Ovarian stimulation for IVF - a balance between efficacy and safety

by

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It is through science that we prove,
but through intuition that we discover

Jules Henri Poincare

Contents

Abstract

Background: To increase the chance of a live birth after in vitro fertilization (IVF) a controlled ovarian hormonal hyperstimulation (COH) is used to collect a certain number of oocytes for fertilization. COH is a potent hormonal treatment with a potential risk of serious adverse events.
Aim: To assess the ovarian response, expressed as the number of oocytes retrieved for IVF that results in an optimal balance between efficacy and safety.
Methods: *Paper I:* A randomized controlled trial (RCT), including 308 patients, comparing the performance of a dosage algorithm based on anti-Müllerian hormone (AMH) to one without AMH for prediction of the desired ovarian response, 5 to 12 oocytes. *Paper II:* A cohort study including 269 serum samples analyzed in a parallel setting investigating the correlation between the two AMH assays used in the RCT. *Paper III:* All fresh IVF cycles performed in Sweden 2007-2013 (n=77,956) and their subsequent frozen embryo transfer (FET) cycles (n=36,270) performed 2007-2014 were included in a population based registry study. Four major outcomes were investigated in relation to the number of oocytes retrieved; live birth rate (LBR), cumulative LBR per fresh and all subsequent FET cycles, incidence of severe ovarian hyperstimulation syndrome (OHSS) and incidence of thromboembolic events. *Paper IV:* All singletons born after fresh IVF cycles in Sweden 2002-2015 (n=27,359) were included in a population based registry study. Five main perinatal outcomes (preterm birth [PTB], very preterm birth [VPTB], small for gestational age [SGA], major birth defects and peri/neonatal death) and two main obstetric outcomes (hypertensive disorders of pregnancy [HDP] and placenta praevia) were investigated in relation to the number of oocytes retrieved. Data was adjusted for maternal age, parity, smoking, BMI, cause of infertility, maternal educational level, maternal country of birth, treatment period, embryo stage, fertilization method (IVF/ICSI), OHSS and vanishing twin.
Results: *Paper I:* There was no significant difference between the two algorithms regarding the primary outcome variable rate of patients with between 5 and 12 oocytes retrieved. *Paper II:* The correlation between the two assays was good, although there were considerable differences between the two assays depending on the actual AMH levels. *Paper III:* LBR after fresh cycles increased by the number of oocytes retrieved, although reaching a plateau at 11 oocytes while cumulative LBR evened out at 20 oocytes retrieved. OHSS increased rapidly if more than 18 oocytes were retrieved. Thromboembolic events were rare and occurred mainly if more than 15 oocytes were retrieved. *Paper IV:* There was no significant association between the number of oocytes retrieved and any of the perinatal outcomes or HDP. There was however a significant association between the number of oocytes retrieved and placenta praevia.
Conclusions: 1. Inclusion of AMH in dose decision did not result in a better prediction of ovarian response. 2. AMH assays have considerable and clinically important methodological problems 3. Ovarian stimulation up to 18 to 20 oocytes retrieved seems optimal from a cumulative live birth perspective, keeping severe adverse events at a reasonable level. 4. Ovarian response was not associated with adverse perinatal outcome though a significant association was found with the risk of placenta praevia.
Keywords: AMH/ovarian response/efficacy outcome/safety outcome

Svensk sammanfattning

Bakgrund: Inför provrörsbefruktning (in vitro fertilisering=IVF) genomgår kvinnan hormonstimulering av äggstockarna som syftar till att mogna fram flera ägg. Tidigare studier har visat att det optimala antalet ägg som innebär störst chans till födsel efter färsk cykel är 6-15. Ett lägre antal ägg innebär risk för att behandlingen får avbrytas och startas om. Ett högre antal ägg innebär ingen högre chans för födsel, däremot en ökad risk för allvarliga komplikationer såsom överstimuleringssyndrom (OHSS) och tromboser. Hormondosen bestäms vanligtvis av en algoritm utifrån t.ex. kvinnans ålder, BMI och antalet synliga antralfolliklar 2-10 mm (AFC), undersökt med ultraljud. Under senare år har Anti-Mülleriskt hormon (AMH) vunnit stor utbredning som prediktor för ovariets svar på stimulering. AFC och AMH är de mest specifika och sensitiva prediktorerna för ovariellt svar och anses likvärdiga. AMH har emellertid visat sig vara behäftat med metodologiska problem beroende på instabilitet i provet orsakat av hanteringen, variationer mellan olika laboratorie-assays samt brist på enhetliga referensintervall.

Vid IVF behandling befruktas äggen med spermier i laboratoriet och odlas i 2-6 dagar. Vanligtvis återförs endast ett embryo till livmodern och eventuella ytterligare embryon av hög kvalitet fryses. Under senare år har teknikerna för odling och frysning av embryon förbättrats snabbt och chansen för födsel efter återförande av fryst/tinat embryo (FET) är numera jämförbar med färskt embryo. Ungefär 1/3 av alla embryoåterföranden i Sverige 2015 utgjordes av FET. Det blir därmed alltmer relevant att undersöka kumulativ förlossningsfrekvens, dvs. chansen för förlossning per äggaspiration inkluderande ett färskt embryoåterförande och alla FET.

Det är tidigare känt att barn födda efter IVF har större risk för låg födelsevikt och för tidig födsel jämfört med barn födda efter spontan konception, även efter justering för kända confounders såsom kvinnans ålder, paritet, flerbörd och längden av bakomliggande infertilitet. Orsaken till denna skillnad är okänd men faktorer som hormonstimulering inför IVF, embryoodling- och frysning kan vara tänkbara orsaker. Studier på syskon har visat att IVF barn har sämre utfall än syskon tillkomna efter spontan konception.

Syfte: Att undersöka om tillägg av AMH i en algoritm för hormondosering ökar chansen för önskat ovariellt svar (5-12 ägg) efter stimulering. Vidare att undersöka associationen mellan antal aspirerade ägg och kumulativ chans till födsel per äggaspiration samt allvarliga komplikationer vid stimulering (OHSS och tromboser). Slutligen att undersöka associationen mellan antalet aspirerade ägg och perinatalt utfall samt obstetriska komplikationer.

Metoder: *Delarbete I:* 308 patienter randomiserades till två olika algoritmer för beslut om startdos av follikelstimulerande hormon (FSH). Algoritm I inkluderade ålder, BMI och AFC, algoritm II inkluderade även serum AMH. Primärt utfall var andel patienter med 5-12 ägg vid äggaspiration. De viktigaste sekundära utfallen var andel patienter med svagt ovariellt svar (<5

aspirerade ägg) och kraftigt ovariellt svar (>12 aspirerade ägg), antal patienter med OHSS, antal brutna behandlingar inklusive orsak, graviditeter och födslar. *Delarbete II:* Serumprover från 269 patienter från den randomiserade studien undersöktes parallellt med två olika AMH assays avseende korrelationen mellan assays och skillnaden i serumnivåer mellan de två metoderna. *Delarbete III:* Alla färska IVF cykler genomförda i Sverige 2007-2013 (n=77,956) med tillhörande FET cykler genomförda 2007-2014 (n=36,270) ingick i en populationsbaserad registerstudie. Data från det nationella kvalitetsregistret för assisterad befruktning (Q-IVF) korskördes med data från nationella patientregistret (NPR). Fyra huvudutfall undersöktes för association till antalet aspirerade ägg; födelsefrekvens efter färsk cykel, kumulativ födselsfrekvens per äggaspiration, incidensen av allvarlig (slutenvårdskrävande) OHSS och incidensen av tromboser. *Delarbete IV:* Alla barn födda i enkelbörd efter färska IVF cykler i Sverige 2002-2015 (n=27,359) inkluderades i en populationsbaserad registerstudie. Data från Q-IVF korskördes med data från data från NPR, medicinska födelseregistret (MFR) och statistiska centralbyrån (SCB), Fem perinatala huvudutfall; prematurbörd före vecka 37 (PTB), prematurbörd före vecka 32 (VPTB), liten för gestationsåldern (SGA), peri/neonatal död samt missbildningar, och två obstetriska huvudutfall; hypertensive disorders of pregnancy (HDP [graviditetshypertoni, preeklampsi och eklampsi]) och placenta previa undersöktes avseende associationen till antalet aspirerade ägg.

Resultat: Delarbete I: Ingen signifikant skillnad observerades mellan algoritmerna avseende primärutfallet andel patienter med 5-12 aspirerade ägg. Bland de sekundära utfallen noterades en signifikant högre andel patienter med svagt ovariellt svar i AMH gruppen medan övriga sekundära utfall inte visade några signifikanta skillnader. Delarbete II: Korrelationen mellan de två undersökta AMH assays var god. Emellertid varierade skillnaden mellan metoderna avsevärt beroende på aktuell serumnivå av AMH. Övergripande var skillnaden mellan metoderna 18 % men för låga AMH nivåer var skillnaden 40 %. Delarbete III: Chans för födsel ökade med antal aspirerade ägg upp till 11 ägg och planade sedan ut. Kumulativ chans till födsel ökade upp till 20 ägg. Samtidigt ökade incidensen av allvarlig OHSS om mer än 18 ägg aspirerades. Antalet tromboser var lågt och förekom framför allt om mer an 15 ägg aspirerades. Delarbete IV: Det fanns inget signifikant samband mellan antal aspirerade ägg och något av de perinatala utfallen eller HDP. Däremot fanns ett signifikant samband mellan antalet aspirerade ägg och placenta previa.

Slutsatser: Inklusion av AMH i beslut av hormondos vid stimulering förbättrade inte prediktionen av ovariellt svar. AMH assays har avsevärda och kliniskt viktiga metodologiska utmaningar och bör användas med försiktighet vid beslut om dosering och behandlingsstrategi. Hormonstimulering resulterande i aspiration av 18-20 ägg förefaller optimalt för maximal kumulativ chans till födsel med en samtidigt acceptabel risk för allvarliga biverkningar.
Antalet ägg påverkade inte det perinatala utfallet. Däremot sågs ett svagt men signifikant samband med risken för placenta previa.

