Obesity, weight reduction treatment and IVF

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To families

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ABSTRACT

Background: Obesity is a growing problem on a global scale and women with obesity have a higher risk for infertility and complications, for both mother and child, in pregnancy and birth. This has been shown both regarding spontaneous pregnancy and in vitro fertilization (IVF). Due to this, being obese may exclude women from qualifying for publicly funded fertility treatments in the Nordic countries. Weight loss has been shown to affect fertility positively in obese women with anovulation but the effect in women scheduled for IVF is not clear.
Aims: To study the effect of a weight reduction intervention on reproductive, obstetric, neonatal, and metabolic outcomes in women with infertility and obesity who were scheduled for IVF treatment.
Methods: *Paper I*: 305 women with World Health Organization (WHO) grade I obesity and an indication for IVF were randomized to weight-reduction with a very low-calorie diet (VLCD) followed by IVF (n=152) or IVF-only (n=153). The primary endpoint was live birth. *Paper II:* The births from Paper I were analyzed for perinatal and maternal outcomes and the primary endpoints were birthweight and deviation from expected birthweight. *Paper III:* 195 women from Paper I having serum samples fulfilling standardized criteria were analyzed as one cohort. Correlation between metabolic and anthropological factors to pregnancy and live birth after IVF was calculated. Metabolic changes of the weight reduction treatment were analyzed.
Results: *Paper I:* The weight-reduction-and-IVF group achieved a significantly higher weight loss compared to the IVF-only group. There was no significant difference in live birth between the groups but there were

significantly more live births achieved through spontaneous pregnancies in the weight-reduction-and-IVF group. *Paper II:* There was no significant difference in birthweight or deviance from expected birthweight between the groups. Perinatal and maternal outcomes were generally good and there was no difference between the groups. *Paper III:* No metabolic or anthropological variables were found to predict pregnancy or live birth after IVF.
Conclusion: A VLCD treatment prior to IVF does not affect the chance of live birth in women with grade I obesity. No detrimental effects of the VLCD on the IVF, maternal, or perinatal outcomes were found. No metabolic or anthropological factors linked to obesity were found to predict pregnancy or live birth after IVF.

Keywords: obesity, infertility, low calorie diet, in vitro fertilization, weight reduction, adipokines

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SAMMANFATTNING PÅ SVENSKA

Fetma är ett växande problem i världen och ungefär en av sju kvinnor i de nordiska länderna bedöms lida av fetma. Fetma är kopplad till ett sämre utfall för mor och barn vad gäller fertilitet, graviditet och förlossning. Detta har framkommit både för spontana graviditeter och de som har åstadkommits med hjälp av provrörsbefruktning. I de nordiska länderna nekas kvinnor med fetma över en viss gräns till statligt finansierad fertilitetsbehandling p.g.a. sämre resultat av behandlingar och ökade risker för mor och barn under graviditet och förlossning. Viktminskning har visat sig förbättra chansen till graviditet hos kvinnor som lider av ägglossningsrubbning men effekten på kvinnor med fetma och planerad provrörsbefruktning är inte klar.
Vi utförde en studie där 305 kvinnor med fetma och planerad provrörsbefruktning delades i två grupper. Den ena gruppen (152 kvinnor) fick en 16 veckors lång viktminskningsbehandling i form av måltidsersättningar med lågt energiinnehåll inför sin planerade provrörsbefruktning. Den andra gruppen (153 kvinnor) fick en provrörsbefruktning direkt. Vi jämförde grupperna med hänsyn till andelen kvinnor som födde barn. Fokus var även på mor och barns hälsa. Vi studerade också om fettvävens distribution och fetmarelaterade ämnen i blod kunde prediktera chansen att bli gravid och få barn genom provrörsbefruktning.
Vi fann att viktminskningsbehandlingen fungerade bra och hjälpte kvinnorna att gå ner i vikt, 9,44 kg i genomsnitt. Detta resulterade i att vid tidpunkten för starten av provrörsbefruktningen var de ungefär 10 kg lättare i genomsnitt än gruppen som gick direkt till provrörsbefruktning. 45 (29,7%) kvinnor i viktminskningsgruppen fick barn medan 42 (27,5%) fick barn i provrörsbefruktning-direkt gruppen. Denna skillnad var inte statistiskt signifikant. Av de kvinnor som fick barn var det signifikant fler i viktminskningsgruppen (16 (10,5%)) jämfört med provrörsbefruktning-direkt gruppen (4 (2,6%)) som fick barn genom en spontan graviditet. Barnen som föddes var jämförbara vad gäller födelsevikt och avvikelse från den förväntade födelsevikten och både de och mammorna var generellt friska i båda grupperna. Vi hittade inga fetmarelaterade ämnen i blodet eller mått som mäter fetma och fettvävs distribution som kunde prediktera chansen att bli gravid och få barn genom provrörsbefruktning.

Sammantagit visar dessa studier att trots att viktminskningen lyckades åstadkomma en avsevärd viktminskning så hade den ingen effekt på chansen att få barn. Den hade heller inte någon effekt på barnens födelsevikt och inga fetma relaterade faktorer kunde hittas som predikterar chansen att få barn. För dessa kvinnor hjälper en effektiv viktminskningsbehandling de inte att skapa bättre chans till att få barn genom planerad provrörsbefruktning.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Einarsson,S, Bergh,C, Friberg,B, Pinborg,A, Klajnbard,A, Karlström,P-O, Kluge,L, Larsson,I, Loft,A, Mikkelsen-Englund,A-L, Stenlöf,K, Wistrand,A, Thurin-Kjellberg,A. Weight reduction intervention for obese infertile women prior to IVF: a randomized controlled trial. Human Reproduction 2017; 32(8): 1621-1630.

II. Einarsson,S, Bergh,C, Kluge,L, Thurin-Kjellberg,A. No effect of weight intervention on perinatal outcomes in obese women scheduled for in vitro fertilization treatment. Acta Obstetricia et Gynecologica Scandinavica 2019; 98: 708-714.

III. Svenson,H, Einarsson,S, Olausson,D, Kluge,L, Bergh,C, Edén,S, Lönn,M, Thurin-Kjellberg,A. Inflammatory and metabolic markers in relation to outcome of In Vitro Fertilization in a cohort of predominantly overweight and obese women. Submitted.

CONTENT

ABBREVIATIONS

AFABP	adipocyte fatty-acid binding protein
ART	assisted reproductive technology
BMI	body mass index
CI	confidence interval
COH	controlled ovarian hyperstimulation
CRP	C-reactive protein
ET	embryo transfer
FAS	full analysis set
FSH	follicle stimulating hormone
hCG	human chorionic gonadotropin
HR	hazard ratio
hsCRP	high sensitivity C-reactive protein
ICSI	intracytoplasmic sperm injection
ITT	intention to treat
IUFD	intra uterine fetal death
IVF	in vitro fertilization
LBR	live birth rate
LCD	low calorie diet
LGA	large for gestational age

LH	luteinizing hormone
OHSS	ovarian hyperstimulation syndrome
OR	odds ratio
PCOS	poly cystic ovary syndrome
PESA	percutaneous epididymal sperm aspiration
PP	per protocol
RCT	randomized controlled trial
RYGB	Roux-en-Y Gastric Bypass
SD	standard deviation
SET	single embryo transfer
SGA	small for gestational age
SMM	severe maternal morbidity
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
WHO	World Health Organization
WHR	waist-hip ratio
WHtR	waist-to-height ratio

1 INTRODUCTION

1.1 OBESITY AND GENERAL HEALTH

Historically, problems related to the nutritional status have concerned lack of good nutrition. Famine and malnutrition were the problems our ancestors had to deal with, and providing food with stability and security was a major challenge. With developments in agriculture and the food industry, prosperity has reached higher levels than ever before, and food is readily available in most societies. The energy density of processed food has also increased while at the same time becoming cheaper than ever before. These conditions have led to obesity becoming a health problem in almost all parts of the world. Overweight and obesity increased globally between 1980 and 2013 by 8.1% (28.8% to 36.9%) for men and by 8.2% (29.8% to 38.0%) for women. Such increases are observed all over the world in both developed and developing countries, the Nordic countries included [1]. The World Health Organization (WHO) has set a goal to halt the rise in obesity on a global scale by 2025. However, trends in recent years make the likelihood of reaching that goal very small, with no nation having succeeded in reversing the growth of obesity in the last three decades [2].

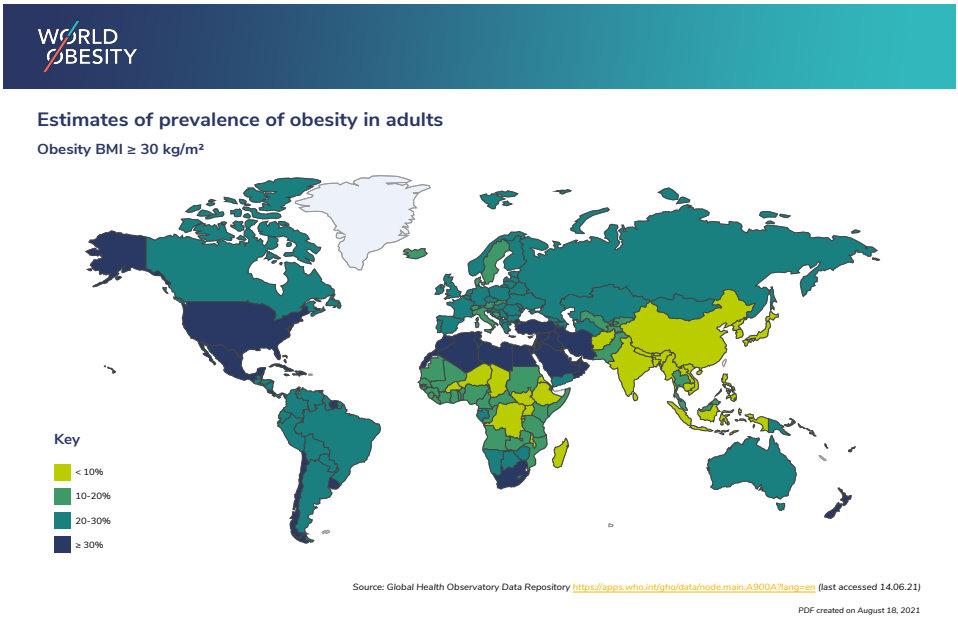

Figure 1 Estimates of prevalence of obesity in adult women

A number of diseases and health problems have been linked to obesity, making obesity a major contributor to the global burden of disease [3]. These include serious and chronic diseases, such as type II diabetes, some cancers and cardiovascular disease [4]. A large study showed a clear association between greater body mass index (BMI) and higher all-cause mortality, hazard ratio (HR) 1.39 (95% confidence interval (CI) 1.34-1.43) per 5 kg/m^2 increase in BMI above 25.0 kg/m^2 [5].

BMI is a measure of nutritional status defined as a person's weight in kilograms divided by the square of the person's height in meters (kg/m^2). Obesity has been defined by the WHO as a BMI of 30.0 kg/m^2 or above. See table 1.

BMI category	Nutritional status
Below 18.5	Underweight
18.5-24.9	Normal weight
25.0-29.9	Pre-obesity / Overweight
30.0-34.9	Obesity class I
35.0-39.9	Obesity class II
Above 40	Obesity class III

Table 1 Nutritional status according to WHO

BMI is the most common method of defining nutritional status in health care today, but other measures exist. Waist-to-height ratio (WHtR) and waist–hip ratio (WHR) reflect the distribution of fat better than BMI, especially in central obesity. WHR adjusted for BMI combines measurements of central and general obesity and has been shown to be more informative than BMI alone regarding the risk of ischemic heart disease mortality [6]. Other methods, such as magnetic resonance imaging, plethysmography, and dual-energy X-ray absorptiometry, that measure body composition with higher precision but require high technology equipment and specially trained personnel and are expensive and not readily available.

