientific findings and outcomes. A consensus meeting, Amsterdam Placental Workshop Group, in 2014 resulted in a document with definitions of different placental lesions and an agreement on lesions with adequate scientific documentation to be considered as definitive manifestations of chronic hypoxia [116]. The definitive lesions which could lead to chronic fetal hypoxia include placenta hypoplasia ≤ the 10th percentile for gestational age, infarctions, retroplacental bleeding and decidual arteriopathy. Villous infarction is the most obvious sign of an event leading to fetal hypoxia and may occur after occlusion of a spiral artery. A small peripheral infarction in the placenta of a full-term pregnancy is a common finding, probably with limited clinical consequences. However, if there are preterm infarctions, central infarctions or if a significant fraction of the placental parenchyma is involved there are potential clinical consequences [112, 116]. Retroplacental bleeding is the pathological sign of a potential placental abruption [112], with the potential to cause fetal hypoxia. Decidual arteriopathy seen when the spiral arteries do not remodel and widening as they should during early pregnancy, which could lead to impaired blood flow. Decidual arteriopathy is seldom found in placental analyses because they are mostly in the maternal part of the decidua [112].

Additionally, there are placental lesions which are signs of compensatory mechanisms of chronic fetal hypoxia. Increased placental size may compensate fetal hypoxia as well as increased angiogenesis seen as increased number of syncytial knots and chorangiosis [112]. Chorangiosis is increased number of capillaries in the terminal villi [112]. Syncytial knots are a marked clustering of syncytial nuclei seen in light microscope.

2.2.6 Placental lesions associated with stillbirth

Placentas from pregnancies complicated by stillbirth are in general smaller than placentas from pregnancies with live-born infants [105]. Increased proportions of signs of both acute and chronic inflammation, chorioamnionitis and villitis and signs of retroplacental bleeding are associated with stillbirth [105]. An umbilical cord that is long for gestational age, a hyper coiled umbilical cord and a velamentous or marginal umbilical cord insertion as well as an umbilical cord knot and umbilical thrombosis are also associated with stillbirth [105, 117, 118]. In addition,

fetal vascular thrombosis and placental infarctions are associated with stillbirth. Especially in pre-term stillbirths immature villous were more common in the placentas compared with placentas from pregnancies with live born infants [119].

2.3 FETAL SIZE, FETAL GROWTH AND SURVEILLANCE OF FETAL GROWTH

There is a linear association between maternal BMI and birthweight [80, 81, 120]. There are different definitions of LGA in different countries. In Sweden LGA is defined as birthweight equal to or above the 97th percentile for gestational age and sex (≥2 SD) and SGA is defined as birthweight equal to or below the 3rd percentile for gestational age and sex (≤-2 SD) [8]. Internationally, LGA is usually defined as a birthweight ≥ the 90th percentile for gestational age and sex and SGA as birthweight ≤ the 10th percentile for gestational age and sex. The 97th percentile may be a more adequate cut-off for defining LGA since previous studies have been able to show increased risk of adverse advents at the 97th percentile however not at the 90th percentile [9, 121]. However, SGA infants with birthweight between the 10th and 5th percentile, and also between the 15th and 10th percentile, had markedly increased risks of mortality and morbidity even if the risks were even higher among infants with birthweight < 5th percentile [121, 122].

Fetal growth is the result of a complex interplay between several factors, e.g. genetics, intrauterine environment, maternal constraint, nutrition and placental function. Insulin and insulin-like growth factor (IGF) are crucial for fetal growth [123-126]. The secretion of fetal insulin is one of the key determinants of fetal growth, most prominent during the third trimester when the largest increase of fetal weight occurs [123]. The Pedersen hypothesis; maternal hyperglycemia leads to increased level of glucose passing the placenta, which in turn stimulates fetal insulin secretion and fetal growth, is one mechanism behind excessive fetal growth [127, 128]. IGF-1 has been shown to be a very important prenatal growth factor, supported by studies of genetic disorders and by animal models [125, 129]. In animal models, IGF-1 deficiency results in severe growth restriction and a high postnatal mortality, and for humans there is a strong correlation between level of IGF-1 in cord blood and birthweight [125, 126, 129]. There are additional mechanisms, for example leptin, an adipokine, regulating the metabolic homeostasis [130]. Maternal leptin levels increase during pregnancy, more pronounced in pregnancies complicated by obesity probably reflecting an increased leptin resistance [130, 131]. Leptin affects placental amino acid transport and affects fetal growth by increased amounts of nutrients transported over the placenta [130].

To be able to take preventive action and make decisions about fetal surveillance during pregnancy and mode of delivery, it is important to identify fetuses at increased risk because of their size [132]. LGA and SGA fetuses are at increased risk of hypoxia/birth asphyxia during labor and have an increased risk of mortality [9, 122]. However, risks can be reduced if clinicians are aware that the fetus is not of appropriate size [133]. The most frequently used screening methods for identification of SGA and LGA fetuses are based on measurements of fundal height. However, measurement of fundal height is imprecise [134, 135]. An abnormal rise of fundal height will warrant an ultrasound examination and an estimation of fetal weight that is repeated every second week if needed. However, primary preventive actions are preferably taken before the pregnancy.

2.3.1 Obesity and large for gestational age infants

Infants born LGA have increased risks of adverse perinatal outcomes including birth asphyxia, shoulder dystocia, and neonatal morbidity [9, 10, 136, 137]. LGA offspring also face excess risks of infant death, future obesity, hypertension and type 2 diabetes mellitus [9, 81, 138, 139]. Approximately one fifth of obese women give birth to an LGA infant, defined as birthweight equal to or above the 90th percentile [81, 120, 140].

The pathophysiology behind LGA in obese pregnancies is not fully understood [141]. Normal pregnancy induces a pro-inflammatory, hyperlipidemic state with increased insulin resistance due to the elevated levels of pregnancy related hormones. These metabolic changes during pregnancy will be exaggerated if the woman is overweight or obese [41]. Several mechanisms behind excessive fetal growth in obese pregnancies may be considered [142]. Obesity is associated with a state of sub-clinical inflammation, impaired insulin signaling, resulting in even more pronounced insulin resistance, increased levels of blood glucose and increased risk of fetal hyperinsulinemia [32, 142]. In addition, obese women have increased levels of lipids; increased levels of triglycerides, increased concentrations of low-density lipoprotein and increased levels of leptin [41, 143]. The high amount of nutrients transported over the placenta will contribute to fetal growth and a high level of triglycerides at the same time as high levels of glucose and insulin will contribute to increased fetal fat mass [142, 144]. High levels of leptin could be associated with maternal fat mobilization and an even higher level of available nutrients [41].

2.3.2 Pregnancy associated plasma protein A and adverse perinatal outcome

Pregnancy associated plasma protein A (PAPP-A) is a proteolytic enzyme, produced in the placenta and in several maternal tissues [145]. PAPP-A cleaves insulin growth factor binding protein (IGFBP), which decreases its affinity to insulin growth factor (IGF) leading to an increased level of physiologically active IGF [145].

PAPP-A is one of the components in the aneuploidy screening in early pregnancy [146]. Low levels of PAPP-A are associated with an increased risk of several pregnancy complications including preeclampsia, SGA and stillbirth. High levels of PAPP-A are associated with risk of LGA [66, 147, 148].

Levels of PAPP-A in early pregnancy are generally lower in women with any type of diabetes [149, 150]. Women with early onset gestational diabetes have, in general, lower levels of PAPP-A than women with late onset gestational diabetes, and women with pre-gestational diabetes have lower PAPP-A levels than women with gestational diabetes, indicating an association between levels of PAPP-A and degree of maternal glucose intolerance [150].

2.4 SWEDISH MEDICAL BIRTH REGISTER

The Swedish Medical Birth register was established in 1973 with the purpose to gather information about antenatal and perinatal factors which could affect the health of the infant. The Swedish Medical Birth Register has been evaluated several times by comparing original medical records with data from the register [151]. Approximately 1.4% of infants in Sweden are not registered in the Swedish Medical Birth Register [151].

BMI is available for 73-88% of women, with the higher values for the later years [151]. Data on parity is valid in 98% of cases of singleton pregnancies. Data on stillbirth or live birth are correct to a very large extent [151]. Birthweight is recorded to a very high degree. In summary the Swedish Medical Birth Register is a validated register with low degree of missing values for many variables [151].

3 AIMS OF THE THESIS

The overall aim was to be able to predict LGA and stillbirth among overweight or obese women and to find factors contributing to the increased risk of stillbirth among obese women.

Specific aims:

Study I	To develop a predictive model for risk of LGA pre-pregnancy and in early pregnancy in obese women.
Study II	To develop a predictive model for risk of stillbirth among overweight and obese women in early pregnancy.
Study III	To investigate if fetuses in pregnancies complicated by obesity suffer from chronic fetal hypoxia to a higher degree than fetuses to normal weight women.
Study IV	To find placental factors mediating the increased risk of stillbirth with increasing BMI.

4 MATERIALS AND METHODS

A brief description of the methods for each study will follow. Comprehensive descriptions of the methods and materials are provided in each of the original articles at the end of the thesis.

4.1 STUDY I

A register-based cohort study including all singletons born alive at term, without fetal anomalies, identified in the first trimester screening data base between the years 2006-2015, crosslinked to the Swedish Medical Birth Register and the Swedish Register of Total Population, including information on 139 277 pregnancies after exclusions.

The outcome, LGA, was defined as birthweight ≥ the 97th percentile (two standard deviations above the mean) for gestational age and sex [8]

Three different predictive models were constructed; one model for all pregnancies complicated by obesity (i.e. both nulliparous and parous women without considering data from previous pregnancies), one model for parous obese women (i.e. all women who had at least one child before the index pregnancy) and one pre-pregnancy model for parous obese women.