List of publications

The thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

I. Magnusson Å, Nilsson L, Oleröd G, Thurin-Kjellberg A, Bergh C. The addition of anti-Müllerian hormone in an algorithm for individualized hormone dosage did not improve the prediction of ovarian response-a randomized, controlled trial. *Hum Reprod* 2017;**1**: 811-819

II. Magnusson Å, Oleröd G, Thurin-Kjellberg A, Bergh C. The correlation between AMH assays differs depending on actual AMH levels. *Hum Reprod Open* 2017;**4**:1-5

III. Magnusson Å, Källén K, Thurin-Kjellberg A, Bergh C. The number of oocytes retrieved during IVF; a balance between efficacy and safety. *Hum Reprod* 2018;**33**:58-64

IV. Magnusson Å, Wennerholm U-B, Petzold M, Källén K, Thurin-Kjellberg A, Bergh C. The association between the number of oocytes retrieved for IVF, perinatal outcome and obstetric complications. *Submitted 2018*

Abbreviations

AFC	antral follicle count
AMH	anti Müllerian Hormone
AOR	adjusted odds ratio
ART	assisted reproductive technology
BMI	body mass index
CI	confidence interval
COH	controlled ovarian stimulation
ESHRE	European Society of Human Reproduction and Embryology
GEE	Generalized Estimating Equations
GnRH	gonadotropin releasing hormone
GQE	good quality embryo
FAS	full analysis set
FET	frozen embryo transfer
FSH	follicle stimulating hormone
hCG	human chorionic gonadotropin
HDP	hypertensive disorders of pregnancy
hMG	human menopausal gonadotropin
ICD 10	International Statistical Classification of Diseases and Related Health Problems-tenth revision
ICSI	intracytoplasmatic sperm injection
IVF	in vitro fertilization
LBR	live birth rate
LBW	low birth weight (<2500 g)
LGA	large for gestational age (more than two standard deviations above Swedish growth standard
MBR	Medical Birth Registry
NPR	National Patient Registry
OR	odds ratio
PCOS	polycystic ovary syndrome
PP	per protocol analysis
PTB	preterm birth (before 37 weeks of gestation)
Q-IVF	The Swedish National Quality Registry for Assisted Reproduction
RCT	randomized controlled trial
SET	single embryo transfer
SCB	Statistics Sweden
SGA	small for gestational age (more than two standard deviations below the Swedish growth standard
TGF	Transforming Growth Factor
VPTB	very preterm birth (before 32 weeks of gestation)

Introduction

Infertility and its implications

Infertility is defined as the inability to conceive despite more than one year of active attempts. The prevalence of infertility in the population is estimated to be approximately 10-12% (Boivin *et al.,* 2007; Datta *et al.,* 2016) with small differences between developed and under-developed countries (Mascarenhas *et al.,* 2012). The increasing trend of postponing pregnancy among women, especially in the western world (Mills *et al*., 2011), has resulted in an increasing demand for infertility counselling and treatment (Leridon and Slama 2008; Birch Petersen *et al.,* 2015).

The causes of infertility may be female factors including ovulation disorders, endometriosis, tubal or uterine conditions, male factors including impaired semen quantity or quality, or a mixture of both. In about 30% of couples no explanation is found and the infertility is regarded as unexplained. (Zegers-Hochschild *et al.,* 2017).

Couples seeking fertility counselling can, after careful medical assesment, be offered assisted reproductive treatment, most frequently in vitro fertilization (IVF). Since the birth of the first IVF baby in 1978 (Steptoe and Edwards 1978), more than 7 million children have been born worldwide after IVF treatment. In Sweden, the first child after IVF was born in 1982 and since then, approximately 65,000 IVF children have been born. Every year around 19,000 fresh and frozen IVF cycles are performed in Sweden and around 4,500 children are born after assisted reproductive technology (ART) which constitutes approximately 3.8% of the yearly birth cohort (Q-IVF 2015; Statistics Sweden 2015).

The IVF procedure

The IVF procedure implies that oocytes collected from the ovaries are fertilized with sperm in the laboratory and that the developing embryo is subsequently trans-ferred to the uterine cavity.

The oocytes present in the ovarian follicles differ in quality, hence, to achieve a mature oocyte of high quality and increase the chance of pregnancy, several oocytes are usually collected, although retrieving only one single oocyte is also possible in IVF natural cycles. In order to achieve multiple oocytes, a controlled ovarian hyperstimulation (COH) with gonado-tropins, in combination with a gonado-tropin releasing hormone (GnRH) agonist or antagonist, is commonly used. Hormone stimulation, given as daily injections of either recombinant follicular stimulation hormone (FSH) or urinary derived human menopause gonadotropin (hMG), starts in the early follicular phase and lasts for approximately 9 to 12 days. The ovarian response to stimulation is monitored using serum estradiol levels and/or vaginal sonography and hormone doses are adjusted to achieve an appropriate number of growing follicles. The stimulation is

stopped when sonograpy reveals 2 to 3 follicles of >17 mm, and a single injection of human chorionic gonadotropin (hCG) is administered for oocyte maturation.

The oocytes are collected approximately 36 hours later and immediately transferred to the laboratory, where they are fertilized using a purified sperm sample. In the case of a normal sperm sample the oocytes are fertilized using the standard technique, i.e sperm is added to the dishes containing the oocytes for fertilization. In case of a reduced number of spermatozoa or reduced sperm motility the fertilization is facilitated by intracytoplasmatic sperm injection (ICSI) of one spermatozoa into each oocyte.

The fertilized oocytes are cultured in the laboratory for 2 to 6 days for embryo development. The embryo morphology is assessed by microscopy or with the new time-lapse technique (Ciray *et al*., 2014). Embryo transfer to the uterus is performed either at cleavage stage, 2 to 3 days after oocyte collection, or at blastocyst stage 5 to 6 days after oocyte collection. Surplus embryos of good quality, in accordance with Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology (the Istanbul consensus workshop on embryo assessment 2011), are cryopreserved.

Since a majority of the obstetric and perinatal complications observed after IVF are related to multiple pregnancies (Bergh *et al.,* 1999; Grady *et al*., 2012; Sazonova *et al.,* 2013; Henningsen *et al.,* 2015) an increasing trend for single embryo transfer (SET) has been observed worldwide. In Sweden SET was performed in 82% of fresh cycles 2015 (Q-IVF).

Surplus good quality embryos (GQE) can be cryopreserved for several years and used for transfer in later cycles. In recent years cryopreservation methods have become increasingly efficient and have, when investigated in randomized trials, resulted in similar (Shi *et al*., 2018; Vuong *et al.,* 2018) or even higher (Chen *et al.,* 2016) live birth rates compared to fresh transfer. Frozen/thawed embryo tranfer (FET) cycles comprise about one third of all IVF cycles performed in Sweden 2015 (Q-IVF).

Predictive factors and biomarkers

Predictors of live birth
The most important predictive factor for the chance of a live birth after IVF treatment is the woman´s age, reflected in the age of the oocyte. The primordial follicle pool, as well as the proportion of good quality oocytes, decreases with age (Baird *et al*., 2005) which is well reflected in treatment results. The chance of a live birth per fresh IVF cycle with SET was approximately 26% in the age cohort 30-35 while the chance was about 6% in women ≥42 years of age in Sweden (Q-IVF 2015). Other well known predictors for live birth are the number of oocytes retrieved, the number of good quality embryos achieved, the number of embryos transferred, previous failed IVF cycles, previous live birth after IVF, smoking, body mass index (BMI) and infertility diagnosis (Templeton *et al.,* 1996;. Arvis *et al.,* 2012). A few studies have suggested a capacity for serum anti-

Müllerian hormone (AMH) (Yates *et al.,* 2011; Brodin *et al.,* 2013; Arce *et al.,* 2013) and the number of sonographically visible antral follicles, the antral follicle count (AFC) (Jayaprakasan *et al.,* 2012) to predict live birth after IVF although, in systematic reviews and a recently published large cohort study, AMH and AFC were found to be poor predictors of live birth (Broer *et al.,* 2013; Iliodromiti *et al.,* 2014).

Predictors of ovarian response

Different biomarkers are also useful in evaluating ovarian function and for predicting ovarian response to hormonal stimulation. Examples of such biomarkers are inhibin B, FSH, AMH and AFC. Of these biomarkers AMH and AFC are considered to be most sensitive and specific, both highly predictive of ovarian response to stimulation (Iliodromiti *et al.,* 2015) and are therefore the predominant biomarkers used in clinical practice today.

AMH

AMH is a glycoprotein belonging to the TGF-β family and is, in the female, produced almost exclusively in the granulosa cells surrounding the growing follicles (Visser and Themmen 2006; Dewailly *et al.,* 2016). However, there is also evidence of AMH production in the endometrial cells (Wang *et al.,* 2009) and AMH expression has been found in the human brain (Cimino *et al.,* 2016) and the placenta (Novembri *et al.,* 2015). AMH can be detected in female fetuses from gestation week 36 (Visser and Themmen 2005). Levels are very low at birth then increase slowly until puberty where AMH-levels even out and peak at approximately 25 years of age. Hereafter a slow decline is seen throughout the fertile period and after menopause serum AMH is almost undetectable (Pankhurst 2017). In the ovary, AMH is detectable from the primary follicle stage and reaches its highest expression in the small (<4 mm) antral follicles. The expression gradually decreases with growing follicle size and is almost undetectable in follicles >10 mm. (Broer *et al.,* 2014).

In the post pubertal ovary AMH inhibits the transition of primordial into primary follicles. Furthermore, AMH inhibits the aromatase driven conversion of androgen to estrogen and decreases the sensitivity of the follicles to FSH (Dewailly *et al.,* 2016). Serum AMH level has been found to correlate significantly to the histologically visible number of primordial follicles present in the ovary (Hansen *et al.,* 2011), thus reflecting the ovarian reserve. Serum AMH has been considered to have minimal intracyclic variations, (van Disseldorp *et al.,* 2010; La Marca *et al.,* 2013) allowing analysis of a simple blood test on any cycle day, and it has consequently become a widely used biomarker in the evaluation of ovarian reserve and for decisions on fertility treatment and hormonal dosage. However, later studies have found significant intracyclic differences in serum AMH levels (Kissell *et al.,* 2014; Gnoth *et al.,* 2015) indicating that treatment decisions might depend on the day of serum sampling (Hadlow *et al.,* 2016;Iliodromiti *et al.,* 2017).

AFC

The number of sonographically visible antral follicles has been shown to have a high degree of correlation to the histologically detectable ovarian primor-

dial follicle pool (Hansen *et al.,* 2011) and AFC is known to decrease significantly with age (La Marca *et al.,* 2011). Hence, AFC is a useful tool for the evaluation of ovarian reserve and for the prediction of ovarian response to stimulation. AFC can easily be assessed in the sonografic evalution done on all patients before start of treatment though, to optimize the quality of the assessment and to minimize inter and intraobserver bias, it is recommended to follow a standard procedure (Broekmans *et al.,* 2010). Besides an experienced examiner, the sonography equipment should be of good quality, have a high resolution, and the probe should have a frequency of minimum 7 Hz. AFC assessment should be carried out on cycle day 2 to 4 and both ovaries should be assessed in two planes. All follicles between 2 and 10 mm are measured in two dimensions and the total number of follicles between 2 and 10 mm constitutes the AFC.

