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ANTENATAL CORTICOSTEROID EXPOSURE
- studies on neonatal and long term outcome

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Antenatal corticosteroid exposure – studies on neonatal and long term outcome

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Till Dan, Siri & Bill

ABSTRACT

Background: Preterm delivery affects six to twelve per cent of all pregnant women each year. Treatment with antenatal corticosteroids (ACS) has contributed greatly to improve outcome after preterm birth. It is administered to women at risk for preterm delivery to reduce the risk of respiratory distress syndrome (RDS) and death of her preterm infant. The protective effect of ACS declines after 7-10 days. Considering that up to 50% of women remain undelivered 7-10 days after ACS administration, and in view of the neonatal benefits, repeat courses of ACS could be considered. However, unresolved concerns about safety still make such treatment regime controversial. Furthermore, it is not clear whether ACS is effective also in extremely preterm gestations.

Objective: The overall objective with this thesis was to investigate the impact of repeat courses of ACS in exposed subjects, both on infant size at birth, and on longer term outcomes (study I-III). Another objective was to explore the association between timing of ACS administration and survival in extremely preterm infants (study IV).

Methods: All studies in the thesis are cohort studies. In study I-III we used a cohort of about 100 children exposed to various courses of ACS in utero. We evaluated them regarding infant anthropometry at birth (study I), and risk factors for cardiovascular disease (study II) and neuropsychological function (study III) at follow up in adolescence/young adulthood. In study IV we evaluated a national population-based cohort of extremely preterm infants (EXPRESS – Extremely Preterm Infant in Sweden Study) regarding ACS administration-to-birth interval and survival.

Results: We found a dose-dependent association between number of ACS-courses and restricted body size at birth (study I). There was no clear correlation between repeat courses of ACS in fetal life and cardiometabolic risk factors at 14-26 years of age (study II). In addition, there was no indication that repeat ACS exposure had an adverse impact on cognitive function or psychological health at follow-up in adolescents and young adults (study III). In study IV we found a significant reduction in mortality among extremely preterm infants after any ACS, with an optimal administration-to-birth interval of 1-7 days.

Conclusions: Although exposure to repeat courses of ACS in utero were found to be related to gradually reduced body size at birth (indicating fetal growth restriction), it seems less likely from our findings that there are clinically important and long-standing adverse effects on cardiovascular and neuropsychological health. Another conclusion from this thesis is that ACS effectively reduces the mortality risk in extremely preterm infants and that timing of antenatal corticosteroids is important also in women delivering extremely preterm.

LIST OF SCIENTIFIC PAPERS

I. NORBERG H, Stålnacke J, Diaz Heijtz R, Smedler AC, Nyman M, Forssberg H, Norman M.
Antenatal corticosteroids for preterm birth: dose-dependent reduction in birthweight, length and head circumference.
Acta Paediatrica. 2011 Mar;100(3):364-9

II. NORBERG H, Stålnacke J, Nordenström A, Norman M.
Repeat antenatal steroid exposure and later blood pressure, arterial stiffness, and metabolic profile.
The Journal of Pediatrics. 2013 Sep;163(3):711-6.

III. Stålnacke J, Diaz Heijtz R, NORBERG H, Norman M, Smedler AC, Forssberg H.
Cognitive outcome in adolescents and young adults after repeat courses of antenatal corticosteroids.
The Journal of Pediatrics. 2013 Aug;163(2):441-6.

IV. NORBERG H, Kowalski J, Maršál K, Norman M.
Timing of antenatal corticosteroid administration and survival in extremely preterm infants: a national population-based cohort study.
BJOG: An International Journal of Obstetrics & Gynaecology. 2017 Mar 15. doi: 10.1111/1471-0528.14545. [Epub ahead of print]

CONTENTS

LIST OF ABBREVIATIONS

11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
ACOG	American College of Obstetricians and Gynecologists
ACS	Antenatal corticosteroid
AI	Augmentation index
BL	Birth length
BM	Betamethasone
BW	Birth weight
Cantab	Cambridge Neuropsychological Test Assessment Battery
CI	Confidence interval
CP	Cerebral palsy
CPAP	Continuous positive airway pressure
D-KEFS	Delis-Kaplan Executive Function System
DX	Dexamethasone
EPICE	Effective Perinatal Intensive Care in Europe
EXPRESS	Extremely Preterm Infants Study in Sweden
FAST	Fetal Antenatal Steroid Treatment
GA	Gestational age
GC	Glucocorticoid
GMFCS	Gross Motor Function Classification System
GR	Glucocorticoid receptor
HC	Head circumference
HDL	High-density lipoprotein
HOMA	Homeostatic model assessment
HPA-axis	Hypothalamic-pituitary-adrenal axis
HR	Hazard ratio
IGF	Insulin-like growth factor
IR	Insulin resistance
IVH	Intraventricular haemorrhage
LDL	Low-density lipoprotein
MBR	Medical birth register
MR	Mineralocorticoid receptor
NDD	Neurodevelopmental disability
NEC	Necrotizing enterocolitis
NICE	National Institute for Health and Care Excellence
OR	Odds ratio

PDA	Patent ductus arteriosus
PVL	Periventricular leukomalacia
RCT	Randomized controlled trial
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SD	Standard deviation
SDS	Standard deviation score
SGA	Small for gestational age
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children

1 BACKGROUND

1.1 PRETERM BIRTH

1.1.1 Definition and epidemiology

The duration of a normal human pregnancy is 40 weeks or 280 days from the first day of last menstrual period.[1] The currently accepted definition of preterm birth is birth before 37 completed weeks of pregnancy, very preterm before 32 weeks and extremely preterm before 28 weeks of pregnancy (figure 1).[2]

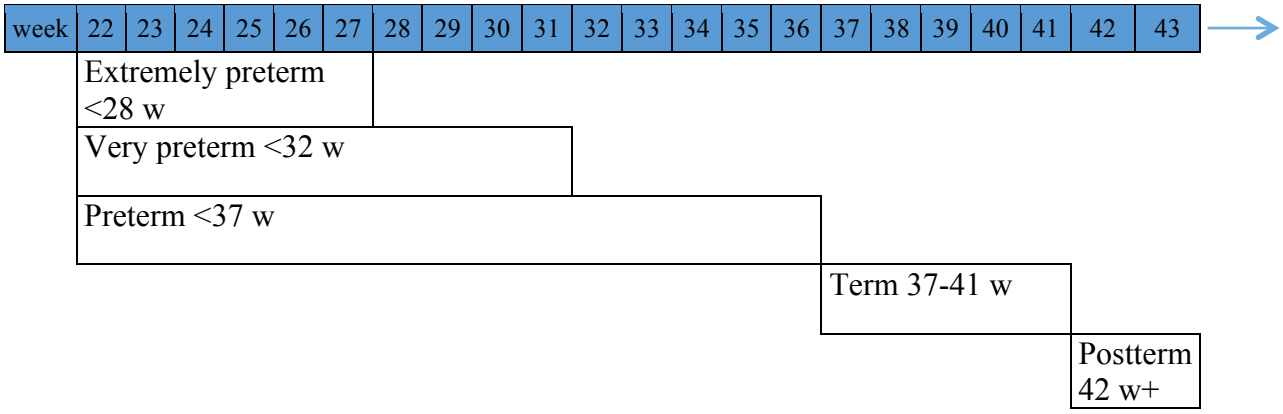

Figure 1. Definition of preterm birth (adapted from Tucker et al.[2])

According to the World Health Organization (WHO), about 15 million babies are born preterm each year, which is more than 1 in 10 babies. In almost all countries the preterm birth rates are increasing. About 1 million babies die each year due to complications of preterm birth, making prematurity the leading cause of death in children under the age of 5 globally.[3]

In Sweden the preterm birth rates look slightly different. 6% of all babies are born preterm (<37 weeks), about 1% are born very preterm (<32 weeks) and approximately 0.3% are born extremely preterm (<28 weeks).[4] Compared to many other countries the preterm birth rates have been rather stable during the last decades.[4]

1.1.2 Survival after preterm birth

The survival rate after preterm birth in Sweden is also different from the global rates. Survival among moderately preterm and very preterm babies in Sweden is almost universal and survival rate after extremely preterm birth is steadily improving. The one-year survival after being born alive at extremely preterm gestational age in Sweden is now approximately 70%[5,6] compared to the early 90´s when the corresponding rate was about 40%[7] (figure 2). The causes for the improved survival are related to many factors in modern obstetric and

neonatal care. The use of antenatal corticosteroids (ACS), exogenous surfactant, advanced ventilator strategies and the centralization of neonatal intensive care are some factors that have contributed greatly to the improved outcome after preterm birth. However, the improved survival must be put in relation to the neonatal morbidity following preterm birth.

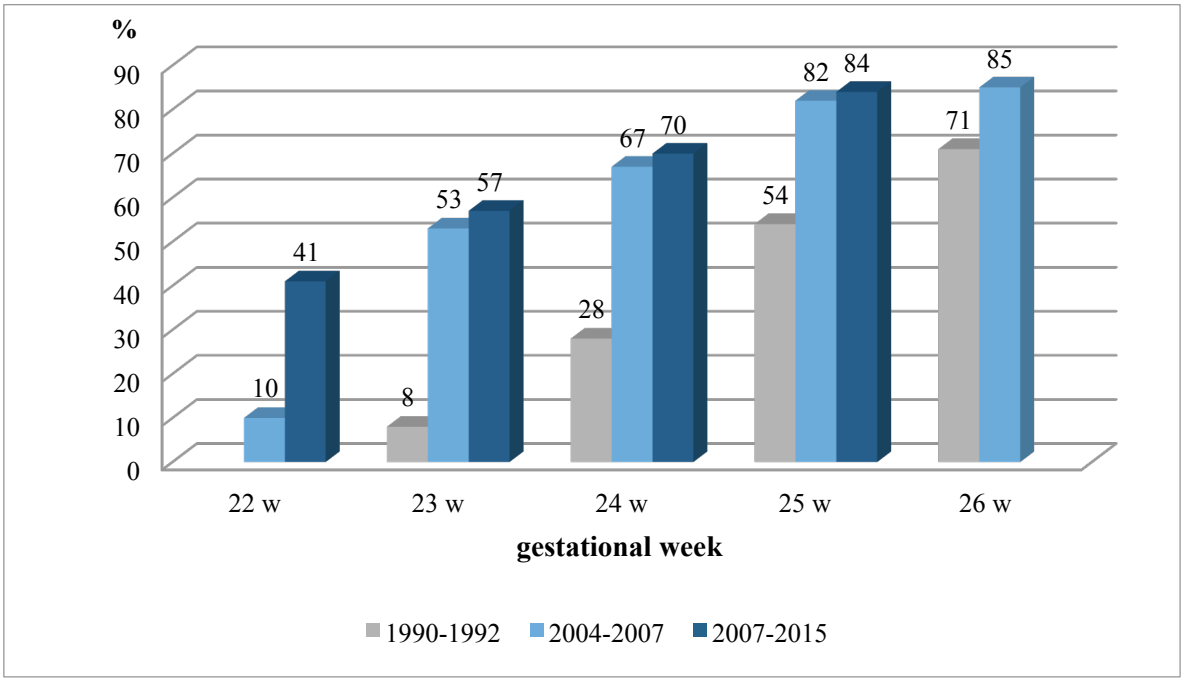

Figure 2. Survival rates after extremely preterm birth in Sweden 1990-1992[7], 2004-2007[5] and 2007-2015[6]

1.1.3 Neonatal morbidities of preterm birth

Infants who are born preterm face an increased risk of neonatal morbidity compared to infants born at term. Most organs are immature in preterm babies leading to a diversity of neonatal illnesses including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), brain injuries such as intraventricular haemorrhage (IVH) and periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), nutritional difficulties, hyperbilirubinemia, renal failure, invasive infections, persisting fetal circulation and patent ductus arteriosus (PDA). One way to get an early indication of possible long-term outcome following preterm birth is to measure *major neonatal morbidities.* The major morbidities are often referred to as; IVH grade III or more, cystic PVL, NEC, ROP stage 3 or more, and severe BPD. The risk of major neonatal morbidities will increase with lower gestational age (GA) as will the risk of poor long-term outcome.[8] The incidence of the major morbidities varies in different settings. In Sweden the chance of surviving one year without any major neonatal morbidity after extremely preterm birth ranged from 2% at 22 weeks of gestation to 54% at 26 weeks (figure 3).[5]

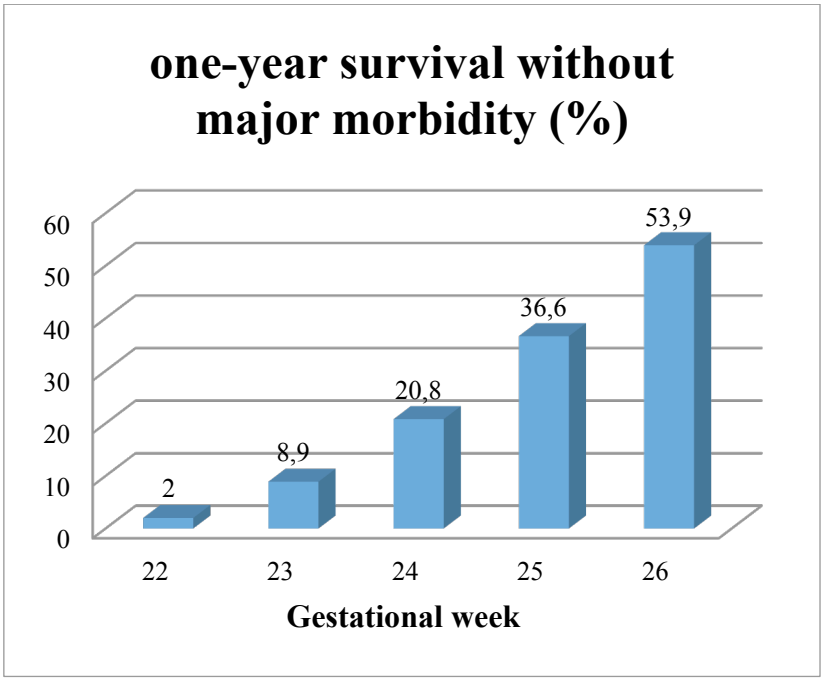

Figure 3. One-year survival without major neonatal morbidity in Sweden 2004-2007[5]

1.2 ANTENATAL CORTICOSTEROIDS (ACS)

One of the most important interventions for improving survival and decreasing morbidity after preterm birth is to treat pregnant women with threatening preterm delivery with antenatal corticosteroids (ACS). In 1972, drs Liggins and Howie conducted a randomized controlled trial (RCT) demonstrating that treatment with ACS markedly reduced the risk of infant respiratory distress syndrome and neonatal mortality.[9] This has since then been confirmed in numerous studies.[10,11] ACS have in addition to its effect on pulmonary maturation been proved to reduce the risk of intraventricular hemorrhage, early systemic infections and necrotizing enterocolitis.[11] ACS reduces the overall risk of neonatal death with an average of 31% (RR 0.69; 95% CI 0.59-0.81).[12] The morbidities ameliorated by ACS are described in more detail below (chapter 1.2.3).

1.2.1 Recommendations for ACS treatment

Antenatal corticosteroids are widely recommended worldwide because of its proven beneficial effects on neonatal outcome after preterm birth. The latest Cochrane review from 2017 concludes that a single course of antenatal corticosteroids should be considered routine for preterm delivery.[12] The World Health Organization recommends ACS for women at risk of preterm birth from 24 weeks to 34 weeks of gestation.[13] The latest guidelines from the American College of Obstetricians and Gynecologists (ACOG) state that a single course of corticosteroids is recommended for pregnant women between 24 0/7 weeks and 33 6/7 weeks of gestation.[14] The UK National Institute for Health and Care Excellence (NICE) published their latest guidelines 2015 in which they recommend offering ACS between 26 0/7 and 33

6/7 weeks of gestation.[15] In Sweden, the current recommendations are to treat pregnant women with threatening preterm labour between 23 0/7 and 33 6/7 weeks of gestation.