Obesity has been shown not only to have a negative effect on the health of the obese individual itself but also to impose an intergenerational effect and affecting the offspring of mothers with obesity. It has been shown that mothers who were born large for gestational age (LGA) have a higher risk of an elevated BMI in adulthood. In turn a high BMI in the mother increases the risk for giving birth to a LGA child [7]. In this way there is a risk of an ever-growing cycle of obesity. In another study the odds of childhood obesity were found to be increased by 264% when the mother was obese before conception [8].

1.2 OBESITY, PREGNANCY, AND BIRTH

1.2.1 COMPLICATIONS DURING PREGNANCY

There are numerous negative effects of obesity on pregnancy and birth, including an elevated risk of miscarriage that is as much as 50-70% higher in obese women compared to women of normal weight [9]. In a study of recurrent early pregnancy loss, obese women had an increased frequency of euploid miscarriage compared to women of normal weight, which suggests that there are some obesity-linked detrimental factors in the environment of the embryo/fetus [10]. In large population-based studies the risk for maternal complications such as pregnancy induced hypertension and pre-eclampsia has been shown to be at least doubled, with further increases for higher BMI values [11-14]. The risk of mild to moderate pre-eclampsia increases by 9.5% per BMI unit. A BMI above 35 kg/m^2, quadruples the risk of mild to moderate preeclampsia and triples the risk of severe preeclampsia [15]. Further, gestational diabetes is clearly linked to a higher BMI with an odds ratio of three in overweight women (BMI 25-30 kg/m^2) rising to 12 at BMI 30-40 kg/m^2 [13]. Antenatal and postpartum depression and anxiety are more common in mothers with a high BMI [16]. Similarly the number and duration of maternal and neonatal admissions to hospital are increased for higher BMI values, increasing the burden on the health service and health service costs [13] [17].

1.2.2 COMPLICATIONS IN LABOR AND DELIVERY

The odds ratio (OR) for induction of labor (1.77, 95% CI 1.73-1.81); instrumental delivery (1.16, 95% CI 1.12-1.21); major postpartum bleeding (1.19, 95% CI 1.15-1.23); and cesarean delivery (1.76, 95% CI 1.72-1.80) is significantly higher for women with obesity grade I as compared to women of normal weight [11].

1.2.3 NEONATAL COMPLICATIONS

The odds ratios for the following neonatal complications, for children of mothers with obesity compared to children of mothers of normal weight, range from 1.58 to 3.55: meconium aspiration; fetal distress; Apgar score below seven; macrosomia (birthweight >4500g); shoulder dystocia; delivery <32 weeks; delivery > 42 weeks; intra uterine fetal death (IUFD); and early neonatal death [11, 12]. The risk of extreme preterm birth (<28 weeks), as compared to women of normal weight, both spontaneous and medically indicated, has been clearly linked to obesity with an OR of 1.58 (95% CI 1.39-1.79) for BMI 30-35 kg/m^{2}and rising to 2.99 (95% CI 2.28-3.92) for BMI >40 kg/m^2 [18]. A higher risk of cerebral palsy in children of obese mothers has been found in a large Swedish study with a hazard ratio (HR) ranging from 1.28 to 2.02 for obesity class I-III. This study was limited to children born at term, with the outcome partly related to asphyxia-related neonatal complications [19]. Congenital anomalies are more common in babies born to mothers with obesity, as compared to mothers of normal weight. In a large national cohort study in Sweden 3.5% of all live-born singletons had a major birth defect. The risk of a major birth defect for offspring of mothers of normal weight was 3.4% and gradually went up to 4.7% for women of obesity class III. This higher risk was evident for birth defects of most organ systems and most prominent for birth defects of the nervous system [20]. Several studies have found a rise in BMI to be an independent risk factor for fetal and infant mortality, with risk doubling when BMI reaches obesity grade II or III [21, 22]. In a meta-analysis by Aune et al. 2014 the relative risk increased by 15-24% per 5-unit increase in maternal BMI (from the reference BMI of 20 kg/m^2) for fetal death, stillbirth, perinatal death, neonatal death, and infant death [21].

1.3 INFERTILITY AND IVF

Infertility is defined by WHO as a disease of the male or female reproductive system that leads to failure of achieving a pregnancy after 12 months or more of regular unprotected sexual intercourse [23]. Infertility has a prevalence of around 9% (5-15%), and of these couples, 56% are seeking medical care for the condition [24]. Approximately one of every four women is expected to experience a subfertility period during her lifetime, 3% of all women are expected to be involuntarily childless, and 6% of parous women are not able to have as many children as they would wish [25]. The underlying reasons for infertility are equally distributed between men and women. The main underlying reasons in women are hormonal problems resulting in anovulation, tubal occlusion, endometriosis, and uterine problems linked to myomas or polyps. The main underlying reasons in men are hormonal problems resulting in decreased sperm production, testicular failure due to genetic, autoimmune or iatrogenic factors and obstruction in the ejaculatory ducts. Lifestyle factors such as obesity, smoking and alcohol consumption are also known to negatively affect fertility in both men and women [26].

In 1978 a major breakthrough in the treatment of infertility was made when the first child was born after in vitro fertilization (IVF) which took place in the Bourn Hall clinic in England [27]. IVF treatment has since evolved and has become an effective and safe treatment that has resulted in more than 9 million children born globally [28]. In 2010 the pioneer Robert G. Edwards received the Nobel prize for the development of in vitro fertilization.

Figure 2 The Bourn Hall Clinic, where the first successful IVF treatment was performed. Source: CC BY-SA 2.0, https://commons.wikimedia.org/w/index.php?curid=191728

In vitro fertilization requires that both sperm cells and oocytes are available. In most cases it is not problematic to get access to the sperm cells. They naturally leave the body at ejaculation and therefore can usually be made available. In around 1% of men no sperm are found in the ejaculate. In approximately 50% of these men, it is possible to retrieve sperm by surgical aspiration of tissue from the testis. In cases with obstruction, it is possible to perform a percutaneous epididymal sperm aspiration (PESA). In this procedure sperm cells are aspirated with a needle from the epididymis, the organ in which the sperm cells are stored after production and until ejaculation. In cases where the problem is related to the testis, sperm cells can be retrieved from tubuli of the testis. This tissue can be accessed by performing either a testicular sperm aspiration (TESA) or testicular sperm extraction (TESE). Both procedures can be performed under local anesthesia and that involve efforts to harvest tubuli tissue from the testis. TESA is

performed by percutaneously aspirating tissue from the testis with a needle. TESE is a procedure that entails opening up the scrotum and the various layers of tissue surrounding the testis to get access to the testicles. With that access the surgeon is able to, macro-, or microscopically inspect the testicular tissue and take focused biopsies in hopes of finding sperm. The success rate for findings sperm is almost 100% for obstructive diseases while much lower for testicular diseases, less than 50%. If no sperm can be found by these means or if no sperm is available for other reasons, donor sperm may be used.

In order for oocytes to be available, in practically sufficient amounts, a controlled ovarian hyperstimulation (COH) must be performed. In COH the growth and maturation of oocytes is stimulated by administering follicle stimulating hormone (FSH) for a period of approximately 10-14 days leading to a number of oocytes responding by developing and becoming mature. In the natural state the pituitary responds to the oocytes maturing by sending out a surge of luteinizing hormone (LH) to drive the final maturation of the oocyte and the ovulation process. In COH it is necessary to control the timing of the final maturation so that the oocytes can be harvested when a sufficient amount is mature. It is therefore necessary to prevent the pituitary of sending the natural LH signal of ovulation. This can be done in two ways; one is administering a gonadotropin releasing hormone (GnRH) agonist, and the other is by using a GnRH antagonist. A GnRH agonist initially stimulates the pituitary to produce the gonadotropins (FSH and LH), but after a few days of treatment, the pituitary downregulates the receptors for GnRH, consequently suppressing the production of gonadotropins in 10-14 days. This protocol, usually called the agonist- or the long protocol, needs to be started 14 days before the start of FSH stimulation. The GnRH antagonist quickly offsets the effect of the GnRH and in this way suppresses the production of gonadotropins. Its administration can be started a few days after the start of FSH stimulation, but it must begin before the oocytes reach maturation. This protocol is referred to as the antagonist- or short protocol. When clinical evaluation of the stimulation shows that the oocytes are mature, an ovulation trigger, usually in the form of human chorionic gonadotropin (hCG), is administered to stimulate final maturation and ovulation of the oocytes. A transvaginal ultrasound guided needle aspiration of the oocytes is performed under local anesthesia and sedation approximately 36 hours after the

ovulation trigger and just before the expected ovulation. By these means the oocytes become available for in vitro fertilization. In the laboratory fertilization is facilitated either by standard IVF, where sperm and oocytes are mixed and fertilization can take place through natural selection, or by intracytoplasmic sperm injection (ICSI), where one sperm is microscopically injected into each oocyte. Even though the mode of fertilization can be either standard IVF or ICSI, the whole process is typically referred to as an IVF treatment in everyday language. In this thesis 'IVF' is used to refer to this kind of treatment irrespective of mode of fertilization. The next step is to culture the fertilized oocytes, which are called pre-embryos if cell division has started, and after 2-5 days the most promising pre-embryo that has a normal maturation pattern and morphology is transferred into the uterine cavity of the woman. Surplus good quality pre-embryos can be frozen and stored for future use. In 2019 the live birth rate (LBR) in Sweden in fresh cycles was 20% per started cycle and 29% for cycles in which an embryo transfer could be performed. Of all embryo transfers, 87% were single embryo transfers (SET) and the remaining 13% were double embryo transfers [29].

1.4 OBESITY AND FERTILITY

Obesity is known to affect the chance of spontaneous pregnancy and also to have numerous other effects on fertility. Women with obesity have a higher incidence of problems with fertility, longer time-to-pregnancy and an almost doubled risk of not conceiving within one year of trying [30-35]. In a group of sub fertile women, the chance of spontaneous pregnancy went down 4% for every rise in BMI unit above 29 kg/m^2 [36]. Being obese in adolescence has been shown to be a risk factor for being childless, for never becoming pregnant [37] and for ovulatory infertility [38]. The mechanism of these effects are complex and not completely understood but are believed to involve the hypothalamic-pituitary-gonad axis, metabolic hormones and inflammatory mediators that affect the ovary and uterus in a negative way [39].

Adipose tissue is considered a highly active endocrine organ that affects and regulates multiple processes such as metabolic homeostasis, inflammation, and reproduction [40]. Adipose tissue produces several different kinds of substances (e.g., hormones, cytokines, proteins) which are commonly called adipokines, that mediate the effect of adipose tissue on other organs. An altered response in the immune system and an elevated state of inflammation are linked to obesity and are a major cause in the development of insulin resistance as well as other problems linked to obesity [41, 42].

Obesity has been identified as a cause of inflammation commonly measured through C-reactive protein (CRP), an inflammatory protein [43]. Lower levels of fecundability has also been linked to higher levels of CRP and this lowered fecundity was also linked to a higher BMI but the causality is unclear however [44]. Raised CRP is thought to be a sign of an altered inflammation pathway and it has been postulated that this change may affect the inflammation that is necessary at the time of ovulation and implantation [45]. A recent systematic review, including eight studies regarding CRP before, during and after IVF treatment found that a state of inflammation, as indicated by increased levels of CRP, seems to have a negative effect on IVF outcomes. However, the present literature is too heterogeneous to be able to draw firm conclusions and further research is needed [46].