Stimulation strategies

There are two main strategies for ovarian stimulation for IVF, the long GnRH agonist and the short GnRH antagonist protocol. Traditionally the long GnRH agonist protocol has been used, initiating approximately two weeks of GnRH agonist treatment for downregulation of ovarian function and synchronizing of the follicles. A starting dose of FSH or hMG , mainly dependent on age, is given and dose adjustment is made according to the ovarian response.

Gradually, as GnRH antagonists have become available on the market, the trend has shifted towards a more widespread use

of the short protocol. The hormone stimulation starts in early follicular phase without downregulation and the GnRH antagonist is added, when follicle growth is once initiated, to prevent spontaneous ovulation. Besides the shorter treatment duration and lower total hormone doses an additional advantage is the avoidance of the hypoestrogenic side effects associated with GnRH agonist treatment.

No significant differences in ongoing pregnancy rates have been reported in individual randomized controlled trials (RCTs) comparing agonist versus antagonist protocols when applied to a general infertile population (Firouzabadi *et al.,* 2010; Guivareh-Leveque *et al.,* 2010; Toftager *et al.,* 2016), polycystic ovary syndrome (PCOS) patients (Lainas *et al.,* 2010; Haydardedeouglu *et al.,* 2012; Kim *et al.,* 2012) or in low reponders (Kim *et al.,* 2011; Prapas *et al.,* 2013). Similar results were found in a Cochrane analysis from 2016 (Al-Inany *et al.,* 2016). However, a recently published Dutch systematic review and meta-analysis found a significantly lower ongoing pregnancy rate in a general infertile population after antagonist protocol, while no differences were found in PCOS patients or poor responders (Lambalk *et al*., 2017). A significantly higher risk of ovarian hyperstimulation syndrome (OHSS) and cancelled cycles due to high response was found in the agonist protocols (Al-Inany *et al.,* 2016; Lambalk *et al.,* 2017), while a higher risk of cancelled cycles due to poor response was found in the antagonist protocols (Al-Inany *et al.,* 2016; Lambalk *et al.,* 2017). The authors from the Dutch review concluded that the agonist protocol might still be considered as the gold

standard for the majority of infertile patients, while PCOS patients and high responders might benefit from the antagonist protocol due to the lower risk of OHSS.

Several studies comparing agonist and antagonist protocols have also reported a significantly lower number of oocytes retrieved after antagonist protocol (Verberg *et al.,* 2009; Lambalk *et al.,* 2017) resulting in a milder stimulation. Mild stimulation has been proposed as a treatment strategy for maintaining live birth rates similar to those resulting from conventional stimulation, though with fewer side effects and less risk of OHSS. However, the concept of mild stimulation has no clear definition and several different protocols have been used. It has been proposed that, even though the rate of cancelled cycles due to poor response is higher, and live birth rate (LBR) per started cycle is lower, the risk of side effects and OHSS is also lower. The mild stimulation approach has been found to require significantly more treatment cycles per couple but the cumulative outcome, including all fresh cycles performed within a year, has been considered comparable to conventional stimulation, both concerning live birth rate and cost-effectiveness (Polinder *et al*., 2008).

OHSS is a feared and serious complication associated with ovarian stimulation and the increased risk correlates with the number of oocytes retrieved (Ji *et al.,* 2013; Steward *et al*., 2014). In severe cases OHSS can develop into a life-threatening condition with an increased risk of thromboembolic events (Rova *et al.,* 2012) and, though rare, deaths have been reported (Braat *et al.,* 2010; Mor and Schenker 2014).

The short protocol has one important advantage, enabling the so called "freeze all strategy" in cases of exessive ovarian respose and impending risk of OHSS. Oocyte maturation is then induced, using a GnRH agonist instead of hCG, and since the severe pathophysiological events observed in OHSS are hCG mediated, the risk of OHSS is almost eradicated (Kol and Humaidan 2013). However, without hCG the luteal phase is inadequate and the endometrium becomes inappropriate for implantation (Humaidan *et al.,* 2005) why all embryos need to be cryopreserved and used for subsequent FET cycles. With modern cryotechniques where embryos are cryopreserved at blastocyst stage after 5 to 6 days of culturing, the freeze all strategy has not affected the LBR negatively (Chen *et al.,* 2016; Shi *et al.,* 2018; Vuong *et al.,* 2018).

In recent years the trend has successively shifted from mild stimulation towards more individually tailored stimulation protocols. Dosage algorithms for predicting ovarian respose to stimulation have been designed, including different biomarkers, most frequently AMH and AFC. The individualized stimulation strategy aims to predict ovarian response, in order to achieve the optimal number of oocytes and to minimize side effects.

A few studies have investigated predictive factors for ovarian response using a multiple regression from a cohort setting (Popovic-Todorovic *et al.,* 2003a; Howles *et al.,* 2006) and subsequently tested the

prediction model in an RCT (Popovic-Todorovic et al., 2003b; Olivennes et al., 2015). In the study by Popovic-Todorovic, the dosage algorithm, based on the strongest predictive factors for oocyte yield, included the total number of antral follicles on cycle days 2 to 5, the total ovarian Doppler score on days 2 to 5, the total ovarian volume on days 2 to 5, age, and smoking status. When comparing the dosage algorithm to standard dosage, significantly higher rates of appropriate ovarian response and ongoing pregnancy were found in the algorithm group. The algorithm proposed by Howles et al., included age, BMI, early follicular phase serum FSH and AFC. When tested against standard dosage in an RCT, a significantly lower number of oocytes were retrieved and a lower rate of OHSS was found in the algorithm group, though no difference in clinical pregnancy rate occurred.

Several models for predicting ovarian response have been suggested as pediments for dosage decisions. Nelson et al., suggested dosage based on categorizing patients into presumptive low, normo or high responders based on serum AMH levels (Nelson et al., 2007; Nelson et al., 2009). Furthermore, dosages based on age, FSH and AMH (La Marca et al., 2012), as well as AMH, AFC and age (Brodin et al., 2015), have been suggested.

A recently published RCT compared individually tailored and standard dosages in an infertile population, but excluded PCOS. The study found a higher rate of patients with the targeted ovarian response, a lower rate of low (<4 oocytes) or excessive (>15 oocytes) reponse and a lower rate of OHSS preventive measures in the individual dosage group. However there were no significant differences between the groups in the number of cancelled cycles, the rate of severe OHSS or live birth rate (Nyboe Andersen et al., 2017).

The optimal number of oocytes

Several large cohort studies (van der Gaast et al., 2006; Hamoda et al., 2010; Sunkara et al., 2011; Fatemi et al., 2013; Stanger and Yovich 2013; Ji et al., 2013; Steward et al., 2014) have shown that the number of oocytes retrieved for IVF is a positive predictor of live birth. Between 5 and 15 oocytes have been found to be optimal for live birth in fresh cycles, with a lower LBR if fewer oocytes are retrieved. However, there is also a plateau (Hamoda et al., 2010; Fatemi et al., 2013; Stanger and Yovich 2013; Stewart et al., 2014) or even a decrease (van der Gaast et al., 2006; Sunkara et al., 2011; Ji et al., 2013) observed, if a higher number of oocytes are retrieved. A possible explanation for the stagnation in live birth rate associated with an increasing number of oocytes might be a negative impact on the endometrium caused by high serum estradiol levels (Valbuena et al., 1999). High gonadotropin doses have also been associated with an impaired embryo quality (Braga et al., 2012), although this association may be biased as high doses are also more common in women of advanced age and diminished oocyte quality. A recently published study did not find any association between gonadotropin doses or the number of retrieved oocytes and the rate of aneuploid embryos (Barash et al., 2017).

A high number of oocytes is also associated with an increased risk of OHSS (Ji *et al.,* 2013; Steward *et al.,* 2014), a feared and serious complication that in severe cases can develop into a life-threatening condition.

In recent years the techniques for culturing and cryopreserving embryos have improved dramatically. Embryos are now frequently cultured for 5 to 6 days and cryopreserved in the blastocyst stage. Transfers of a frozen/thawed blastocyst have a higher chance of resulting in a live birth than transfers of a frozen/thawed cleavage stage embryo. However blastocyst culture results in fewer embryos available for cryopreservation compared to cleavage stage embryos. It is also still unclear if the cumulative live birth rate, including all fresh and frozen transfers, after one oocyte retrieval, is higher after blastocyst culture than after cryo-preservation on day 2 (Glujovsky *et al.,*2016). As FET cycles become more common it is of increasing relevance to evaluate the optimal number of oocytes for the cumulative live birth rate per fresh and subsequent FET cycles. A few small single center studies (Ji *et al*., 2013; Stanger and Yovich 2013;) have reported increasing cumulative live birth rates with a higher number of oocytes retrieved.

Perinatal and obstetric outcomes

A continuous follow up on treatment efficacy and safety is of crucial importance for patients and professionals working with ART. Besides the most important efficacy variable LBR, safety variables such as serious adverse events during hormone stimulation, obstetric complications and perinatal outcome have to be evaluated.

In Sweden all ART treatment results, both from private and public clinics, are reported to the National Quality Registry of Assisted Reproduction (Q-IVF, www.qivf.se). This Quality Registry started in 2007 and includes all started ART cycles in Sweden and with the full identities of the women participating. Before 2007 IVF cycles were reported in two ways. Since 1982 all cycles and their results have been reported as aggregated data to the National Board of Health and Welfare but without personal identity numbers. Furthermore, all cycles leading to delivery have been reported to the National Board of Health and Welfare, with identified data (MBR-IVF 1982-2006), for research purposes. Full identity, which includes the woman's social security number, makes it possible to cross-link treatment data with other health registries such as the Medical Birth Registry (MBR), the National Patients Registry (NPR), the Causes of Death Registry, the Registry for Birth Defects and the Drug Registry. Furthermore, cross-linkage is possible to the approximately one hundred existing Swedish National Quality Registries, and to Statistics Sweden (SCB) for socio-economic data.

It is well known that children born after IVF, as opposed to spontaneous pregnancies, have a higher risk of adverse neonatal outcome such as preterm birth (PTB) small for gestational age (SGA), low birth weight (LBW) and perinatal mortality (McDonald *et al.,* 2009; Bergh and Wennerholm 2012; Pinborg *et al*., 2013) and that IVF pregnancies are associated with an increased risk of

obstetric complications (Sazonova et al., 2011; Luke et al., 2017).

The majority of obstetric and perinatal complications are associated with multiple pregnancies. However, the risk of obstetric complications such as hypertensive disorders of pregnancy (HDP) [which includes gestational hypertension, pre-eclampsia and eclampsia], gestational diabetes, placenta complications and peripartal hemorrhage, as well as perinatal complications such as PTB, LBW, SGA and perinatal mortality are still higher in IVF singleton pregnancies than in singletons from spontaneous pregnancies (Wennerholm et al., 2013; Qin et al., 2016; Luke et al., 2017). Furthermore, ART singletons have a higher risk of congenital malformations (Adjusted odds ratio [AOR] between 1.3 and 1.6) (Källén et al., 2010; Pandey et al., 2012; Hansen et al., 2013; Henningsen et al., 2018).