The most used treatment regimen is two doses of 12 mg of betamethasone (BM) intramuscularly 24 hours apart. This regimen is one example of a complete course of ACS. When only one of the two doses has been administered before birth, it is referred to as an incomplete course. In a minority of settings, dexamethasone (DX) is used instead of betamethasone and there are still insufficient evidence to support the use of one corticosteroid over the other.[12]

The well-described positive effects of ACS have though been accompanied by concerns regarding potential adverse effects on the fetus, affecting both short- and long-term health. Those concerns have mainly risen from in vitro studies and studies in animals, showing unwanted side effects on different tissues and organs. Concern for side effects of ACS are of particular concern in pregnancies that do not end preterm. In such cases, little if any benefit from ACS may be outweighed by potential adverse effects.

1.2.2 Corticosteroid´s mechanism of action

To be able to understand how ACS can exert their positive, and potential negative, effects on the fetus we have to look at the function of glucocorticoids in general.

1.2.2.1 Glucocorticoids during pregnancy

The main circulating endogenous glucocorticoid (GC) in humans is cortisol. Cortisol is produced and released from the adrenal cortex. This is regulated by the hypothalamic-pituitary-adrenal axis (HPA-axis) (figure 4).

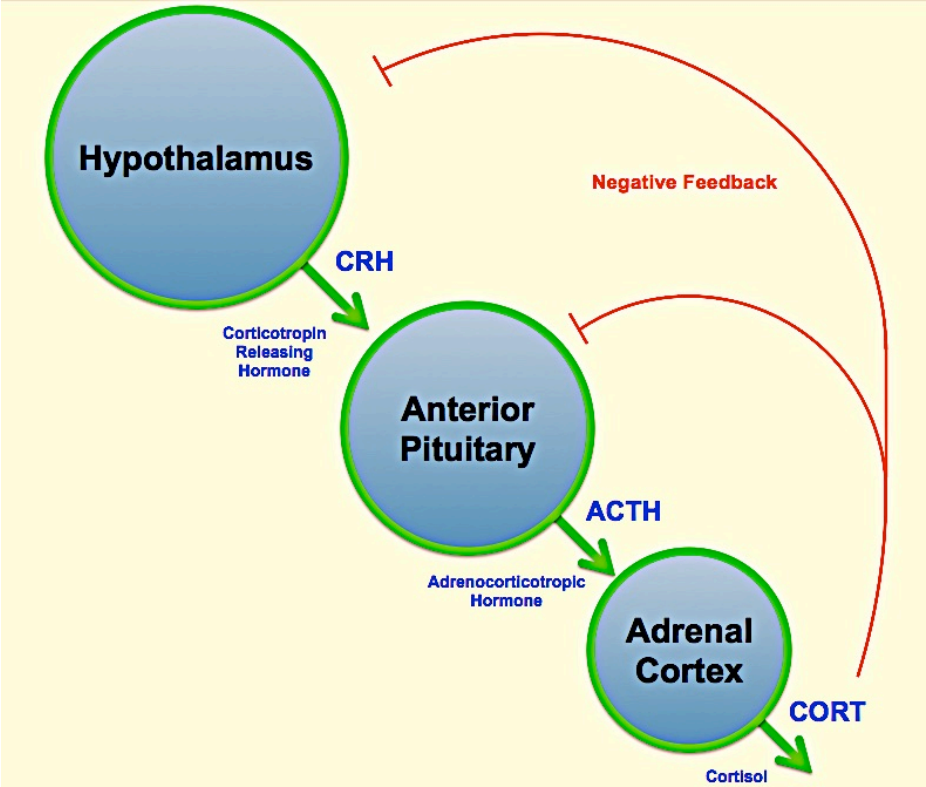

Figure 4. The hypothalamic-pituitary-adrenal axis (HPA-axis)

Glucocorticoids are involved in the expression of a large variety of genomes in many organ systems and they regulate metabolic processes and stress responses.[16] Their pharmaceutical use derives from their anti-inflammatory and immunosuppressive activities.

Glucocorticoids play a very important role in the normal fetal development, especially regarding pulmonary maturation, brain development and fetal growth (see chapter 1.4.2.2). Nevertheless, excessive transmission of glucocorticoids to the immature fetus could have a negative impact on developmental programming, narrowing physiological boundaries for health and increasing the risk of subsequent disease later in life (see chapter 1.4.2.3).[17] During pregnancy, the fetus is protected from high levels of endogenous cortisol from the mother via 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the placenta and fetal tissue. 11β-HSD2 converts active cortisol to inactive cortisone and prevents the entry of maternal cortisol to the fetal compartment. In addition, the HPA-axis in the immature fetus is inactivated which results in low fetal output of cortisol. Hence, the fetal levels of cortisol are approximately 10% of those in maternal blood.[16] In contrast, the synthetic glucocorticoids betamethasone and dexamethasone used in ACS treatment, are not inactivated by 11β-HSD2, which results in higher concentrations of synthetic glucocorticoids in fetal blood (30% of those in maternal blood).[16]

Endogenous glucocorticoids exert their effects mainly through the glucocorticoid receptor (GR) but also through the mineralocorticoid receptor (MR), which act as transcription factors to alter gene expressions. In addition, GR and MR can also mediate fast non-genomic actions via membrane-located receptors.[18] The synthetic GCs BM and DX only have affinity to the glucocorticoid receptor and bind to the GR with 25-fold higher affinity than cortisol.[19]

1.2.2.2 Glucocorticoids and normal fetal development

One of the most vital roles of glucocorticoids in the normal fetal development is their effects on pulmonary maturation, lowering the risk of respiratory distress in preterm infants (chapter 1.2.3.1). Glucocorticoids also play a vital role in fetal brain development by initiating terminal neuronal maturation, remodeling of axons and dendrites and affecting cell survival.[20,21] Besides its effects on the fetal lungs and brain, GC have important effects on other organ systems such as the heart, circulation, kidneys, liver, adipose and thyroid, all contributing to improve postnatal adaptation.[17,22] Glucocorticoids also affect systems that regulates fetal growth[23] and profoundly influence the development and lifelong function of the HPA-axis.[24]

1.2.2.3 Glucocorticoids and developmental programming

The ability of the early environment to program physiological function throughout life was identified already in the 1950s.[25] In the 1980s, Barker et al discovered that low birth weight was associated with increased risk of cardiovascular disease in adulthood.[26] He postulated that low birth weight could be seen as a surrogate marker of fetal malnutrition, and that there was a causative relationship between adverse fetal environment and suboptimal health in

adulthood. Since then, animal experiments as well as observational human data has provided support for the Barker hypothesis.[27-29] This field of research, exploring the association between environmental challenges during pregnancy, altered fetal growth and development, and later pathophysiology, is referred to as the *developmental origins of health and disease* or *developmental programming*.

Glucocorticoids are, as mentioned above, an important developmental switch, driving changes in gene regulation that are necessary for normal fetal growth and maturation of many organ systems. However, excessive transmission of GC to the fetus or at an incorrect stage of maturation have been suggested to be major contributing mechanisms for adverse developmental programming, resulting in altered physiological function throughout life.[17,30,31] Synthetic glucocorticoids effect on the HPA-axis is of special concern since the HPA-axis has a critical role in the regulation of metabolic, cardiovascular, reproductive and neurological systems, and dysregulation of the HPA-axis is associated with a number of chronic diseases.[17,32-34]

1.2.3 Neonatal morbidities affected by ACS

1.2.3.1 Respiratory distress syndrome (RDS)

RDS is one of the most serious complications of preterm birth and the primary cause of early neonatal death.[35] It affects up to 80-90% of extremely preterm infants and about half of the very preterm infants, but can also affect infants born at later gestations.[36] RDS usually presents at birth or shortly thereafter. The symptoms are progressive and include grunting, chest wall retractions, nasal flaring, and increased work of breathing. In the infant lung, surfactant is produced by type II pneumocytes in the alveolar airspaces. Surfactant forms a film in the alveoli, lowering alveolar surface tension thus increasing lung compliance and preventing atelectasis. The pathophysiology behind the disease can briefly be explained by the structurally immature and surfactant-deficient lung that has a tendency to collapse. The presence of relatively well-perfused but poorly ventilated areas of the lung results in ventilation/perfusion mismatch with hypoxemia and hypercarbia. Left untreated, the condition may lead to respiratory failure and death. The treatments for RDS include surfactant replacement therapy, continuous positive airway pressure (CPAP), and mechanical ventilation.

The use of ACS to enhance pulmonary maturation has markedly reduced the incidence of RDS. Reviews have shown that ACS reduces the risk of RDS with an average of 34% (RR 0.66; 95% CI 0.56-0.77) and the need for mechanical ventilation with an average of 32% (RR 0.68; 95% CI 0.56-0.84).[12]

The mechanism behind ACS´ positive effect on the fetal lung is that glucocorticoids increase surfactant production by increasing phospholipid synthesis and stimulating the production of surfactant-associated proteins.[37] In addition, GCs promote fetal lung development by

stimulating cell maturation and differentiation, stimulating antioxidant enzymes, thinning of the alveolar septae and regulating pulmonary fluid metabolism.[37-39]

1.2.3.2 Intraventricular hemorrhage (IVH)

The risk of developing IVH is inversely proportional to gestational age. It mainly affects preterm infants born before 32 weeks. The incidence ranges from about 10-50% in different centers. 90% of the cases occur in the first 3 days after birth. IVH is a hemorrhage from the germinal matrix located in the lateral ventricles of the brain. The hemorrhage is commonly graded I-IV where grade I is a small hemorrhage in the subependymal area or in the matrix, grade II includes blood in the ventricle but without ventricular dilatation, grade III includes ventricular blood with ventricular dilatation whereas grade IV includes parenchymal engagement.[40] The prognosis for IVH grade I-II is generally considered good whereas grade III and IV increase the risk of poor neurological outcome such as cerebral palsy (CP), lower cognitive function and impaired motor function[41] and is associated with white matter damage (PVL).[41,42]

The use of ACS has in systematic reviews been found to reduce the incidence of IVH with an average of 45% (RR 0.55; 95% CI 0.40-0.76).[12]

Glucocorticoids role in prevention of IVH is likely due to increased circulatory stability and vascular resistance,[43,44] maturation of cerebral microvasculature[45,46] and improved lung function reducing the need for mechanical ventilation.

1.2.3.3 Necrotizing enterocolitis (NEC)

NEC is a rare but potentially very serious complication after preterm birth. It is an inflammatory condition in the gut mainly affecting the most immature infants or infants small for gestational age (SGA). The incidence varies from about 5-10% in different settings. The etiology is not fully understood but some predisposing factors are enteral feeding, decreased oxygenation of the gut, decreased intestinal blood flow and invasion of pathogenic bacterial flora. The clinical symptoms include a distended abdomen, blood in the stools and signs of pain in the infant. The disease is characterized by acute inflammation of the intestinal wall, necrosis, perforations and septicemia. Milder cases are often treated conservatively with antibiotics and withheld feeds whereas more severe cases require surgical intervention. Mortality rates can be up to 50% in severe cases.[47]

The use of ACS has been found to reduce the incidence of NEC with an average of 50% (RR 0.50; 95% CI 0.32-0.78).[12]

1.2.3.4 Neonatal infections

Systemic infections are common complications after preterm birth and can be divided into two subgroups; early onset (within 48 hours after birth) and late onset (>48 hours after birth). The incidence of septicemia in extremely preterm infants varies from 25-60%.[48]

Early neonatal infections are mainly contracted in utero or at birth and the most common microorganisms are group B streptococci. Late onset infections are mainly nosocomial and the most common microorganism is coagulase-negative staphylococci. Systemic infections are treated with antibiotics and antibiotic treatment is often given on wide indications before bacterial infection can be excluded.

The use of ACS has been found to reduce the incidence of early systemic infections with an average of 40% (RR 0.60; 95% CI 0.41-0.88).[12]

1.2.4 Long-term outcomes of preterm birth

1.2.4.1 The Barker hypothesis

In addition to the increased neonatal morbidity, survivors after preterm birth also face an increased risk of illness later in life. Barker et al discovered in the 1980´s that low birth weight as a surrogate marker of adverse fetal environment, was associated with increased risk of cardiovascular disease in adulthood.[26] This has then been observed in numerous studies and it is now widely known that both low birth weight and prematurity are predisposing factors for developing pulmonary, cardiovascular and metabolic diseases as well as neurological and neurodevelopmental conditions later in life. Besides fetal malnutrition, excess exposure to glucocorticoids in utero could be a candidate mechanism underlying the associations between low birth weight and increased risk of cardiometabolic and other diseases in adulthood.

1.2.4.2 Cardiovascular and metabolic outcome after preterm birth

Most studies reporting data in historical cohorts before the modernization of perinatal and neonatal medicine show no increased risk for coronary heart disease or hypertension among elderly people born preterm.[49,50] However, these results are biased because of the very selective survival after preterm birth during that time period. In the modern era of perinatal and neonatal medicine (after the 70´s) with a more universal survival, there are emerging numbers of studies showing an association between prematurity and increased risk of cardiovascular and metabolic diseases later in life. For example, preterm birth has been correlated to hypertension[51-53], altered structure and function of the heart and the arterial tree[54,55], diabetes[56,57], increased levels of plasma low-density lipoproteins (LDL)[53], overweight[58] and stroke[59,60] in adults.

Since most women delivering preterm nowadays receive ACS, antenatal corticosteroid exposure could be one explanatory factor for these associations. In animals, ACS exposure has been associated with increased risk factors for cardiometabolic disease including enhanced fat deposition,[61] impaired metabolism of visceral fat,[62] higher blood pressure[63] and decreased insulin sensitivity.[64] In contrast to animal studies, the adverse effects of ACS in humans appear to be less prominent. A long-term follow-up in adult subjects has found that those exposed antenatally to synthetic GCs compared with placebo had similar blood

pressure, adiposity, blood lipids, fasting insulin concentrations, glucose tolerance and morning cortisol concentrations.[65] Results from human observational studies have been a bit conflicting, with some showing no association between ACS exposure and later risk factors for cardiometabolic disease,[66,67] while others have showed a small increase in blood pressure,[68] slight decrease in renal clearance[66], impaired β -cell function and evidence of increased aortic stiffness.[69] In addition, two observational studies demonstrated increased stress reactivity in term-born children exposed antenatally to glucocorticoids.[70,71]

1.2.4.3 Neurodevelopmental outcome

It is well known that survivors of preterm birth face an increased risk of impaired neurological function. Major neurological disabilities such as cerebral palsy (CP), hearing and visual impairment are often used as markers for quality of care but cognitive difficulties and behavioral problems are also important outcome measures. Neurodevelopmental disability (NDD) is often categorized into mild, moderate or severe depending on the status of hearing, vision, IQ and Gross Motor Function Classification System (GMCFS). The incidence of NDD varies greatly between countries. A recent meta-analysis including studies from eight developed countries showed that the proportion of moderate to severe NDD among survivors of preterm birth ranged from 43% in infants born at 22 weeks of gestation to 24% in infants born at 25 weeks of gestation.[72] In the Swedish EXPRESS-cohort born before 27 weeks of gestation, the incidence of moderate to severe NDD at 6.5 years of age was 34%.[73]

Major neurological disabilities are often identified early in life whereas milder deficits affecting behavioral, intellectual and educational outcome may become obvious with increasing age. These more subtle cognitive dysfunctions reported in children born preterm include lower intelligence, visual motor problems, deficient memory, delayed language skills, executive dysfunctions and social and emotional difficulties.[74] Increased prevalence of learning disabilities, ADHD and autism spectrum disorders have also been reported.[75]

In animal studies, exposure to ACS has been associated with reduced brain mass,[76,77] delayed myelination, decreased maturation of the retina and peripheral nerves[78,79] and impaired programmed apoptosis.[80] Some of these effects persisted into adulthood,[81] raising concerns that ACS treatment could contribute to adverse long-term neurodevelopment in individuals born preterm. However, in human studies, the outcomes after ACS exposure are much more reassuring. In RCTs, there are no significant correlation between ACS and adverse effects on later cognitive ability or neurosensory disability, including cerebral palsy.[82,83] [84,85] Other results in human studies are contradictive. For example, several studies have suggested adverse effects on emotional regulation after ACS exposure,[86,87] whereas in longer term follow-up of clinical trials there have been no correlation to clinically significant disturbances in early childhood behavior, executive function or adult psychiatric illness.[84,88,89]