Leptin is an adipokine secreted by adipocytes and has a positive correlation to the amount of adipose tissue. Sometimes referred to as the satiety hormone, it signals the energy reserve of the body stored in adipose tissue to

the hypothalamus. There it functions by raising metabolism and inhibiting hunger and food intake [47]. With respect to fertility, leptin stimulates the hypothalamic-pituitary axis, and thereby facilitating the production of gonadotropins. It is thought to play a major role in puberty by signaling sufficient energy reserves. In addition, deficiencies in leptin production or signaling due to mutations have been shown to cause hypogonadotropic hypogonadism [48].
In obesity there is a higher level of leptin, which is thought to produce a state of leptin-resistance that prevents the hormone from controlling satiety and stimulation of the hypothalamic-pituitary axis. The lack of stimulus on the hypothalamic-pituitary axis is one of the possible pathways that can lead to anovulation, which is the most common cause of infertility in women with obesity [49]. Leptin not only affects the hypothalamus but also has a direct effect on the ovary and the uterus. Leptin receptors are found in the theca and granulosa cells of the follicle, in the oocyte itself and in the endometrium, suggesting an important role in oocyte maturation, sex hormone synthesis and implantation [49].
A systematic review studied the effect of leptin on female fertility in general and IVF in particular [49]. The review found indications that high serum leptin levels had a detrimental effect on female fertility in general and conflicting results on the effect on pregnancy outcome in IVF. The main conclusion of the review was that the studies were very heterogeneous, making it difficult to draw reliable conclusions on the effect of leptin on different parameters of the IVF process.

Both plasma and follicular fluid concentration of free fatty acids are higher in women with obesity. An excess of free fatty acids has been shown to correlate with morphological changes and detrimental maturation of the oocytes [50] [51, 52]. This effect is believed to be mediated through the toxic effect of excess free fatty acids (lipotoxicity) and has also been shown to affect the embryo and the implantation process [45, 53]. The adipocyte fatty-acid binding protein (AFABP) is an adipokine that has been shown to be a lipid chaperone that is thought to mirror the amount of free fatty acids in blood. It has also been directly linked in multiple ways to the development of metabolic syndrome and atherosclerosis [54].

1.5 OBESITY AND IVF

Obesity has been shown to effect IVF results negatively in many ways. A response to the stimulation of the ovaries is harder to achieve, which is shown by the need for higher doses of gonadotropins, and accompanied by a higher rate of cancelled cycles [55-59]. The number of oocytes and embryos have been shown to be lower [56, 60] the size of the oocytes smaller, and the embryos′ development faster, there are fewer cells in the developed blastocysts, lower glucose consumption, different amino acid metabolism and higher levels of endogenous triglycerides [61]. Studies on the quality of the embryos have been conflicting, with some showing obesity to have a negative effect [61, 62] and others not [60, 63, 64]. Lower chance of clinical pregnancy [59, 60, 65] and a higher risk of miscarriage [57, 59] have been shown in a number of studies. In all, the live birth rate of women with obesity is lower, at least 15-20%, compared to women of normal weight [56, 59, 65-68].

How exactly obesity affects the results of IVF is not fully understood but there are indications and evidence for both an effect on the oocyte or pre-embryo prior to implantation and on the receptivity of the endometrium. The negative effect of obesity on the receptivity of the uterus has been shown in studies in which oocyte recipients receive donor oocytes from donors of normal weight. In these studies women with obesity had a significantly lower live birth rate as compared to normal-weight oocyte recipients, with a relative difference of 20-25% [69, 70]. Another study, examining the effect of the BMI of the donor on the result of oocyte donation treatments, adjusted for the BMI of the recipient, showed a negative effect of a higher donor BMI on live births [71]. This result suggests an effect on the oocyte prior to fertilization and implantation.

Adipose tissue seems to affect fertility differently depending on its distribution. Studies have shown that a lower chance of success in IVF is not only linked to overweight and obesity defined by BMI but also to a larger waist circumference and a higher WHR [72] [73].

There are only a few studies reporting on complications in IVF in general, and the existing evidence regarding the effect of BMI on complications in IVF is therefore limited. A systematic review 2012 studied complications in IVF and found sufficient data to draw firm conclusions only with regard to ovarian hyperstimulation syndrome (OHSS), multiple pregnancies and

extrauterine pregnancies. This study found no convincing evidence that more of these complications were linked to IVF treatments for women with obesity compared to women of normal weight [74].
A study of the effect of the combination of high BMI and IVF on preeclampsia found that overweight and obesity were, as expected, independently linked to preeclampsia, while not IVF by itself. However, there were signs of IVF compounding the risk seen in the overweight and obesity groups [75]. Another study by the same author showed that IVF was associated with a doubled risk of severe maternal morbidity (SMM), defined as a severely ill woman who dies or experiences a near-fatal event during, or within, 6 weeks of the cessation of pregnancy. Women who were overweight or obese had a 25% higher risk for SMM than women of normal weight. There were, however, no indications of a compounding effect of obesity on the higher risk for SMM linked to IVF, or vice versa [76].
Given the above-mentioned negative effects of obesity on pregnancy and birth, and the fact that weight loss for obese women having anovulation often resolves infertility, BMI limits have been advocated for treatment with IVF, with public clinics in Sweden setting a BMI limit of 30 kg/m^2 or 35 kg/m^2.

1.6 WEIGHT INTERVENTION

In general, for weight reduction intervention, compliance and drop out is a problem. This is illustrated by a 30-94% drop out rate at one year [77] [78, 79]. A systematic review covering 26,455 patients undergoing different types of weight intervention treatments found that simply recommending weight loss or exercise alone resulted in minimal or no weight loss. As shown in Figure 3, methods involving reduced energy in the diet or weight-management medication, all resulted in weight loss for the first 6 months, with losses still present at 24, 36 and 48 months. These methods can be expected to result in a weight loss of approximately 5-8.5 kg at 6 months, 4.5-7.5 kg at 12 months and 3-4 kg at 24, 36 and 48 months. Such sustainable weight loss is, despite being modest, beneficial for lowering the risk of developing chronic health problems [77]

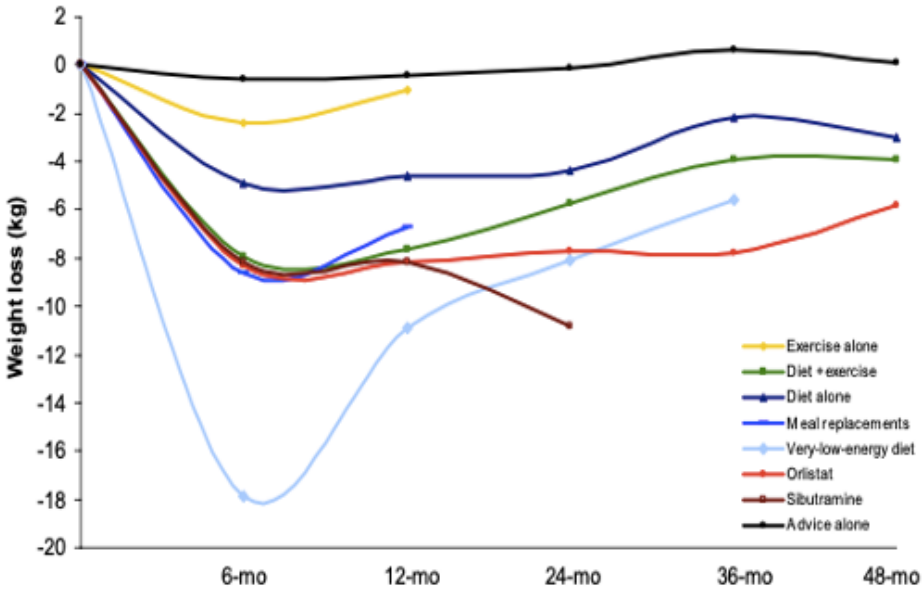

Figure 3 Average weight loss of subjects completing a minimum 1-year weight-management intervention; based on review of 80 studies (N=26,455; 18,199 completers [69%]).

*Reprinted from the Journal of the American Dietetic Association, **107**(10), Franz, M.J., et al., Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up, p. 1755-67, Copyright (2007), with permission from Elsevier.*

A low-calorie-diet (LCD) consists of 1000-1200 kcal/day and a very-low-calorie-diet/very-low-energy-diet (VLCD/VLED) approximately 800 kcal/day [80]. In a systematic review and meta-analysis VLED combined with behavioral programs were shown to achieve greater long-term weight loss than behavioral programs alone. They also seemed to be well tolerated and to lead to few adverse events. Compared to behavioral-only programs, weight loss was substantial at 12 months at 10.3 kg versus 6.4 kg. However, neither approach achieved substantial sustainable weight losses at 24 months and 38-60 months, with weight loss for VLED plus behavioral programs versus behavioral-only programs merely 4.2 kg vs. 2.8 kg and 3.4 kg vs. 2.1 kg [81].

Treating obesity with bariatric surgery is a very radical approach, and understandably there have been major concerns as to whether or not the short- and long-term risks of this invasive treatment outweigh the benefits of weight loss for women with obesity WHO grade I-II. Bariatric surgery has evolved over the past decades, and several surgical methods have been abandoned due to unacceptable adverse effects, high risk of complications and a modest or unsustainable weight loss. The most often performed, best documented, effective, and safe procedures today are the sleeve gastrectomy and the Roux-en-Y Gastric Bypass (RYGB) with a risk lower than 6% of short-term serious adverse events and perioperative mortality of 0.03-0.2%. Guidelines generally recommend consideration of bariatric surgery at a BMI of 40, or at 35 where there is a serious obesity-related comorbidity [82, 83]. At 5-year follow-ups only 3% approximately of the patients that had a RYGB, and a little bit less than 15% of those who had a gastric sleeve, had rebounded to within 5% of their initial weight [83]. The long-term weight loss at 10-year follow-ups has been shown to be around 60% of excess weight for both sleeve gastrectomy and RYGB [84]. Excess weight is calculated as ([initial weight] – [postop weight]) / ([initial weight] – [ideal weight]), where ideal weight is defined as the weight corresponding to a BMI of 25 kg/m^2. Bariatric surgery has been shown to give better results than best-available medical treatment for a number of diseases, for example, type 2 diabetes, dyslipidemia and hypertension. Observational studies also show a reduced risk of all kinds of cancer by as much as 50% and a 35-40% lower 5-10 year all-cause mortality [83].

1.7 WEIGHT INTERVENTION AND INFERTILITY

It has been shown that women with obesity and anovulation start ovulating and gain good fertility through lifestyle changes that mediate weight losses exceeding 5% [85, 86]. Based on these results, weight loss is considered the first-line treatment for anovulatory infertility in women with obesity.
A little more than ten years ago a number of small, randomized studies indicated that there were beneficial effects of lifestyle interventions on the chance of pregnancy and live birth [87, 88]. However, these studies were underpowered, and therefore not able to provide a reliable conclusion. The opposite result was shown in a large Dutch well-designed randomized study of 574 patients published in 2016. This Dutch study compared an intervention group that received a 6-month lifestyle modification followed by the relevant infertility treatment indicated, with a control group that directly received infertility treatment. The trial resulted in modest weight loss of 4.4 kg in the intervention group, as compared to 1.1 kg in the control group. The primary endpoint of the study was vaginal birth of a healthy singleton at term, which was found to be significantly lower in the lifestyle modification group, but with no significant differences in adverse maternal or neonatal events [89]. A subgroup analysis was also performed, but it failed to identify any subgroup of patients that got a higher healthy live-birth rate [90]. Further analysis of the results also did not show any cost benefit of the lifestyle modification program [91]. A meta-analysis that included the above-mentioned trials, did show a positive effect of lifestyle intervention on ovulation, weight loss and natural conception, but no significant effect on IVF conception, miscarriages and most importantly, live births [92]. A recent report by the Cochrane Library found that pre-conceptional advice on weight compared to current routine care led to weight loss of approximately 1.3 kg, but it could not conclude with certainty that it led to more live births or fewer adverse events [93]. A major concern in weight-reduction intervention is the drop-out rate. In a systematic review of 15 studies of lifestyle intervention programs for obese infertile women, the drop-out rate was found to be 24% [94]. This high rate is obviously a major problem and limits the potential of such programs for this group of patients.