Both subfertility per se and IVF treatment, including ovarian stimulation and embryo culture techniques, have been suggested as risk factors for adverse perinatal and obstetric outcomes. An increased risk of PTB has been described in singletons born after IVF, but also in pregnancies resulting from ovarian stimulation without IVF. It has also been described in spontaneous pregnancies with a time to pregnancy >1 year, compared to spontaneous pregnancies with no history of subfertility (Pinborg et al., 2013). Subfertility followed by spontaneous conception has been defined as a risk factor for adverse obstetric as well as perinatal outcomes (Luke et al., 2017).

There is a difference in perinatal and obstetric outcome when comparing fresh embryo transfer and FET. In general, a better outcome has been observed for singletons after FET cycles compared to children born after fresh IVF cycles (Sazonova et al., 2012; Wennerholm et al., 2013; Maheshwari et al., 2018) as the risk of PTB, SGA and LBW is reduced. However, after FET a higher risk of large for gestational age (LGA) and macrosomia (Wennerholm et al., 2013; Ishihara et al., 2013; Maheshwari et al., 2018) as well as a higher risk of stillbirth (Henningsen et al., 2014) has been observed. As for obstetric outcomes, a higher risk of HDP has been found in ART pregnancies, in particular following cryopreservation (Opdahl et al., 2015; Chen et al., 2016; Maheshwari et al., 2018) and a higher risk of postpartum hemorrhage (Sha et al., 2018) has been reported after FET compared to fresh transfer.

Studies investigating the impact of embryo stage at fresh transfer on perinatal outcome have presented conflicting results. Several studies have reported a higher risk of PTB (Källén et al., 2010; Kalra et al., 2012; Dar et al., 2013; Alviggi et al., 2018) following blastocyst compared to cleavage stage transfer, while the difference in the risk of LBW and SGA seems less pronounced (Kalra et al., 2012; Martins et al., 2016; Ginström Ernstad et al., 2016; Alviggi et al., 2018). Even though an, increased risk of perinatal death has been reported after blastocyst transfer compared to fresh cleavage stage embryo transfer (Ginström Ernstad et al., 2016), this was not

confirmed in a recently published large review and metaanalysis (Alviggi *et al.,* 2018).

Earlier studies have described a higher risk of congenital malformations after blasto-cyst transfer (Källén *et al.,* 2010; Dar *et al.,* 2014) though, a recently published large cohort study found no increased risk of birth defects in singeltons after blastocyst transfer compared to singeltons after cleavage stage transfers. (Ginström Ernstad *et al.,* 2016).

A very encouraging finding, when comparing perinatal outcomes over time, is the observation of a significant decline in perinatal complications for singletons after ART, which has been noticed in most outcomes (Henningsen *et al*., 2015).

Aims of the thesis

➢ To investigate the performance of serum AMH, as part of a dosage algorithm, to predict the targeted number of oocytes collected for IVF

➢ To investigate the correlation between serum AMH values measured with two frequently used assays

➢ To investigate the association between the number of collected oocytes for IVF and live birth rate/cumulative live birth rate

➢ To investigate the association between the number of collected oocytes and the serious adverse reactions, severe OHSS and thromboembolic events

➢ To investigate the association between the number of collected oocytes, perinatal outcome and obstetric complications

Methodological considerations

The thesis comprises one randomized controlled trial, one retrospective observational study and two population based registry studies. An overview of the thesis is given in Figure 1.

The Regional Ethics Committee at Gothenburg University approved all studies (Dnr 219-12, Dnr 811-14, Dnr T 144-17).
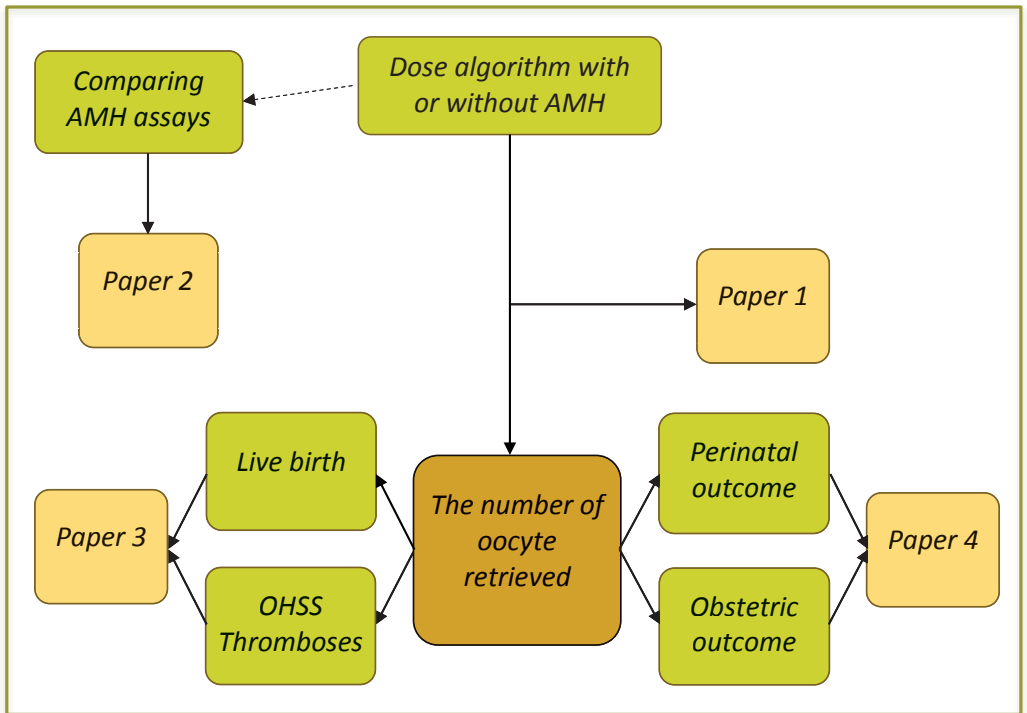

Figure 1. Overview of the thesis

National registries

The Swedish National Quality Registry of Assisted Reproduction (Q-IVF). Between 1982 and 2006 and after the birth of the first IVF child in Sweden, data on all assisted reproductive treatments was reported to The Swedish National Board of Health and Welfare. During these years, 1982 to 2006, only clustered data was reported for IVF cycles. In addition, in collaboration with The Swedish National Board of Health and Welfare, at three occasions during this period, full identification data for deliveries resulting from IVF was collected for research purposes. Since this data file is stored at the Medical Birth Registry (MBR), for the purpose of this study it was decided to name this file, the Medical Birth Registry/IVF (MBR/IVF). In 2007 the National Quality Registry for Assisted Reproduction (Q-IVF) was established and funded by the Swedish Association of Local Authorities and Regions (SKL). This registry includes results of all started IVF cycles in Sweden along with full personal identification, i.e. patient´s social security number. All IVF clinics, public as well as private, report treatment characteristics and results to Q-IVF and the results are public and posted on the Q-IVF website (www.qivf.se). All patients are informed about the Q-IVF and may choose not to have their data included, although this is very rare.

The Swedish National Patient Registry (NPR) includes information on International Statistical Classification of Diseases and Related Health Problems - Tenth Revision (ICD 10) codes for all specialized out-patient and in-patient care in Sweden. Primary health care is not included. The Registry was launched in 1964, reached full coverage in 1987 and since 2001, both public and private specialized healthcare units are included. The registry was validated in 2011 and was shown to have a high validity for most diagnoses making the registry suitable for large-scale population-based research (Ludvigsson *et al.,* 2011).

The Medical Birth Registry (MBR) started in 1973 and includes all pregnancies leading to birth. Data includes variables on pregnancies, deliveries and new borns. The registry was validated in 1990 and found to have high validity and includes virtually all deliveries in Sweden (Cnattingius *et al*., 1990; MBR 2003).

Statistics Sweden (SCB), a large national database including, for example, data on demographics, socio-economic conditions and educational levels in the Swedish population.

Aim 1
To investigate the performance of serum-AMH, as part of a dosage algorithm, to predict the targeted number of oocytes collected for IVF.

Background
In recent years, numerous observational studies (Nelson *et al*., 2007; Nelson *et al*., 2009; Brodin *et al*., 2015; Nelson *et al*., 2015) and systematic reviews (La Marca *et al*., 2010; Broer *et al*., 2013; La Marca *et al*., 2014; Ilidromiti *et al*., 2015) have reported that the biomarkers AMH and AFC are good predictors of ovarian response. Following these results, many IVF clinics in Sweden and elsewhere have included AMH measurements as part of

their routine fertility work-up despite the fact that no randomized trial has proven their efficacy.

Methodological considerations

AFC and AMH are considered to have similar performance in sensitivity and specificity for ovarian response and have a high rate of correlation (Brodin *et al*., 2015). One RCT comparing a dose algorithm based on AFC to one based on AMH found no difference in the primary outcome variable, the desired ovarian response (Lan *et al.,* 2013). However, no RCT has compared the performance of AMH-based dosage with standard dosage with the primary aim of predicting ovarian response. Furthermore, no RCTs have investigated whether combining AMH and AFC into the same algorithm would result in a better prediction of ovarian response than using only one of them.

We investigated the performance of an AMH-based algorithm against usual dosage procedure in a randomized controlled setting. Thus, the FSH dose was adjusted to patient characteristics including age, BMI and AFC and further adjusted in the intervention group according to the AMH value. The reason for this particular study design was to be able to include all women, independent of expected poor, normal or high responses. Another possibility would have been to test an AMH-based dose algorithm against a fixed FSH dose in patients with expected normal ovarian response. Although randomized trials comparing different FSH doses in women with expected poor response have not shown any benefits of a higher FSH dose on the number of oocytes retrieved or live birth rate (Lefebvre *et al.,* 2015; Basfu

et al., 2016; van Tilborg *et al*., 2017), the same might not be true for women with an expected high response. Ovarian stimulation in high responders is more complicated as it is associated with an increased risk of OHSS, and new knowledge on how to optimize stimulation for these patients is important.

The intention was therefore to study the model in a generally infertile population; hence all patients eligible for IVF with standard technique, going through their first treatment cycle and using own gametes, were asked to participate. The conventional dosage algorithm used in everyday practice in our department, including AFC, age and BMI, was used for the control group. The primary aim was to investigate whether the addition of AMH to the conventional dosage algorithm would increase the rate of patients having the targeted number of oocytes retrieved and thereby reduce the rate of poor and excessive responses.