4.2 STUDY II

A register-based cohort study including all singletons born at gestational week 28+0 or later, with women without pre-gestational diabetes, between 2006 and 2015, identified in the first trimester screening database (KUB-database), cross-linked to the Swedish Medical Birth Register and to the Swedish Register of Total Population. The final study cohort comprised 145,319 pregnancies, after exclusions. The predictive models were based on all pregnancies to women with BMI ≥ 25 and without pre-gestational diabetes.

The outcome was stillbirth from gestational week 28+0 in singleton pregnancies.

The predictive model was based on risk estimates from the logistic regression analysis. The final model for prediction of stillbirth included predictors significantly associated with stillbirth in the multivariable regression model, (e.g. BMI, PAPP-A, maternal age, smoking status, the maternal country of birth and parity).

4.3 STUDY III

A prospective cohort study with consecutively collected placentas and cord blood samples from 89 women with BMI ≥ 30 and 91 women with BMI 18.5-24.9, all with term, singleton uneventful pregnancies. Women with a diagnosis of pre-gestational diabetes, a diagnosis of GDM, preeclampsia, hypertension, fetal malformations or intrauterine fetal growth restriction defined as a birthweight ≤ 10e percentile for gestational age and sex [8] were excluded.

Immediately after delivery cord blood was sent to a research laboratory, Studiecentrum at Karolinska University Hospital Solna, for analysis of erythropoietin. The placentas were sent to the Department of Perinatal Pathology at Karolinska University Hospital, Huddinge. Two senior perinatal pathologists performed the placental analyses following a standardized protocol, blinded for maternal BMI [152]. Placenta samples were prepared for histopathological examination with focus on lesions associated with chronic fetal hypoxia including: placental infarction, placental abruption, decidual arteriopathy, placental size, chorangiosis and proportion of syncytial knots.

Linear regression analyses were used to assess the impact of maternal BMI on erythropoietin concentrations, adjusted for the potential confounders maternal age and parity and in addition, the mediators gestational age [49, 100, 153] and mode of delivery. There were outliers with high erythropoietin concentrations, which were scrutinized to be able to find a reason for that.

Placental lesions were analyzed one at a time and in addition, two composite variables associated with chronic fetal hypoxia. One with lesions which could lead to chronic fetal hypoxia; placental hypoplasia, retroplacental bleeding, infarctions and decidual arteriopathy and one composite variable of lesions which could be compensatory of chronic fetal hypoxia; placental hyperplasia, chorangiosis and increased number of syncytial knots.

4.4 STUDY IV

A case control study with all singleton stillbirths, without pregnancies complicated by pre-gestational diabetes or GDM and without major fetal malformations, diagnosed at term with a BMI ≥ 30 or a BMI 18.5-24.9 identified in the Stockholm Stillbirth database between 2002 and 2018 were selected as cases, n=87 and n= 264 respectively. Information about the pregnancies and maternal characteristics have been prospectively collected for all cases of stillbirth. All placental analyses were reevaluated by two senior perinatal pathologists.

Placentas from women with live-born infants, the controls, were collected from 106 full term singleton pregnancies with BMI ≥ 30 consecutively, and 113 pregnancies with BMI 18.5-24.9 consecutively at Södersjukhuset, Stockholm, Sweden. Placentas were analyzed by two senior perinatal pathologists following a standardized protocol, blinded for maternal BMI and other maternal characteristics [152].

Frequencies of the placental variables and birthweight were compared between cases and controls stratified by BMI. Since controls were selected with a pre-determined distribution of obesity, the proportion of obese women among the controls was corrected by weighting, to ascertain an equal proportion of obese women as in the general population. The true proportions of normal weight women and obese women in the Stockholm region were collected from the National Board of Health and Welfare [154]. A logistic regression with BMI as exposure and stillbirth as outcome was conducted. Potential confounders, i.e. maternal age, smoking habit, parity and maternal country of birth were added to the logistic regression. Different groups of placental variables, indicating potential mediators of the effect of BMI on stillbirth, were added to the adjusted logistic regression analysis. These were: A) variables indicating abnormal umbilical cord: velamentous insertion, umbilical thrombosis, umbilical knot, an umbilical cord long for gestational age and a high coiling index, B) variables indicating inflammation, chorioamnionitis, and C) variables indicating maternal circulatory disorders, retroplacental bleeding, infarcts and placental hypoplasia [105, 116].

5 METHODOLOGICAL CONSIDERATIONS

5.1 STUDY I AND STUDY II

The validity of a study might be threatened by systematic errors like selection bias, misclassification and confounding bias. Systematic errors will continue to exist regardless of the size of the sample if the study design is not appropriate. In addition, the reliability of a study might be threatened by random errors. However, random errors will decrease with the size of the sample. Calculating the P-value and the confidence interval are methods to assess the degree of uncertainty due to random errors.

5.1.1 Systematic errors

5.1.1.1 Selection bias

Study I and study II were based on the first trimester screening database, (KUB-database). PAPP-A has been advocated as a valuable predictor for both LGA, SGA and stillbirth [155].

The first trimester screening database (KUB-databasen) collects a large part of women undergoing the first trimester screening in Sweden which include PAPP-A and b-hCG levels in early pregnancy. That was the reason to base study I and study II on the first trimester database. However, the first trimester database suffers from selection bias. When first trimester screening was first offered it was only women 35 years or older or with a special reason or a special request who were offered the first trimester screening. Rather soon the population who were offered the first trimester screening increased, especially in the Stockholm region. Guidelines about offering first trimester screening as well as midwives educated in first trimester screening differ throughout the country. Women in the first trimester screening database have a higher educational level, higher age and lower BMI than the general obstetric population in Sweden [12]. Hence, the first trimester database is based on a selected population. Incidences and absolute numbers will be affected by the selection bias. When performing a prediction, the area under the curve (AUC) will be based on the strength of the associations between the predictors and the outcome. If the strength of the associations differs in the sample and the general obstetric population the AUC will be biased. A weaker association in the sample would have biased the AUCs downwards and a stronger association would have biased the AUCs upwards (towards 1). However, it is not obvious whether the predictor-outcome associations are different in our sample compared with the general obstetric population. The absolute numbers of stillbirth are lower in our sample, however the ratio between incidence of stillbirth among normal weight women and incidence of stillbirth among obese women is in line with previous studies [3]. Further, predictions generally become better when the predictors have high variability (e.g. include both low and high values), the relatively low variability of predictors in our sample are likely to have biased the AUCs downwards (towards 0.5). In both study I and study II the predictions are made for certain BMI levels, ≥ 30 and ≥ 25, respectively. In study I the AUC would have increased if women over all BMI levels were included in the predictions in accordance with the reasoning above. In study II the associations between the potential predictors and the outcome were non-existing or very weak among the normal weight women, in contrast to in women with BMI $\geq$ 25. Because of that the AUC decreased for predictions including women from all BMI levels in study II. The variables associated with stillbirth among women with BMI ≥ 25 that were not associated with stillbirth among normal weight women were BMI, PAPP-A, maternal age, smoking status, maternal country of birth and parity.

If I would have re-done study I and study II, I would not have restricted the data to pregnancies with known value of PAPP-A. In that case I would have had data from the Swedish Medical Birth register which would not have suffered from selection bias. It would

then have been possible to do the predictions among un-selected pregnancies and compare the results between these two samples. The associations between PAPP-A and LGA and PAPP-A and stillbirth are weak, however with low P-values since there is a large sample size. The predictive capacity of PAPP-A is weak for LGA, SGA and stillbirth [66, 147, 156-159].

5.1.1.2 Cross-validation

When doing predictions confounding is not a problem since we are not measuring a causal effect. However, there are other concerns. It is possible to over-fit the model to the data, which would lead to a very good prediction in our specific sample, but not in a new random sample. To obtain an unbiased estimate of the predictive accuracy, the dataset was randomly divided in two parts where one part was used to fit the model and the predictions were made in the other part, a cross-validation. The cross-validation was done 1000 times for all the different predictive models in study I and study II. For predictions of LGA the results were virtually the same after cross-validation, indication a good internal validity. After cross-validation for predictions of stillbirth the AUC slightly decreased.

5.1.1.3 Misclassifications

Study I and study II were based on the first trimester screening database cross-linked with the Swedish Medical Birth register. The exposure was BMI and the outcomes were LGA and stillbirth, respectively. BMI is based on measured weight during the first trimester and self-reported height. Self-reported height may be overestimated and may be overestimated to a higher degree among overweight and obese women. In addition, initially there were approximately 400 cases with extremely high BMI values. Height and weight had been switched when BMI was calculated. By calculating BMI from the height and the weight variables these extreme values disappeared. The correct BMI values in cases with misclassified, extremely high BMI values, varied between 15 and 38. If this would not have been corrected it would have had the potential to dilute the results since the misclassification was unrelated to the true BMI. The LGA variable is easily counted from the birthweight, the gestational age and the infants' sex, which are all reliable variables with almost no missing values. Stillbirth is also a reliable variable, with a low degree of missing value [151]. The Swedish medical birth register is a reliable and validated source [151]. The risk of misclassification is low in both study I and study II. If there were misclassifications anyway, they were probably random and would then have the possibility to dilute the results.