1.2.4.4 Pulmonary outcome

The most severe pulmonary complication after preterm birth is the development of bronchopulmonary dysplasia (BPD). BPD is often defined by the need of supplemental oxygen at 36 weeks postmenstrual age and is graded into mild, moderate or severe depending on how much oxygen needed. The risk of developing BPD increases with decreasing GA, prolonged mechanical ventilation and oxygen therapy. BPD is a combined restrictive and obstructive disease where the restrictive component tends to normalize during the first years whereas the obstructive component tends to predominate later in life with asthma-like symptoms.[90,91] Severe cases will be dependent on supplemental oxygen for several months to years. Individuals being born preterm but with no or only mild BPD also have an increased risk of respiratory symptoms and decreased lung function later in life than the general population.[92-94]

Although ACS has many beneficial effect on the fetal lung, some experimental studies in rats exposed to ACS have shown a correlation to larger and fewer alveolar air spaces in adulthood,[95,96] raising concern that later lung growth may be impaired also in humans. However, in clinical studies, ACS exposure did not affect spirometric measures of lung volume or expiratory flow in childhood and adulthood.[97 98]

1.2.5 Repeat courses of ACS

Although the beneficial effects of ACS on the fetus are very well described, many studies have suggested a transient effect from ACS. [9,11,99] It seems like the maximum benefit from ACS on the fetus occurs 24 hours to 7 days after a complete course has been given. After 7 days the positive effects have diminished. This suggests that timing of ACS administration is crucial. Diagnosing actual preterm labour has been proved to be difficult, with 30-80% of women with symptoms suggesting preterm labour remaining pregnant 14 days later.[100,101] This has raised the question whether or not to repeat the ACS course to women who remains undelivered 7 days after the initial course. In Danderyd hospital, Stockholm, Sweden, and many other settings, it was routine during the 1980´s and 90´s to repeat the ACS course weekly to undelivered women with threatening preterm delivery until delivery occurred or until pregnancy reached 34 weeks. In some settings a *rescue course* of ACS is used, meaning administering a second course to patients whose pregnancies continue more than a week or 2 beyond their original course and in whom delivery has again become likely.[102] Studies have shown that repeat courses of ACS add positive effects on neonatal outcome compared to a single course. The latest Cochrane review from 2015 found that repeat courses of ACS to pregnant women still undelivered 7 days after an initial course was associated with 17% reduction in RDS and 16% reduction in serious infant outcome and the absolute benefit of repeat doses was similar to that of an initial course (numbers needed to treat to prevent respiratory distress syndrome: single course 12; repeat courses 17).[103]

The suggested beneficial effects from repeat courses of ACS must however be put in relation to the concerns of potential negative effects from excess glucocorticoid exposure on fetal

growth and development, as well as long-term health. The rationale for these concerns mainly rises from results from experimental animal studies, showing that repeat courses of ACS could have negative effects on a variety of functions including fetal growth[104], brain development[81], behaviour[105], vascular function[106], fat/glucose metabolism[107,108] and HPA-function[104]. In human studies however, the effects from repeat courses of ACS appears to be less harmful:

1.2.5.1 Effects on fetal growth after repeat courses of ACS

Some studies in humans have shown a reduction in fetal growth after repeat courses of ACS[109-112] whereas others have not[113,114]. Reductions in both birth weight, birth length and head circumference at birth have been reported. However, the observed reductions in some measures of growth seems to be transient with the observed differences in birth size no longer significant at hospital discharge[112] or at later follow-up of the child[87,115]. Furthermore, many of the studies where a difference in birth size has been observed have not adjusted for gestational age, making the results difficult to interpret.[103]

Several mechanisms have been suggested to contribute to the glucocorticoid-induced retardation of fetal growth. These include altered placental function and nutrient transfer,[116] decreased DNA synthesis and cell division,[117] reduced fetal tissue water content[118] and increased protein catabolism.[119] It is likely that altered expression and action of insulin-like growth factors (IGF) underlie many of these changes.[120]

1.2.5.2 Long term outcome after repeat courses of ACS

The largest randomized controlled studies investigating the effects of repeat courses of ACS have reported outcomes in early childhood (2-8 years) and the results are reassuring.[87-89,115,121,122] As shown in table 1, there is no increased risk for any of the reported outcomes following repeat courses of ACS. However, one large American RCT reported higher rates of CP in the repeat group (five of six cases), although not statistically significant (p=0.12).[115] In addition, the Australasian RCT reported that more individuals in the repeat group were assessed for attention problems than in the single course group at the 2-year follow-up.[87] At the follow-up at 6-8 years of age there were however no reported differences in neurodevelopmental outcome (table 1).[121]

Follow-ups into adolescence and adulthood are however lacking and there is a concern that possible negative effects of excessive glucocorticoid exposure could be revealed later in life. The latest Cochrane review states that there is a need for follow-up studies into adulthood after repeat courses of ACS.[12]

Table 1. Outcomes after repeat courses of ACS reported in randomized controlled trials. Red text indicates adverse outcomes, arrows indicate direction of an effect after repeat ACS courses versus a single course.

Author/group	Neonatal outcome	Outcome at follow-up
Asztalos et al/ MACS (Multiple Courses of Antenatal Corticosteroids Study)	• Mortality, severe RDS, IVH 3-4, PVL, BPD, NEC ➔ • Birth weight, birth length, head circumference ↓	**2 years** • Death, neurologic impairment ➔ **5 years** • Death, survival with neurodevelopmental disability ➔
Crowther et al/ ACTORDS (The Australasian Collaborative Trial of Repeat Doses of Corticosteroids)	• RDS, severe lung disease, oxygen therapy, mechanical ventilation ↓ • Birth weight, head circumference ↓ - no difference at discharge from hospital	**2 years** • Body size, blood pressure, respiratory morbidity, behavior score, survival free of major morbidity ➔ • Assessed for attention problems ↑ **6-8 years** • Body size, fat mass, blood pressure, spirometry, insuline sensitivity, estimated GFR, neurodevelopment, cognitive function, behaviour, health-related quality of life, use of health services ➔
Wapner et al	• Surfactant administration, mechanical ventilation, CPAP ↓ (≥4 courses) • Birth weight ↓ (≥4 courses) • Small for gestational age ↑ • Composite neonatal outcome ➔	**2-3 years** • Body size, neurocognitive function tests ➔ • (Cerebral palsy ↑ (p=0.12))
Peltoniemi et al	• RDS ↑	**2 years** • Survival without severe developmental impairment, cerebral palsy, growth, rehospitalisation ➔

➔ = no difference, ↓ = decreased, ↑ = increased

1.2.5.3 Recommendations about repeat courses of ACS

The clinical recommendations regarding use of repeat courses of ACS are diverging as illustrated in figure 5. More studies about the effects and possible adverse outcomes after repeat courses of ACS are warranted before a more conclusive recommendation can be stated.

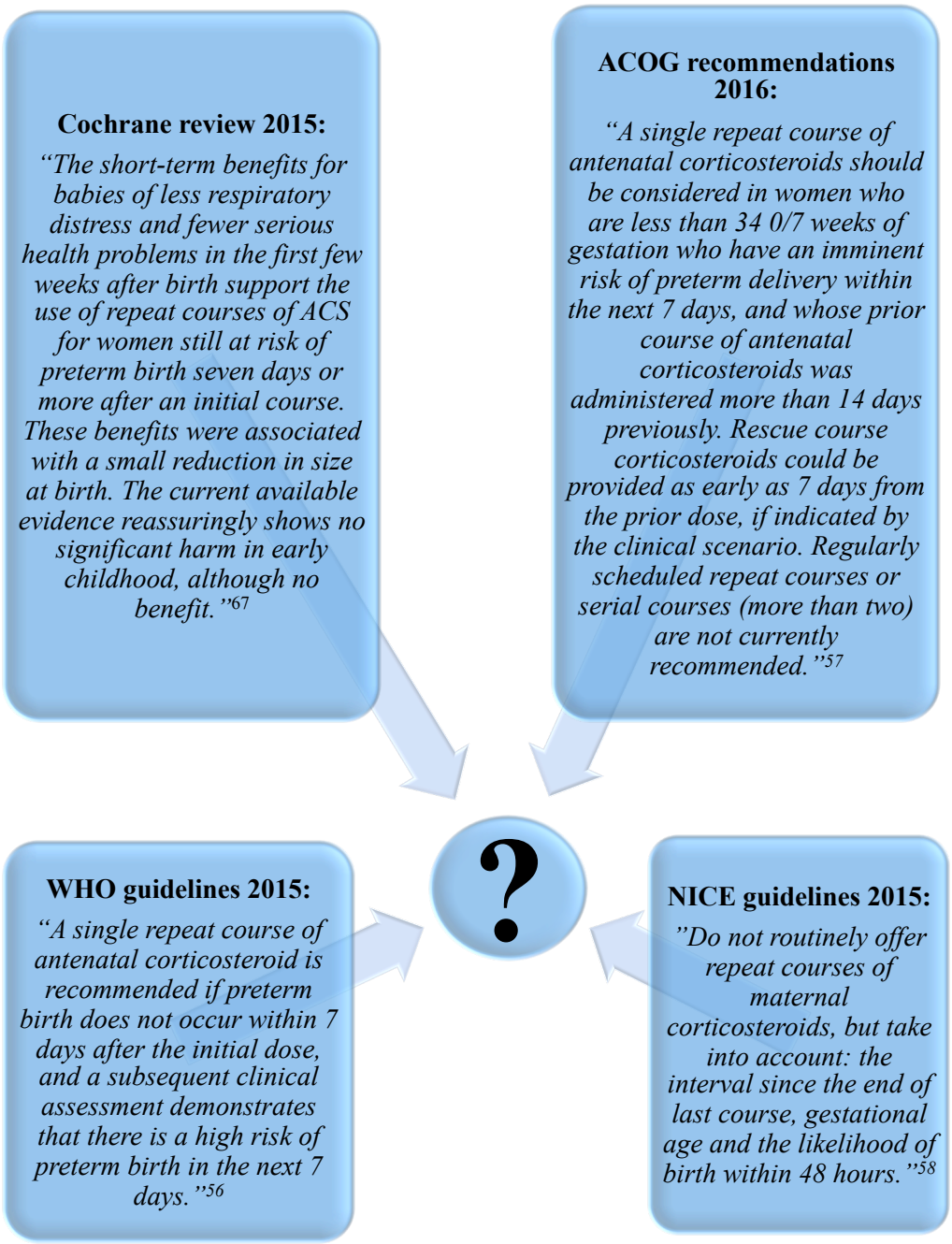

Figure 5. Recommendations about repeat courses of ACS

1.2.6 ACS in extremely preterm gestations

Most studies evaluating the effect of ACS on neonatal outcome have been conducted in moderately preterm or very preterm gestations. There are very sparse data on ACS´s effects in extremely preterm gestations. It is important to note that the evidence supporting ACS at <26 weeks gestation is based mainly on laboratory studies and non-RCTs. The only RCT in this field is from the pre-surfactant era and only had 49 participants in that group.[9] A previous meta-analysis evaluating ACS in extremely preterm gestations showed no reductions of neonatal mortality and morbidity prior to 26 weeks gestation[123] whereas more recent studies have indicated similar[124,125] or even more pronounced benefits[126,127] from ACS for extremely preterm births. In a Swedish population-based prospective observational study of extremely preterm infants born 2004-2007 (EXPRESS), ACS-treatment was associated with a significantly lowered mortality (OR=0.4; 95% CI=0.2-0.8).[5] In the same study they showed that ACS is a very common treatment also among extremely preterm infants (Figure 6). This is however not true for many other settings where treatment with ACS in extremely preterm gestations is more restricted. For example, in the French EPIPAGE-2 cohort study with extremely preterm infants born in 2011, only 2% of neonates at 22 weeks, 12% at 23 weeks, 57% at 24 weeks and 78% at 25 through 26 weeks gestation were treated with ACS (figure 6).[128] The reason to refrain from ACS treatment at extremely low gestations most likely reflect an anticipation of a very poor prognosis in which case ACS would not be administered and management of the infant after birth would be limited to compassionate care. But another explanation could be that use of ACS at the lower end of gestations was judged as pointless.

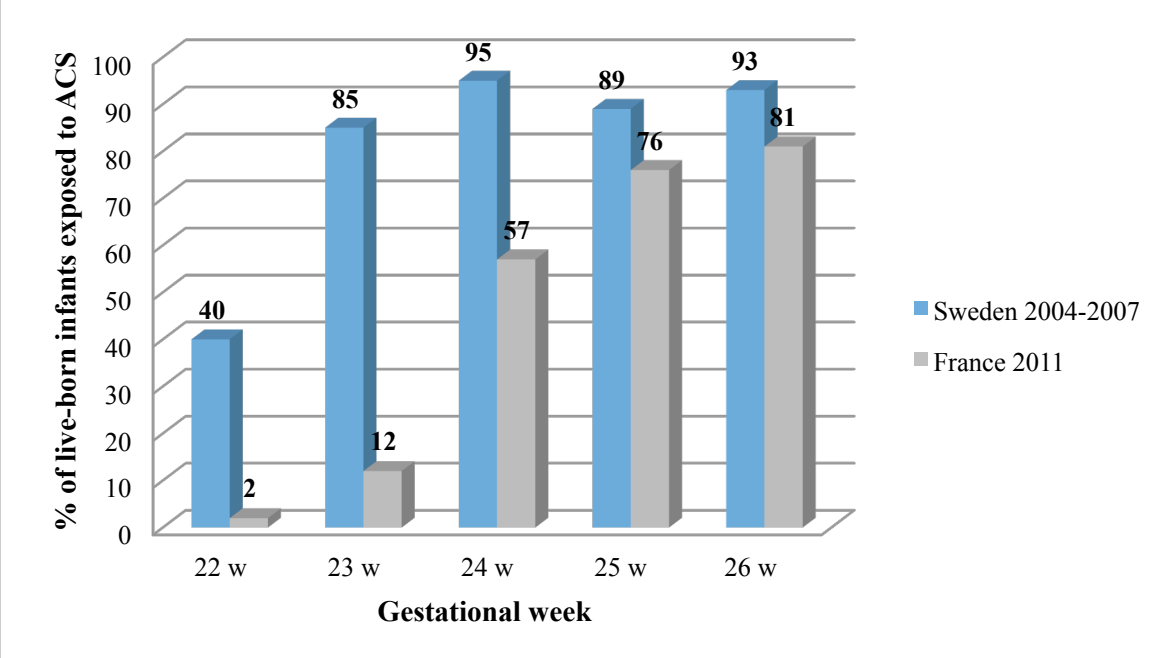

Figure 6. ACS treatment among extremely preterm infants in Sweden 2004-2007 (EXPRESS)[5] and in France 2011 (EPIPAGE-2)[128]

The most recent recommendations for ACS treatment include the possibility to treat even more immature infants. The ACOG recommendations from 2016 states that ACS may be considered for pregnant women starting at 23 0/7 weeks of gestation who are at risk of preterm delivery within 7 days, based on a family's decision regarding resuscitation.[14] The NICE guidelines also states that ACS could be considered from week 23 0/7.[15] In the new Swedish national guidelines from 2016, ACS should be considered from 22 0/7 weeks of gestations.