The most important outcome variable for successful treatment is live birth. Ideally, the design of the present study would have included non-inferiority for live birth and superiority for optimal ovarian response. Such a design would, however, have required a considerably higher number of women in the study. Several studies have found that between 5 and 15 retrieved oocytes result in optimal LBR after fresh cycles (van der Gaast *et al.,* 2006; Hamoda *et al*., 2010; Fatemi *et al.,* 2013; Sunkara *et al.,* 2011; Steward *et al.,* 2014). The number of retrieved oocytes, between 5 and 12, chosen as the desired response in this study, was decided on as giving an appropriate balance between efficacy and

safety (Chen *et al*., 2015).

Statistics

The flow chart of the study is illustrated in Figure 2. Statistical analyses were performed using a Full Analysis Set (FAS). The FAS consisted of all randomized patients who had at least one follow up variable. In addition, a per protocol (PP) analysis was performed excluding patients with protocol violations. The statistical methods used are summarized in Table 1.
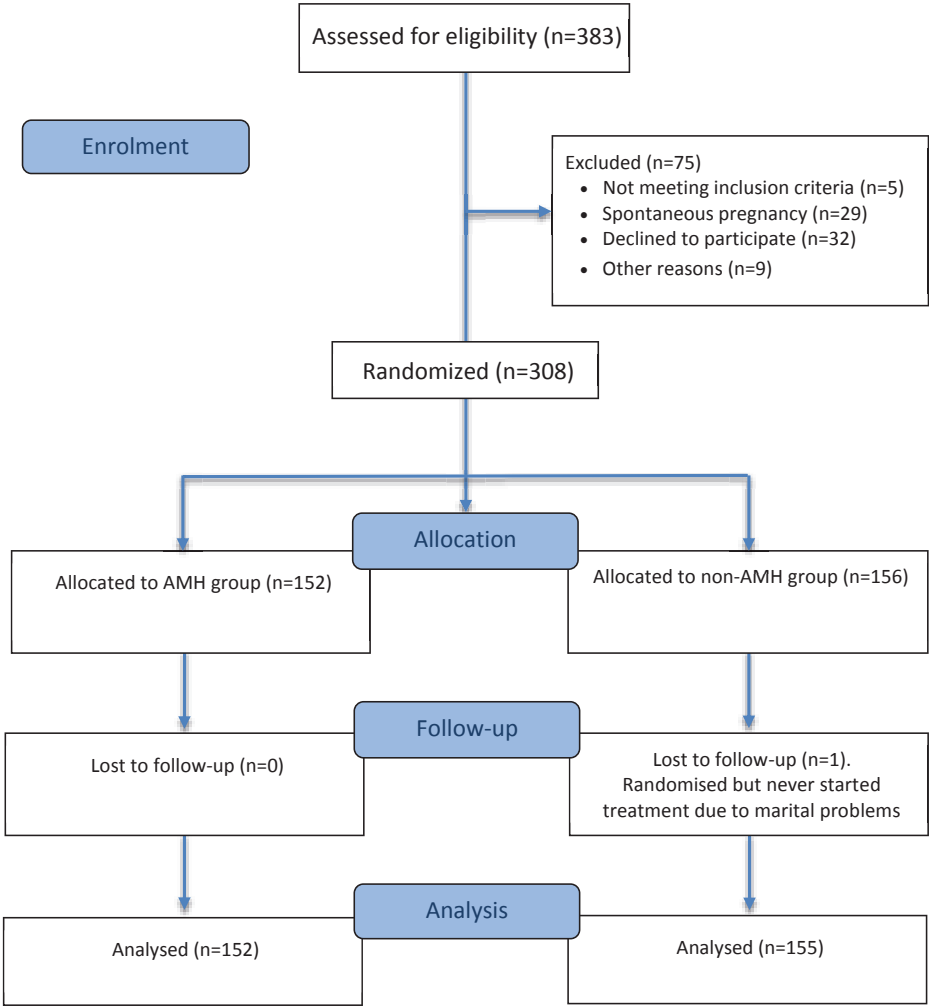
AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone

Figure 2. Flow chart of a randomized trial comparing two dose algorithms for starting dose of FSH in IVF.

Table 1. Overview of statistical methods

	Paper I	Paper II	Paper III	Paper IV
Study design	Randomized controlled trial	Retrospective cohort study	Population-based registry study	Population-based registry study
Sample size	308	269	77,956	27,359
Study period	2013-2016	2013-2016	2007-2014	2002-2015
Cross-linking			Q-IVF, NPR	Q-IVF, NPR, MBR, MBR-IVF, SCB
Statistical methods				
Association between dependent and independent variables			Generalized Estimated Equation. OR and AOR 95% CI	Univariable and multivariable logistic regression analysis OR and AOR 95% CI
Descriptive continuous variables	Mean, SD, median, minimum and maximum		Mean, SD, median, minimum and maximum	Mean, SD, median, minimum and maximum
Descriptive dichotomous variables	Numbers and percentage		Numbers and percentages	Numbers and percentages
Analyses dichotomous variables	Fisher's exact test 95% CI			
Analyses ordered categorical variables	Mantel-Haenszel chi-square test			
Analyses non-ordered categorical variables	Pearson's chi-square test			
Analyses continuous variables	Fisher's non-parametric permutation test $p<0.05$			
Correlation between AFC and AMH	Spearman´s rank correlation coefficient			
Correlation between AMH assays		Spearman´s correlation coefficient Bland Altman Plot Passing-Bablok regression		
Interobserver agreement AFC assessment	Wilcoxon Signed Rank test			

SD, standard deviation. CI, confidence interval. OR, odds ratio. AOR, adjusted odds ratio

Aim 2
To investigate the correlation between serum AMH values measured with two frequently used assays.

Background
Blood samples from the first 26 patients randomized to the AMH group were analysed using The Beckman Coulter Gen II original assay. In 2013, Beckman Coulter reported instability in their assay, due to complement interference. Since complement interference appeared to be a problem in serum samples stored at room temperature but not in frozen samples (Welsh *et al*., 2014), we regarded the AMH values as reliable. For the remaining 126 patients randomized to the AMH group, the modified Beckman Coulter Premix method was used.

The aim of this study was, in a parallel setting, to investigate the relationship between AMH values analysed simultaneously with the Gen II original assay and the Premix method assay, in a secondary analysis of a randomized, controlled trial of well characterized infertile women.

Methodological considerations
The study was a retrospective observational study. At the first visit a blood sample was taken from all patients participating in the RCT. Serum was immediately stored in -70º C. If the patient was randomized to the AMH group the sample was thawed and analysed for AMH. For the first 26 patients the Gen II original assay was used. For the remaining 126 patients randomized to the AMH group the sample was analysed in a parallel setting using both the Gen II original assay and the Premix method,

although the results from the Premix method were used for AMH classification.

After study termination all samples from the non AMH group were thawed and analysed in a parallel setting using both the Gen II original assay and the Premix method. In total 269 serum samples were analysed with both assays. In twelve patients samples were missing.

The statistical method used is listed in Table I.

Aim 3
To investigate the association between the number of collected oocytes for IVF and LBR /cumulative live birth rates.

Aim 4
To investigate the association between the number of collected oocytes and the serious adverse reactions OHSS and thrombo-embolic events.

Background
The optimal number of oocytes for live birth in fresh IVF cycles has been found to be between 5 and 15 oocytes (van der Gaast *et al.,* 2006; McAvey *et al*., 2011; Ji *et al*., 2013).

As SET has become more widespread (Ishihara *et al.,* 2015), particularly in the Scandinavian countries (Thurin *et al*., 2004; Pandian *et al.,* 2013; McLernon *et al.,* 2010), more embryos have become available for freezing and thawing and FET cycles now constitute a third of all IVF cycles performed yearly in Sweden. Consequently it is of increasing relevance to evaluate the optimal number of oocytes for cumulative live birth rate, including one

fresh cycle and all subsequent FET cycles from the same oocyte pick up.

Since a high number of oocytes is associated with an increased risk of severe OHSS (Ji *et al.,* 2013; Steward *et al*., 2014) it is of importance to find the balance between efficacy, i.e. cumulative live birth rate and the risk of serious complications.

The study addressed two aims. First, to investigate the association between the number of oocytes retrieved and LBR resulting from fresh IVF cycles. Furthermore the cumulative LBR after one fresh and all subsequent FET cycles from one oocyte retrieval.

Second, to investigate the relationship between the number of retrieved oocytes in fresh cycles and the serious side effects, severe OHSS and thromboembolic events.

Methodological considerations
From 2007 data has been collected on all started IVF cycles performed in Sweden, both cycles leading to pregnancy and live birth, and cycles not resulting in pregnancy. Data, including deliveries after fresh cycles performed between 2007 and 2013 and FET cycles performed between 2007 and 2014 were available in 2016, at the start of this study.

A strength of the study is the large study population and the population-based cohort design where all cycles except oocyte donation cycles are included. Thus, no selection bias is present. Furthermore, all cycles are linked to the patient´s social security number and it is thereby possible to cross-link to other health and quality registries, in this case the NPR.

The study period included all fresh cycles performed between 2007 and 2013 and FET cycles performed between 2007 and 2014. In Sweden the cryopreservation of embryos is allowed for five years. A weakness of the study is that for fresh cycles performed between 2010 and 2013 all FET cycles may not have been included in the cumulative data. Hence, there is a possibility that the cumulative live birth rate was underestimated. However, an analysis of data from earlier in the study period showed that a majority of cryo-preserved embryos were thawed and transferred within a year after the fresh cycle. The addition of further years to the cumulative data would probably have increased the cumulative live birth rates slightly.

Registry studies have some further disadvantages. Consequences of missing data and outliers, i.e. unreasonable variable values, have to be analyzed in relation to the impact on study outcomes. A further weakness in the IVF registry is that the Q-IVF registry does not include data on in-fertility diagnoses or variables concerning embryo quality.

Statistical method
Generalized Estimating Equations (GEE) were used to analyze data with repeated measurements. The GEE estimates the population mean associations and can be used for different types of outcome measurements. We used GEE to estimate the association between the number of oocytes retrieved and deliveries. Hosmer-Lemeshow tests were used to find the best model (linear, second degree, or third degree polynomial) representing the number of oocytes fitting the predicted

calculation of outcome to the actual observed outcome. Adjustments were performed for the known confounding factors maternal age, year of treatment, previous failed IVF cycles, previous IVF children and fertilization method used (conventional IVF/ICSI) (Templeton *et al*., 1996; Arvis *et al.,* 2012), although in population-based studies it is possible that there are additional unknown confounders.

The statistical methods used are summarized in Table I.

Aim 5

To investigate the association between the number of collected oocytes, perinatal outcome and obstetric complications.