1.8 WEIGHT INTERVENTION AND IVF

In 2006 a small pilot study was published with 10 patients who received a VLCD intervention prior to IVF. Four of the patients withdrew and for three of the six patients that completed the weight intervention, there were either no oocytes at oocyte pick up or no fertilized oocytes [95]. This poor result warranted caution, and in the conclusion of the article VLCD was not recommended.

Table 2 Summary of randomized controlled trials of weight reduction treatment for obese women in order to improve live birth rates

Study and country	Study	No. of patients (intervention vs. control)	Change in weight (kg ±SD) - intervention vs. control	Live birth - intervention vs. control	p-value
Moran, et al., 2011 Australia	RCT Dietary and exercise treatment + IVF vs. IVF only	38 (18 vs. 20)	-3.8 ±3.0 vs. -0.5 ±1.2	7/18(38.9%) vs. 5/20 (25.0%)	0.483
Sim, et al., 2014 Australia	RCT VLCD and exercise + IVF vs. IVF only	49 (27 vs. 22)	-6.6 ±4.6 vs. -1.6 ±3.6	12/27 (44.4%) vs. 3/22 (13.6%)	0.02
Becker, et al., 2014 Brazil	RCT Hypocaloric diet + IVF vs. IVF only	26 (14 vs. 12)	-4.5 ± 0.8 vs. +0.7 ±0.8	3/14 (21.4%) vs 0/12 (0.0%)	Not available
Mutsaerts, et al., 2016 The Netherlands	RCT Lifestyle intervention + fertility treatment vs. fertility treatment only	564 (280 vs. 284)	-4.4 ±5.8 vs. -1.1 ±4.3	123/280 (43.9%) vs. 153/284 (53.9%)	Rate Ratio 0.82 95% CI 0.69-0.97

Later two randomized studies of weight intervention with hypo-caloric diet and lifestyle change, compared to no weight intervention, in infertile women with overweight or obesity, planning IVF, ICSI or cryo-stored embryo transfer treatment, showed a positive effect of the intervention on weight, hormones, pregnancy and live births [96, 97]. Another similar study also

showed a significantly larger weight loss in the intervention group, but in this study no statistically significant difference was seen between the groups, in relation to pregnancies or live births [88]. All these studies were small and underpowered for the clinically relevant endpoint of live births.

Surgery

Because an invasive treatment like bariatric surgery on fertility is not easily examined using well-designed prospective randomized studies, the evidence is not robust. Most studies are small but most do show a favorable effect on sex-hormones, ovulation, and fertility.

In a prospective study of 29 women who had a gastric bypass, post-operatively there was a significantly shorter follicular phase, a lowering of testosterone and estrogen, a rise in SHBG and an improvement in sexual function [98].

A systematic review and meta-analysis on the effect of bariatric surgery for women with polycystic ovary syndrome (PCOS), identified and analyzed 13 case studies which included 2130 women [99]. The surgery had a positive effect on the menstrual irregularity that is a marker for anovulation. Irregular cycles went from 56.2% pre-operatively to 7.1% at the end of the study. It also showed that a pre-operative infertility of 18.2% decreased significantly to 4.3%.

Two recent studies have been concluded on the outcome of IVF after bariatric surgery. One identified 83 women and the other 153 women that had a history of bariatric surgery followed by IVF. Both studies compared the group of women who had undergone surgery with those who had not, all BMI matched using the post-operative BMI of the first group. The studies showed that after the first IVF cycle both groups had comparable cumulative live birth rates [100] [82]. Further, in one of the studies [100] a comparison was also made to a matched group of severely obese women who had not undergone surgery. With respect to cumulative live birth the group of severely obese women had poorer outcomes and with respect to live birth per embryo transfer, significantly poorer outcomes. The cohort was however small, and a much larger group would be needed for a study to have the necessary power to show a significant difference in cumulative live births.

Encouraging results were shown in a retrospective study of 40 women with a prior IVF failure. In this study the women had an IVF treatment both prior to and after a gastric-sleeve operation. Surgery showed a positive and

significant effect with respect to the need for gonadotropin, number of oocytes, number of good-quality embryos and resulted in a favorable outcome with 14/40 live births [101].
However, a prospective cohort study of 48 women of fertile age, that showed a significant lowering of AMH by 40%, measured 12 months postoperatively after RYGB, has given rise to some concerns [102]. Unfortunately, the effect of this change in AMH on the fertility of these women is unknown.
The effect of bariatric surgery on complications of pregnancy and birth has shown reductions in the risk of gestational diabetes, excessive fetal growth, instrumental delivery, cesarean section, obstetric anal sphincter injury and postpartum hemorrhage, but also increases in the risks for moderately preterm birth (>32 – <37 weeks) and small-for-gestational-age infants [103-106]. There is no robust evidence concerning the nutritional effect of bariatric surgery on the intrauterine fetus or the optimal timeframe post operatively for conception and pregnancy. There are also concerns regarding the intrauterine growth of the fetus during rapid weight loss and potential malnutrition linked to bariatric surgery. Given these concerns and the known higher risk of SGA, women are advised to postpone pregnancy for 12-18 months post operatively [107, 108].

1.9 COST OF FERTILITY TREATMENTS FOR WOMEN WITH OBESITY

Economic analyses have shown a substantially higher cost linked to obesity in fertility treatment, pregnancy, and birth. This higher cost is explained by a lower chance of live birth and a higher risk for complications as shown in a higher number of hospital admissions (obese +45% and morbidly obese +88%) and a longer duration of stay per admission (obese +9% and morbidly obese +12%) [13]. These factors have been estimated to increase the cost per live birth by 70-100% [17].

A cost-effectiveness analysis of a randomized controlled trial (RCT) testing a lifestyle intervention program prior to infertility treatment showed it not to be cost effective. It was less costly but also less effective in terms of healthy LBR as compared to direct infertility treatment [91]. Despite the direct infertility treatment being more expensive per patient it was more effective and resulted in substantially more healthy live births (35% vs. 27%) within the timeframe of the study resulting in the same cost per live birth.

2 AIM

The overall aim of this thesis is to study the effect of a weight- reduction intervention on the reproductive, obstetric, neonatal, and metabolic outcomes in women with infertility and obesity that were scheduled for IVF treatment.

Paper I
To evaluate whether weight-reduction treatment in infertile women with WHO class I obesity scheduled for IVF improved the outcome, assessed as live births, compared with women who received IVF treatment without a weight-loss treatment.

Paper II
To investigate whether birthweight, and deviation from expected birthweight, were affected by weight intervention before IVF treatment. A secondary aim was to study the effect of such an intervention on other perinatal and maternal outcomes.

Paper III
To study the correlation of anthropometric factors and adipose tissue-related inflammatory and metabolic markers to pregnancy and live birth.

3 MATERIAL AND METHODS

Table 3 Overview of the study design of the studies

Paper	Design	Data collection	Participants	Analyses
I	Randomized controlled trial	Health records	305 infertile women with obesity scheduled for IVF. Intervention group received 16 weeks weight reduction treatment prior to IVF and control group received IVF only.	Fisher´s exact test. Mann-Whitney U-test. Mantel-Haenszel chi-square test. Pearson's chi-square test. Multivariable logistic regression. ANCOVA.
II	Secondary analysis of a randomized controlled trial	Health records from the study and from antenatal care and birth of children born within the study	All women with a live singleton birth within the study and all singletons born within the study (45 in the intervention group and 41 in the control group)	Fisher´s exact test. Mantel-Haenszel chi-square test. Chi Square test. Mann-Whitney U-test. Kruskal-Wallis test.
III	Observational prospective cohort study. Post hoc analysis of study I.	Health records. Blood samples at randomization (both groups) and after weight reduction treatment (intervention group)	195 women who fulfilled criteria for blood samples	Multiple logistic regression. Logistic regression. Spearman's rank correlation. Wilcoxon's signed rank test.

3.1 STUDY DESIGN - PAPER I

3.1.1 SETTING

We estimated that approximately 10% of the patients to be eligible to the study and 50% of these to be willing to participate. Thus, to recruit 316 patients to the study, a base of more than 6000 patients was needed. As the largest IVF clinics in the Nordic countries, perform approximately 1000 IVF treatments per year, and since the patients can receive more than one treatment per year, it very soon became clear that we had to reach out to our colleagues in other IVF-clinics in Sweden and the other Nordic countries in order to complete the study within a reasonable time. We therefore performed a multicenter, multidisciplinary, prospective, randomized controlled trial at nine infertility and five obesity clinics in Sweden, Denmark, and Iceland. The recruitment started in Sweden in 2010, Denmark started recruiting in 2012, and Iceland in 2014. The participating IVF- and obesity treatment units were as follows: Sahlgrenska University Hospital in Gothenburg; Karolinska University Hospital in Stockholm; Skåne University Hospital in Malmö; Örebro University Hospital; Rigshospitalet in Copenhagen; Herlev Hospital in Copenhagen; Hvidovre Hospital in Copenhagen; Holbæk Hospital in Holbæk; University of Copenhagen, Deparment of Nutrition, Exercise, and Sports in Copenhagen; and ART Medica in Reykjavik.
Data was gathered locally from each patient´s clinical records and data concerning live birth was gathered from maternal health care and delivery records for mother and child. All data was gathered into an electronical case report form.

3.1.3 STUDY POPULATION AND RANDOMIZATION

Eligible for the trial were infertile women between the ages of 18 and 38, with indications for IVF/ICSI and scheduled to start their first, second or third IVF/ICSI treatment and having WHO grade I obesity. The exclusion criteria were divided into two categories. The first was contraindication for VLCD and included insulin dependent diabetes mellitus and binge eating disorder as defined by Questionnaire of Eating and Weight Patterns-Revised [109]. The second category covers our perception of the extreme mental load

and unusual circumstances which included planned oocyte donation, planned pre-implantation genetic testing, or a husband having azoospermia known at randomization. Not having adequate knowledge of the local language was also an exclusion criterion. Only one cycle per patient was included in the study. We included first transfers using cryopreserved embryos for those patients that needed to have all embryos frozen on a medical indication. Randomization was performed by the doctor or the study nurse locally at each fertility clinic at a first IVF visit for those about to start their first treatment or at an in-between-treatments consultation for second- and third-cycle patients. A computerized randomization program with concealed allocation of patients and in the proportion of 1:1 ensured that the intervention group and the control group would have the same number of patients. In case of a technical problem making computer randomization impossible, closed opaque envelopes for emergency randomization were provided to all randomizing clinics. Optimal allocation was applied according to Pocock's minimization technique for sequential randomization [110] which took into account the following known confounding factors to minimize bias:

Age (<30 years/≥ 30 years and as continuous variable)
Parity (0/>0)
PCOS (yes/no)
Fertilization method planned (IVF/ICSI)
Tubal factor (yes/no)
Smoking (yes/no)
Waist – continuous variable
BMI- continuous variable

Due to the nature of the intervention blinding was not possible for patients, physicians or the nurses studying and treating the patients. Blinding was however used for the embryologists, who took care of the gametes, evaluated the quality of the pre-embryos, and chose them for transfer to the uterus. The statisticians were blinded as well and unaware as to which group the patients were allocated.