1.2.7 Are the recommendations implemented?

Knowing the recommendations on ACS treatment, one can ask how well the recommendations are implemented in clinical practice. In a recent European study, the use of ACS varied greatly between different European settings. The proportion of pregnant women delivering at 24-32 weeks gestation, receiving ACS varied between 70 and 98% (figure 7).[129]

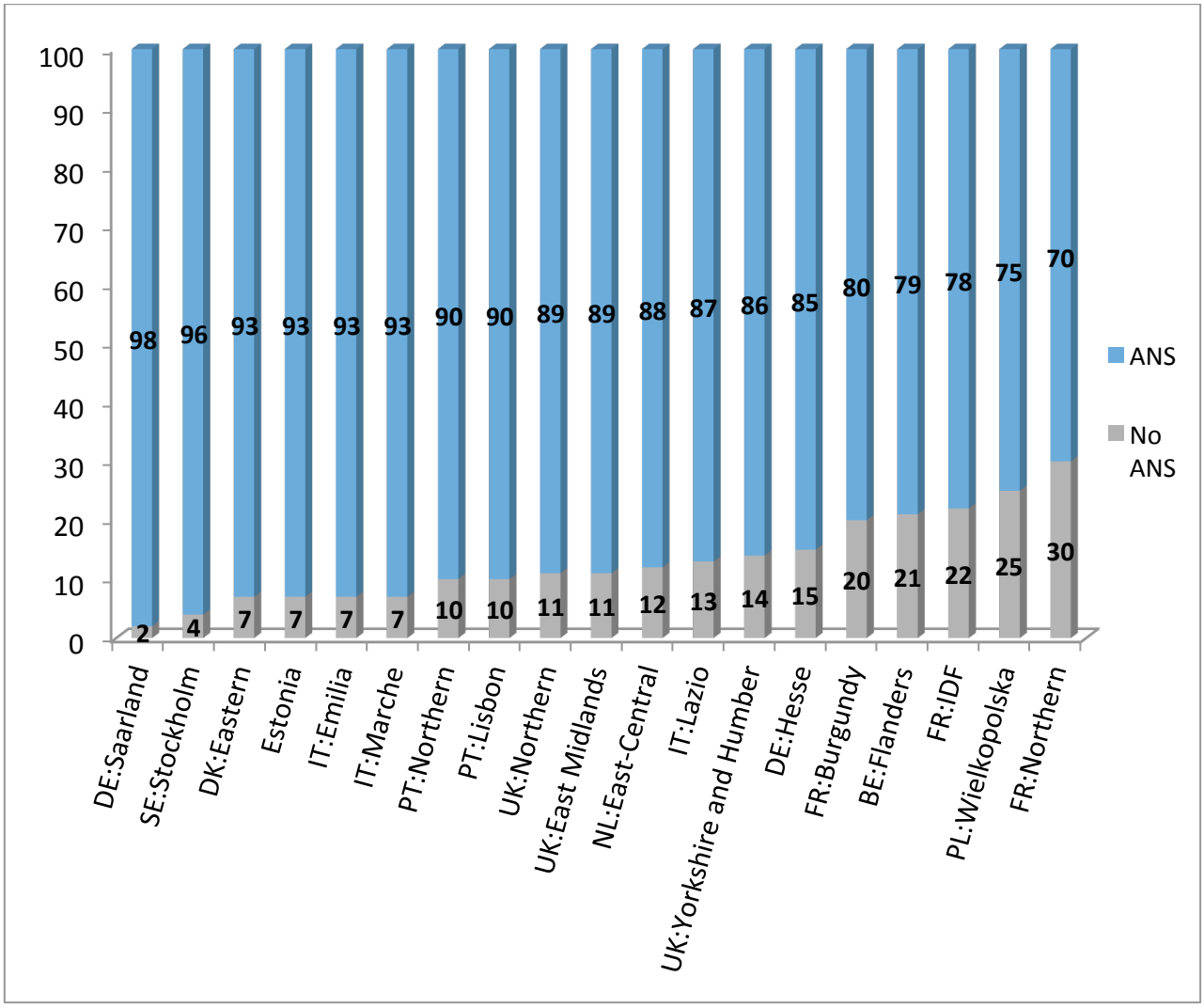

Figure 7. Treatment rates of antenatal corticosteroids (ANS) in European settings[129]

Recent corresponding figures from the US show that an average of 69.4% of infants born from 23 0/7 to 34 6/7 weeks gestation had been exposed to ACS.[127] Rates of exposure to ACS were lower among infants at the higher and the lower ends of the recommended gestational age range (figure 8).

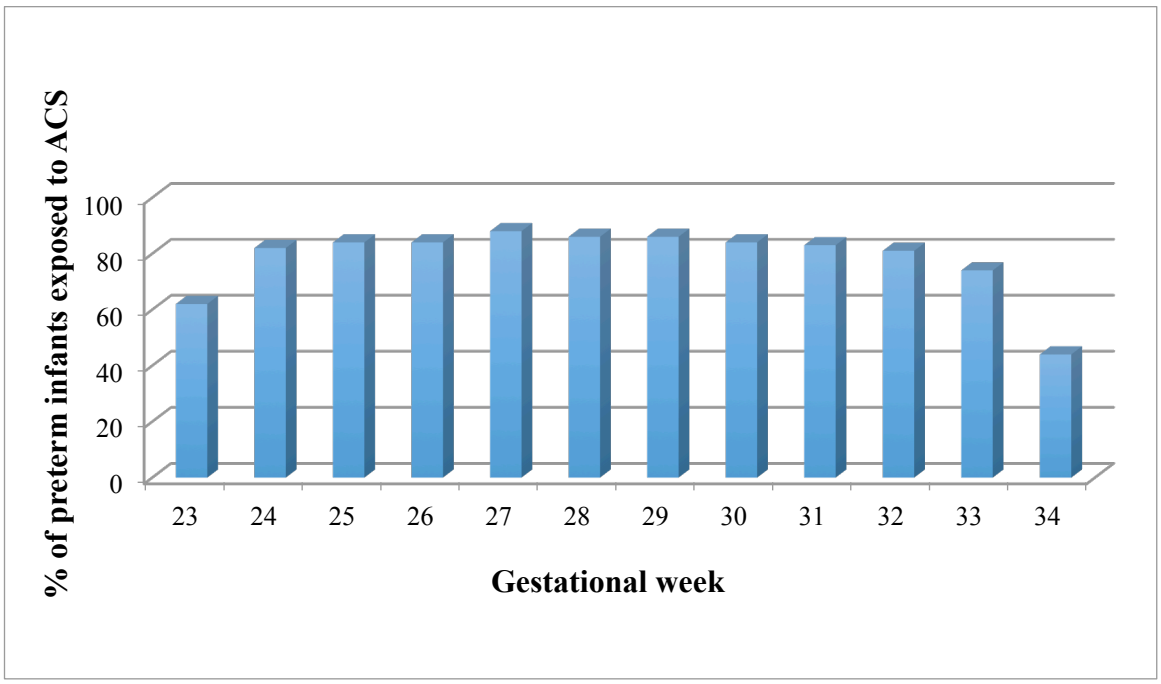

Figure 8. Treatment rates of antenatal corticosteroids by gestational age in the United States[127]

2 AIMS OF THE THESIS

The overall objective with this thesis is to investigate the safety and efficacy of antenatal corticosteroids in both a short and long term perspective.

The specific aims of the included studies are:

Study I:

- To investigate if the effects of ACS on birth size are dose dependent.
- To investigate if the length of gestation at start of ACS therapy is correlated to birth size.

Study II:

- To investigate the relationship between repeat courses of ACS and risk factors for cardiovascular and metabolic disease in adolescents and young adults.

Study III:

- To investigate the relationship between repeat courses of ACS and cognitive and psychological functioning in adolescents and young adults.

Study IV:

- To investigate the impact of the administration-to-birth interval of ACS on survival of exremely preterm infants

3 METHODS

3.1 STUDY DESIGN

Figure 9 presents an overview of the included studies. The two cohorts will be described thoroughly in the next chapter. All studies in the thesis are cohort studies.

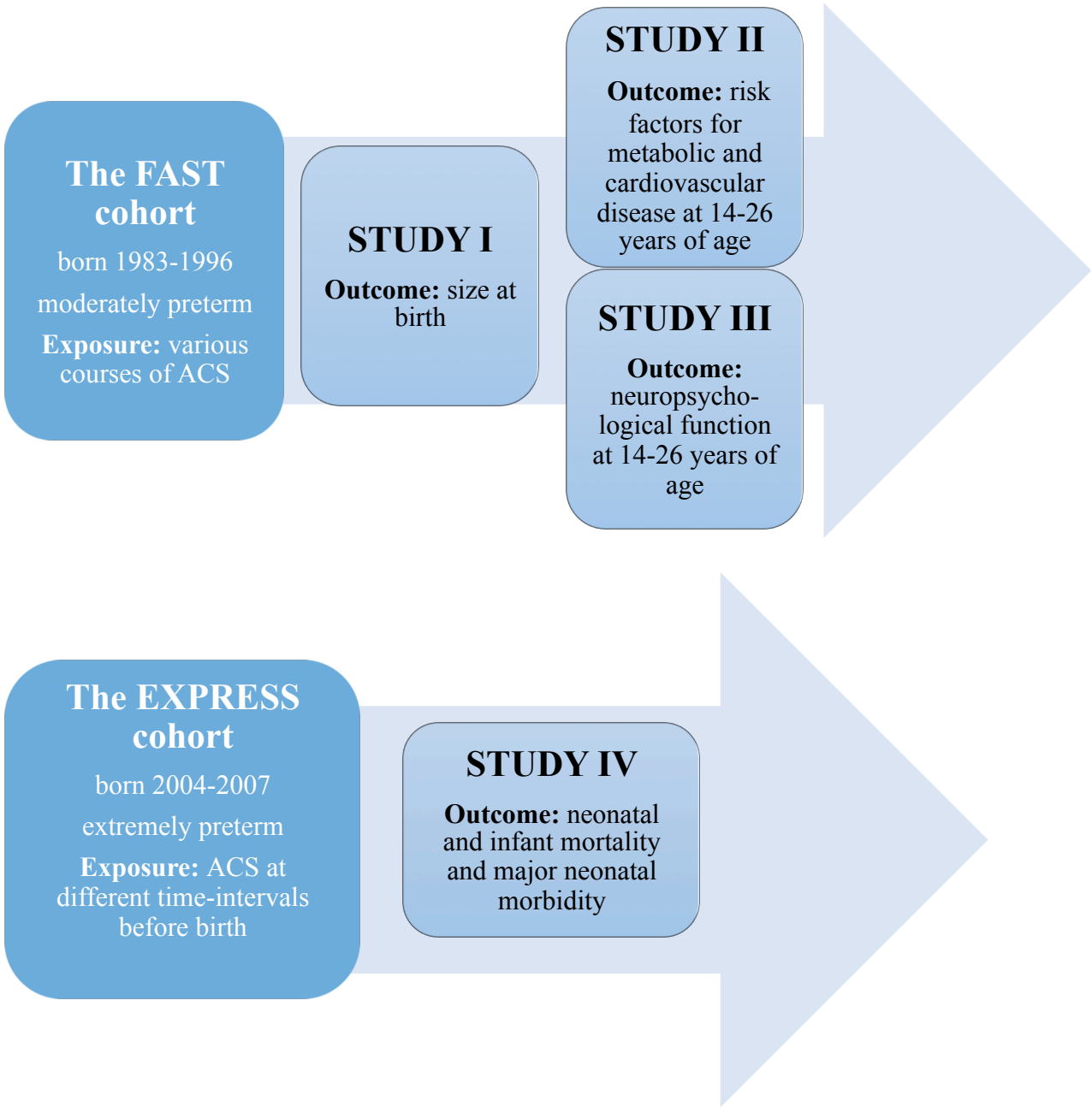

Figure 9. An overview of study I-IV

3.2 STUDY POPULATIONS

3.2.1 The FAST cohort

The Fetal Antenatal Steroid Treatment (FAST) study was a cross-disciplinary venture assessing both psychological and medical aspects after corticosteroid exposure in utero. All subjects in this cohort were born at Danderyd Hospital, Stockholm, Sweden, between the years 1983 and1996. This hospital was among the first in Sweden implementing the use of ACS to pregnant women with threatening preterm delivery. During the study period, standard ACS treatment consisted of an initial induction of betamethasone 24 mg intramuscular (8 mg x 3 with 8 hour intervals), followed by a weekly course of 12 mg betamethasone, continued until delivery or until pregnancy reached 34 gestational weeks, if the threat of preterm delivery still remained. This treatment regimen is referred to as *multiple* or *repeat courses* of ACS. All infants exposed to repeat courses of ACS were eligible for inclusion in the study. Predefined exclusion criteria were fetal anomalies, maternal steroid use for other medical conditions, congenital viral infections and chromosomal aberrations. Infants exposed to repeat courses of ACS in utero (two to nine courses) were categorized into three groups depending on the number of exposures (figure 10).

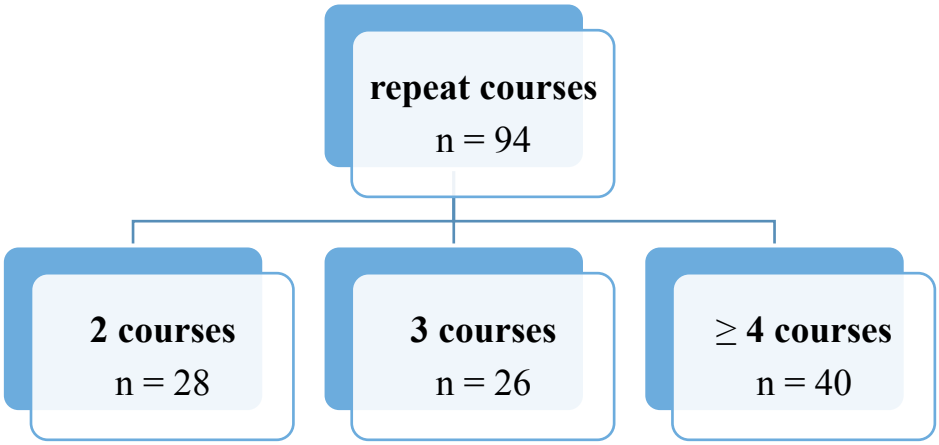

Figure 10. Flow chart of the FAST cohort

The majority of the infants in the cohort were born moderately preterm or term (mean gestational age 34.0 weeks) and with no major neonatal complications (table 2). During the study period, pregnant women were offered free healthcare visits throughout the pregnancy, and almost all (98%) attended their midwife appointments. Further description of the cohort is found in paper I-III.

Table 2. Neonatal characteristics of the FAST-cohort

	2 courses n = 28	3 courses n = 26	≥ 4 courses n = 40
Mean gestational age, w	34.9	33.2	34.4
Mean birth weight, g	2381	2192	2292
Boys, n (%)	15 (54)	18 (69)	26 (65)
IVH/PVL, n (%)	0	0	0
NEC, n (%)	0	0	0
ROP, n (%)	0	1 (4)	0
BPD, n (%)	0	1 (4)	1 (3)

3.2.2 The follow-up studies (II & III)

For the follow-up of the FAST-cohort, four of the 94 subjects could not be found or had moved from Sweden. The remaining 90 were invited to participate in the follow-up, 58 accepted and completed the assessment. At follow-up, participants´ ages ranged from 14-26 years with a mean age of 18 years, 62% were boys.

A control group of individuals unexposed to ACS and matched for gestational age, sex and year of birth were identified from the same hospital´s birth registry. Of the 103 controls invited to follow-up, 44 accepted and completed the assessment. For dose-response analyses, we also included a group of subjects exposed to a single course of ACS by using the same criteria as for the control group. Of the 48 subjects invited in this group, 25 accepted and completed the assessment. Figure 11 illustrates the included groups in the follow-up studies.