Background

It is well known that singletons born after IVF have a higher risk of adverse neonatal outcome (McDonald *et al.,* 2009; Pinborg *et al*., 2013; Wennerholm *et al.,* 2013; Qin *et al*., 2016) and IVF pregnancies are associated with an increased risk of obstetric complications (Sazonova *et al*., 2011; Qin *et al*., 2016; Luke *et al*., 2017). Only a few studies, and with conflicting results, have investigated the association between the number of oocytes retrieved and adverse perinatal and obstetric outcome (Sazonova *et al.,* 2011; Sunkara *et al.,* 2015)

Methodological considerations

From 2002, births after IVF were identified in MBR/IVF and later in the Q-IVF registry. Using social security numbers data was cross-linked to MBR, NPR, and SCB. Data on perinatal and obstetric morbidity was collected from MBR and NPR. Socioeconomic data was collected from SCB. Data on fresh cycles performed in 2015 was available when the study started in 2017.

In the study period, 28,059 singleton babies were born after ovarian stimulation and IVF, excluding cycles with oocyte donation. In 700 cases (2.5%), data on the number of oocytes retrived was missing, leaving a study population of 27,359 singletons.

Statistical methods

Uni- and multivariable logistic regression analyses were used to explore the association between the number of oocytes retrieved and outcome variables. Adjustments were performed for known and possible confounders such as maternal age, parity, smoking, BMI, cause of infertility, maternal educational level, maternal country of birth, treatment period, embryo stage, fertilization method (IVF/ICSI), OHSS and vanishing twin. Every confounder was tested using a backward elimination procedure to find the final multivariable model. A Hosmer-Lemeshow test and visual inspection was used to find the best model (linear, second degree, or third degree polynomial) representing the number of oocytes fitting the predicted calculation of outcome to the actual observed outcome.

Missing data

Missing data for different variables are presented in the Table 6 concerning patient characteristics. In the regression analyses missing data are replaced with mean values.

The statistical methods used are summarized in Table 1.

Results and comments

Paper I

The addition of anti-Müllerian hormone in an algorithm for individualized hormone dosage did not improve the prediction of ovarian response -a randomized, controlled trial.

Prior to the decision regarding starting dose, 308 patients, aged 18 to 40 years and with a BMI of 18.0 to 35.0, starting their first IVF treatment with their own gametes and using standard technique, were randomized to one of two algorithm groups. Algorithm 1 (AMH group), included serum AMH, BMI, age and AFC. Algorithm 2 (non AMH group) included BMI, age and AFC, representing the usual base for dosage decisions in our clinic.

The primary outcome variable was the rate of patients in each group having the targeted number of oocytes (between 5 and 12) retrieved.

Secondary outcome variables were:
- the rate of moderate/severe OHSS
- the rate of OHSS preventing strategies (coasting or freezing of all embryos)
- live birth rate
- biochemical pregnancy rate
- the rate of patients with poor response (<5 oocytes retrieved)
- the rate of patients with excessive response (>12 oocytes retrieved)
- the rate of cancelled cycles due to excessive response
- the rate of cancelled cycles due to poor response
- the rate of cancelled cycles other reasons
- the total dose of gonadotropin
- the number of follicles $\geq$12 mm at 0-2 days before hCG
- the number of oocytes retrieved
- the rate of dose adjustments
- fertilization rate
- the number of good quality embryos on day 2
- the number of transferred embryos
- day of embryo transfer
- the number of cryopreserved embryos day 2 and/or 5
- miscarriage rate

Results
There were no significant differences between the groups concerning demographic background variables (Table 2).

Results are presented In Table 3.

Table 2. Summarized demographic and baseline characteristics by randomized group (FAS population).

Variable	AMH (n=152)	No AMH (n=155)	p-value	Difference between groups Mean (95% CI)
Age at first dose (years)	32.3 (4.0) 32.4 (21.4; 39.3)	32.3 (3.8) 32.5 (20.2; 39.3)	0.94	-0.04 (-0.92; 0.85)
Duration of infertility (months)	32.4 (14.7) 30 (1; 120)	32.0 (14.9) 30 (0; 108)	0.83	0.38 (-2.92; 3.69)
PCOS				
No	127 (83.6)	126 (81.3)	0.71	2.3 (-6.9; 11.4)
Yes	25 (16.4)	29 (18.7)		-2.3 (-11.4; 6.9)
BMI (kg/m^2)	23.6 (3.7) 22.8 (18.1; 35.1)	23.5 (3.6) 22.9 (18.0; 35.0)	0.82	0.09 (-0.72; 0.91)
AFC	21.6 (12.0) 19 (3; 73)	21.3 (11.3) 18 (6; 70)	0.85	0.26 (-2.37; 2.88)
AMH	4.03 (3.53) 2.95 (0.20; 18.20) n=148			
AMH				
Low <1.55 ng/ml	36 (24.3)			
Normal 1.55-2.95 ng/ml	38 (25.7)			
High >2.95 ng/ml	74 (50.0)			

AFC, antral follicle count; AMH, anti-Müllerian hormone; PCOS, polycystic ovarian syndrome
For categorical variables, n (%) is presented.
For continuous variables, the mean (SD)/median (min; max)/n= is presented.

Table 3. Summarized Efficacy analyses by randomized group (FAS population).

Variable	AMH (n=152)	No AMH (n=155)	p-value	Difference between groups Mean (95% CI)
5-12 oocytes	81 (53.3)	96 (61.9)	0.16	-8.6 (-20.3; 3.0)
<5 oocytes	39 (25.7)	17 (11.0)	0.0013	14.7 (5.5; 23.9)
>12 oocytes	32 (21.1)	42 (27.1)	0.27	-6.0 (-16.2; 4.1)
Cancelled cycles poor response	7 (4.6)	4 (2.6)	0.52	2.0 (-2.8; 6.8)
Cancelled cycles excessive response	0	0	1.00	
Moderate/severe OHSS	5 (3.3)	6 (3.9)	1.00	-0.6 (-5.4; 4.2)
OHSS preventing strategies	6 (3.9)	12 (7.7)	0.24	-3.8 (-9.7; 2.1)
Dose adjustment	83 (54.6)	81 (52.3)	0.77	2.3 (-9.5; 14.2)
Total dose gonadotropin (IU)	1685	1604	0.41	80.9 (-112.1; 273.3)
Number of good quality embryos	2.74 (2.69) 2 (0; 14) n= 138	2.87 (2.27) 3 (0; 11) n= 141	0.67	-0.13 (-0.72; 0.46)
Number of cryopreserved embryos	1.88 (2.51) 1 (0; 12) n= 138	2.15 (2.10) 2 (0; 10) n= 140	0.35	-0.27 (-0.81; 0.28)
Biochemical pregnancy	64 (46.7)	62 (44.6)	0.82	2.1 (-10.4; 14.6)
Live birth/cycle	48 (31.6)	42 (27.1)	0.46	4.5 (-6.3; 15.3)

For categorical variables, n (%) is presented.
For continuous variables, the mean (SD)/median (min; max)/n= is presented.
For comparisons between groups, Fisher's exact test was used for dichotomous variables and Fisher's non-parametric permutation test for continuous variables.

The targeted number of oocytes
The rate of patients having 5 to 12 oocytes retrieved was 53.3% in the AMH group and 61.9% in the non AMH group, p=0.16, difference (−8.6 [95% CI−20.3; 3.0]). Hence no significant difference was seen in the primary outcome variable when comparing the two algorithms.

Pregnancy and live birth
Biochemical pregnancy rate was 46.7% in the AMH group and 44.6% in the non AMH group, p=0.82. Live birth rate per started cycle was 31.6% in the AMH group and 27.1% in the non AMH group, p=0.46.

Excessive response
The rate of patients having >12 oocytes retrieved was 21.1% in the AMH group and 27.1% in the non AMH group, p=0.27. The majority of patients with excessive response were classified as high responders and consequently had a low starting dose of 75 or 100 IU (Figure 3). The number of patients with severe OHSS was 5 (3.3%) in the AMH group and 6 (3.9%) in the non AMH group, p=1.00. In the AMH group 6 (3.3%) patients underwent OHSS prevention strategies compared to 12 (7.7%) patients in the non AMH group. The difference was not statistically significant, p=0.24.

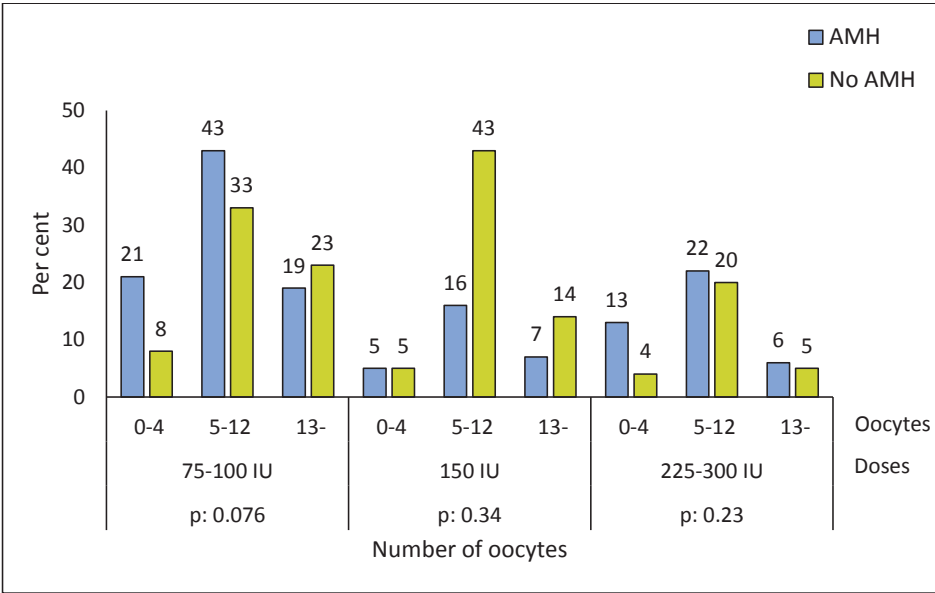**Figure 3.** Percentage of patients is shown on the y-axis and number of patients is shown above each bar. The p-values refer to significance tests in distribution of oocytes between AMH and non-AMH groups.

Poor response

The rate of patients having <5 oocytes retrieved was 25.7% in the AMH group and 11.0% in the non AMH group (14.7 (5.5; 23.9]), p=0.0013. One might assume that the cause of this difference is that patients with high AMH received too low a starting dose. However, when dividing the patients according to the starting dose (low, normal, or high dose), no significant differences in the distribution of oocytes were seen between AMH and non-AMH groups (Figure 3). There was no significant difference between the groups in the rate of cancelled cycles due to poor response.

Conclusions and comments

In conclusion there was no difference between the two algorithms in predicting the targeted number of oocytes retrieved.