5.1.2 Random errors

Random errors occur as an imbalance between the compared groups caused by chance. Random errors will decrease with increased size of the sample. Study I and study II are based on large samples and random errors was not a significant problem. Both the P-values and the confidence intervals are used to show the precision of the results and the possibility of pure chance as a cause of the results. The P-values are a measurement of the possibility to have the same or a more extreme result if there were no real differences between the compared groups (i.e. if the null-hypothesis was true). 95 % of the confidence intervals will contain the true parameter value, if the trial was hypothetically repeated and the confidence interval was recalculated for each trial. Since the sample was large even small differences between groups became strongly significant. The predictive capacity is mainly based on the magnitude of the effect of the associations and not on the P-value.

5.1.3 External validity

External validity refers to the possibility to apply the conclusions from the study also to other populations. The external validity in study I and study II will depend on the possible differences in effect by the predictors on the outcomes in the sample compared to another populations. E.g. will an increase in BMI from 30 to 32 increase the risk of giving birth to an LGA infant to a higher or lower degree in the general obstetric population than in the sample? This question is hard to answer. However, there is no obvious reason to believe that there would be a substantial difference. The risk of stillbirth among overweight or obese women divided with the risk of stillbirth among normal weight women (2.6/1.6=1.6) is in line with results from a large meta-analysis [3].

5.2 STUDY III AND STUDY IV

5.2.1 Power calculation

Study III compared differences in proportion of placental lesions. Previous researchers have been reporting conflicting results [107-109]. A power calculation depends on assumptions about differences between the compared groups. Because of previous conflicting results these assumptions were hard to make and a power calculation hard to do.

Cord blood erythropoietin levels associated with chronic fetal hypoxia have been suggested to increase 10-15 U/L in different studies [100, 104]. Erythropoietin concentration of <30 U/L is suggested as a normal erythropoietin concentration [97]. A power calculation to be able to have

80% chance to find a difference of mean erythropoietin level between 30 U/L and 40 U/L gives a sample of 60 persons in each group.

Study IV was a case control study with a fixed number of cases collected from the Stockholm stillbirth database. It would not have been possible to collect more cases. Hence, the reason to conduct a power calculation was not to be able to collect enough cases, it was to evaluate the possibility to be able to have a reasonably good chance to answer the research question. The aim of study IV was to explore the potential mediating role of placental lesions on the causal pathway from maternal obesity to term stillbirth. Towards this aim the association between maternal obesity and term stillbirth was compared before and after adjusting for placental lesions.

5.2.2 Systematic errors

5.2.2.1 Selection bias

For study III placentas and cord blood samples were collected at the delivery ward and at the unit of elective cesarean section. This sample of women and placental analyses were, in addition, used as controls in study IV. Elective cesarean sections are pre-planned. From the day the inclusion started almost all women who fitted the inclusion criteria were included. One woman was not willing to participate. However, the proportion of normal weight women was higher, and normal weight women were included in a shorter time-period than women with BMI ≥ 30. Since the cesarean sections were elective it was possible to know that only a small number fitting the inclusion criteria was missed for inclusion. The faster inclusion of normal weight women is not likely to have affected the sampling. Spontaneous labors are un-planned. There had been information distributed about the intended inclusion of women, however the probability to be included in the study was higher when the author was present at the delivery ward. There is a higher probability that a woman with a labor lasting for many hours should be identified and included than a woman who only was present a short time at the delivery ward, a difference which may have been even more pronounced among normal weight women since it was easier to remember to include women with BMI ≥30. If a larger proportion of normal weight women had a longer duration of labor, it may have increased some of the erythropoietin concentrations. In that case, it would lead to a decreased difference between normal weight and obese women and it would have decreased the magnitude of the results. Length of labor would not affect the results of the placental analyses. Nulliparous women, however, generally have labors lasting for a longer period of time and could have

been overrepresented. Normal weight women were included during a shorter time-period than women with BMI ≥ 30.

The erythropoietin levels may have been affected by mode of delivery, with the highest levels among the vaginal births. Even an un-complicated vaginal delivery may cause fetal hypoxia which could lead to increased erythropoietin production [74]. There was a tendency to a longer duration of time from cervical dilatation of 7 cm to delivery among normal weight women, which could be explained by a higher number of nulliparous women among the normal weight women. The longer duration of labor may have increased erythropoietin levels among normal weight women, in that case the difference of mean erythropoietin level between normal weight and obese women would decrease. However, both parity and mode of delivery were adjusted for in the analyses. The odds ratio for BMI without mode of delivery in the model was 0.9745 and was virtually the same with mode of delivery in the model OR 0.9735, indicating that the effect of obesity on level of erythropoietin was not depending on mode of delivery. The association between BMI and erythropoietin was, in addition, analyzed stratified on mode of delivery. Inevitably, stratification by mode of delivery leads to analyses in smaller parts of the data and a larger statistical uncertainty. However, there was a significant and borderline significant association between BMI and erythropoietin among women with vaginal delivery and women with elective cesarean section, respectively (P-value 0.04 and 0.08, respectively).

Study IV compared placental lesions from pregnancies with stillborn and live-born infants stratified on BMI. The women with live-born infants, the controls, were almost the same as in study III and their selection was discussed above, however placenta pathology should not have been affected by mode of delivery. The Stockholm stillbirth database was used to identify cases, women with stillborn infants. The Stockholm stillbirth database contains virtually all cases of stillbirth in the Stockholm region from 1998, numbers controlled by comparison of numbers reported from the Board of Health and Welfare [154]. All cases of term stillbirth without a diagnosis of diabetes and with BMI 18.5-24.9 or BMI ≥ 30 from Jan 1st, 2002 were included in study IV. The reason to exclude cases of stillbirth before 2002 was an increased number of missing values and difficulties with reaching the placenta analyses which were crucial in this study. There were only ten placental analyses missing among the cases, four in pregnancies with obesity and six in pregnancies with normal weight. The criteria for GDM changed in 2016 and there may be cases of stillbirth, who would have been diagnosed with GDM today, however did not get the diagnosis the year they gave birth. Previous studies have shown that women with diagnosed GDM do not have an increased risk

of stillbirth as compared to women without GDM [160, 161]. However, previous studies have also shown that women with increased risk of undiagnosed GDM, (i.e. at increased risk of GDM without a diagnosis) are at increased risk of stillbirth compared to women without GDM, also when adjusted for BMI [160]. One possible explanation could be that women with a diagnosis of GDM are closely monitored and their glucose concentrations are treated to become as close to normal as possible, which could compensate for the increased risk.

There is no selection bias among women with stillborn infants, since all these women were included. The controls were collected during a much shorter time period. The proportion of obese women has increased during the time of case collection [12]. The incidence of stillbirth has been stable [65]. Hence, year of birth is not a confounder, only taking exposure and outcome into consideration. The pathways between BMI, stillbirth and year of birth are illustrated in figure 4. If these assumptions are correct the adjustment for year of birth in the logistic regression with obesity as exposure and stillbirth as outcome would not affect the estimate of obesity in relation to stillbirth, however, increase the P-value. The greatest "risk factor" of stillbirth becomes year of birth since no infant was live born between 2002 and 2017 among cases and controls in our sample. However, this risk factor is an artefact of the study design and in the adjusted analysis, risk estimates remained stable also after adjustment of year of birth.

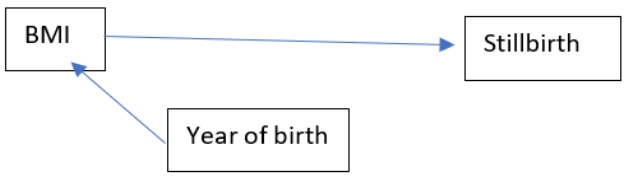

Figure 4: A direct acyclic graph (DAG) is a tool to visualize potential causal pathways. In this case the DAG is used to visualize the pathways between BMI, stillbirth and year of birth. Prevalence of obesity has increased during the years of the study; however, incidence of stillbirth has been stable.

However, there are other circumstances which have changed during these years, which would have been valuable to be able to adjust for. The most important change may be the stricter diagnostic criteria for GDM. There may be cases who would have been diagnosed with GDM in year 2018 however, were not diagnosed with GDM the year they gave birth. In this case GDM could be a potential mediator for the effect of BMI on the risk of stillbirth. Since the risk of misclassification is largest among cases, this potential misclassification will be differential. It could potentially affect the effect of BMI on stillbirth.

When conducting the logistic regressions in study IV, there was a positive association between parity and stillbirth. However, it is well known that nulliparity increases the risk of stillbirth, hence the association should have been negative [26]. One could wonder if there was a selection bias among the controls or if the risk associated with nulliparity differs between different BMI categories or with different gestational ages among stillbirths. Study IV include term stillbirths and certain BMI categories. However, in the whole Stillbirth database, all BMI categories included, there are 39% nulliparous women with term stillbirths and 37% nulliparous women with pre-term stillbirth and 45% nulliparous women with term stillbirth among normal weight women. Among controls in study IV, there were 55% nulliparous normal weight women and 42% nulliparous obese women. The controls comprised a small sample of women with potential of random errors. However, according to the Board of Health and Welfare there has been a stable level of 48% nulliparous women among normal weight women over the past years [154]. Hence, also compared to all women in the target population there was a positive association between parity and stillbirth among normal weight women. Among obese women the proportion of nulliparous women, according to the Board of Health and Welfare has been 37% the last years [154]. Among obese women with stillborn infants there were 38 % nulliparous women, hence compared to all obese women in the target population there was a negative association between parity and stillbirth, as expected, although weak.