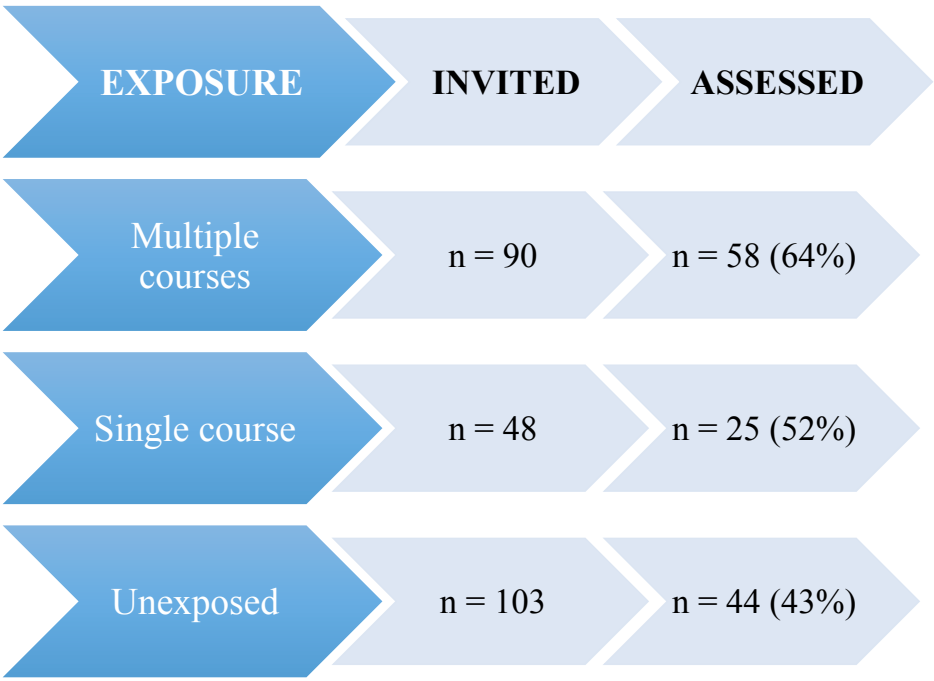

Figure 11. Flow-chart of the participants in the follow-up studies

3.2.3 The EXPRESS cohort (IV)

The Extremely Preterm Infants in Sweden Study (EXPRESS) included all pregnant women residing in Sweden and delivering extremely preterm infants, born at 22–26 completed weeks of gestation, from April 1st 2004 to March 31st 2007. This cohort was used in study IV and consisted of all live-born infants (n = 707), including multiple births and infants with malformations, whereas stillborn infants (n = 304) were excluded. Among live born infants, 520 survived the neonatal period and 497 were alive at 1 year old (figure 12).

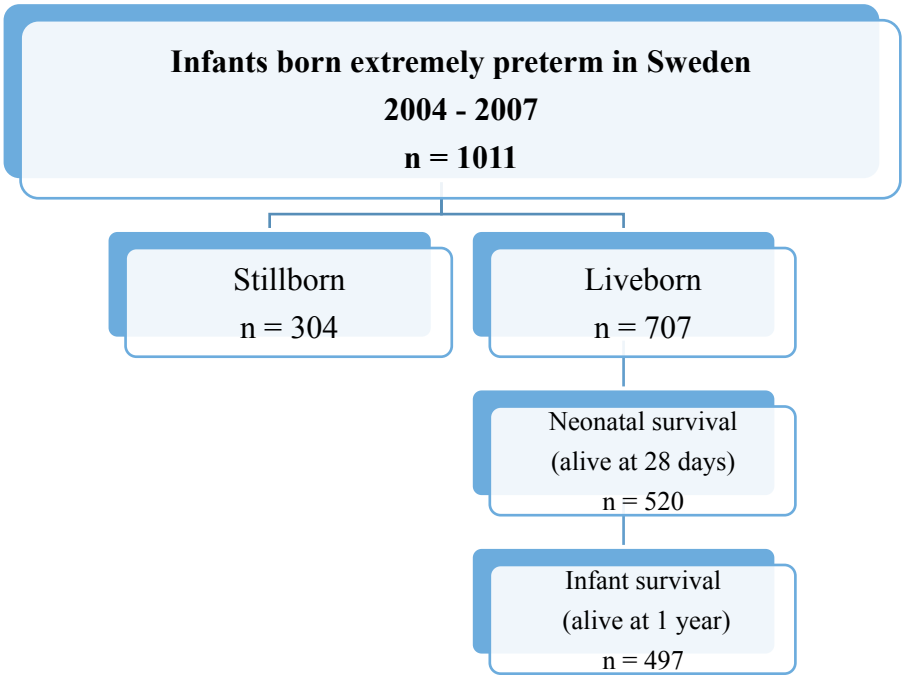

Figure 12. Flow-chart of the EXPRESS-cohort[5]

In the EXPRESS-cohort, 84% were exposed to antenatal corticosteroids in utero. A complete course with ACS to the pregnant mothers consisted of two doses of 12 mg betamethasone 24 h apart. In the EXPRESS-database, time from the first ACS dose to delivery in hours had been prospectively collected. We categorized all live-born infants into four categories according to the time-interval from the first ACS dose to delivery: <24 h; 24–47 h; 48 h to 7 days; and >7 days (figure 13). Further description of the cohort characteristics is found in paper IV.

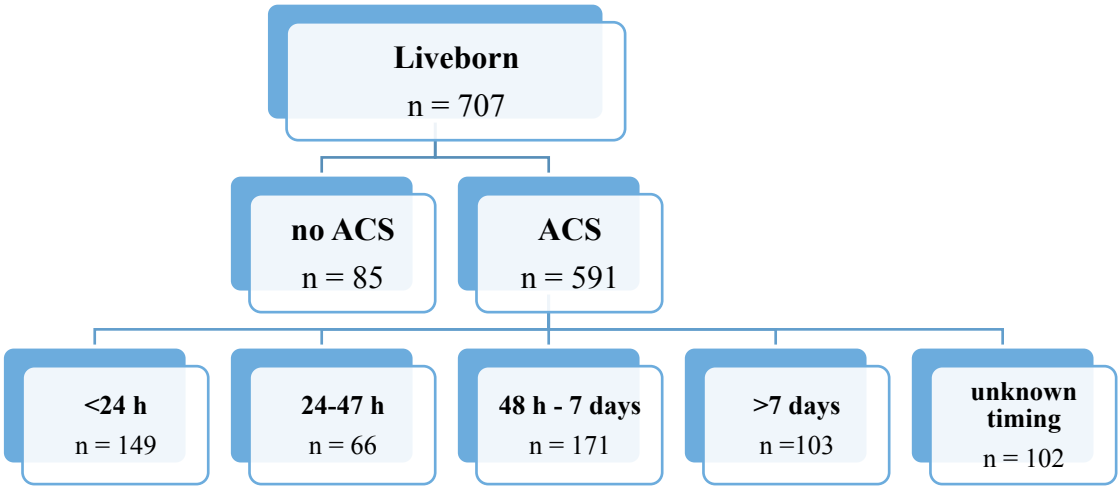

Figure 13. Categorization of ACS exposure in the EXPRESS cohort

3.3 DATA COLLECTION

3.3.1 Study I-III (FAST)

During the study period, comprehensive data from all mothers undergoing antenatal care and infants admitted for neonatal care were prospectively collected in a hospital registry. From this registry, we identified all infants born between 1983 and 1996, admitted for neonatal care, and whose mothers had received multiple courses of ACS (n = 81). In addition, we scrutinized the records from the antenatal clinic, and in total, we identified 94 infants exposed to multiple courses of ACS. Information on maternal age, height, parity, multiple pregnancy, smoking habits as well as infant sex, birth weight (BW), birth length (BL) and head circumference (HC) at birth were retrieved from the Swedish Medical Birth Register (MBR). All MBR data were validated by scrutinizing maternal and infant hospital records. Maternal blood pressure disease, gestational age and neonatal morbidity were also recorded from these records. Standard deviation scores (SDS) for birth size were calculated according to national reference data for normal fetal growth.[130]

3.3.1.1 The Swedish Medical Birth Register (MBR)

All birth units in Sweden report to the Medical Birth Register (MBR). The register contains information on more than 99% of all births in Sweden. The information is collected prospectively during pregnancy on standardized forms and forwarded to the register. Validation of the MBR has proved high quality.[131]

3.3.2 Study IV (EXPRESS)

The EXPRESS-study was a national collaboration where all obstetric and pediatric departments in Sweden participated. The study was descriptive with no attempts in the study framework to standardize treatment. During the study period, Sweden had seven healthcare regions, each served by a regional level III hospital. The general policy was to

centralize extremely preterm deliveries to these regional hospitals. In each of the 7 health care regions, one obstetric and one pediatric study coordinator were responsible for data acquisition and quality control. Data on mothers and stillborn infants were collected at the time of delivery. Data on live-born infants were collected prospectively during the first 180 days of hospitalization or until discharge or death. Mother and infant data were cross-linked with the Medical Birth Register to ensure accuracy. Information on infant deaths after discharge home until 1 year was obtained from the National Population Register. Data collection continued for all infants who were transferred between hospitals. All data were collected by local staff on standard study forms in accordance with a manual defining the variables. Regional data were electronically transmitted to a central database and again checked for quality and completeness.[5]

3.4 CLINICAL ASSESSMENTS

At the follow-up of the FAST-cohort 14 to 26 years after birth we assessed cardiovascular and metabolic functions (study II) as well as neuropsychological function (study III). The follow-up took place at Astrid Lindgrens Children´s Hospital in Stockholm, Sweden, from October 2008 to April 2010.

3.4.1 Cardiovascular and metabolic assessment (study II)

All cardiovascular and metabolic assessments were performed by one registered and trained research nurse.

3.4.1.1 Anthropometric measures

The participants´ weight, height and waist circumference were registered. From these measures, Body Mass Index (BMI) was calculated. Overweight, obesity and elevated waist circumference were defined (see details in paper II).

3.4.1.2 Blood pressure

After at least 5 minutes of sitting rest, systolic BP (SBP) and diastolic BP (DBP) were measured in the left arm with an appropriately sized arm cuff. Three consecutive measures were performed at 2-minute intervals, and mean SBP and DBP values were calculated. Elevated blood pressure was defined for the different age groups (see details in paper II).

3.4.1.3 Pulse wave analyses

A pulse wave analysis system was used together with applanation tonometry to noninvasively acquire the radial artery pressure waveforms. Central aortic waveforms were derived from those obtained from the radial artery using a validated data transfer algorithm. From the aortic waveforms, central aortic SBP and DBP were determined. Identification of early and late systolic peaks in the aortic pressure curve allows quantification of an augmentation index

(AI, %). The AI is related to the speed of the central and peripheral pressure wave reflections and an increasing AI reflects increasing arterial stiffness. The mean value of 3 recordings, each comprising 10 consecutive pressure waves, was taken. Elevated AI was defined as AI above 10% and 17% for men and women, respectively (see paper II for more details).

3.4.1.4 Blood sample analyses

From each participant, 5 mL blood was sampled to analyse glucose, insulin, triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A1 (Apo A1) and apolipoprotein B (Apo B), lipoprotein(a), and cortisol concentrations in plasma or serum. From these analyses, HDL/LDL-ratios and Apo B/Apo A1-ratios were calculated. All blood samples were morning fasting samples.

Homeostatic model assessment (HOMA) is a method for assessing insulin resistance (IR) from basal fasting glucose and insulin. HOMA-IR was calculated using a HOMA-calculator.[132]

3.4.2 Neuropsychological assessment (study III)

All neuropsychological tests were performed by one licensed and trained psychologist who was blinded to exposure group and gestational age at birth. The assessments were performed in the same room and at approximately the same time of the day for all participants. The entire assessment process lasted 2.5 to 3 hours. The neuropsychological tests were grouped into five broadly defined domains including; i) General cognitive ability; ii) Memory and learning; iii) Working memory; iv) Attention and speed; and v) Cognitive flexibility and inhibition. The last three domains reflect executive functions. The tests included in the neuropsychological test battery are presented in table 3. Measures on psychological health were obtained from self-report forms including the Achenbach Adult or Youth Self-reports, the World Health Organization´s screen for ADHD and a Quality of Life Inventory (QoLI).

Table 3. Neuropsychological test battery. Tests listed in order of administration.

Test	Source	Brief description	Cognitive domain
MOT, Motor Screening	Cantab	Screens for visual, movement and comprehension difficulties.	
RTI, Reaction Time	Cantab	Measures speed of response and movement and 5-choice paradigms.	Reaction time Attention
RVP, Rapid Visual Processing	Cantab	Measures visual sustained attention and is a sensitive measurement of general performance.	Attention
SWM, Spatial Working Memory	Cantab	Measures the ability to retain spatial information and to manipulate items in working memory.	Working memory
SST, Stop Signal Task	Cantab	Uses staircase functions to generate an estimate of stop signal reaction time and measures the ability to inhibit a prepotent response.	Response inhibition
IED, Intra/Extra Dimensional Set Shift	Cantab	Measures rule acquisition, reversal featuring visual discrimination, attentional set formation, maintenance, shifting and flexibility of attention.	Cognitive flexibility
TMT, Trail Making Test	D-KEFS	Connect by drawing a line digits and letters in numeric/alphabetical orders.	Cognitive flexibility Speed
RAVL-R		Examiner reads a list of 15 words and participant repeat all words he/she remembers directly and after 30 minutes.	Verbal learning/ retention
Block Design	WISC	The participant views a constructed model or picture and use blocks to re-create the design within a time limit.	General ability (non-verbal)
Vocabulary	WISC/ WAIS	The participant names or defines words of increasing difficulty.	General ability (verbal)
Coding	WISC	The participant transcribes digit-symbol codes within a time limit.	Attention Speed
Digit Span	WISC	The participant repeats an increasing number of digits in the same and the opposite order.	Working memory
Symbol Search	WISC	The participant is asked to mark symbols within a time limit.	Attention Speed
Design Fluency	D-KEFS	The participant connects 5 dots with lines to create unique patterns within a time limit.	Cognitive flexibility
Verbal Fluency	D-KEFS	The participant lists as many words as possible within a time limit with given beginning letters and semantic categories (e g animals, boy names).	Cognitive flexibility

Cantab, Cambridge Neuropsychological Test Assessment Battery; D-KEFS, Delis-Kaplan Executive Function System; WISC, Wechsler Intelligence Scale for Children; WAIS, Wechsler Adult Intelligence Scale

3.5 STATISTICAL METHODS

3.5.1 Study I

Group differences were tested using ANOVA and chi-square test. Correlation coefficients and linear regression were used to study associations between ACS timing and dose, and outcomes. To adjust for possible confounders or covariates, we used multiple linear regression analyses. A p-value <0.05 in the final model was considered statistically significant. Group characteristics are presented as mean and standard deviations (SD) or proportions (%). Outcomes are presented as mean (95% confidence interval). Considering that this was an observational study we had to be cautious about interpretations of a causative relationship and although several confounders were controlled for in the analyses, confounding cannot be excluded.

3.5.2 Study II and III

To test for group differences we used chi-square test or Student´s t-test. Simple linear regression was used to study associations between GA at first exposure and outcomes. To adjust for possible confounders or covariates, we used multiple linear regression analyses. Binary outcome variables were analysed using logistic regression. A p-value <0.05 in the final model was considered statistically significant. Data are presented as numbers, proportions (%), or mean and standard deviations. With our given sample, we had a power of 0.8 to detect a group difference of 0.56 or more (i.e., medium effect size). The small size of our cohort and the observational study design are limitations and the interpretation of results has been performed with caution. Furthermore, we cannot exclude selection bias in study participation.

3.5.3 Study IV

Group differences were presented using descriptive statistics. Cox proportional hazards regression analysis was used to estimate hazard ratios (HR) for survival across the ACS administration-to-birth interval categories. Based on available knowledge on optimal timing for ACS administration, the category 48 h to 7 days was considered as reference category, i.e. HR = 1.00. HRs for neonatal and infant survival in relation to ACS administration-to-birth intervals were evaluated in a multiple Cox proportional hazards regression model adjusting for possible confounders or covariates. Cumulative neonatal and infant survival adjusted for covariates was also determined using Kaplan–Meier survival analyses. The odds for surviving without major neonatal morbidity were analysed using a multiple logistic regression model. A p-value <0.05 was considered statistically significant. The large sample size allowed for adjusted analyses of survival in relation to timing of ACS-administration and several potential confounders. The observational study design means we have to be careful interpreting a causative relationship and we cannot exclude that other confounding factors than those included herein were unevenly distributed between categories and may have affected our results.

3.6 ETHICAL CONSIDERATIONS

The studies included in the thesis were approved by the Ethical Review Board in Stockholm (study I-III, 2007/898-31) and in Lund (study IV, 42-2004).

3.6.1 Register studies

In study I and IV, data from different registers were used and study subjects did not have to do any extra tests. The ethical considerations are related to the registration itself. Individuals could be uncomfortable knowing that there is information about them available in registers. All patient material in the presented studies has been handled with a high level of confidentiality. For the EXPRESS-registry, the parents provided oral informed consent for data acquisition.

3.6.2 Follow-up studies

Study II and III were follow-up studies and participation required written informed consent. The ethical considerations are related to the potential discomfort of being identified as an individual at risk. One can assume that the majority of the invited individuals exposed to ACS in utero were not even aware of the exposure or the possible negative effects from the exposure. We made sure to always answer questions and worries from the participants. As a rule, we did not give the participants individual feedback on assessment results but information was given if asked for.