There was, however, a significantly higher rate of patients with poor ovarian response in the AMH group, although there was no difference in cancelled cycles due to poor response or pregnancy rates or LBR between the groups. A post hoc analysis of all poor responders revealed a positive correlation between poor response and BMI, indicating that a high BMI requires a higher starting dose, despite AMH and/or AFC indicating a presumed high response.

Furthermore, in a post hoc analysis of the AMH group, 19/39 patients with poor responses would have had a higher starting dose if decisions had been made without AMH, while 17/32 patients with excessive responses would have had a higher starting dose without AMH. This might indicate that AMH is a more useful tool in avoiding

excessive rather than poor responses. However, the rate of excessive response and moderate/severe OHSS was not significantly different between the two group.

Paper II

The correlation between AMH assays differs depending on actual AMH levels. Using the original Beckman Coulter Gen II Eliza assay and the modified premix Beckman Coulter assay, 269 serum samples from infertile women, originating from the RCT presented in Paper I, were analysed in a parallel setting.

The aim was to investigate the correlation between the assays, since both of them were used in the RCT.
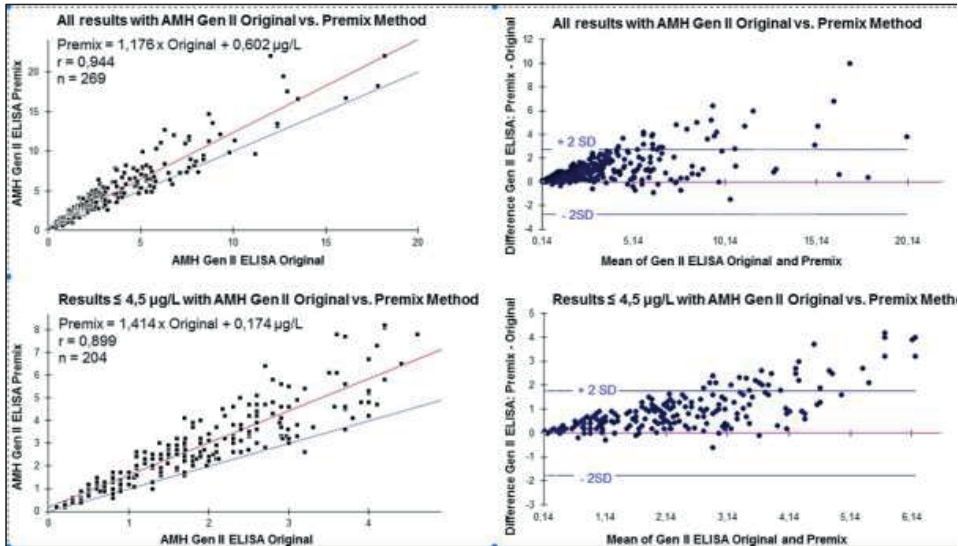
Results
The correlation coefficient between the two assays was 0.94 (Spearman´s correlation coefficient). A Bland Altman plot for analysis of the difference between the two assays revealed the modified assay giving

higher AMH values than the original assay. The difference increased with increasing means. This was confirmed in the linear Passing-Bablok regression where the overall difference between the assays was 18%, although for lower AMH values (up to 4.5 μg/L with original assay) the difference was approximately 40%. The results are presented in Figure 4.

Comments
The results might partly explain the surprisingly high rate of patients with poor response (<5 oocytes retrieved) found in the group of patients randomized to the AMH algorithm. Some of these patients were probably incorrectly classified as high responders and consequently received a starting dose that was too low.

Hence, the results indicate a risk of misclassification of patients according to presumed ovarian response and thereby the risk of an incorrect dosage. It is urged that careful attention be paid when AMH values are translated between AMH assays for dose decisions.

All results with AMH Gen II Original vs. Premix Method

Premix = 1,176 x Original + 0,602 µg/L
r = 0,944
n = 269

All results with AMH Gen II Original vs. Premix Method

Results ≤ 4,5 µg/L with AMH Gen II Original vs. Premix Method

Premix = 1,414 x Original + 0,174 µg/L
r = 0,899
n = 204

Results ≤ 4,5 µg/L with AMH Gen II Original vs. Premix Method

r, correlation coefficient; n, number of samples.

Figure 4 Comparison of the results from the Beckman Coulter AMH Gen II ELISA, using the Original method and the modified Premix method. The upper charts show all anti-Müllerian hormone results (up to about 20 µg/L) and the charts below show results where the Original method concentrations are up to 4.5 µg/L (corresponding to an average of about 6.5 µg/l with the Premix method). The left panels represent the linear least squares regression plots and the right panels represent the Bland–Altman plots.

Paper III

The number of oocytes retrieved during IVF; a balance between efficacy and safety.

Between 2007 and 2013, 39,387 women underwent 77,956 fresh IVF cycles in Sweden. Between 2007 and 2014, 36,270 FET cycles were performed with embryos originating from these fresh cycles. Demographic data and treatment characteristics are presented in Table 4.

Results

The association between the number of oocytes retrieved, LBR and cumulative LBR

The LBR increased by the number of oocytes retrieved in fresh cycles up to 11 oocytes and then evened out. A slight decrease was observed if more than 19 oocytes were retrieved. The highest observed LBR was 30.3% for the mean age 34 years (Figure 5).

In 2,178 out of the 77,956 (2.8%) oocyte aspirations all embryos were cryo-preserved. Excluding the "freeze all" cycles resulted in fewer cycles in the denominator, hence the graph for LBR after fresh cycles was slightly increased and showed a highest level in LBR of 32%. The cumulative LBR was of course not affected.

A plateau, at 20 oocytes, was also observed in the cumulative LBR where the highest rate observed was 45.8% (Figure 5).

The cumulative LBR increased significantly by the number of oocytes (Adjusted odds ratio [AOR] 1.06, 95% Confidence interval [CI]: 1.06; 1.07).

In the multivariate analysis, maternal age, previous failed cycles and ICSI (vs IVF) affected LBR negatively, while the year of treatment and live birth of a previous IVF child affected LBR positively (Table 5).

The association between the number of oocytes retrieved and OHSS
The total number of severe (hospitalized) OHSS cases was 371 (0.5%). The risk of severe OHSS increased significantly by the number of retrieved oocytes (Odds ratio [OR] 1.13, 95% CI: 1.12; 1.15). Adjustment for age only changed the estimate marginally (AOR 1.12, 95% CI: 1.08; 1.14). The increase in incidence had a steeper slope from 18 oocytes where the rate of OHSS was 1%, the incidence reaching 2.5% at 25 oocytes retrieved. The incidence of OHSS, LBR and cumulative LBR in relation to the number of oocytes retrieved is illustrated in Figure 6 and the incidence in relation to the number of

oocytes retrieved as a categorized variable is illustrated in Figure 7. The distribution of early (starting <9 days after oocyte retrieval) and late (starting ≥10 days after oocyte retrieval) OHSS was similar when <15 oocytes were retrieved, while early OHSS was numerically more common if ≥15 oocytes were retrieved. Out of the 371 patients with severe OHSS, 171 (46.1%) achieved a live birth.

The association between the number of oocytes retrieved and thromboembolic events
The total number of patients who experienced thromboembolic events was 14. Two patients were registered for two thromboembolic events each, in the same fresh cycle, resulting in a total number of events of 16. The incidence of thrombo-embolic events in relation to the number of oocytes retrieved as categorized variable showed a significant increase in the higher categories of 15 to 19 and >20 oocytes, compared to the low categories <10 and 10 to 14 oocytes (p for trend ≤0.008), (Figure 8). The most common ICD 10 diagnosis was deep thrombosis in pregnancy (ICD 10 code O22.3). Of patients who had experienced thromboembolic events, 8/14 (57.1%) had a live birth.

Comments
The condition of OHSS has only one ICD 10 code, N98.1 which is used to describe a spectrum from mild discomfort to serious conditions demanding intensive care. We considered it most clinically relevant to include only serious OHSS cases, which is why we chose to include only patients treated in in-patient units. Some serious cases may have been treated in out-patient

units and might consequently be missing from our data, although we consider such cases to be rare.

It would be of great interest to relate LBR to the FSH doses given per oocyte retrieved i.e. the ovarian sensitivity to stimulation. Since 2009, data on gonadotrophin doses is reported to the Q-IVF registry and will eventually be available for future studies. Taking both efficacy (live births) and safety into account, ovarian stimulation up to between 18 and 20 oocytes retrieved seems optimal from a cumulative live birth perspective, keeping severe adverse events at a reasonable level.

Table 4. IVF/ICSI data characteristics at cycle and woman level, respectively (Sweden 2007–2013)

| Characteristics (cycle level)	N=77,956	
	n	(%)
Maternal age		
18-34 years	39555	(50.7)
35-37 years	18404	(23.6)
38-39 years	11068	(14.2)
40 years and over	8929	(11.5)
Previous failed fresh cycles		
0	40157	(51.5)
1	18921	(24.3)
2	9930	(12.7)
3 or more	8948	(11.5)
Any previous IVF child	5083	(6.5)
Treatment type		
IVF	39226	(50.3)
ICSI	37886	(48.6)
Oocytes retrieved		
Median [IQR]	9	[5-12]
Year of cycle start		
2007-2009	31912	(40.9)
2010-2011	23057	(29.6)
2012-2013	22987	(29.5)
Maternal age at first IVF treatment (woman level)		
	N=39,387	
18-34 years	22493	(57.1)
35-37 years	8637	(21.9)
38-39 years	4543	(11.5)
40 years and over	3714	(9.4)
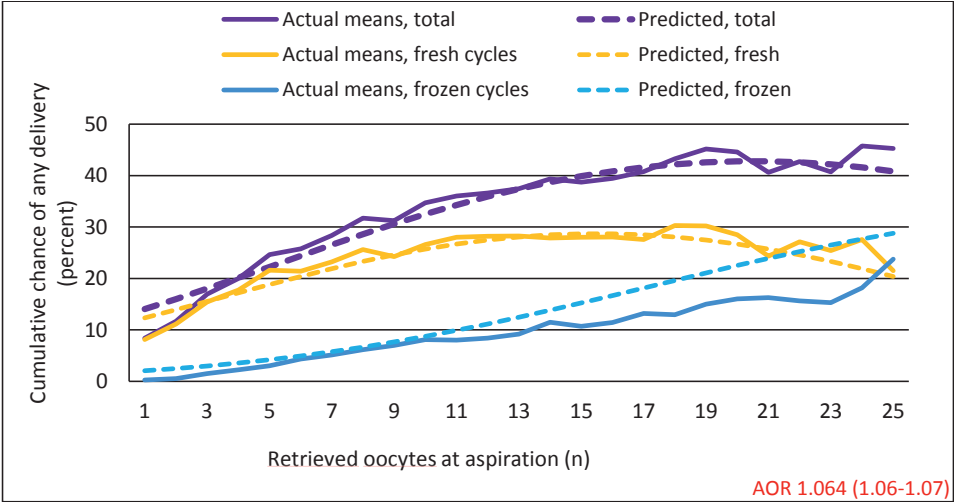
Figure 5. Percentage live birth in relation to the number of oocytes retrieved (considering fresh, subsequent frozen and cumulatively, respectively). Actual percentages and results from general estimation equations (GEE) analyses using models with one linear and one quadratic term.
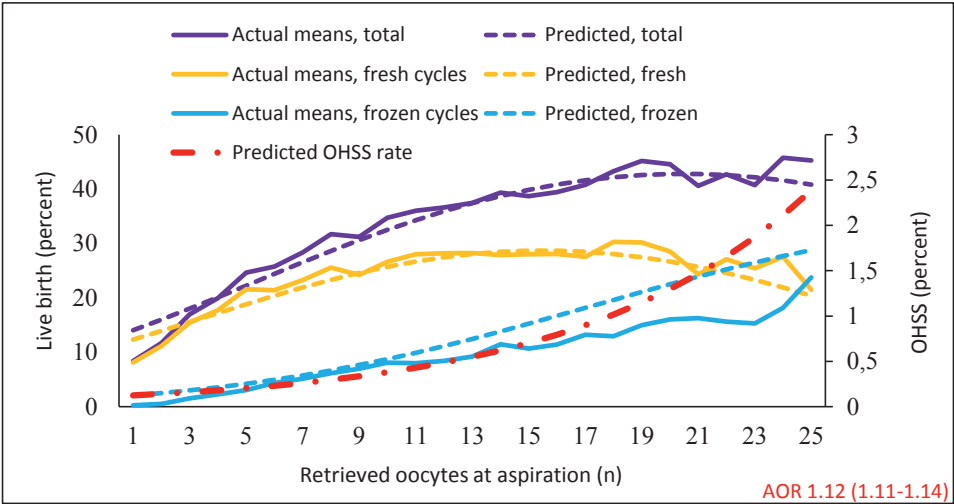