The association between maternal age and stillbirth was negative in the logistic regression in study IV. It is known that high maternal age increases the risk of stillbirth as well as low age. It is not possible to get the median age in different BMI categories from the Board of Health and Welfare, without a special application. Median age of women giving birth to their first child in Stockholm was 30.4 years in 2018, according to the Board of Health and Welfare [154]. Median age in the Stockholm stillbirth database was 31.8 years, both nulliparous and parous women included, no difference in maternal age between stillbirths at term and preterm. In our sample of women, both nulliparous and parous women giving birth at Södersjukhuset, a median maternal age between 32-33 years does not seem unlikely. However, cases of stillborn infants were collected during a period of 16 years and median age among women giving birth have increased during the years. Taken together, the controls consist of a small sample which implicates an increased risk of imbalance due to pure chance, however, without obvious signs of selection bias.

A possible explanation of the opposite direction of the association between parity and stillbirth and between maternal age and stillbirth could be a higher degree of surveillance

among nulliparous women and older women. The knowledge about risk factors among older women and nulliparous women may have influenced guidelines in a way to compensate for the increased risks, figure 5. Nulliparous women have a larger number of antenatal controls and maternal age is taken into consideration when discussing possible induction of labor.

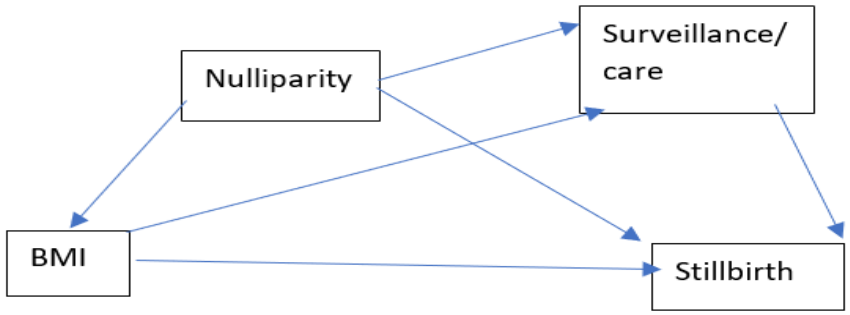

Figure 5: BMI has a positive association with stillbirth. Nulliparity is a confounder with a negative association with BMI and a positive association with stillbirth. However, nulliparity could have a positive association with surveillance and surveillance a negative association with stillbirth, protecting nulliparous women and potentially changing direction of the overall association between nulliparity and stillbirth.

5.2.2.2 Confounders and mediators

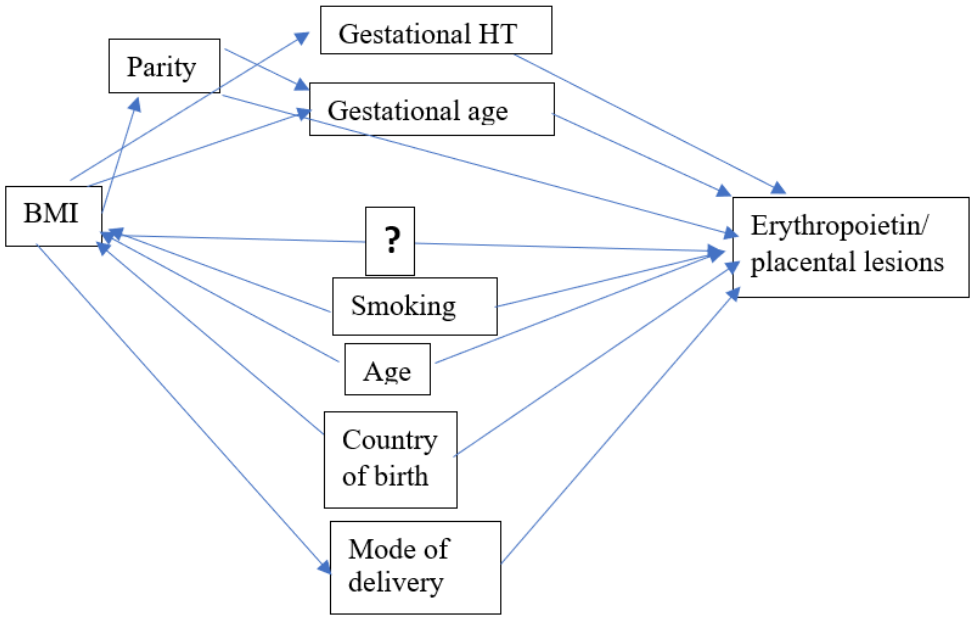

Figure 6: DAG for the causal pathways in study III: gestational age, gestational hypertension and mode of delivery were mediators and maternal age, smoking habit, country of origin and parity were potential confounders.

A DAG is a tool to visualize potential causal pathways, which can be used to select confounders to adjust for in the statistical analysis [162]. Confounding is an absolute concept, defined as the presence of common causes of the exposure and outcome [163]. Confounder is a relative concept defined as "the common causes of exposure and outcome that, were they all measured, would be sufficient to adjust for confounding" [163]. Which variables we may consider as confounders thus depend on which other variables we have adjusted for. A DAG was used to visualize the causal pathways in study III, figure 6. Gestational age was a mediator on the pathway from BMI to concentration of erythropoietin. Mode of delivery was a mediator, fixed by study design and therefore adjusted for in the analyses. Gestational hypertension was also a mediator. However, the final analyses were not adjusted for gestational hypertension since the adjustment for gestational hypertension did virtually not affect the association between BMI and concentration of erythropoietin or the association between BMI and placental variables. The analyses were also adjusted for maternal age and parity, two potential confounders. However, the final analyses were not adjusted for country of birth, educational level or smoking, which were also potential confounders. Adjustment for country of birth, educational level and smoking did not affect the associations between BMI and concentration of erythropoietin or BMI and placental variables.

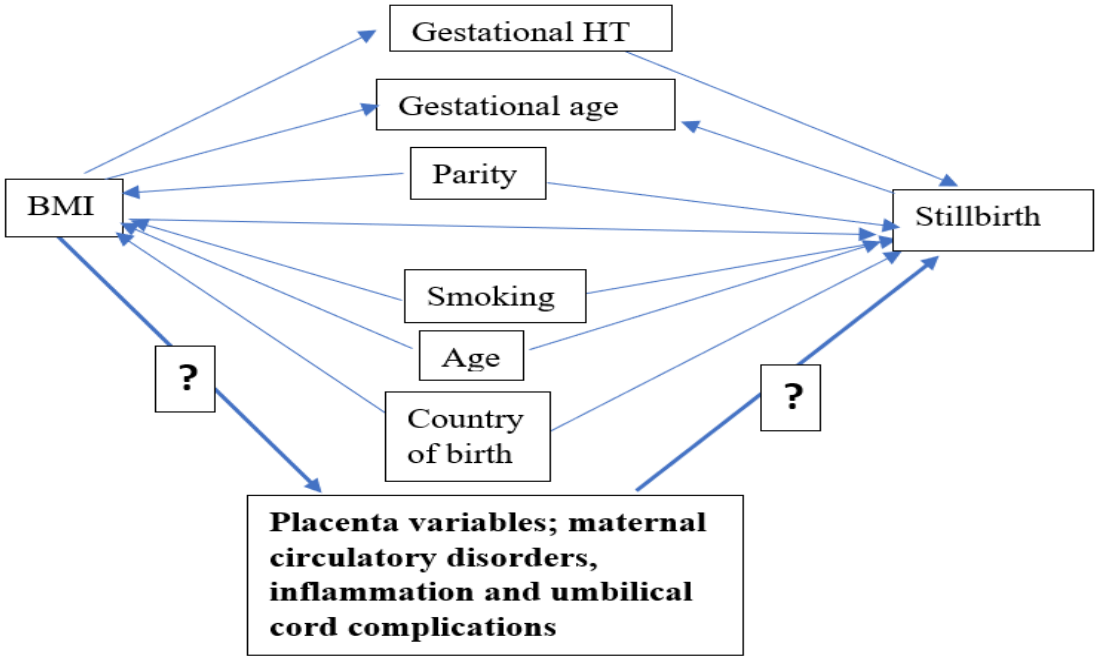

Figure 7: A DAG to visualize the causal pathways in study IV. Parity and gestational hypertension were mediators whereas maternal age, smoking and country of birth were potential confounders. Gestational age was a collider since the delivery will be induced after the diagnosis of stillbirth. Placental variables were mediators adjusted for in order to evaluate their effect.

A DAG was used to visualize the causal pathways in study IV, figure 7. It is well known that there is a causal pathway from maternal BMI to stillbirth [3]. To secure that the group of women with BMI > 30 was large enough, controls were collected specified on BMI. The proportion of obese women among the controls was corrected by weighting, to become equal to the proportion of obese women in the general population. Numbers of deliveries stratified on BMI was collected from the Board of Health and Welfare [154]. A logistic regression was conducted adjusted for the potential confounders, parity, country of birth, maternal age and smoking status. There was virtually no difference when adjusting for the potential mediator gestational hypertension, hence this was not done in the final analyses. Country of birth was used as a dichotomous variable. The sample was small, and it would have been hard to draw any conclusions from a more divergent variable. However, one could argue that categorization or dichotomization of variables will lead to loss of information, which in turn may lead to loss of power [164] and/or residual confounding [165]. The effect of BMI on stillbirth decreased slightly after adjusting for parity, county of birth, maternal age and smoking status. Pathological placental signs were mediators on the casual pathway between BMI and stillbirth. The analysis was adjusted for different groups of placental variables, i.e. umbilical cord abnormalities, chorioamnionitis and signs of maternal circulatory disorders. The adjustment for different groups of placental variables was done to be able to evaluate which proportion of the effect of BMI on stillbirth could be mediated by that group of placental variables.