In all the studies, results were presented at group level and no individual result could be revealed.

4 RESULTS

4.1 REPEAT ACS AND BIRTH SIZE (STUDY I)

4.1.1 Description of study population

The group characteristics of the study population in study I are presented in table 4.

Table 4. Group characteristics of study I

	2 courses n 28	3 courses n 26	≥4 courses n 40	*p*
MATERNAL DATA				
Age, years, mean (SD)	31.5 (±4.9)	31.6 (±6.0)	30.8 (±4.6)	ns
Height, cm, mean (SD)	166.8 (±6.3)	167.2 (±6.2)	168.4 (±5.0)	ns
Primipara, n (%)	16 (57)	19 (73)	26 (65)	ns
Smoker, n (%)	10 (36)	2 (8)	4 (10)	0.01
Prepregnancy hypertension/preeclampsia, n (%)	0 (0)	6 (23)	5 (13)	0.03
PREGNANCY DATA				
Gestational age, n (%)				
<32 w	5 (18)	8 (31)	6 (15)	ns
32-36 w	18 (64)	15 (58)	29 (73)	
≥ 37 w	5 (18)	3 (12)	5 (13)	
GA at start of ACS, w, mean (SD)	30.0 (±1.7)	29.2 (±2.2)	27.4 (±1.5)	<0.001
Total dose ACS, mg, mean (SD)	36 (±3)	48	74 (±15)	<0.001
Multiplets, n (%)	11 (39)	13 (50)	16 (40)	ns
INFANT DATA				
Boys, n (%)	15 (54)	18 (69)	26 (65)	ns
Year of birth, n (%)				
1983-1987	8 (29)	6 (23)	4 (10)	ns
1988-1992	12 (43)	13 (50)	15 (38)	
1993-1996	8 (29)	7 (27)	21 (53)	

4.1.2 Smaller birth size after ACS exposure

We found that increasing doses of ACS was associated with a significant reduction in standard deviation scores for BW, BL and HC (table 5). The mean BW-SDS after two courses of ACS was -0.21 which was significantly higher than the mean BW-SDS observed after 4 or more ACS courses (-1.01, p = 0.04), (table 5 and figure 14). The mean BL-SDS was -0.19 after two courses of ACS and -1.04 after four or more courses (p = 0.07), and the mean HC-SDS was 0.25 after two courses of ACS and -0.23 after four or more courses (p = 0.04) (table 5).

In the final multiple regression model adjusting for maternal age, height, parity, smoking, and blood pressure disease, multiple pregnancy, gestational age and infant sex we saw that exposure to four or more ACS courses was associated with a birth weight of -0.36 SDS (corresponding to -151 g for girls and -156 g for boys at term equivalent age) compared to those exposed to two or three courses of ACS ($p = 0.007$, $R^2 = 0.15$). The corresponding numbers for birth length was -0.43SDS (= -0.75 cm in girls and -0.81 cm in boys at term equivalent age) ($p = 0.01$, $R^2 = 0.15$) and for head circumference -0.22 SDS ($p = 0.04$, $R^2 = 0.10$).

Table 5. Neonatal anthropometry in relation to number of ACS courses. Data are mean (95% CI)

	2 courses n 28	**3 courses n 26**	**≥ 4 courses n 40**	***p***
Birth weight, SDS	-0.21 (-0.61, 0.19)	-0.47 (-0.96, 0.02)	-1.01 (-1.46, 0.56)	0.04
Birth length, SDS	-0.19 (-0.66, 0.28)	-0.32 (-0.87, 0.23)	-1.04 (-1.60, -0.48)	0.07
HC, SDS	0.25 (-0.14, 0.64)	0.26 (-0.09, 0.61)	-0.23 (-0.52, 0.06)	0.04

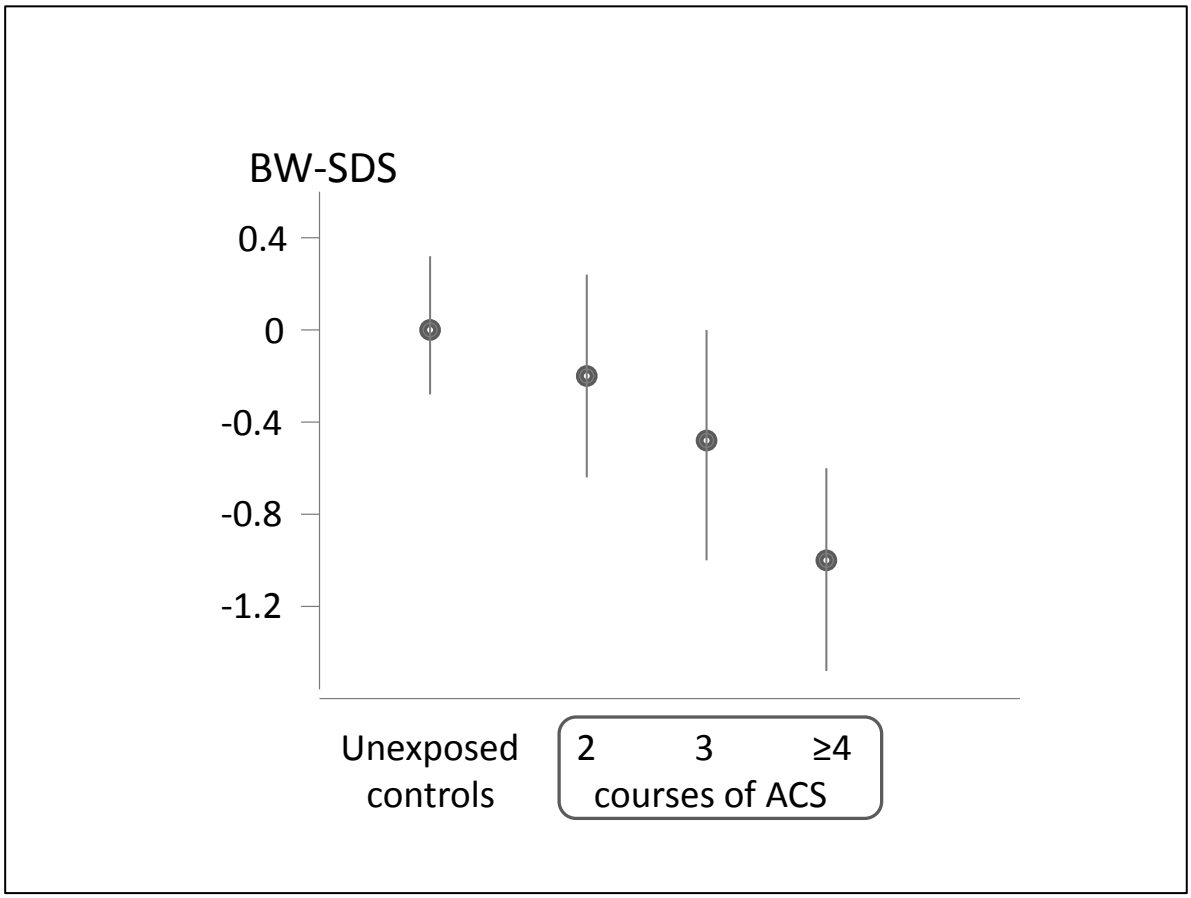

Figure 14. Standard deviation scores for birth weight in relation to ACS exposure. Data are mean (95% CI).

4.1.3 Gestational age at start of ACS and birth size

We found no association between GA at start of ACS and size at birth. After adjusting for GA at birth and sex, there was no correlation between GA at the start of ACS therapy and BW-SDS ($p = 0.85$), BL-SDS ($p = 0.26$) and HC-SDS ($p = 0.076$).

4.2 REPEAT ACS AND LATER CARDIOMETABOLIC AND NEUROPSYCHOLOGICAL OUTCOME (STUDY II & III)

4.2.1 Description of study population

The characteristics of the participants in the follow-up studies are presented in table 6.

4.2.2 Cardiovascular risk profile in adolescence and young adulthood after repeat courses of ACS (study II)

There were no significant differences between the groups regarding body mass index, systolic or diastolic blood pressures, arterial stiffness measured by augmentation index, blood lipids, insulin resistance, or morning cortisol levels, either in simple regression or in multivariable models (table 7).

However, subjects with an elevated AI had been exposed to a mean of 4.2 courses of ACS compared with a mean of 1.9 courses of ACS in participants with normal AI ($p = 0.0048$), and although not statistically significant ($p = 0.06$), there were more subjects with elevated AI who had been exposed to repeat courses of ACS in fetal life ($n = 7$) compared with unexposed subjects ($n = 1$).

In addition we found that glucose, insulin, and IR correlated weakly to GA at start of ACS (glucose $\beta = -0.06$ mmol/L per week longer gestation at start of ACS, $r = -0.29$, $p = 0.007$; insulin $\beta = -12.9$ pmol/L, $r = -0.36$, $p = 0.001$; and HOMA-IR $\beta = -0.17$ per week longer gestation at start of ACS, $r = -0.32$, $p = 0.004$).

In this study, 13% of the participants were overweight and 4% were obese, which is consistent with national statistics on overweight and obesity among young adults in Sweden (Statistics Sweden, Survey of living conditions, 2010-2011). Furthermore, as expected at young adult age, we found limited number of individuals with high BP, elevated blood lipids, or IR, indicating that the cohort is representative for Swedish conditions.

Table 6. Group characteristics of study II and III. Data are mean ±SD or n (%).

	Unexposed n=42	1 course of ACS n=25	≥2 courses of ACS n=58	*p*
Invited, n	103	48	90	
Parental data				
Maternal age, years	30.9 ±5.3	28.7 ±4.8	32.0 ±5.1	0.03
Primipara	20 (48)	18 (72)	26 (45)	0.06
Maternal smoking	7 (17)	3 (13)	7 (12)	0.79
Parental education[A]				
High school or less	9 (21)	10 (40)	19 (33)	
Professional	6 (14)	6 (24)	6 (10)	0.16
Academic	27 (64)	9 (36)	33 (57)	
Exposure data				
GA at 1st ACS course, weeks	_	30.6 ±1.3	28.7 ±2.1	<0.001
Total ACS dose, mg	_	23.2 ±2.8	55.4 ±20.7	-
Infant data				
Twin/triplet	9 (21)	5 (20)	29 (50)	0.002
Male sex	23 (55)	14 (56)	36 (62)	0.74
Gestational age at birth, weeks	34.7 ±2.9	32.2 ±2.9	33.6 ±2.5	0.002
Birth weight, g	2 534 ±702	1 909 ±593	2248 ±638	0.001
Birth weight, SDS	-0.2 ±1.1	-0.8 ±1.2	-0.4 ±1.1	0.10
Major perinatal complications [B]	5 (11.4)	6 (24.0)	7 (12.1)	0.29
Characteristics at follow-up				
Age, yrs	18.5 ±2.8	18.0 ±3.4	17.8 ±3.0	0.58
Menarche, yrs	12.9 ±1.2	12.4 ±4.2	12.4 ±1.0	0.68
Smoking [C]	4 (10)	2 (8)	9 (16)	0.12
Snuff [C]	4 (10)	3 (12)	1 (2)	0.21
Oral contraceptives among girls	6 (32)	5 (45)	2 (7)	0.07
CVD or DM in 1st-2nd grade relative	29 (69)	13 (52)	43 (74)	0.15
Swedish not first language	5 (11.4)	1 (4.0)	2 (3.4)	0.23

[A] The parent with highest education, [B] Includes at least 1 of the following: mechanical ventilation, neonatal seizures, sepsis, bronchopulmonary dysplasia, any degree of retinopathy of prematurity, or intraventricular hemorrhage, [C] On a daily basis
CVD = cardiovascular disease, DM = diabetes mellitus

Table 7. Anthropometry, BP, arterial stiffness, and metabolic outcomes in relation to ACS exposure. Data are mean ±SD if not stated otherwise

	Unexposed n=42	≥2 courses of ACS n=58	*p*
Anthropometry			
Weight, kg	66.4 ±11.1	65.4 ±13.8	0.68
Height, cm	173.8 ±9.1	173.7 ±8.5	0.94
BMI, kg/m^2	22.0 ±3.3	21.4 ±3.5	0.35
Overweight, n (%)	7 (17)	6 (10)	0.36
Obese , n (%)	1 (2)	3 (5)	0.47
Waist, cm	82.7 ±10.3	81.3 ±9.5	0.48
(>88cm/>102cm,♀/♂)	7 (17)	5 (9)	0.23
Blood pressure and arterial stiffness			
SBP, mmHg	121 ±13	120 ±12	0.83
>95th percentile, n (%)	3 (7)	4 (7)	0.96
DBP, mmHg	71 ±9	70 ±9	0.62
>95th percentile, n (%)	1 (2.4)	1 (1.7)	0.82
AoSBP, mmHg	102 ±10	101 ±10	0.60
AoDBP, mmHg	72 ±8	71 ±9	0.51
AI, %	2.2 ±6.6	2.3 ±9.8	0.95
elevated, n (%)	1 (2)	7 (12)	0.06
Lipid profile			
triglycerides, mmol/L	0.8 ±0.4	0.9 ±0.7	0.62
elevated, n (%)	1 (2)	4 (7)	0.29
cholesterol, mmol/L	4.1 ±0.7	4.1 ±0.8	0.81
elevated, n (%)	3 (7)	4 (7)	0.96
HDL, mmol/L	1.3 ±0.4	1.3 ±0.4	0.94
LDL, mmol/L	2.5 ±0.7	2.4 ±0.6	0.55
LDL/HDL	2.2 ±0.9	2.1 ±0.8	0.96
elevated, n (%)	0	0	
ApoA1, g/L	1.5 ±0.2	1.5 ±0.3	0.99
ApoB, g/L	0.8 ±0.2	0.8 ±0.2	0.83
ApoB/ApoA1	0.54 ±0.15	0.53 ±0.14	0.86
elevated, n (%)	1 (2)	0	0.19
Lipoprotein(a), mg/L	270 ±290	331 ±437	0.71
elevated, n (%)	3 (7)	5 (9)	0.79
Glucose/insulin profile			
Glucose, mmol/L	4.9 ±0.4	5.0 ±0.4	0.18
Insulin, pmol/L	66 ±33	70 ±70	0.90
HOMA-IR	1.2 (0.6)	1.2 (1.0)	0.84
Cortisol			
Cortisol, nmol/L	490 ±203	475 ±187	0.79

4.2.3 Neuropsychological functioning in adolescence and young adulthood after repeat courses of ACS (study III)

We found that exposure to repeat courses of antenatal corticosteroids was not associated with general deficits in higher cognitive functions, self-reported attention, adaptability, or overall psychological function. However, among the 21 outcome measurements included to tap executive functions, four revealed significant univariate group differences between unexposed subjects and subjects exposed to repeat courses of ACS (table 8.) All four were found in the domain of attention and speed. After adjusting for covariates, only two measures remained significant, the other two reached near statistical significance. In addition, the two group differences were not dose-dependent; the subgroup exposed to two courses had the lowest means and the group exposed to three courses had the highest.

There were no significant associations between repeat ACS exposure and verbal or nonverbal ability. Memory and learning were also unrelated to repeat exposure to antenatal corticosteroids.

There were no differences between groups in scores from self-report inventories on psychological health. Self-reported ADHD-symptoms were not increased in the exposed groups; neither was there any differences regarding life satisfaction obtained by self-rated quality of life inventory.