3.1.3 EXPOSURE

Weight management intervention

The aim of the weight reduction was to reach a BMI as close to normal as possible during a time period of approximately 16 weeks. The intervention started with 12 weeks of a strict liquid formula VLCD, with a daily energy intake of 880 kcal supplied by a powder that could be mixed with hot or cold water (Modifast, Nutrition & Santé, France). A recommendation was also made to drink 2 liters of water daily. During the VLCD period, all patients had scheduled visits with a health professional (dietician or nurse) at the start of treatment and every 2-4 weeks up until week 12. After finishing the 12-week VLCD period, the patients were scheduled for individual visits with a dietician for a period of two to five weeks, for the re-introduction of a normal balanced diet and weight stabilization. The rationale behind this stabilizing period was to have the patient at the start of the IVF treatment in weight-stable state and following a normal healthy diet, which we found to be a reasonably cautious measure before trying to help the patients to become pregnant. Prior to IVF treatment, the patient met the dietician again for a follow-up visit. If a patient was unable to complete the VLCD treatment, they received individualized weight-loss counselling based on a normal healthy solid-food diet until the start of IVF treatment. Patients started IVF after the weight-intervention period regardless of the weight reduction achieved. During and after IVF treatment all patients in the weight intervention group were offered complementary dietary counselling by the dietician for one year from randomization to prevent rebound and to support weight stability.

IVF treatment

In this study all patients were treated with the long protocol which at study start was the standard of care in the Nordic countries. They received a GnRH agonist, and the ovaries were stimulated with follitropin alfa (Gonal-f, Merck) in individualized doses. Their cycles were monitored locally in the clinics with serum-estradiol measurements and/or vaginal ultrasound. Ovulation was induced with choriogonadotropin alfa (Ovitrelle, Merck) and 36 hours later oocyte retrieval was performed by an ultrasound guided transvaginal needle puncture. Fertilization was carried out using either standard IVF or if indicated, ICSI according to standard procedures. Embryo transfer (ET) was mostly performed using two- or three-day cleaving stage pre-embryos and luteal-phase support was given from oocyte retrieval with

progesterone by vaginal route. Two weeks after ET, hCG was measured in serum, with a level of more than 5 IU/L considered positive for pregnancy, in which case vaginal ultrasound for confirmation of clinical pregnancy was performed approximately four weeks after ET.

3.1.4 PRIMARY AND SECONDARY OUTCOMES

The primary outcome in our study was live birth, defined as at least one child born alive regardless of gestational age. Secondary outcomes were pre-specified and regarded pregnancy related or IVF related or concerned embryological or dietary related measurements. Weight change, compliance to weight intervention, the COH (e.g. the number of cancelled cycles, total dose of gonadotropins, number of oocytes retrieved, the rate of ovarian hyperstimulation syndrome (OHSS)), the quality of the pre-embryos (e.g., number of good quality and frozen embryos) and the early pregnancy outcome (e.g., rate of pregnancy, biochemical pregnancy, clinical pregnancy, miscarriage, ectopic pregnancy).

3.1.6 STATISTICS

Descriptive statistics is presented by mean, standard deviation, median, and range. Categorical variables are given as numbers and percentages. For comparison between groups Fisher´s exact test was used for dichotomous variables and Mann-Whitney U-test for continuous variables.
Mantel-Haenszel chi-square test was used to test the statistical significance of stratified or matched categorical variables. This test was used for ordered categorical variables. Pearson's chi-square test was used to test the differences of categorical data. This test was used for all non-ordered categorical variables. Multivariable logistic regression was used to test for independent predictors after adjusting for relevant confounders. ANCOVA was used for continuous variables.
The primary analysis was performed on the full analysis set (FAS) population. The FAS population consisted of all randomized women having at least one follow-up variable and having started the IVF treatment (defined as having started the COH) or achieved a spontaneous pregnancy. We also performed a per protocol (PP) analysis on the population of all randomized subjects having completed the study without significant protocol deviation.

Even in a population of infertile women with an indication for IVF, spontaneous pregnancies can be expected, and these occurring after randomization were included for both study groups in FAS and PP analyses. For the primary variable, live birth, and for important secondary variables, risk differences and risk ratios with 95% confidence intervals and exact 95% confidence intervals for the estimated proportions were calculated. All significance tests were two sided and conducted at the 5% significance level. Two subgroup analyses were performed for the primary efficacy variable and for selected secondary variables; one for PCOS patients and one for patients completing the diet program and reaching a BMI $\leq$25 kg/m^2 or lowering their BMI by at least five units.

The sample size was based on data from a previous study of 364 women from our own clinic [111], where the live birth rate after IVF/ICSI in women with obesity was 12.5 % (7/56) and for women with a normal weight was 26.3% (81/308). We thus chose to base our power calculation on a difference of 13% (12% to 25%). We calculated that 152 patients were needed in each group, giving a total of 304 patients. As is customary in clinical research, we set the significance to 5% and the power to 80%. Based on the clinical experience we estimated a low dropout rate. To compensate for dropouts the sample size was increased to 316 and no loss of follow up was expected.

A futility analysis after approximately half of the planned patients had been included was done by a steering committee, consisting of an experienced statistician and an experienced researcher without any other involvement in the study. The aim of the futility analysis was to analyze and identify if there were signs of such a big difference between the groups regarding live birth or serious adverse events that it would be unethical to continue offering or not offering the studied intervention. The steering committee found no such signs and recommended that the study should continue.

3.1.7 METHODOLOGICAL CONSIDERATIONS PAPER I

We found it very important to design our study to test a clinically significant difference in live birth rates since a weight intervention is time consuming and both physically and psychologically demanding. Our goal was to test a model that could be applied in normal clinical practice. We judged that a substantial difference was needed for clinicians to abandon their everyday clinical routine, and to prescribe such a time consuming and expensive

treatment. Even more important was that the difference should be able to motivate the patients to accept the treatment. One needs to keep in mind, that the women having a planned IVF treatment ahead of them usually already had been trying to conceive for 1-2 years at a minimum and had used up large parts of their patience already [112]. In our experience, any measures that postpone or lengthen the time to treatment and live birth, is psychologically very demanding and unwanted to this group of patients.

We limited our study group to WHO grade I obesity because most public clinics in the Nordic countries do not treat women if they have a BMI above 35 kg/m^2 and we therefore anticipated major practical obstacles in performing the study if we would have chosen to include women with a higher BMI. We made an effort to include a population, as close to the "normal patient" as feasible, thus we restricted the exclusion criteria as much as possible.

In planning this study a number of possible weight interventions were considered and evaluated through a literature review, and by expert opinion from colleagues at the obesity clinic at the Sahlgrenska University hospital. Lifestyle intervention in the form of calorie restriction and a physical exercise program was not found feasible, due to the rather modest and slow weight loss that can be expected. Bariatric surgery was neither found feasible, due to the invasive nature of the treatment, and the fact that it has been recommended to wait at least 12 months post operatively before trying to conceive.

We aimed to conform as closely as possible to the intention-to-treat (ITT) principle. ITT entails analyzing all the randomized patients, in the group they are allocated to, irrespective of how their participation in the study turns out. However, as this can be difficult to achieve in practical terms, we adhered to the principle for the main analysis on the FAS population, which comes as close to the ITT population as possible.

Making the patients go through the weight reduction program takes them four months longer then going directly to IVF. This is a strain on a group of patients that have already used up almost all their patience. It is also known, that soon after the age of 30 years the female fertility diminishes. This has been shown by estimations of fecundity going down by 12%, and the chance of having a healthy baby by 3.5%, for every year after the age of 30 [113]. It is therefore possible that the time spent on weight reduction has a negative effect on the fertility and therefore the chance of live birth in the study and later. As this was a large study, we found it ethical to evaluate the data by performing a futility analysis after approximately half of the planned patients had been included.

3.2 STUDY DESIGN - PAPER II

3.2.1 STUDY POPULATION

The study population consisted of all women participating in paper I who had a singleton live birth (45 in the weight-reduction-and-IVF group and 41 in the IVF-only group) and the singletons themselves.

3.2.2 EXPOSURE

As this study was a secondary analysis of the data from paper I the exposure was the same as in paper I, namely the VLCD weight-reduction treatment.

3.2.3 OUTCOMES

Birthweight, and deviation from the expected birthweight, were the main outcomes of paper II. Secondary outcomes were other perinatal outcomes like preterm birth, low birth weight (<1500 g,<2500 g), small for gestational age (< 2 SD), large for gestational age (>2 SD), birth weight > 4500 g, Apgar score < 7 at 5 min and major birth defects. The maternal variables were preeclampsia, eclampsia, gestational diabetes, gestational hypertension, post-partum hemorrhage > 1000 mL and caesarean section.

To evaluate the deviation from the expected birthweight we used a calculation method developed in a study of estimations of intrauterine growth and birthweight, where corrections are made for gestational age and gender [114].

3.2.4 STATISTICS

This study was a secondary analysis of the data from paper I. According to two post hoc power calculations, based on the mean birth weight and the mean deviation from expected birthweight in the control group (45 singletons), it was possible to find a difference in mean birthweight of 320g and a difference in mean deviation from expected birthweight of 6.1% (significance 5%, power 80%).

For comparison between the two randomized groups Fisher´s exact test was used for all dichotomous variables, the Mantel-Haenzel Chi-Square test was used for ordered categorical variables, the Chi-Square test was used for non-ordered categorical variables and the Mann-Whitney U-test and the Kruskal-Wallis test were used for continuous variables.
A p-value less than 0.05 or a 95% confidence interval not including 1.0 was considered significant.

3.2.5 METHODOLOGICAL CONSIDERATIONS

There have been concerns regarding safety of VLCD weight intervention for women in the immediate pre-conceptional period [95] and a lower risk of excess fetal growth and higher risk of retarded fetal growth in pregnancies after weight loss after bariatric surgery has been shown [103]. Thus, we wanted to study the effect of the VLCD weight intervention on the intrauterine growth of the fetus, which is in some extent an indicator of future somatic and psychomotor development of the child [114].

3.3 STUDY DESIGN - PAPER III

3.3.1 STUDY POPULATION

This was a post hoc analysis of paper I. To understand the effect of inflammation and adipokines on the outcomes of IVF, we studied this well-defined group in a prospective cohort manner. We identified all women from paper I who met specific criteria for the serum samples obtained shortly before the IVF treatment, namely that they were obtained after a fasting period of at least six hours and before administration of the GnRH-agonist. This resulted in a cohort of 195 women (131 from the weight-reduction-and-IVF group and 64 from the IVF-only group).

3.3.2 OUTCOMES

The main outcome was correlation of anthropometric factors and adipose tissue-related inflammatory and metabolic markers with pregnancy and live birth.

3.3.3 STATISTICS

Multiple logistic regression, applying full models and backward stepwise selection, with $p<0.05$ as condition in each step for staying in the model, was used to identify factors independently associated with IVF outcomes (pregnancy and live birth, respectively). Candidate variables were age, hsCRP, leptin, AFABP, BMI and waist circumference. Alternative analyses were also performed where WHtR was substituted for waist circumference. The linearity assumption for continuous variables was checked by calculating the difference in -2 log likelihood between the models with and without the variable in question squared included. Logistic regression was also used to analyze possible interactions between randomization group and candidate variables regarding outcome. Spearman's rank correlation was used to evaluate associations between variables and between changes in leptin and AFABP during intervention. The Wilcoxon's signed-rank test was used for testing the effect of the intervention within the intervention group on leptin and AFABP levels All tests were two-sided and p values below 0.05 were considered statistically significant.