5.2.2.3 Misclassification

There may be inter-individual and intra-individual differences in classifying placental lesions, which could lead to misclassifications (8). The two senior perinatal pathologists who did the placental analyses discussed the classifications in case of uncertainty and came to a conclusion. BMI was blinded to the perinatal pathologists which would make the potential misclassifications non-differential (i.e. the same risk of misclassification among exposed and non-exposed). The misclassifications would then possibly dilute the results. Data on maternal and infant characteristics came from the antenatal records. There is a risk of misclassification in the records, and in addition a risk of misclassification during the work of variable extraction. However, these misclassifications would be non-differential with the possibility to dilute the results and would not force the results in any specific direction. The variable "educational level" was extracted from the antenatal records and was only noted as type of occupation from the beginning. This variable was not completely reliable, since it is not always obvious which educational level is hidden behind a certain occupation. The variable

"maternal country of birth" was also extracted from the antenatal records. Country of birth was the variable with largest proportion of missing values both in study III and study IV, in study IV both among cases and controls. Since there were 15% missing values for "country of birth" in study IV multiple imputation was used to predict the value of missing variables based on the other variables. The multiple imputation was used for all missing values in all variables used in the logistic regression. The analyses were re-run with multiple imputation with virtually the same results.

5.2.3 Random errors

Study III and study IV were small studies with potential problems with random errors. Estimates derived from a study with a small sample size will have wider confidence intervals and larger P-values indicating the larger degree of statistical uncertainty. In study III there were wide confidence intervals for the linear regressions of the effect of BMI on erythropoietin concentration. In study IV there were wide confidence intervals in general and especially for the measurement of difference in effect of obesity on stillbirth when different groups of placental variables were added. The small sample size is one reason for that. Another reason is that the proportion of difference of effect of obesity on stillbirth includes a division, which will amplify the degree of uncertainty.

5.2.4 External Validity

There are no obvious reasons for the results of study III not to be valid in other comparable populations. However, small studies need other studies pointing in the same direction in order to be able to provide solid evidence. Study III is a rather small study in which the results constitute a small piece in a large puzzle. The Stockholm Stillbirth database is unique in number of cases. It includes virtually all stillbirths in the Stockholm region since 1998. One limitation with study IV is the selection of cases over long time. However, with such a rare outcome, collection of cases over time is necessary. The target population in study IV was all women giving birth in the Stockholm region during 2002-2019. There are no obvious reasons that the results from study IV would not be valid for other comparable populations. However, it is unclear to what degree changes over time occurred and what implications that could have.

6 RESULTS

A brief summary of the results for each study will follow. A more comprehensive description of the results is provided in each of the original articles added at the end of the thesis.

6.1 STUDY I

There were in total 12 704 pregnancies from 11 580 unique women with BMI ≥ 30 in the study cohort and the prevalence of LGA, defined as birthweight ≥ the 97th percentile, was 8.5 % (n=1 078). In normal weight women as well as women with BMI ≥ 30, the median PAPP-A values were higher in case of an LGA infant than if the infant was non-LGA.

The final prediction for the pre-pregnancy model in parous women with BMI ≥ 30 had an AUC for the ROC curve of 0.80 (95% CI; 0.78 to 0.82), with a sensitivity of 46 % at a fixed 90% specificity. The final prediction of the predictive model for LGA, in early pregnancy, in parous women with BMI ≥ 30 had an AUC for the ROC curve of 0.81 (95% CI; 0.79 to 0.82), with a sensitivity of 48 % at a fixed 90% specificity. The AUC for the ROC curve for the final predictive model for all women with BMI ≥ 30 (including both nulliparous and parous women) was 0.66 (95% CI; 0.64 to 0.67) with a sensitivity of 21 % at a fixed 90% specificity

Results remained essentially unchanged when models were re-run taking all possible two-way interactions into account and in the cross-validation procedure.

6.2 STUDY II

There were in total 45,859 pregnancies from 41,010 unique women with overweight or obesity and without pre-gestational diabetes. The prevalence of stillbirth was 2.6/1000 births in pregnancies complicated by overweight/obesity and 1.6/1000 births in normal weight women. The median gestational age at stillbirth was 37 weeks both in pregnancies complicated by overweight or obesity and in normal weight pregnancies. For the whole group of pregnancies with stillborn infants, the median PAPP-A levels were lower, and the proportion of SGA markedly increased as compared to pregnancies with live born infants. The potential predictors BMI, smoking status, maternal age, parity, maternal region of birth and preeclampsia were all significantly associated with stillbirth among women with overweight or obesity, however they were not associated with stillbirth in pregnancies of women with normal weight. The AUC for the ROC curve of the final predictive model was 0.69 (95% CI 0.64-0.74). The sensitivity was 28 % at a fixed 90% specificity. The AUC after the cross-validation procedure for the final predictive model was 0.65.

6.3 STUDY III

Median BMI among lean and obese women were 22.2 kg/m^2 and 32.7 kg/m^2 respectively. A larger proportion of obese women were born outside Sweden compared to normal weight women.

The adjusted linear regression analysis showed a significant positive association between maternal BMI and cord blood erythropoietin concentration, *P*-value 0.01. Normal erythropoietin cord blood concentrations < 30 U/L (*P*-value 0.01) were significantly more frequent in offspring of lean mothers, in the adjusted logistic regression. The adjusted linear regression for BMI on erythropoietin concentration stratified on mode of delivery showed a significant positive association among women with vaginal delivery, (P-value 0.04) and a borderline significant association for women with elective cesarean section (P-value 0.08).

There were nine pregnancies with erythropoietin concentrations above 100 U/L. Six of those had a BMI ≥30, except from BMI they had all uncomplicated, normal pregnancies. Seven of those women had a vaginal birth and five were primiparous. Five women gave birth in gestational week 41+0 or later. The arterial cord blood pH was between 7.14 to 7.20 in four cases. None of these infants had a cord blood pH < 7.14. All infants had a birthweight appropriate for gestational age.

Placentas to women with BMI ≥ 30 had significantly longer cords for gestational age. There were no differences comparing placentas from obese and lean women with respect to placental lesions associated with chronic hypoxia.

6.4 STUDY IV

The Stockholm stillbirth database contains information on 1,694 stillbirths delivered between 2002 and 2018. After exclusion of fetuses with major malformations, multiples, stillbirths diagnosed before gestational week 37+0 and fetuses to mothers with diabetes, our final cohort included 531 cases. 91 of these were born to obese women and 270 to women of normal weight. There were four cases of stillbirth to obese women with missing placenta and another six cases of stillbirth to normal weight women with missing placenta. In total there were n=87 cases of stillbirths complicated by maternal obesity and n= 264 stillbirths to women of normal weight.

A long umbilical cord was more common among obese women compared to normal weight women. A long and hyper coiled umbilical cord, vasculitis and umbilical cord thrombosis had a stronger association with stillbirth in obese women than in normal weight women.

Velamentous or marginal cord insertion were more common among stillbirths to obese women than in stillbirths to normal weight women. Causes of term stillbirth according to the Stockholm stillbirth classification [61] did not differ between pregnancies of normal weight women and obese women.

When the crude logistic regression was adjusted for the potential confounders maternal age, parity, country of birth and smoking, there was a reduction of the effect of obesity on the risk of stillbirth. Placental variables, potentially mediating the effect of obesity on the risk of stillbirth were added to the adjusted logistic regression analysis, one group at a time. When umbilical cord abnormalities were added to the logistic regression, the effect of obesity on the risk of stillbirth decreased with 38 % (CI 0.01-2.17), from OR 1.83 (CI 1.09-3.12) to OR 1.45 (CI 0.83-2.56) and the risk of stillbirth was no longer significantly increased. When chorioamnionitis was added to the logistic regression model, there was a 15 % (CI -0.02-0.67) reduction of the effect size, OR 1.68 (CI 0.99-2.88), with a borderline significant association between obesity and stillbirth. Signs of maternal circulatory disorders did not impact the effect of obesity on the risk of stillbirth. In total, for the logistic regression adjusted for confounders, there were 20 % of observations with missing values. Data on maternal country of birth was missing in 15 % of the women, parity was missing in 8 % of women, maternal age in 0.8% of women and information on smoking habits was missing in 0.5% of women. Thus, all analyses were re-run with multiple imputation of all missing data with virtually the same results, not shown.

7 RELATED FINDINGS

7.1 STUDY I AND STUDY II

A low level of PAPP-A probably reflects an impaired placentation [150]. It has been argued that PAPP-A is a valuable predictor for both SGA, LGA and stillbirth [150, 155]. It is true that PAPP-A has a strong statistical association with these outcomes in large populations [156]. However, how valuable a predictor is, depends on the strength of the association, not only how certain the association is. The normal distribution of the mean level of PAPP-A has a discrete shift to the left for SGA infants and stillborn infants and a discrete shift to the right for LGA infants, figure 8. There is no obvious cut-off level in the concentration of PAPP-A associated with LGA or stillbirth. A strong association between the predictor and the outcome is needed for a valuable prediction. The associations between PAPP-A and the outcomes SGA, LGA and stillbirth do not include an obvious cut-off level and the associations are weak. The

possibility to predict SGA is not higher in comparison to the possibility to predict LGA, according to our data. The value of PAPP-A as a predictor in these situations is limited [66, 147, 158, 159, 166].