Table 8. Selected neuropsychological outcomes after repeat courses of ACS. Data are mean ±SD

	Unexposed (n=44)	Repeat ACS (n=58)	*p*	Adjusted mean difference [95% CI]	Adjusted *p*
General Cognitive Ability					
Block Design	47.7 ±9.8	46.4 ±11.5	0.54		
Vocabulary, normed z-score	-0.19 ±1.2	-0.20 ±0.99	0.96		
Memory and Learning					
RAVL	54.0 ±6.6	54.9 ±7.0	0.51		
RAVL - Retention	12.5 ±1.9	12.8 ±1.8	0.43		
Working Memory					
Spatial Working Memory, Between errors	10.9 ±8.8	12.6 ±11.1	0.41		
Digit Span Backward	7.0 ±1.9	6.4 ±2.0	0.11		
Attention and Speed					
Rapid Visual Processing	0.90 ±0.05	0.88 ±0.05	0.02	-0.02 [-0.04 to 0.00]	0.05
Trail Making Test, TMT-A, Digits	28.7 ±11.1	32.6 ±12.5	0.11		
Digit Span Forward	9.7 ±1.9	8.7 ±1.8	0.007	-0.91 [-1.65 to -0.17]	0.02
Symbol Search	40.3 ±6.5	35.8 ±7.8	0.003	-3.88 [-6.76 to -1.01]	0.009
Coding	74.2 ±13.5	67.1 ±16.1	0.02	-5.63 [-11.73 to 0.48]	0.07
Cognitive Flexibility and Inhibition					
Trail Making Test, TMT-B	64.6 ±23.5	72.2 ±21.4	0.10		
Verbal Fluency 2, Category	42.6 ±9.0	42.7 ±9.2	0.95		
Design fluency 2, Total	31.1 ±6.7	29.1 ±6.7	0.15		
Intra/extradimensional shift, Adjusted errors	21.6 ±18.3	26.6 ±19.4	0.19		
Signal stop time, Signal stop reaction time	178 ±33	190 ±47	0.16		

4.3 ACS IN EXTREMELY PRETERM GESTATIONS (STUDY IV)

4.3.1 Description of study population

Characteristics of the study group are presented in table 9.

Table 9. Perinatal characteristics of live births according to the administration-to-birth interval of ACS. Data are numbers (%).

	Administration-to-birth interval of ACS						
	no ACS n=85	**<24h n=149**	**24-47h n=66**	**48h-7d n=171**	**>7d n=103**	**ACS but unknown timing n=102**	***p***
Maternal characteristics							
Smoker	9 (10.6)	15 (10.1)	5 (7.6)	27 (15.8)	12 (11.7)	16 (15.7)	0.74
Hypertensive disease*	3 (3.5)	12 (8.1)	9 (13.6)	29 (17.0)	12 (11.7)	21 (20.6)	<0.01
Obstetric characteristics							
Born at level III hospital	26 (30.6)	107(71.8)	62 (93.9)	157(91.8)	98 (95.2)	85 (83.3)	<0.001
Tocolytic treatment	23 (27.1)	119(79.9)	42 (63.6)	111(64.9)	61 (59.2)	55 (53.9)	<0.001
PPROM	12 (14.1)	11 (7.4)	11 (16.7)	31 (18.1)	20 (19.4)	18 (17.7)	<0.001
Placenta previa	4 (4.7)	2 (1.3)	3 (4.6)	6 (3.5)	7 (6.8)	5 (4.9)	0.33
Placenta abruption	13 (15.3)	9 (6.0)	5 (7.6)	14 (8.2)	13 (12.6)	13 (12.8)	0.15
Infant characteristics							
Gestational age at birth, w							
22	29 (34.1)	11 (7.4)	1 (1.5)	3 (1.8)	0	5 (4.9)	
23	14 (16.5)	28 (18.8)	14 (21.2)	26 (15.2)	4 (3.9)	13 (12.8)	
24	7 (8.2)	32 (21.5)	13 (19.7)	38 (22.2)	25 (24.3)	22 (21.6)	<0.001
25	22 (25.9)	40 (26.9)	22 (33.3)	52 (30.4)	29 (28.2)	32 (31.4)	
26	13 (15.3)	38 (25.5)	16 (24.2)	52 (30.4)	45 (43.7)	30 (29.4)	
SGA	6 (7.1)	12 (8.1)	13 (20.0)	41 (24.0)	20 (19.4)	22 (21.6)	<0.001
Male gender	44 (51.8)	78 (52.4)	32 (48.5)	92 (53.8)	58 (56.3)	67 (65.7)	0.23
Multiplets	15 (17.6)	41 (27.5)	18 (27.3)	30 (17.5)	23 (22.3)	26 (25.5)	0.18
Surfactant <2 h after birth	27 (31.8)	94 (63.1)	32 (48.5)	84 (49.1)	57 (55.3)	60 (58.8)	0.001

*essential hypertension and/or preeclampsia and/or eclampsia.
PPROM = preterm prelabor rupture of membranes; SGA = small for gestational age (birth weight >2SD below the mean of national standard).

4.3.2 Neonatal and infant survival in relation to ACS and administration-to-birth intervals

The outcomes evaluated in this study were neonatal (alive at 28 days) and infant (alive at 365 days) survival and infant survival without major morbidity (survival without any of the following; IVH ≥3, cystic PVL, NEC, ROP ≥3 or severe BPD). We found clear associations

between timing of ACS and survival in extremely preterm infants (table 10 and figure 15). In the final model, adjusting for maternal smoking, maternal blood pressure disease, placenta previa, placental abruption, PPROM, regionalization of care, gestational age, SGA, infant gender and surfactant therapy within 2 h after birth, we found that the lowest HRs for neonatal and infant survival were found among infants unexposed to ACS. Extremely preterm infants born <24 h after ACS as well as those born more than 7 days after ACS administration also had lower HRs for survival than the reference category (infants born 48 h to 7 days after ACS administration). Survival in infants born at 24–47 h did not differ from that in infants born 48 h to 7 days after ACS administration (table 10).

In the fully adjusted model, there were no statistically significant difference between the exposed groups regarding infant survival without major morbidity, however odds ratios in infants unexposed to ACS were significantly lower as expected (table 10).

Table 10. Association between the administration-to-birth interval of ACS and survival among live-born infants

	Administration-to-birth interval of ACS					
	no ACS n=85	**<24h n=149**	**24-47h n=66**	**48h-7d n=171**	**>7d n=103**	**ACS but unknown timing n=102**
	Adjusted hazard ratio (95% confidence interval)					
Neonatal survival 28 days	0.22 (0.12-0.38)	0.44 (0.26-0.74)	2.08 (0.80-5.47)	reference	0.54 (0.29-1.03)	0.46 (0.26-0.83)
Infant survival 365 days	0.26 (0.15-0.43)	0.53 (0.33-0.87)	1.60 (0.73-3.50)	reference	0.56 (0.32-0.97)	0.53 (0.31-0.90)
	Adjusted odds ratio (95% confidence interval)					
Infant survival without major morbidity	0.33 (0.16-0.69)	0.65 (0.39-1.08)	0.67 (0.35-1.29)	reference	0.59 (0.35-1.02)	0.75 (0.43-1.30)

In summary, we found that shorter or longer administration-to-birth intervals than 24 h to 7 days were associated with an approximately doubled risk for neonatal and infant mortality. The lowest survival was found in infants unexposed for ACS – their adjusted infant mortality risk was five times higher than in infants in the reference group exposed to ACS 48 h to 7 days before birth.

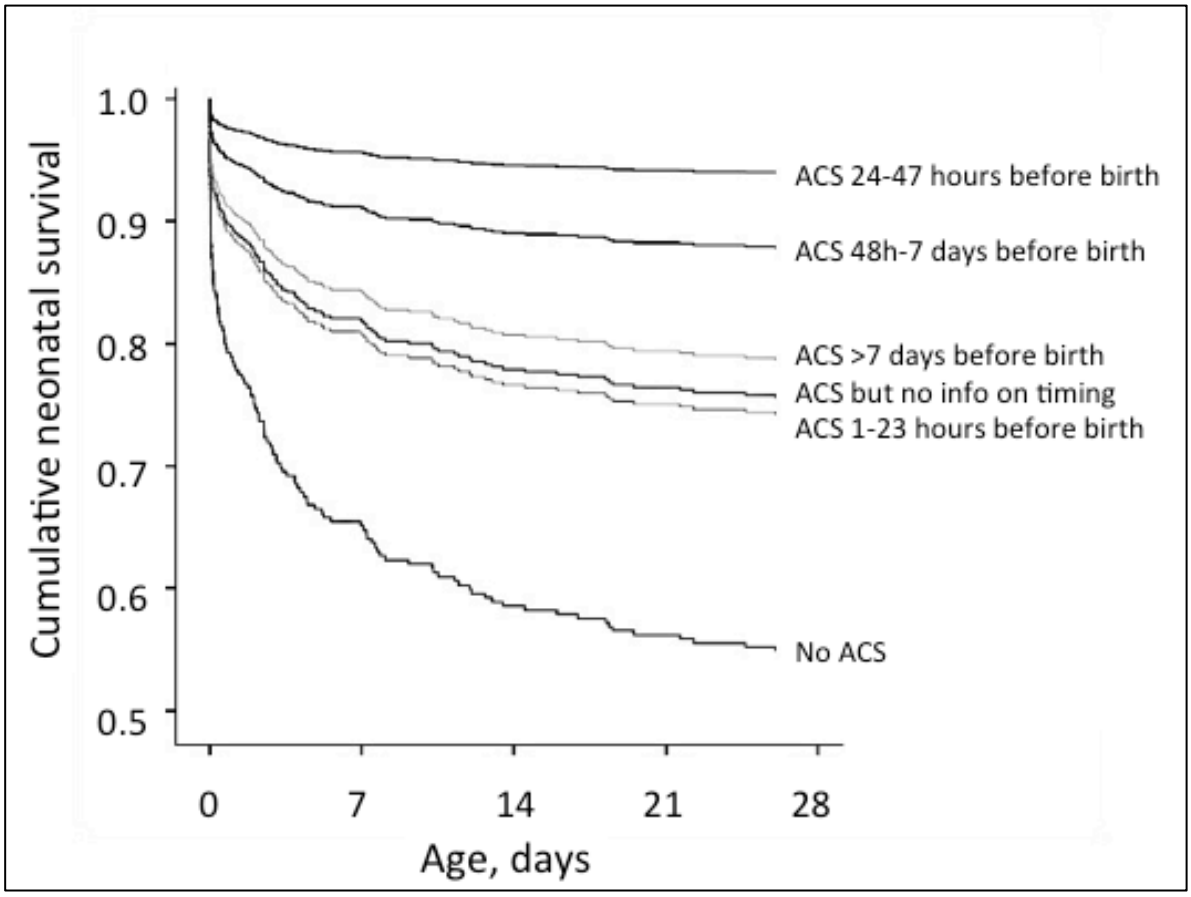

Figure 15. Adjusted survival curve for extremely preterm infants related to ACS treatment and administration-to-birth intervals.

5 DISCUSSION

5.1 REPEAT ACS AND BIRTH SIZE

A vast amount of studies on animals have previously shown a correlation between ACS exposure and restricted fetal growth. For example, birth weights of preterm lambs decreased with 11–15% after one course, with 19% after two and with 25–27% after 3 weekly courses of betamethasone.[104,133] In rabbits, Pratt et al showed a significant reduction in birth weight with increasing doses of antenatal betamethasone. Late treatment resulted in a greater decline in birth weight than did the same doses given at an earlier gestational age.[134] Studies in humans however have showed more conflicting results where some have found a correlation between ACS and size at birth whereas others have not (chapter 1.2.5.1). The reasons for the different results in animal and human studies could be explained by differences between species in sensitivity to the glucocorticoid receptors, and differences in dosing and type of glucocorticoids (dexamethasone instead of betamethasone) and stages of pregnancy among studies. The conflicting results in human studies could possibly be explained by limitations in power or the use of actual birth weight as an outcome instead of BW-SDS – corrected for sex and gestational age – which is a more appropriate outcome measure. In order to more accurately assess the effects of ACS on fetal growth, repeated assessments of fetal body size in utero would be a better way forward.

Results from our study are in line with results from animal studies, showing that increasing courses of ACS were associated with a significant reduction in SDS for BW, BL and HC, even after adjusting for possible confounders. There was a clear trend towards a linear dose–response relationship between increasing number of ACS courses and smaller size at birth, whereas GA at the start of ACS was not associated with birth size.

The strengths of this study include the use of predefined criteria for exposure and outcome data, eliminating problems with recall or other forms of recording bias. The cohort consisted of almost 100 exposed infants, which allowed for analyses with sufficient power to discover clinically important effects. Confounders of fetal growth and birth size were controlled for. By investigating anthropometry in a group of unexposed, healthy, term infants born at the same hospital and during the same time period as the infants in the exposed study group, we can conclude that the anthropometry of our subjects are representative for Swedish conditions. Finally, the majority of infants were born moderately preterm without major pregnancy complications, refining the possibility to disclose effects of ACS exposure without significant interference from severe maternal or perinatal morbidity. The limitations include the fact that although an association between increasing number of ACS courses and reduction in birth size was found, we still have to be cautious about interpretations of a causative relationship. This is an observational study, and although several other risk factors were controlled for in the analyses, confounding cannot be excluded.

5.2 REPEAT ACS AND LATER METABOLIC/CARDIOVASCULAR FUNCTION

Excess glucocorticoid exposure in fetal life has, as mentioned in previous chapters, been suggested to be a major mechanism for adverse early programming. Previous studies in animals have shown that both single and repeat courses of ACS can have lasting adverse effects on neuroendocrine function, blood pressure and glucose homeostasis.[135] The mechanisms behind this could be that excessive exposure to glucocorticoids in utero have been shown to negatively alter nephron development, renin-angiotensin-aldosterone system, baroreceptor function, elastin synthesis, and vascular function in animal studies,[106,136,137] as well as altered neurohormonal regulation, altered insulin signalling and reduced β -cell function.[64,138,139] The impact of excessive fetal glucocorticoid exposure on later cardiovascular and metabolic function in humans are however not as obvious. A few human studies have shown a possible correlation between ACS and later adverse cardiovascular risk profile. For example, Doyle et al showed that ACS to pregnant women was associated with higher blood pressure in their 14 year old offspring[68], and Dalziel et al showed higher insulin levels 30 years after ACS exposure.[65] Most of the follow-up studies in humans after ACS exposure have, as mentioned in previous chapters, not been able to show such a correlation. Accordingly, ACS administered to human fetuses appears to be less harmful than when administered to experimental animals. Besides species differences, exposure to corticosteroids earlier in pregnancy in animal studies, as well as use of higher doses and of dexamethasone instead of betamethasone, may be part of the explanation to why experimental and clinical research have come to different conclusions. It is also possible that postnatal influences might be more influential in humans, making the effects of antenatal steroids more difficult to detect.

Results from our study are in line with results from RCTs reported in preschool children and extends this knowledge to adolescence and young adulthood, showing that also at older age, repeat courses of ACS are not associated with changes in body weight/BMI, BP, blood lipids, glucose, insulin, or cortisol.

Arterial stiffness, as measured by mean AI, did not differ between groups, however, the number of subjects with an elevated AI was higher (n = 7) in the group exposed to repeat ACS courses compared with the unexposed group (n = 1). In a fairly recent observational study of slightly older adults exposed to ACS and born at an earlier GA, increased aortic arch stiffness was associated with use of ACS.[69] Aortic narrowing and alterations in aortic elasticity are in line with recent experimental research showing that lambs born preterm had thicker aortic walls and a smaller aortic lumen, and that elastin deposition was markedly increased in the aorta of lambs born preterm.[106] In that experimental study, it was not possible to determine the role of ACS. To do so, aortic structure and function should receive specific attention in forthcoming follow-up studies after randomized controlled trials testing efficacy and safety of repeat ACS courses. In addition, differences in pulse wave reflection and AI may reflect structural and functional differences in other parts of the vascular tree distal to the large arteries.

Our study showed no difference in glucose, insulin, or IR between unexposed and exposed subjects. However, glucose, insulin, and IR were inversely associated with GA at start of ACS. This finding supports previous suggestions that sensitivity to ACS, as regards glucose metabolism later in life, is dependent on GA.[69] Given that earlier ACS exposure could contribute to higher glucose and insulin levels 20 years later, this finding, although not clinically relevant in our study group, may have special implications for the discussion around safety of repeated ACS courses in older individuals than those studied herein and in pregnancies with extremely short duration.