3.4 ETHICAL CONSIDERATIONS

The studies were all approved by the Regional Scientific Ethical Committee of Gothenburg, Sweden, the Regional Scientific Ethical Committee of Copenhagen, Denmark, and the Scientific Ethical Committee of Iceland.

4 RESULTS

4.1 PAPER I

During a period of five years and 4 months (October 2010 to January 2016), 962 patients were assessed for eligibility. Of these, 317 (33%) patients met the inclusion criteria and were willing to participate and were therefore randomized to one of the two groups.

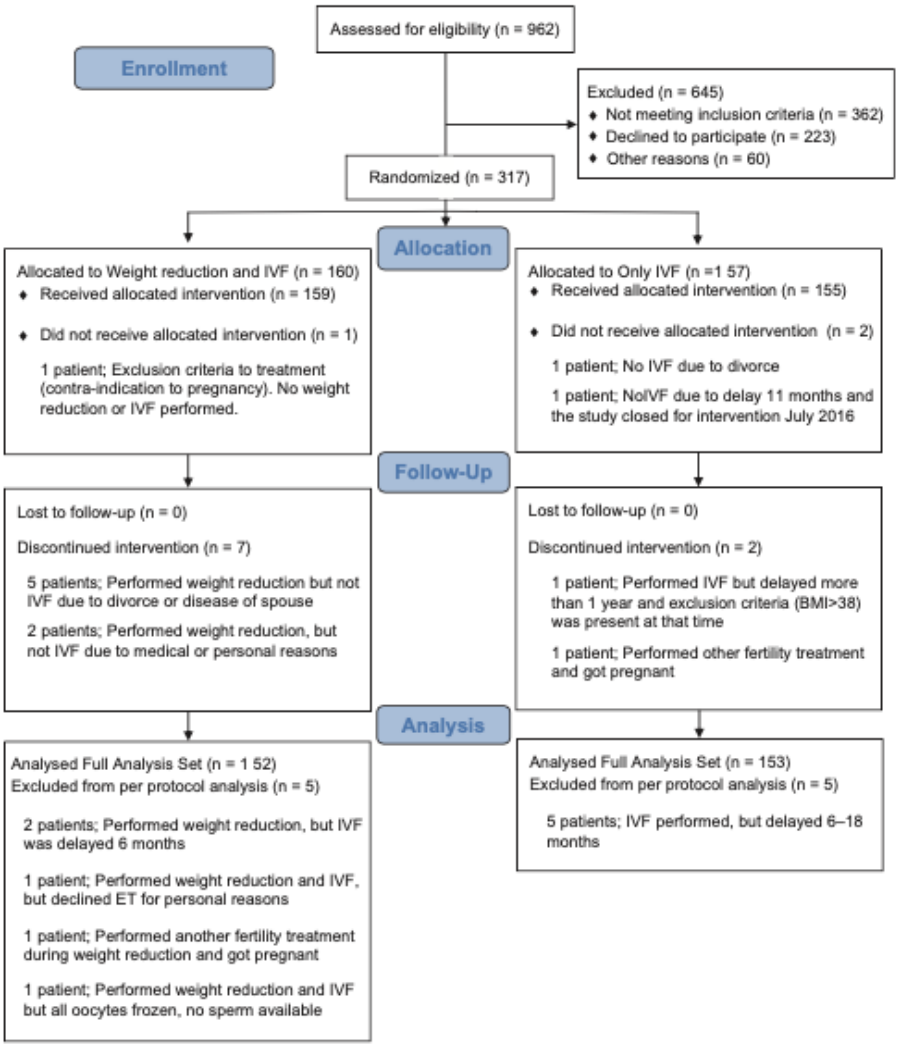

Figure 4 Flow chart of eligibility, randomization, and follow-up

The groups were shown to be similar regarding baseline characteristics. At randomization the BMI and weight were comparable between the groups with the weight-reduction-and-IVF group having a mean BMI of 33.1 kg/m^2 and a mean weight of 92.4 kg as compared to 33.0 kg/m^2 and 91.0 kg respectively for the IVF-only group. The weight reduction was sharp and effective and the weight change between randomization and last documented weight within the study (as close to oocyte retrieval as possible) was – 9.44 kg and – 3.25 BMI units in the weight-reduction-and-IVF group as compared to +1.19 kg and + 0.449 BMI units in the IVF-only group (see figure 5). The spread in weight change was large from – 23.3 kg to +7.9 kg in the weight-reduction-and-IVF group and -3.3 kg to + 9.6 kg in the IVF-only group. The majority in the weight-reduction-and-IVF group reached a substantial weight reduction with 73.7% achieving a weight reduction of more than 5% weight reduction and 54.6% achieving a weight reduction of more than 10%. No patient in the control group reached a weight reduction of more than 5%.

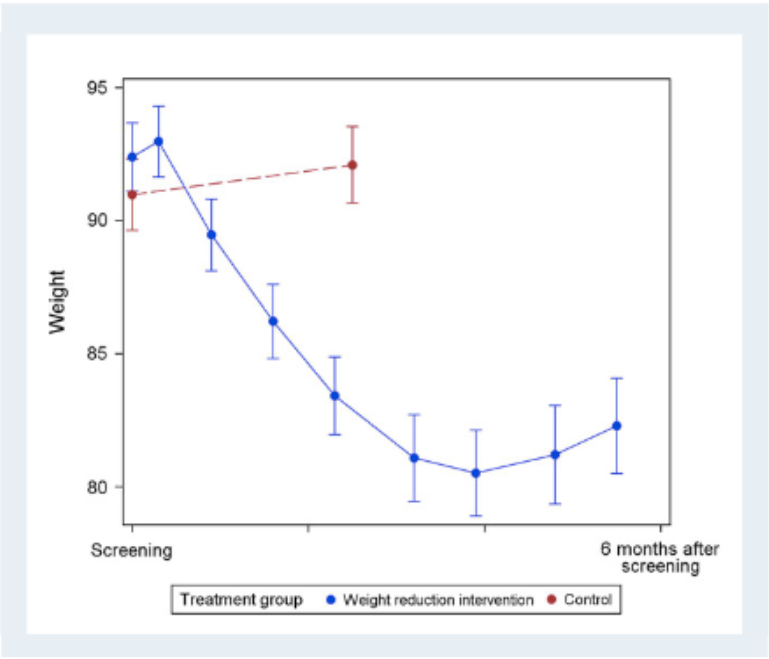

Figure 5 Graph of weight change for the two study groups from randomization to oocyte pick-up.

The live birth rate was 29.6% (45/152) in the weight-reduction-and-IVF group and 27.5% (42/153) in the IVF-only group. The difference was not statistically significant. We did not find any statistically significant difference on other IVF outcomes, but we found significantly more spontaneous pregnancies leading to live birth in the weight-reduction-and-IVF group. See table 4.

Table 4 Outcomes according to study group in the full analysis set.

Variable	**Weight-reduction-and-IVF group (n=152)**	**IVF-only group (n=153)**	**p-value**
Spontaneous pregnancy leading to live birth	16 (10.5%)	4 (2.6%)	0.0089
No. of oocytes retrieved per patient	8.56 (5.28) 7.00 (1.00;25.00) n=133	9.00 (5.85) 8.00 (0.00;32.00) n=139	0.63
No. of good quality embryos day 2	2.43 (2.57) 2.00 (0.00; 14.00) n=131	2.64 (2.59) 2.00 (0.00; 12.00) n=137	0.51
Clinical pregnancy	53 (34.9%)	47 (30.7%)	0.52
Miscarriage/positive pregnancy test	8/66 (12.1%)	5/56 (8.9%)	
Live birth (including spontaneous pregnancies)	45 (29.6%)	42 (27.5%)	0.77

For categorical variables n (%) is presented. For continuous variables mean (SD)/median (min; max)/n = is presented.

We found that five patients in each group did not fully comply with the protocol and so performed a PP analysis on the remaining 147 patients in the weight-reduction-and-IVF group and the 148 patients in the IVF-only group. Similar results were obtained as in the FAS analysis, with no significant difference in LBR but more live births following spontaneous pregnancies in the weight-reduction-and-IVF group.

We performed two pre-defined subgroup analyses. The first compared outcomes for live birth rates in patients with a PCOS diagnosis in both groups. The live birth rates were 27.5% (11/40) in the weight-reduction-and-IVF group and 22.0% (9/41) in the IVF-only group. This difference was not statistically significant. The second analysis compared the control group to the group of patients in the weight-reduction-and-IVF group that followed the weight reduction protocol and therefore reached normal weight (BMI 18.5-25 kg/m^2) or the expected weight reduction of 5 BMI units. This goal was reached by 38 patients, of whom 18.4% (7/38) of them had a live birth as compared to 27.5% (42/153) in the IVF-only group. This difference was not statistically significant.

The cost of the weight reduction program is estimated to be 15.000 SEK. Because the cost of the VLCD powder is equivalent to or even lower than the cost of a normal diet, this part of the weight reduction treatment was considered cost neutral compared to normal eating habits. The cost of the weight reduction treatment is therefore mainly related to the cost of consultations and control visits to health professionals. The cost of IVF treatment was at a market price of 40.000 SEK and the cost of medication was estimated to be 15.000 SEK per treatment. We found the cost per live birth to be 216.889 SEK in the weight-reduction-and-IVF group compared to 195.119 SEK in the IVF-only group. See figure 6.

Weight-reduction-and-IVF group (n=152):

Weight intervention only (live birth through spontaneous pregnancy) (n=16)
16 patients x 15.000 SEK/patient = 240.000 SEK

Weight reduction and IVF (n=136)
136 patients x 70.000 SEK /patient (weight intervention 15.000 SEK + IVF 40.000 SEK + medication 15.000 SEK) = 9.520.000 SEK

Total cost for 152 patients: 9.520.000 SEK + 240.000 SEK = 9.760.000 SEK

Cost per live birth: 9.760.000 SEK: 45 live births = **216.889 SEK/live birth**

IVF-only group (n=153):

No IVF (live birth through spontaneous pregnancy) (n=4)
4 patients x 0 SEK/patient = 0 SEK

IVF (n=149)
149 patients x 55.000 SEK/patient (IVF 40.000 SEK + medication 15.000 SEK) = 8.195.000 SEK

Total cost for 153 patients: 0 SEK + 8.195.000 SEK = 8.195.000 SEK

Cost per live birth: 8.195.000 SEK: 42 live births = **195.119 SEK/live birth**

Figure 6 Calculation of cost per live birth according to randomization group

4.2 PAPER II

We included 86 singleton live births in our analysis. There was one more live birth in the study, a twin birth in the IVF-only group. This twin birth was not included in the analysis. The twins were born near their due date and both mother and children were healthy.

We found the two groups of women to be comparable in relation to demographics, BMI, and weight at randomization. There was however a significant difference in change in BMI and weight from randomization to last measurement of BMI prior to oocyte pickup, with the weight-reduction-and-IVF group lowering their BMI by 2.88 kg/m^2 and their weight by 8.00 kg, but the IVF-only group went up 0.35 kg/m^2 and their weight by 0.96 kg.