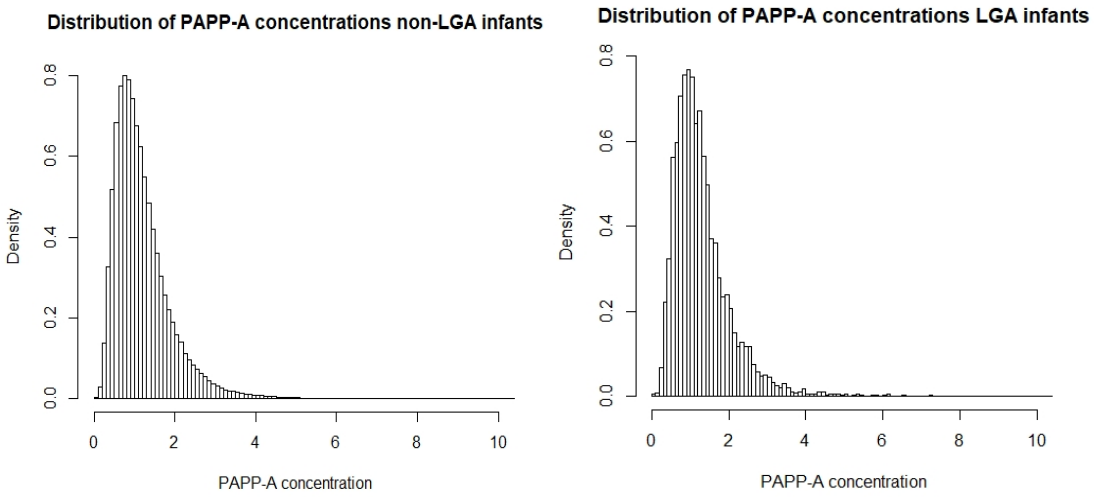

Figure 8: Distribution of PAPP-A concentration during first trimester among non-LGA infants and LGA infants, median 1.03 and 1.14 respectively and mean 1.19 and 1.31 respectively. A weak but strongly significant association without a cut-off for the outcome. Hence, PAPP-A is a weak predictor.

In general, the level of PAPP-A is lower among women with overweight or obesity, P-value < 0.001, however the effect was small. In general, the distribution of PAPP-A levels was narrower among obese women than among normal weight women. Decreased levels of PAPP-A have also been reported in pregnancies with maternal diabetes. Both among women with diabetes and women with obesity there were an association between PAPP-A concentration and LGA infants [150]. Abnormal glucose metabolism may negatively impact placentation by influencing trophoblast invasion [167] and it has been suggested that the concentration of PAPP-A may reflect the degree of maternal glucose intolerance with the lowest concentrations among women with diabetes type 1 and gradually values closer to the normal among women with diabetes type 2, early on-set GDM and late-onset GDM [150]. Thus, it may be speculated that the lower levels of PAPP-A observed in women with diabetes and/or BMI ≥ 30 reflect a suboptimal placentation [150]. This is in line with the finding of high rates of LGA infants in diabetic pregnancies with tight glycemic control at time of conception [168]. The increased levels of PAPP-A in pregnancies with LGA infants may reflect an undisturbed placentation and as a result of an undisturbed placentation in combination with increased levels of blood glucose later in pregnancy, an increased risk of an LGA infant. It is

possible that insulin resistance in non-diabetic pregnancies with BMI ≥ 30, with only slightly elevated glucose levels affect placentation and PAPP-A levels.

The risk of giving birth to an LGA infant was significantly decreased if the woman had a lower BMI at the start of the next pregnancy (OR 0.9 CI 0.83-0.99, P-value 0.04), in line with earlier studies [24].

In study II the associations between the potential predictors and the outcome were non-existing or very weak among normal weight women, in contrast to women with BMI ≥ 25. The predictors BMI, PAPP-A, smoking status, maternal age, parity, maternal region of birth and pre-eclampsia were not statistically associated with stillbirth among normal weight women, however among overweight or obese women. One can speculate about the reasons for this difference. One explanation could be that risks increase when they are added, however there was only a significant interaction between BMI and parity and no other significant interactions between predictors and BMI. Another explanation could be that obese women are handled different than normal weight women or that the extra burden of obesity does not lead to increased surveillance in sufficient degree.

7.2 STUDY III AND STUDY IV

Placental hypoplasia and placental hyperplasia were defined as a placental weight <10th percentile and placental weight > 90th percentile for gestational age, respectively, according to a well-known and well-used table for placental weight [169]. There was a high incidence of placental hypoplasia among both normal weight and obese women, approximately 25 % and 21 %, respectively. The table of placental weights and their percentiles was made in the USA during the 1990s, based on 787 placentas of different gestational age [169]. Either the table of placental weights is not reliable for conditions in Stockholm or Sweden or our material was biased in some way we cannot understand, with a much larger proportion of placental hypoplasia than it should be among uncomplicated pregnancies, both among normal weight and obese women. In addition, there was a large proportion of pathological placental lesions among normal weight women with uncomplicated pregnancies, however, this was in line with earlier studies [107, 108]. The fact that even an uncomplicated pregnancy may have a large proportion of pathological placental lesions needs to be considered when evaluating placental lesions.

There was a strong association between gestational age and erythropoietin among term pregnancies, figure 9. The association between gestational age and erythropoietin was un-affected by adjustment of potential confounders; i.e. maternal age, BMI, pregnancy induced

hypertension, parity and mode of delivery. The finding was in accordance with a recent study from Finland [100].

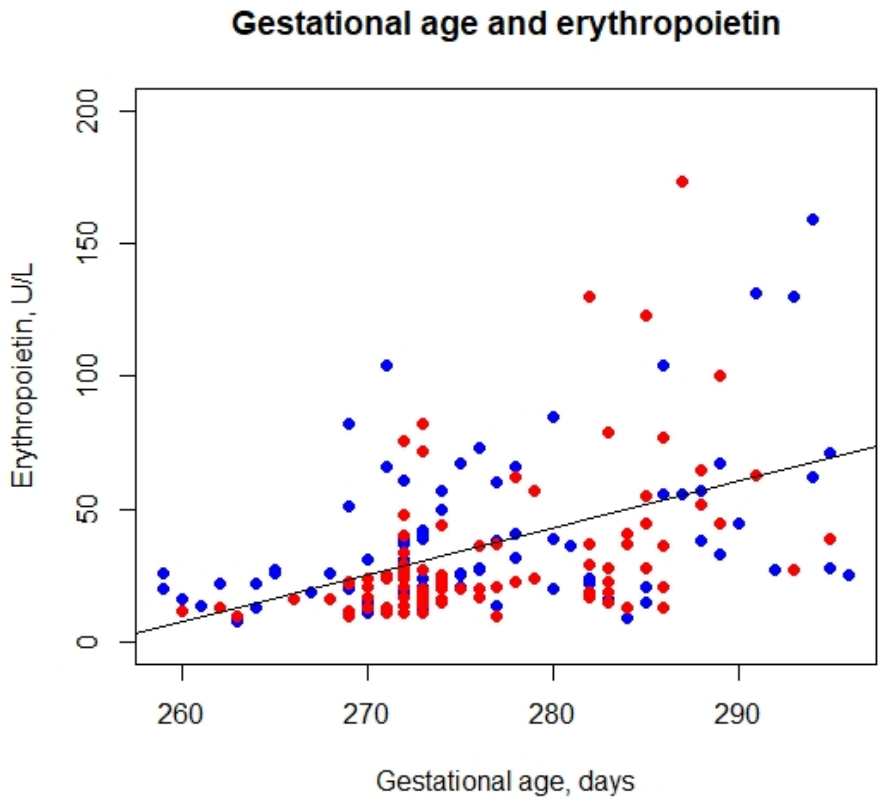

Figure 9: Erythropoietin and gestational age. Red dots: elective cesarean section, blue dots: vaginal birth. Linear regression adjusted for maternal age, BMI, mode of delivery, gestational hypertension and parity.

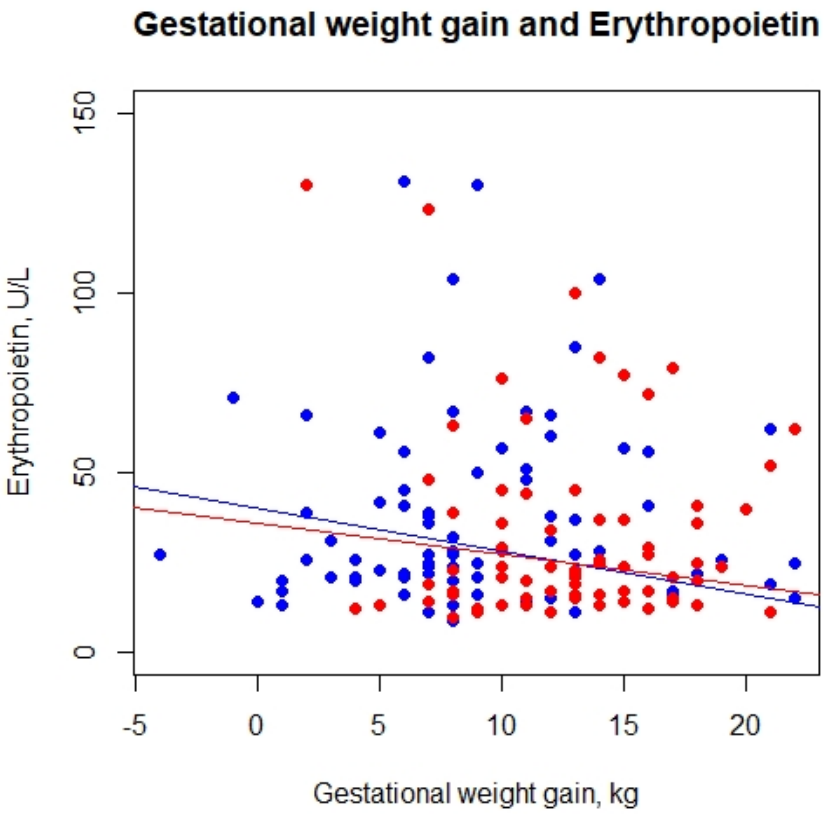

Figure 10: Gestational weight gain had a negative association to erythropoietin concentration. Blue dots are obese women and red dots are normal weight women.