The major strengths of this study include the cohort of individuals of both sexes exposed to varying numbers of weekly courses of ACS, and the long-term and detailed follow-up into adolescence or adulthood. Other strengths include the use of predefined criteria for exposure, eliminating problems with recall bias. All registry data have been prospectively collected and the assessment at follow-up was blinded to perinatal exposures. Finally, the majority of the infants in the study groups were born moderately preterm without major pregnancy or neonatal complications, refining the possibility to disclose effects of ACS exposure without significant interference from maternal and perinatal morbidity or postnatal steroid exposure. The small size of our cohort and the observational study design are limitations. Furthermore, we cannot exclude selection bias in study participation. There was an overrepresentation of individuals with low birth weight SDS among nonparticipants, and such bias may influence the lack of association between exposure and outcome. In addition, our findings do not apply to extremely preterm infants exposed to repeat courses of ACS and born before 28 weeks of gestation.

5.3 REPEAT ACS AND LATER NEUROPSYCHOLOGICAL FUNCTION

There is a large collection of evidence from animal studies suggesting that excessive GC exposure in utero affects the developing brain in many ways resulting in subtle or drastic changes in subsequent function. As described in chapter 1.2.4.3, ACS reduces brain mass and delays myelination in animals. In addition, it is known that the hippocampus highly expresses glucocorticoid and mineralocorticoid receptors and is particularly vulnerable to glucocorticoid manipulations[140] and changes in hippocampal structure and function have in numerous studies been shown to alter cognitive ability, behaviour and the risk of psychological disorders later in life.[141,142] In human studies, excessive exposure to synthetic GC has reassuringly not been found to cause as much adverse effects on neuropsychological function as feared. In a western Australian cohort, repeat courses of ACS was not associated with changes in general cognitive ability or in internalizing behaviour, however, they found an increased risk of aggressive-destructive behaviour, hyperactivity and distractibility at 3 and 6 years of age.[86] In addition, Spinillo et al found that repeat courses of ACS could have adverse effects on neurodevelopmental outcome in 2-year-old children, but that was true primarily for those exposed to dexamethasone rather than betamethasone.[143] The RCTs on repeat courses of ACS have, as mentioned before, showed no obvious correlation between ACS exposure and neuropsychological function in preschool/early school years (table 1).

The results from our study are in line with those from the RCTs indicating that repeat courses of ACS are not associated with deficits in higher cognitive functions or psychological health. However, mean scores obtained on 2 tests, Symbol Search and Digit Span Forward, were significantly lower for the repeat group compared with the unexposed group. Both measures fall within the executive domain of attention and speed, which reduces the likelihood that they are mere statistical artefacts attributable to multiple comparisons. Although deficits in test scores cannot be equated with behaviour problems in everyday life, the modest group differences in test scores observed in our cohort may be functionally related to the behaviour problems found in preschool children in other studies mentioned above. Previous studies in nonhuman primates investigating the long-term effects of fetal glucocorticoid overexposure on prefrontal cortex gene expression have found significant effects of this treatment on the expression of glucocorticoid receptors and that these effects were more pronounced in neonates than in adults.[144] It is tempting to speculate that the modest effects observed in our cohort of adolescents and young adults are remnants of deficits that were more clearly expressed in the younger preschool children in previous studies but have been compensated for in later development. In our dose-response analysis, we did not find any relationship between the number of corticosteroid courses and outcome. These findings are reassuring, given the dose-dependent reduction in neonatal anthropometrics previously reported to occur in this cohort. The lack of a dose-response effect could though be a result of sampling bias, given the small number of participants in each subgroup.

The strengths and limitations of this study are similar to those of study II (see chapter 5.2). Worth noticing is that this study does not address the issue of long term cognitive outcome in subjects exposed to repeat courses of antenatal corticosteroids and born before 28 weeks of gestation, it is feasible that exposure earlier in fetal life could have a different outcome.

5.4 ACS IN EXTREMELY PRETERM GESTATIONS

It is important to note that the evidence supporting ACS at <26 weeks' gestation is based mainly on laboratory studies and non-RCTs. However, given the results from many observational studies[145] suggesting a strong positive effect of ACS also in extremely preterm gestations, it would seem non-ethical to conduct RCTs on this subject.

Animal and in vitro lung tissue studies have shown that ACS might have beneficial effect on the lungs even at very early gestations.[146,147] This is supported by the findings in our study showing that the lowest survival was found in infants unexposed to ACS – their adjusted infant mortality risk was five times higher than in infants in the reference group exposed to ACS 48 h to 7 days before birth.

Animal and in vitro studies have also shown the greatest benefits from ACS on surfactant production when delivery occurs between 24 h and 7 days after a complete course of ACS.[148,149] However, human studies are contradictory. Some studies have found a decline in the effectiveness of ACS over time[126,150,151], whereas others failed to do so.[152-154] In addition

there is new evidence supporting a much faster effect of ACS on the fetus than previously known. Results from the EPICE (Effective Perinatal Intensive Care in Europe)-cohort showed that infant mortality decreased significantly when ACS was administered only a few hours before birth.[129] Previous knowledge has suggested that ACS given prior to 24 hours before birth had poor effectiveness. In our study we found that shorter or longer administration-to-birth intervals than 24 h to 7 days were associated with a doubled risk for infant mortality. These results are in line with a recent study where the authors found that ACS for preterm birth at 24–34 weeks of gestation had maximum beneficial impact on neonatal morbidity and mortality when administered 1– 7 days before birth. In that study, the decline in effectiveness of ACS after 7 days was primarily observed among neonates born at early gestations (24–28 weeks).[126]

The strengths of our study include prospective enrolment of all extremely preterm births in Sweden over a 3-year period. The study was one of the largest and the only population-based cohort evaluated for effects of ACS, and of timing of ACS, in all live-born infants born at 22–26 weeks of gestation. The ACS administration-to-birth interval was prospectively collected in hours. The outcomes were robust and clinically highly relevant. The sample size allowed for adjusted analyses of survival in relation to timing of ACS-administration and several potential confounders.

It is worth noting that we excluded stillborn infants. Among stillborn infants alive at onset of labour, a minority (n = 22/66; 30%) had been treated with ACS, meaning that these fetuses had been proactively managed. Excluding intrapartum mortality may therefore have introduced some bias, overestimating the protective effects of ACS at the time it was administered. However, under the premise that the infant was live-born, our risk estimates of no or untimely ACS are valid. The EXPRESS-database does not contain specific information on reasons for not treating with ACS; however, a recommendation to withhold treatment rested mainly on regional guidelines about management of extremely short gestations or severe malformations. Among infants born at 22 weeks of gestation, 29/49 (59%) did not receive ACS, at 23 weeks of gestation the corresponding proportion was 14/99 (14%), and in infants born at 24 weeks of gestation the proportion not receiving ACS was 7/137 (5.1%). Accordingly, crude estimates of survival in relation to ACS exposure and administration-to-birth intervals are likely to be confounded by indication. To resolve this issue, we only presented mortality data adjusted for gestational age. Another limitation is the small numbers of 22-week fetuses, with only 20 exposed to ACS and five survivors, making generalizability of our findings to this group limited. Further, we cannot exclude that other confounding factors than those included in the study affected our results. Moreover, our results cannot be extrapolated to other drugs or dosing regimens than the ones used in Sweden. Finally, the included infants were born a decade ago. Since then, neonatal care has improved even more and the evidence for less invasive ventilation strategies has become clearer.[155] Still, we think our findings are generalizable for the current extremely preterm population as in recent national statistics, the proportions of infants born at 22–26 weeks of gestation in Sweden who

were intubated at birth and mechanically ventilated were similar to those in EXPRESS. (Data from the Swedish Neonatal Quality registry).

5.5 GENERAL DISCUSSION

The studies included in this thesis have contributed to the knowledge about antenatal corticosteroid treatment. Both about its life-saving effects in extremely preterm infants, and its potentially adverse effects in a short and longer term perspective.

It is very well established that ACS is one of the most important interventions for improving outcome after preterm birth and that one single course of ACS is of no harm to the exposed child. Therefor, it is utterly important to optimize the administration of ACS, making sure that the treatment is given to all eligible mothers with threatening preterm delivery. The treatment rate could be improved by a more proactive management also in extremely preterm gestations and by considering ACS to women at risk for imminent preterm delivery in the next hours.

Whether or not to repeat the ACS course to women who remain undelivered seven or more days after the initial course is still unclear. Summarizing the evidence from previous studies and adding the knowledge from the papers included in this thesis, one can conclude that despite the emerging body of evidence linking fetal glucocorticoid exposure to permanent changes in homeostasis and organ function in experimental studies, there are little evidence of harm following exposure to repeat courses of ACS in humans. In view of the additive neonatal benefits following repeat ACS it would seem appropriate to consider at least one single rescue course of ACS to women who didn´t deliver one week or more after the initial course and who again are at risk of preterm delivery.

6 CONCLUSIONS

Repeat antenatal corticosteroids and birth size:

- Repeat courses of antenatal corticosteroids (ACS) are associated with a decline in birth size, which may have implications for later development and health.
- The reduction seen in birth size after repeat courses of ACS is dose-dependent.
- Gestational age at start of ACS exposure is not related to reduced size at birth.

Repeat antenatal corticosteroids and later metabolic/cardiovascular function:

- Repeat courses of ACS are not associated with changes in body weight/body mass index, central or peripheral blood pressure, blood lipids, glucose, insulin, or cortisol at follow-up 14 to 26 years after birth.
- Long-standing effects on the arterial tree and glucose metabolism, the latter dependent on gestational age at ACS exposure, cannot be excluded.

Repeat antenatal corticosteroids and later neuropsychological function:

- Repeat courses of ACS are not correlated to adverse effects on higher cognitive functions and behaviour at follow-up 14 to 26 years after birth.
- Repeats courses of ACS may have an impact on some aspects of executive functioning regarding attention and speed.

Antenatal corticosteroids in extremely preterm gestations:

- Exposure to ACS has a clear positive effect on morbidity and survival also in extremely preterm infants.
- The optimal time-interval for administration of ACS is 24 hours to 7 days before birth.
- Shorter or longer administration-to-birth intervals than 24 hours to 7 days are associated with a markedly increased risk of neonatal and infant mortality.

7 FUTURE PERSPECTIVES

Although much research has been conducted on this subject there are still many unanswered questions and evidence gaps to be filled. Future research areas should include testing:

- **The lowest effective glucocorticoid dose**
  - It is not known if a lower dose of glucocorticoids could be as effective as the current dosing regime. If a lower dose of ACS can be shown to be equally effective, side effects may be reduced and it could possibly lead to a more proactive management of repeating the courses to women undelivered after the initial course of ACS.
- **The optimal timing of glucocorticoid administration before preterm birth**
  - It is not fully clear how fast ACS can exert its effect on the fetus and how long the effect lasts. Very recent data challenge the current thinking about optimal timing of ACS[129] and more research on this area is warranted.
- **The effect of ACS in low-resource settings**
  - Almost all studies on ACS have been conducted in high-income countries; therefore, the results may not be applicable to low-resource settings with high rates of infections. A recent cluster-randomised trial in low- and middle-income countries surprisingly showed that ACS led to increased neonatal mortality in the population.[156] More research on this area is crucial.
- **The effect of ACS in multiple pregnancies and other high-risk obstetric groups**
  - There are insufficient data regarding risks and benefits of ACS on high-risk obstetric groups such as multiple pregnancies, premature rupture of membranes and hypertension syndromes.[12]
- **The possible adverse effects of repeat courses of ACS into adulthood**
  - Although results from follow-up studies into childhood after repeat courses of ACS are reassuring there is a need for follow-up into adulthood before we can conclude that repeat courses of ACS is a safe treatment. The RCTS on this subject will have to wait decades until the included children have become adults; hence, observational studies are also warranted.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Att födas för tidigt är vanligt i Sverige och resten av världen. Tack vare stora förbättringar i vården av den gravida kvinnan och det nyfödda barnet kan numera barn som är födda så tidigt som graviditetsvecka 22-23 överleva. Majoriteten av de för tidigt födda barnen överlever utan några bestående men medan en andel av framförallt de extremt för tidigt födda barnen drabbas av olika funktionsnedsättningar. Exempel på sådana funktionsnedsättningar kan vara försämrad syn, nedsatt lungfunktion, neurologisk påverkan eller nedsatt kognitiv funktion. Dessutom löper de för tidigt födda barnen en ökad risk för folksjukdomar såsom diabetes och hjärt-kärlsjukdomar senare i livet.

En stor förklaring till att fler överlever tidig födsel är att man på 70-talet införde behandling med kortison till gravida kvinnor som riskerar att föda sitt barn för tidigt. Behandlingen kallas för *antenatal kortisonbehandling* och fungerar bl a så att kortisonet får fostrets lungor att mogna snabbare. När barnet sedan föds är det bättre rustat att andas själv och får mer sällan och mindre allvarlig lungsjukdom än barn vars mammor inte fått kortison. Det är även visat att behandlingen minskar risken för hjärnblödning, infektioner och allvarliga tarmåkommor hos det nyfödda barnet. Pga de positiva effekterna är antenatal kortisonbehandling en väldigt vanlig behandling över hela världen. Dock finns det en oro för att behandlingen eventuellt kan ge biverkningar på barnet. Denna oro har uppstått pga att forskningsstudier på djur har visat att det finns starka kopplingar mellan antenatal kortisonbehandling och bieffekter på bl a fostertillväxt och hjärnans utveckling samt ökad risk för hjärt-kärlsjukdomar och neuropsykologiska funktionsnedsättningar senare i livet. Huruvida denna koppling även finns hos människor är inte lika tydligt och det behövs mer forskning inom området för att ta reda på om så är fallet.

Huvudsyftet med denna avhandling var dels att undersöka om det finns något samband mellan antenatal kortisonbehandling och olika biverkningar på barnet på kort sikt samt senare i livet. Vi har därför undersökt en grupp om ca 100 individer födda på Danderyds sjukhus under åren 1983-1996 och som under fosterlivet exponerats för varierande doser av kortison. I studie I undersökte vi om behandlingen påverkar tillväxten av fostret och vi fann att det finns ett sådant samband. Barn som hade utsatts för höga doser kortison i fosterlivet var både lättare, kortare och hade ett något mindre huvudomfång vid födseln än normalt. Man vet sedan tidigare att en hämmad fostertillväxt kan leda till hälsoproblem senare i livet varför detta är en potentiellt allvarlig biverkan. I studie II och III följde vi upp dessa barn i tidig vuxen ålder (14-26 år) för att undersöka om kortisonbehandlingen var kopplad till ökad risk för antingen hjärt-kärlsjukdom (studie II) eller neuropsykologiska funktionsnedsättningar (studie III). Vi fann inga tydliga samband mellan kortisonbehandling i fosterlivet och senare ohälsa i vår studiegrupp vilket är en viktig iakttagelse för att kunna fortsätta rekommendera behandlingen utan oro för framtida biverkningar. Det behövs dock fler studier på fler individer och i äldre åldrar för att kunna befästa denna iakttagelse.

I studie IV undersökte vi en grupp barn som fötts extremt för tidigt (före graviditetsvecka 27) i Sverige. Vi ville undersöka om antenatal kortisonbehandling var lika effektiv även bland dessa väldigt tidigt födda barn vilket inte har studerats i någon större utsträckning tidigare. Ett annat syfte med studien var att undersöka om det är viktigt att ge kortisonbehandlingen vid ett visst tidsintervall före förväntad födsel. Vi fann att bland de ca 700 barnen i vår undersökta grupp hade antenatal kortisonbehandling en tydlig och stark positiv effekt på barnens överlevnad. Våra resultat visade även att för störst skyddseffekt så ska behandlingen ges 1-7 dygn före beräknad förlossning.

Denna avhandling har bidragit till ökad kunskap angående antenatal kortisonbehandling. Sammanfattningsvis kan vi från resultatet av våra studier konstatera att:

- Antenatal kortisonbehandling påverkar fostrets tillväxt negativt men effekten tycks vara övergående då inga skillnader i längd och vikt sågs vid långtidsuppföljning.
- Till skillnad från djurstudier verkar inte antenatal kortisonbehandling hos människa leda till ökad risk för metabol ohälsa eller neuropsykologiska funktionsnedsättningar i ung vuxen ålder.
- Antenatal kortisonbehandling har en livräddande effekt även på extremt för tidigt födda barn.
- Det är viktigt att ge kortisonbehandlingen vid rätt tidpunkt för att uppnå optimal effekt.

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