No statistically significant differences between the groups regarding birthweight and deviation from expected birthweight were found. Mean birth weight in the weight-reduction-and-IVF group was 3486 g (SD 523) and in the IVF-only group 3584 g (SD 509) (P= 0.46), with a mean difference of -98.6 g (95% CI -320.3 to 123.2). Deviation from expected birth weight was 0.25% (SD 10.4) in the weight-reduction-and-IVF group and 0.87% (SD 12.9) in the IVF-only group, with a mean difference of 1.1% (95% CI: -6.1 to 3.9).

Generally, maternal and perinatal outcomes (see table 4) were good in both groups and no statistically significant differences between the groups were observed. We also analyzed the groups after categorizing the women into those who at the last measure before oocyte pickup, had a BMI < 30 kg/m^2 versus those with a BMI ≥ 30 kg/m^2. However, we found no significant differences between the groups there either. The size of the cohort did not permit firm conclusions to be drawn on these outcomes.

No children born within the study needed treatment in a neonatal intensive care unit. Six children were born with birth defects. Five (5.8%) had major birth defects, three in the weight-reduction-and-IVF group and two in the IVF-only group (OR 1.39, 95% CI 0.22.-8.79).

Table 5 Maternal and perinatal outcome according to study group

Variable	Weight intervention and IVF (n = 45)	IVF only (n = 41)	Odds ratio/ mean difference between groups	95% CI	P-value
Cesarean section	13 (29)	10 (24)	1.14	0.45-2.94	
Preeclampsia	5(11)	4(19)	1.19	0.30-4.76	
Gestational diabetes	1 (2)	2 (5)	0.45	0.04-5.21	
Gestational hypertension	1(2)	2(5)	0.45	0.04-5.21	
Gestational age (days)	277.9 (15.1) 281.0 (218.0; 296.0)	279.8 (9.6) 280.0 (253.0; 295.0)			0.95
Birthweight (g)	3486 (523) 3560 (1820; 4384)	3584 (509) 3638 (2140; 4820)	−98.6	−320.3 to 123.2	
Deviation from expected birthweight, %	0.25 (10.4)	0.87 (12.9)	1.1	−6.1 to 3.9	
Major birth defects	3 (7)	2 (5)	1.39	0.22-8.79	

IVF, in vitro fertilization. For categorical variables n (%) is presented. For continuous variables mean (SD)/median (min; max)/n are presented.

4.3 PAPER III

Of the 195 women from whom blood samples were obtained correctly prior to IVF eight conceived spontaneously before starting IVF. We therefore studied the remaining 187 that underwent IVF. The majority of the women were obese (78.3%), some were overweight (20.1%) and only three (1.6%) were of normal weight. The pregnancy and LBR achieved with the help of IVF in the cohort was 35.8% and 24.6% respectively.

None of the studied variables was found to be a predictor of pregnancy or live birth after IVF. Similar result was obtained when the original control and intervention groups were analyzed separately.

We compared measurements at randomization and at the examination closest to IVF (after the weight reduction treatment) in the group of women belonging to the original intervention group. A reduction was found in all parameters on a group level except AFABP, which was unchanged. However, we found the change in AFABP in the individual patient to correlate negatively with the change in leptin.

5 DISCUSSION

We conducted this study to investigate if it was possible to improve live birth rate in women with obesity through IVF by first receiving weight reduction treatment, and further to study safety for mother and child. We were also interested in understanding factors that might affect the outcomes in these women. We conducted a large multicenter randomized trial of 305 patients, finding a substantial and quick weight-loss effect of the VLCD treatment. This resulted in a significant weight difference between the weight-reduction-and-IVF and the IVF-only groups immediately before the start of the IVF treatment. We did not, however, find a significant difference between the groups in the primary outcome of live births. But the live births in the weight-reduction-and-IVF group were to a larger extent achieved through spontaneous pregnancies. Despite the fact that more patients in the weight-reduction-and-IVF group achieved a live birth through a spontaneous pregnancy, and therefore did not need an expensive IVF treatment, the cost per live birth was lower in the IVF-only group. No difference was found in LBR in two subgroup analyses, one in women with PCOS in both groups, and the other in women in the weight-reduction-and-IVF group reaching the pre-defined weight-loss goal (normal BMI or a weight loss of 5 BMI units or more). We further found that the mean birthweight and the mean deviation from the expected birthweight were comparable between the groups, and the observed differences were small without any clinical significance. The outcomes regarding maternal and perinatal outcomes were generally good and did not differ significantly between the groups. Similar results were found when the total study group was analyzed after categorization into groups with BMI <30 kg/m^2 and BMI >30 kg/m^2. In the last part of this study, we examined a well-defined cohort from paper I to determine if pregnancy or live birth could be predicted from adiposity or distribution of adipose tissue, or inflammation or metabolites from adipose tissue. No such indications were found. In the weight-reduction-and-IVF group we found a reduction after the weight-reduction treatment in all anthropological and blood test measurements, apart from AFABP which remained unchanged.

5.1 WEIGHT REDUCTION TREATMENT

Only 33% of the eligible patients, identified at the nine participating infertility clinics, were randomized to the study. The study was originally planned to be finalized within two years, with an estimation of 50% of the eligible patients to be randomized. The result of the recruitment shows how difficult it is to motivate patients to accept such a demanding treatment. The fact that almost three out of four patients in the weight reduction group lost more than 5% and one out of two lost more than 10% of their initial weight clearly shows that the VLCD is an effective weight reduction treatment and works for most patients. The weight-reduction-and-IVF group had a mean weight loss of 9.44 kg at 3-4 months and were at the start of the IVF 10.3 kg lighter than the IVF-only group. A VLCD is not as effective as bariatric surgery, for which a weight loss of > 20 kg can be expected [100] but in this study it was substantially more effective than the 6-month lifestyle weight reduction program that was tested in a well-conducted Dutch study in a similar setting. In that study the intervention resulted in only a 4.4 kg weight loss, which was only 3.3 kg more than the control group [89]. Despite the clear effectiveness of the VLCD treatment its weight loss may be insufficient to affect the underlying mechanisms responsible for a poorer outcome of IVF in women with obesity. A large retrospective cohort study from the United States showed a relatively small difference with regard to live births, namely 1.6% between the reference group of women of normal weight versus women with overweight and 3.4% between the reference group and women with obesity class I [67]. The fact that the differences were so subtle in this large data set (239.127 fresh autologous cycles) indicates that within these weight classes the underlying factors, related to adipose tissue, may be hard to detect. Inflammation measured through CRP and leptin are both correlated with BMI [43, 115] and have been linked to a detrimental effect on fertility [46] [116]. In our study the weight reduction treatment resulted in a decrease in hsCRP and leptin, but AFABP, which acts as a chaperone for free fatty acids [117], did not decrease and was furthermore found to have a delta negatively correlated with the delta leptin. This may result may indicate that a steady state was not reached after the weight loss and therefore free fatty acids were still circulating. A longer steady state may have been better for the metabolism, but it is not known how long that steady state would have needed to be.

5.2 LIVE BIRTHS

In our power calculation we used a study from our own clinic as a reference. This study showed an LBR of 12.5% for women with class I obesity as compared to 26.3% for women of normal weight [111]. In our study the LBR in both the control (29.6%) and the intervention group (27.5%) exceeds these numbers, and the result in both groups are good, as can be confirmed by comparison with the general result of 28% LBR in the National Swedish Quality Registry for this period [118]. We expected a much lower LBR in the control group. It is possible that in conducting our study with its focus on treatment for women with obesity, better knowledge of and understanding and experience in treating this patient group may have led to better treatments and results than in the reference study. It is also possible that the results from the reference study were misleading and that the true difference in LBR between women with overweight and women with obesity grade 1 is lower than we thought. In a large U.S. cohort study from 2016 the implantation and live birth rate declined with increasing BMI [67]. In this study the implantation rate for women with overweight and women with grade 1 obesity was 28.3% and 26.9% respectively, and the live birth rate 29.8% and 28.0% respectively. These differences are quite small, and the clinical relevance is limited. These results are more in line with our results and provide minimal support for a demanding weight loss treatment.
Four times more live births were achieved through spontaneous pregnancies in the weight-reduction-and-IVF group. This result may be linked to the weight reduction itself, but it must be kept in mind that the groups were not comparable regarding the possibility of achieving a spontaneous pregnancy. Directly after the randomization the weight-reduction-and-IVF group started the weight-reduction program which lasted for approximately 16 weeks, before they did their IVF. The control group on the other hand started their IVF treatment as soon as possible after randomization which led to most of them starting the treatment within 2-4 weeks. It has been shown that couples on an IVF waiting list have a 9-25% chance per year of achieving an treatment-free ongoing pregnancy [119]. Some pregnancies are therefore to be expected while waiting for IVF. It has also been shown that a weight loss can facilitate ovulation in women with PCOS [86], suggesting possible mechanism behind the increased number of live births through spontaneous pregnancies. It should be noted that while the weight-reduction-and-IVF group achieved four times more live births through spontaneous pregnancies

than the IVF-only group, this group also had at least three to four times longer time for that to happen, making it more likely that the increase in live births through spontaneous pregnancies was related to the longer timeframe rather than the weight intervention itself.

It has been shown that in PCOS weight loss is favorable for metabolic factors [120, 121]. In light of this we were interested in knowing if the outcome of the weight-loss intervention would be different for this group. Our subgroup analysis of women with PCOS found no statistical difference between the groups with respect to the live birth rate. The size of this group was small, however, resulting in an analysis of limited statistical power.

We performed two other analyses, a PP analysis and a subgroup comparing the IVF-only group and those patients who achieved the desired/expected effect of the weight intervention compared to the control group. These analyses aimed to determine if compliance with the protocol and achievement of the optimal and expected weight loss of the intervention affected the live birth rate. The main analysis of the study was done on the FAS population, which constituted of all patients starting the study, whether they followed the protocol or not. The FAS population is representative of how this intervention and treatment would work in a real-life clinical practice setting. What interests clinicians and patients, of course, are the chances and risks for all patients, without any knowledge of an individual's adherence to the protocol. It is equally important to know what the chances and risks are for individuals who can adhere to the protocol and reach the desired/expected goal of the intervention. Only a small fraction of the patients (five in each group) did not comply with the protocol and this group had similar result as the FAS population. The goal of desired/expected weight loss was reached by 38 patients, with a live birth rate of 18.4% (7/38) compared to 27.5% (42/153) in the IVF-only group. This difference was not statistically significant but as with the other subgroup analysis the size of this group was small and had therefore the analysis of limited statistical power.

5.3 COST

Although there was no statistically significant difference in the total live birth rate between the groups there were statistically significantly more live births achieved through spontaneous pregnancies in the weight-reduction-and-IVF group 10.5% (16) as compared to 2.6% (4) in the control group. Regardless of cause, this difference warrants further examination. It must be viewed as beneficial for the individual to achieve a live birth through a spontaneous pregnancy and therefore not need to go through an expensive and demanding IVF treatment. This is also of interest from a health economic perspective. What effect on the cost does it have that fewer IVF treatments were needed in the weight-reduction-and-IVF group to achieve a comparable live birth rate? This perspective was not a predefined outcome of the study, but we did a simplified between-group cost-effectiveness analysis. Even though more live births were achieved through spontaneous pregnancies in the weight-reduction-and-IVF group, with a smaller number of patients needing to go through the planned IVF treatment, our analysis shows that at the group level planning to have IVF treatment without weight reduction is less costly than starting weight reduction first (195.119 SEK/live birth vs. 216.889 SEK/live birth). This result is in line with that from the economic analysis of another RCT in a similar setting, which found a lifestyle intervention prior to infertility treatment not to be cost effective [91]. Our analysis took into account only the cost of the weight-reduction treatment and the IVF but not, as has been done in earlier studies, also the costs linked to maternal and perinatal care [13, 17, 122]. However, since we found no difference in the maternal and perinatal outcomes, it is likely that these costs also do not differ between the groups.