When gestational weight gain was added to the linear regression of BMI and erythropoietin, there was a significant negative association between gestational weight gain and erythropoietin concentration, figure 10. Women with low gestational weight gain were mostly women with obesity, who are also recommended to gain less weight during pregnancy. Recommendations of optimal gestational weight gain differs with pre-gestational BMI and are 5-9 kg and 11.5-16 kg for obese and normal weight women, respectively. Women with low gestational weight gain, under the recommendation for BMI category, had higher mean erythropoietin concentrations than women with recommended gestational weight gain and higher than women with high gestational weight gain, both among obese and normal weight women.

In study IV we try to find placental explanations for the increased risk of term stillbirth with increasing BMI. However, the number of placental findings among healthy pregnancies are also obvious. It is true that placental hypoplasia and delayed villous maturation are more common in pregnancies complicated by term stillbirth, however also present in one third to one fourth of the uncomplicated controls. A long cord, velamentous or marginal cord insertion and hyper coiled umbilical cord were associated with stillbirth, although hypo coiled

cord was much more common and did not have a positive association with stillbirth. Placental infarctions and placental thrombosis are common both in placentas with live-born and stillborn infants in this sample, not significantly associated with stillbirth, table 3. However, there will be cases with massive infarctions or massive placental thrombosis possibly causative of stillbirth. Umbilical cord thrombosis was associated with stillbirth among obese women, however not among normal weight women.

Table 3: Placental variables comparing placentas from term live born and stillborn infants

Fetal weight and placental characteristics	Live born N=222	Stillborn N=358	P-value
Fetal weight, g	3480 (3240,3830)	3220 (2877.5,3567.5)	<0.001
SGA <10th percentile	15 (6.76%)	88 (24.79%)	<0.001
Placental weight, g	495 (429.25,569.5)	429 (365.75,498.5)	<0.001
Fetal weight/Placental weight	7.26 (6.6,7.81)	7.56 (6.65,8.62)	0.002
Placental hypoplasia (n, %)	61 (27.48%)	186 (51.96%)	<0.001
Placental hyperplasia (n, %)	29 (13.06%)	18 (5.03%)	0.001
Abnormal shape of placenta (n, %)	27 (12.16%)	58 (16.62%)	0.181
Cord long for gw (n, %)	26 (11.71%)	117 (35.03%)	<0.001
Margin cord incision (n, %)	14 (6.31%)	54 (15.34%)	0.002
High coiling index (n, %)	4 (1.8%)	31 (8.96%)	0.001
Low coiling index (n, %)	61 (27.48%)	51 (14.74%)	<0.001
Umbilical cord knot (n, %)	4 (1.82%)	27 (7.74%)	0.005
Deficient villous maturation (n, %)	60 (27.03%)	139 (39.49%)	0.003
Placental Infarction (n, %)	43 (19.37%)	91 (25.42%)	0.114
Intervillous Thrombosis (n, %)	84 (37.84%)	121 (34.47%)	0.466
Thrombosis Placenta (n, %)	57 (25.68%)	19 (29.23%)	0.681
Thrombosis Umbilical cord (n, %)	19 (8.6%)	44 (12.54%)	0.184
Chorioamnionitis (n, %)	56 (25.23%)	149 (42.45%)	<0.001
Vasculitis (n, %)	31 (14.03%)	58 (16.52%)	0.494
Funisitis (n, %)	5 (2.25%)	23 (6.55%)	0.033
Villitis (n, %)	21 (9.5%)	85 (24.29%)	<0.001
Increased fetal red blood cell count (n, %)	11 (5%)	37 (10.54%)	0.03
Placental abruptio (n, %)	4 (1.8%)	32 (9.12%)	<0.001

8 DISCUSSION

8.1 STUDY I

The main result of study I was a reasonably good predictive model for risk of LGA infants among obese multiparous women, pre-pregnancy or in early pregnancy. The predictor contributing most to the predictive capacity was previous child's birthweight. The predictive model of LGA infants among obese primiparous women in early pregnancy was weak.

The interpretation of a predictive model cannot be based on the AUC only. The incidence or prevalence of the outcome as well as consequences of false negative and false positive results must be considered. The incidence of LGA infants is high. LGA infants and their mothers, are at increased risk during delivery and during life of the LGA infant. However, there is no easy solution to decrease the risk of being born LGA. Predictive models may be used to choose which women would benefit from an ultrasound with estimation of fetal weight in gestational week 36-37. However, our predictive models were not meant to be guiding obstetricians in the decisions about mode of delivery or time of delivery. They were meant to predict which women would benefit from interventions during pregnancy to decrease the risk of the infant to become LGA in the first place. However, no such interventions during pregnancy have been able to show a decreased incidence of LGA or affect maternal or neonatal outcome except a slightly decreased gestational weight gain and a slight decrease in birth weight [55, 170]. The decreased concentrations of PAPP-A in early pregnancy among obese women, compared to normal weight women, indicate that obesity will affect the pregnancy already from conception. To be able to decrease risks of both mother and infant, interventions before pregnancy are needed [25, 171]. The pre-pregnancy predictive model for obese parous women was equally good as the predictive model in early pregnancy. Already after the first pregnancy, it is possible to perform a good prediction for risk of LGA for the next pregnancy. The predictive capacity is even better if the prediction is made over all BMI categories, figure 11. Additionally, weight loss between pregnancies decreases the risk of an LGA infant in next pregnancy. This is the window of prevention.

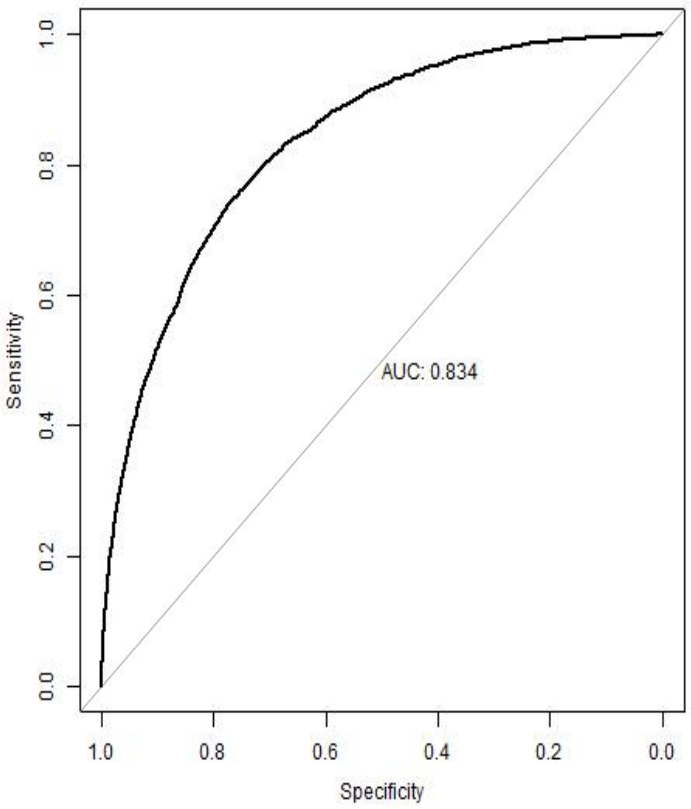

Figure 11: Prediction for risk of LGA for all women who have given birth before.

In our sample LGA infants (birthweight > 97th percentile or >2SD) and macrosomic infants (birthweight >4 500 g) were almost equally many but to a large part different individuals. The most important "predictor" of the macrosomic infants was gestational age, which is impossible to use as a predictor since it is not known when the prediction should be made. However, the macrosomic infants will, to a large extent be born following a prolonged pregnancy as opposed to an LGA infant.

8.2 STUDY II

The main result of study II was a predictive model of stillbirth among overweight or obese women. The predictive capacity was medium good. However, if SGA (≤10th percentile) was introduced in the predictive model the predictive capacity increased to a good capacity, indicating a better predictive capacity with estimated fetal weight in the model. SGA infants are at increased risk of stillbirth and other adverse outcomes. Fetuses small for gestational age are at increased risk even with a broader definition of SGA than the one used in Sweden [122]. Infants with birthweight between the 5th-10th percentile are at increased risk of mortality and asphyxia related injuries even if the risks are more prominent among infants with a birthweight < 3rd percentile, which is the definition used in Sweden [8]. Obese women have a

decreased risk of SGA infants, however SGA infants to obese women are at increased risk of mortality and asphyxia related injuries compared to SGA infants to normal weight women [82].

There was not a significant association between a slower fetal growth between gestational weeks 10 and 20 and stillbirth. However, the highest predictive capacity in the cross-validation was when fetal growth between gestational weeks 10-20 was included in the model. Thus, indicating a stronger model if differences in early fetal growth were included in the model.

The incidence of stillbirth is low which will result in a low positive predictive value (the proportion of women with a positive test who will suffer from a stillbirth). For women with increased risk of adverse outcome the degree of surveillance could be increased. Whatever surveillance method used it has to be performed for a large number of women of whom the vast majority will never suffer from stillbirth.

8.3 STUDY III

The main result in study III was the positive association between BMI and concentration of erythropoietin in cord blood.

When analysis was stratified on mode of delivery there was still a positive significant association between BMI and erythropoietin among infants born vaginally. The findings indicate that fetuses to obese women have an increased risk of chronic hypoxia before delivery, which may make them more vulnerable during delivery and at the end of pregnancy. Our knowledge about risk factors and specific findings during pregnancy and delivery will affect our decisions about time of delivery, mode of delivery and surveillance during pregnancy and delivery, hopefully decreasing risks of infants and women.