5.4 MATERNAL AND PERINATAL OUTCOME

We found no statistically significant difference between the groups in birthweight or deviation from expected birthweight. The birthweight was 98 grams lower in the weight-reduction-and-IVF group (3486g vs 3584g) and both groups were very close to the 3499g mean birthweight in Sweden in 2016 [123]. The 95% CI showed the true difference likely to lie between -320g to +123g which is a rather small and irrelevant relevant difference from a clinical perspective. In births for women who have had bariatric surgery, an effect on birthweight has been noted in the form of a lower risk of macrosomia (birthweight >4500g) and LGA, and a higher risk of SGA [103]. It is not clear if this is an effect of the weight loss or the surgical alteration of the nutritional uptake capacity of the gut. In bariatric surgery the anticipated weight loss is at least double the weight loss we observed in our studies. A reduction of that magnitude is probably needed to observe a change in birthweight. We did not observe any statistically significant difference between the groups on other maternal and perinatal outcomes, which were generally good and in line with the expected outcomes for this cohort. However, our data did not have sufficient power to permit firm conclusions to be drawn regarding these secondary outcomes. A lifestyle weight intervention that resulted in a weight loss of approximately 3 kg also did not show any significant difference in these outcomes [89]. These results strengthen our view of the weight-reduction treatment being safe for the mother in pregnancy and birth and not negatively influencing the intrauterine environment of the fetus.

5.5 PREDICTIVE FACTORS

Previous cohort studies have clearly shown a negative association between adiposity, pregnancy, and live births [55, 64, 68-70, 124, 125] a result we did not see in our study. Most patients in our study had overweight or class I obesity according to the WHO classification when their IVF was performed. In a large U.S. cohort study of 239.127 fresh autologous IVF cycles the overweight and obesity class I groups had respectively a 1.2% and 2.6% lower implantation rate and a 1.6% and 3.4% lower live birth rate as compared to women of normal weight [67].

Most studies that show a negative association between obesity and the outcome of IVF are based on treatments with fresh transfers but a number of studies on frozen-thawed embryo transfers have shown similar live birth rates in women across BMI categories [126-128]. This may be due to a different hormonal milieu at the time of implantation, which could affect the endometrium, optimizing it for implantation. Not only has obesity measured by BMI been linked to less favorable outcomes for IVF treatments [67] but also the distribution of body fat, mainly as waist circumference [129]. We investigated both BMI, which is a very common measure on nutritional status and waist circumference and waist-height ratio, both of which better represent distribution of adipose tissue. However, no relationship to pregnancy or live births could be seen. In our cohort we found that hsCRP and leptin were decreased after weight-reduction treatment, but AFABP did not. As AFABP is a chaperone of free fatty acids from adipose tissue, we expected to find a decrease because of the reduction of adipose tissue. It has been shown that AFABP is correlated with the dynamic phase of weight loss [130]. It is possible that the period of weight stabilization was not sufficiently long to establish a stable metabolic environment in our study. The fat mobilized by the weight-reduction treatment may thus still have been circulating, resulting in an unchanged level of AFABP. This mechanism may have affected the results, but it is not known how much time is needed after a dramatic weight loss for a stable metabolic state to be reached.

5.6 STRENGTHS AND LIMITATIONS

The main strength of paper I and II is that they are based on a large multicenter, randomized trial which is the optimal study design for intervention to minimize bias. The study has high external validity through its wide inclusion criteria, narrow exclusion criteria, and the fact that it was performed at multiple centers in three different countries. Another strength is that the weight-reduction treatment effectively managed to reduce patients weights, creating a substantial difference between the groups. Paper III is based on the patients from this same trial and therefore the cohort consists of a well-defined group of patients.

There are a number of limitations to the study. Only approximately half of the eligible patients were included in the study. The vast majority of the eligible women that did not get randomized declined participation. This can raise doubts regarding validity but shows the reluctance of patients in this situation to undergo a demanding weight reduction treatment that postpones the start of IVF treatment and therefore possibly the resolution of their infertility. The study was setup to detect a difference of 13% in LBR, which is a rather large difference in this context. It is possible that we missed a smaller true difference that a larger study population would have been able to find. We however firmly believed that to be able to motivate clinicians to recommend, and patients to accept, such a physically- and mentally demanding and time-consuming treatment, a large difference was necessary.

In Paper I the groups were not completely comparable because the weight-reduction-and-IVF group started their IVF treatment later then the IVF-only group. This gave the women longer time to achieve a spontaneous pregnancy, but also caused them to be 3-4 months older at the time of the IVF treatment, which can affect fertility negatively. We found this a realistic setup, however, for a model that would be applied in everyday clinical work.

Paper II is a study of secondary outcomes of the trial and paper III is a post-hoc analysis from that same study. Because the power calculation in paper I was based on the primary outcome, the study group of women giving live birth, together with the children born within the study, is small and therefore the power limited.

It is possible that the time from finishing the weight reduction treatment until the start of the IVF treatment was not sufficient to accomplish a steady metabolic state, which may have affected the results.

The randomized controlled trial included only women with WHO grade I obesity and therefore the results cannot be generalized to all women with obesity. It is possible that women with another level of obesity would get a different result in studies like these.

6 CONCLUSION

Based on the results presented in this thesis it can be concluded that VLCD is an effective and fast way of achieving a substantial weight loss in a cohort of women, with WHO grade I obesity scheduled for IVF.

In infertile women, with WHO grade I obesity scheduled for IVF, a VLCD weight reduction treatment prior to IVF does not have a substantial effect on the LBR.

In infertile women, with WHO grade I obesity scheduled for IVF, more live births after a spontaneous pregnancy occurred in a group that received a VLCD weight reduction treatment prior to IVF as compared to a group that received IVF only.

In infertile women, with WHO grade I obesity scheduled for IVF, the cost of fertility treatment per live birth is lower in a group of women that received IVF only as compared to a group that received a VLCD weight-reduction treatment prior to IVF.

In infertile women, with WHO grade I obesity scheduled for IVF, a VLCD weight reduction treatment prior to IVF does not affect birthweight or deviation from the expected birthweight of the newborns, as compared to a group that received IVF only.

In a cohort of infertile women scheduled for IVF none of the following was found to be a predictive factor for pregnancy and live birth: low grade inflammation, adipokines, presence of obesity, and distribution of adipose tissue.

7 FUTURE PERSPECTIVES

When starting the preparations for the studies in this thesis we had observed a clear negative effect of obesity on live births in our own clinic. We were very hopeful that our treatment model, which included a weight-reduction treatment with proven efficacy, would alleviate some of the negative effect of obesity on the outcome of IVF and possibly help the patients to accomplish a change that would also give them a positive general health effect. At that time, infertile women with obesity in the Nordic countries were not able to get publicly-reimbursed IVF treatments if their BMI was above 35 kg/m^2. In some places the limit was even set lower at a BMI 30 kg/m^2. These decisions were understandable as retrospective cohort studies available to decision makers at the time clearly showed that there was a negative association between obesity and live birth after IVF. The logical conclusion was therefore that weight loss should be advocated, and BMI limits set. Even though obesity had been recognized as a problem there were, however, in this situation no treatments, programs or support offered to these patients. This was the case in most public clinics in the Nordic countries in 2010, and still is. A pre-determined idea seemed to prevail of weight loss conferring a positive effect and being easily acquired by the patient herself, who therefore needed no help from the clinic. It´s hard to not wonder if obesity is thought of as a self-inflicted condition caused by bad choices in nutrition and exercise (or lack there off) and that it is well within the power of the patients to turn things around by taking responsibility for her own choices and starting to show discipline and resilience. Is it possible that obesity is seen not as a disease or a social-health problem but rather as a sign of a weak character and bad individual choices? Patients with obesity are well aware of their problem and have made many attempts to lose weight [131]. Lifestyle and diet weight interventions in various forms have been shown in a meta-analysis of 26.000 patients to accomplish a weight loss of approximately 3-10% at 6 months and 1-4% at 2-4 years [77]. This is a modest effect in the short term and minimal in the long term and clearly illustrates how difficult it is to lose weight. A modest weight loss may be beneficial in terms of general health, but it is nowhere close to moving patients between WHO weight categories, as is probably needed to affect fertility, pregnancy, and birth. Bariatric surgery is still the only weight intervention able to accomplish a weight loss large enough to move the patient into a different WHO BMI category. Bariatric

surgery has shown a positive effect on maternal and perinatal outcomes, but it is very invasive and complications can be very serious.

Even though the vast majority of patients in our trial receiving the weight-reduction treatment showed admirable treatment compliance and achieved a substantial weight loss we did not observe an effect on live birth, the ultimate goal of IVF treatments. We also did not recognize any effect on the maternal and perinatal outcomes, and the weight-reduction treatment model was more expensive calculated per live birth. These results are consistent with the those of Mutsaerts et al. who found the same to be true regarding a lifestyle-based weight-intervention prior to infertility treatments of various kinds [89]. The two large, randomized studies on weight intervention for infertile women with obesity both found no effect from substantive weight-intervention programs on LBR or maternal- and perinatal complications. It must be kept in mind that these weight interventions are expensive, demanding and time consuming, first and foremost for the patients themselves, but also for health professionals, with detrimental effects also incurred for society.
In pregnancies of women with obesity the risk for maternal and perinatal complications is elevated, with potentially serious consequences, which we as caregivers have a duty to prevent if possible. But are the consequences so great that they warrant denying women with obesity fertility treatment? Comparing these complications in women with obesity to those in oocyte donation (OD) recipients, the risk level is quite similar. There most of the complications already mentioned in the Introduction are common to both groups, with the risk level approximately doubled or tripled compared to other IVF pregnancies or spontaneous pregnancies [11, 132]. In conditions such as pregestational diabetes and chronic kidney disease, the risk of numerous maternal and perinatal complications is also substantially elevated, with some complications having far higher risks than in obesity. In contrast to obesity, the chronic nature of the disease and the elevated maternal and perinatal risks are accepted, and decision makers have not categorically denied these patients treatment. Every patient is evaluated individually, and the focus is on optimizing the underlying condition pre-conceptionally [133, 134].
The current situation is that if you are a woman with infertility and grade I or II obesity you cannot be sure of getting a publicly-funded infertility treatment in the Nordic countries. The reasons for denying you treatment are lower

chances of live birth, higher treatment costs, and higher risks of complications for you and your child in pregnancy and birth. You therefore get the advice to lose weight so that you can qualify for treatment, but without receiving any help in accomplishing the weight loss. Any help you did get would probably be through a lifestyle- or diet-based weight intervention which is known to have a modest effect on your weight and confer no better chance of a live birth. In fact, it is more likely that it would delay the live birth and cost more. Furthermore, there is no evidence that a weight-reduction intervention lowers the risk of complications for yourself and your unborn child in pregnancy or in birth. That risk is substantially higher than in women without any health issues and supports decision makers in withholding treatment. However, in the context of other underlying reasons like in oocyte donation, diabetes, and chronic kidney disease it is often regarded as an acceptable and manageable risk.

In my opinion decision makers need to ask themselves; what is the reason for denying women with obesity grade I and II infertility treatment? Is it based on the best available evidence and care for the women and their unborn children, or is it something else? It is time to reevaluate the current limits for treatment and consider accepting the problem of obesity for what it is, as is done with many other conditions, or evaluate offering more effective weight reduction treatments such as bariatric surgery.

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