The hypothesis of study III was that the metabolic situation in obese women, with for example increased blood glucose levels, and in addition, their larger body mass, with increased oxygen demand, would lead to less oxygen transported to the placenta and to the fetus. There were no differences in placental lesions associated with fetal hypoxia comparing obese and normal weight women. Results were somewhat conflicting, with a positive association between obesity and erythropoietin however no differences in placental lesions associated with fetal hypoxia comparing obese and normal weight women. If that depends on small differences between normal weight women and obese women with uneventful pregnancies, if placental analyses are to blunt or if differences in individual pregnancy

adaptation and differences in placental response to hypoxia make the placental picture more diverged, is an open question.

8.4 STUDY IV

The main result of study IV was that umbilical cord abnormalities may explain one third of the effect of obesity on risk of stillbirth. Chorioamnionitis could explain approximately 10-15 % of the effect of obesity on risk of stillbirth.

Mechanisms behind the association between obesity and stillbirth is multifactorial [67]. Increased incidence of umbilical cord abnormalities and chorioamnionitis are two possible explanations. Our findings indicate that more focus on umbilical cord abnormalities could be valuable. To be able to take umbilical cord abnormalities into account in decisions about surveillance of the pregnancy or time of delivery, more precise methods of measurement of umbilical cord abnormalities are needed. With a reliable method to find umbilical cord knots, measure umbilical cord length and coiling index in utero, such findings could be included, among other risk factors considered, when deciding about time of delivery or labor and pregnancy surveillance.

Obese women have an increased degree of inflammation perhaps affecting the increased findings of chorioamnionitis. To be able to use the knowledge about chorioamnionitis as a part of the explanation of the association between obesity and stillbirth a reliable marker of chorioamnionitis during pregnancy would be needed. The finding of chorioamnionitis as explanation is previously shown and, in that study, it was also shown that c-reactive protein was not a good marker for chorioamnionitis [78].

8.5 OBESITY

Obesity is a modifiable risk factor with several pregnancy related complications [3, 26]. First trimester PAPP-A concentrations are lower in obese women as compared to normal weight women. Obesity increases the risk of velamentous cord insertion, congenital heart disease and miscarriage [7, 172, 173]. Hence, obesity will affect the pregnancy as early as already from conception. Obesity is still a modifiable risk factor. However, weight loss before pregnancy is the method needed to modify risks. During pregnancy, a limited gestational weight gain can lead to decreased additional risks. Increased surveillance can potentially find additional risks apart from BMI, for example an SGA fetus, GDM or velamentous cord insertion. An individual evaluation of each pregnant woman is important. Which are her risk factors?

Which are the risk factors of her fetus? A fetus with an estimated weight 10 % below the expected weight may be an additional risk of one fetus but not of another.

Knowledge about risk factors, methods to evaluate risk factors, a thorough individual evaluation of each woman's risks combined, and reasonable decisions based on this knowledge, may be a recommendation to decrease stillbirth incidence among obese women.

8.6 FUTURE RESEARCH

The incidence of stillbirth has been stable for decades. It is a challenge to make the incidence continue to decrease. To be able to do that more knowledge about certain risk groups, causal pathways and mechanisms behind stillbirths is needed.

Ideas of future research projects:

To investigate placental characteristics in preterm stillbirths stratified on obesity compared to live born preterm babies.

To investigate how risk factors for stillbirth have changed during the two past decades in the Stockholm region.

To investigate the risk of stillbirth among women born in different parts of the world and if there are specific risk factors for women with different origin.

9 ETHICAL CONCIDERATIONS

9.1 INTEGRITY AND AUTONOMY

Study I and study II are register based studies. Data are de-identified, so that information about a unique woman and her infant is not possible to reach. The participating women haven't given their informed consent to their contribution, which is common in register-based studies. These two studies will not affect the women or their infants as long as the information about women and infants are de-identified.

Study III and study IV include comparison between placental lesions from pregnancies complicated by obesity compared to placentas from pregnancies with normal weight women. These studies include collection of placentas and cord blood samples. The participating women were given both oral and written information about the studies. They were given easy understandable information and had the possibility to ask questions. After the information, the woman decided whether she would like to participate or not. A decision which did not

affect the care for her or her infant. The participating women signed a written informed consent. All information about the woman and her infant were de-identified as soon as possible.

9.2 RISK-BENEFIT

For study I and study II the risks for the participants are negligible as long as the information about them are de-identified and data handled in a safe way. Good predictive models for both LGA and stillbirth is a prerequisite for preventive action.

In study III and study IV, placentas and cord blood were collected. Most women are not interested in the placenta after the delivery. When we were not collecting the placentas, they would normally be wasted. Right after the infant is born routine blood samples are taken from the cord. The infant can't feel the stitch in the cord. The blood we will analyze would have been wasted if not used in the study.

Study III and study IV aim to gain more knowledge about the mechanisms to explain the well-known association between BMI and stillbirth. We were not allowed to ask about the women's participation before the labor started since it could potentially cause unnecessary anxiety because of the increased risk of stillbirth. The women from whom we collected placentas and cord blood were healthy controls, a fact which could potentially reduce anxiety. Stillbirth is a severe complication which we would like to prevent in all possible cases. The number of obese pregnant women are increasing. Thus, it is important to learn more about the mechanisms causing the association between BMI and stillbirth in order to be able to take preventive action against stillbirth.

In conclusion; the first two studies carry very small risks. The study design makes it possible to give response to the aim of the studies. Study III and study IV have very small risks for the mother and infant, and we analyzed placentas and blood samples that would have been wasted in normal cases. Deeper knowledge about the mechanisms leading to stillbirth in obese women is important. These studies will add a piece of new knowledge to the puzzle about the link between stillbirth and obesity.

9.3 THE POLICY OF EQUALITY

All women and their infants have been treated equally independent of whether they participate in the studies or not. If someone withdrew their participation in the studies, it did not affect the treatment of woman or infant.

10 SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Kvinnor med högt BMI löper en ökad risk för komplikationer i samband med graviditet och förlossning. En av de vanligaste komplikationerna är att föda ett stort barn och en av de allvarligaste komplikationerna är att fostret dör i livmodern. Det finns många studier som visar på ökade risker för kvinnor med förhöjt BMI, men det finns få studier som belyser möjliga orsaker till att riskerna ökar. För att en ska kunna minska riskerna för kvinnor med högt BMI under graviditet och förlossning så behöver en ha större kunskap om varför deras risker ökar. Vilka är de bakomliggande mekanismerna? Vad skulle behöva åtgärdas för att minska riskerna? Vi skulle också behöva veta om det går att säga vilka av dessa kvinnor som löper störst risk att drabbas av oönskade utfall.

Avhandlingen innefattar fyra studier som syftar till att finna de grupper av kvinnor som löper högst risk att drabbas av att få ett dödfött barn eller ett barn som är stort för graviditetsveckan, samt att finna kopplingar som kan ligga bakom den ökade risken att föda ett dödfött barn som ett högt BMI innebär.

De två första studierna avser att tidigt i graviditeten kunna prediktera dödfödda barn respektive barn som föds stora för tiden hos kvinnor med BMI ≥30 respektive BMI ≥ 25. Dessa studier baseras på en screeningdatabas från tidig graviditet som sammanlänkats med medicinska födelseregistret samt ett register från statistiska centralbyrån. Utifrån data i dessa register har prediktiva modeller byggts upp baserade på blodprover tagna tidigt i graviditeten (PAPP-A och B-hcg), moderns ålder, BMI, rökvanor, utbildningsnivå, inkomstnivå och födelseland. De prediktiva modellerna visar att man med god precision kan prediktera stora barn hos kvinnor som tidigare fött barn och att förra barnets födelsevikt är den bästa prediktorn. För förstföderskor var den prediktiva kapaciteten svag. Prediktionen för risk att föda ett dödfött barn var medelgod. Den gick dock att förbättra genom att en låg födelsevikt lades till i modellen vilket indikerar att modellen skulle fungera bättre om en la till uppskattad fostervikt.

Den tredje studien utreder risken att fostret ska drabbas av kronisk syrebrist i livmodern kopplat till mammans BMI som i sin tur skulle kunna öka risken för fostrets död före förlossningen. Den fjärde studien försöker hitta bidragande orsaker till den ökade risk för fosterdöd som finns hos kvinnor med BMI ≥ 30. I den tredje studien har analyser av moderkakor jämförts mellan kvinnor med BMI 18.5-24.9 och kvinnor med BMI ≥ 30. Koncentrationen av erythropoietin, ett protein som ökar vid syrebrist, i navelsträngsblod har

också jämförts mellan dessa grupper. Generellt är erytropoetinkoncentrationen lite högre hos barn till kvinnor med BMI ≥ 30, vilket talar för en ökad förekomst av en låggradig långvarig syrebrist. Det var inga tydliga skillnader i analysen av moderkakorna. I den fjärde studien jämförs analyser av moderkakor mellan normalviktiga kvinnor med levande födda barn och dödfödda barn och kvinnor med BMI ≥ 30 med levande födda barn och dödfödda barn med syfte att förklara skillnaden i risk. Resultaten visar att långa navelsträngar som är fästa långt ut i moderkakan och snurrade många varv kan förklara ungefär en tredjedel av riskökningen hos kvinnor med BMI ≥ 30. Akut inflammation i moderkakan kan förklara ca 10-15 % ytterligare av riskökningen hos kvinnor med BMI ≥ 30.

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