Figure 6. Percentage live birth in fresh cycles and cumulatively (left y-axis) and percentage OHSS (right y-axis) in relation to the number of oocyte retrieved.

Table 5. Odds Ratios for live birth considering both fresh and subsequent frozen cycles. Results from generalized estimating equations (GEE) analyses (linear models).

|	Crude estimates		Estimates multivariate model	
	OR	95% CI	OR	95% CI
Oocytes retrieved (per one step increment, linear model)	1.073	1.070-1.077	1.064	1.061-1.067
Maternal age (one year increment)	0.923	0.920-0.926	0.936	0.932-0.939
Year (one year increment)	1.021	1.013-1.029	1.031	1.022-1.039
Previous failed fresh treatments (one step increment)	0.866	0.855-0.877	0.900	0.888-0.912
Previous children with IVF (one step increment)	1.218	1.143-1.298	1.462	1.373-1.556
ICSI (vs IVF)	0.839	0.812-0.866	0.819	0.792-0.846

OR, odds ratio; CI, confidence interval
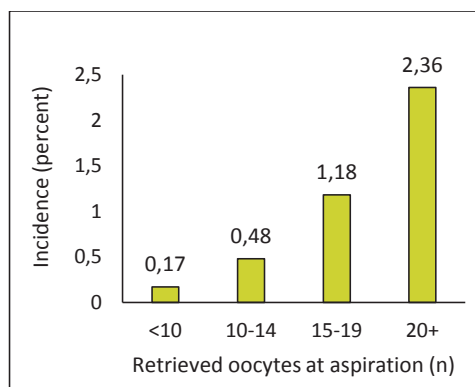
Figure 7 Incidence of OHSS in relation to the number of oocytes retrieved (categorzed). $p<0.0001$ (Chi square for trend).
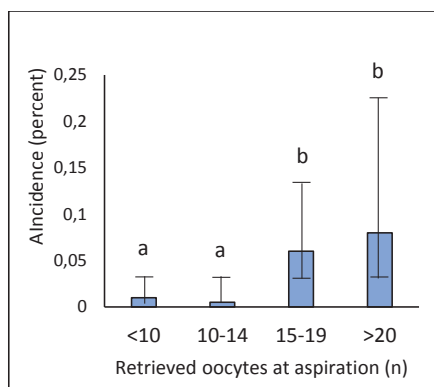

Figure 8 The incidence of thromboembolic events by the number of retrieved oocytes (categorized). Incidents and 95% confidence intervals (CI). P = 0.008 (Chi-square for trend). P<0.05 (unlike letters differ significantly, Fisher's exact test).

Paper IV

The association between the number of oocytes retrieved for IVF, perinatal outcome and obstetric complications.
Between 2002 and 2015, 28,059 singelton babies were born after fresh IVF cycles in Sweden. In 700 cases no information on the number of oocytes retrieved was available, leaving a study cohort of 27,359 children with complete data for analysis.

Demographic data and treatment characteristics are listed in Table 6.

Descriptive statistics on outcomes are summarized in Table 7.

Results
The association between the number of oocytes retrieved and perinatal outcome
The perinatal outcomes are summarized in Table 8. No significant associations were found between the number of collected oocytes and the main perinatal outcomes PTB (AOR 1.002, 95% CI 0.994 to 1.011), very PTB [VPTB] (AOR 1.013, 95% CI 0.994 to 1.032), SGA (AOR 0.998, 95% CI 0.988 to 1.009), birth defects (AOR 1.009, 95% CI 0.998 to 1.020) or peri/neonatal death (AOR 1.008, 95% CI 0.975 to 1.043). However, in the descriptive data a skewed gender distribution was observed and a secondary analysis showed a significant association between >20 oocytes retrieved and a higher rate of males (AOR 1.126, 95% CI 1.014 to 1.249). Blastocysts were also independently associated with a higher rate of males and after adjustment for embryo stage (cleavage stage/blastocyst) the association was still significant.

Subgroup analysis for poor responders (≤3 oocytes retrieved) showed no significant association for any of the perinatal outcomes.

Subgroup analysis for OHSS revealed that OHSS was independently associated with PTB (AOR 1.56, 95% CI 1.23 to 1.97), VPTB (AOR 1.65, 95% CI 1.02 to 2.69) and SGA (AOR 1.51, 95% CI 1.12 to 2.03).

Subgroup analysis for anovulatory infertility/PCOS showed that anovulatory infertility/PCOS was independently associated with PTB (AOR 1.41, 95% CI 1.25 to 1.59), VPTB (AOR 1.50, 95% CI 1.16 to 1.92) and peri/neonatal death (AOR 0.18, 96% CI 0.09 to 0.33).

The association between the number of oocytes retrieved and obstetric complications
The incidence of placenta praevia in relation to the number of oocytes retrieved as categorized variable is illustrated in Figure 9. A significant association was found between the number of oocytes and placenta praevia (AOR 1.020, 95% CI 1.004 to 1.036) independent of embryo stage, though blastocysts were also independently associated with placenta praevia. For HDP a negative association was found with the number of oocytes retrieved as continuous variable (OR 0.99, 95% CI 0.98 to 1.00) though no association was found when adjusting for confounders (AOR 0.99, 95% CI 0.98 to 1.00). (Table 8).

No significant associations were found between the subgroups poor responders

(≤3 oocytes), anovulatory infertility/PCO or OHSS and any of the obstetric outcomes.

Comments

While no association between the number of oocytes retrieved and main perinatal outcome was found, an association was observed between OHSS and PTB. Since OHSS is more common in IVF treatments due to anovulatoy infertility/PCOS this observation might reflect the increased risk of PTB also observed in PCOS patients after spontaneous conception (Roos *et al.,* 2011).

An association between placenta praevia and IVF has previously been described (Qin *et al***.,** 2016; Luke *et al.,* 2017), especially after blastocyst transfer (Sazonova *et al.,* 2011; Ginström *et al.,* 2016). However the present study showed an independent, though weak, association between the number of oocytes retrieved and placenta praevia.

Previous studies have reported an association between blastocyst transfer and a higher rate of male gender (Dean *et al.,* 2012; Maalouf *et al.,* 2014; Ginström *et al.,* 2016). The correlation between >20 oocytes retrieved and the higher number of males found in the present study remained significant after adjustment for embryo stage. This might well be a random finding and should be interpreted with caution.

Table 6. Characteristics of study population.

	Number of oocytes retrieved							
	<10 N=13 431		10–14 N=8552		15–19 N=3843		≥20 N=1533	
	n	(%)	n	(%)	n	(%)	n	(%)
Year of treatment								
2002-2004	2348	(17.5)	1490	(17.4)	710	(18.5)	289	(18.9)
2005-2007	2589	(19.3)	1745	(20.4)	858	(22.3)	395	(25.8)
2008-2010	3303	(24.6)	2120	(24.8)	977	(25.4)	427	(27.9)
2011-2013	3620	(27.0)	2197	(25.7)	919	(23.9)	305	(19.9)
2014-2015	1571	(11.7)	1000	(11.7)	379	(9.9)	117	(7.6)
Maternal age								
Mean [SD]	33.39	[4.3]	32.63	[4.2]	32.21	(4.1)	31.95	[3.9]
18-34 years	7735	(57.6)	5624	(65.8)	2682	(69.8)	1108	(72.3)
35-37 years	3222	(24.0)	1788	(20.9)	774	(20.1)	311	(20.3)
38-39 years	1570	(11.7)	779	(9.1)	270	(7.0)	78	(5.1)
40 years and over	902	(6.7)	361	(4.2)	116	(3.0)	36	(2.3)
Not known	2	(0.0)	0	(0.0)	1	(0.0)	0	(0.0)
Paternal age, Mean [SD]	37.13	[5.8]	36.61	[5.7]	36.42	[5.8]	36.13	[5.4]
Parity								
0	9784	(72.8)	6293	(73.6)	2836	(73.8)	1140	(74.4)
≥1	3647	(27.2)	2259	(26.4)	1007	(26.2)	393	(25.6)
Previous Cesarean Section	803	(6.0)	474	(5.5)	222	(5.8)	68	(4.4)
Maternal smoking								
Yes	315	(2.3)	203	(2.4)	78	(2.0)	35	(2.3)
Not known	685	(5.1)	426	(5.0)	185	(4.8)	77	(5.0)
BMI								
<18.5	204	(1.5)	145	(1.7)	63	(1.6)	17	(1.1)
≥25	4643	(34.6)	2717	(31.8)	1193	(31.0)	432	(28.2)
Non known	1101	(8.2)	707	(8.3)	326	(8.5)	116	(7.6)
Maternal education								
≤9 years	344	(2.6)	208	(2.4)	98	(2.6)	32	(2.1)
10-12 years (high school)	2898	(21.6)	1880	(22.0)	854	(22.2)	348	(22.7)
≤2 years post high school	1334	(9.9)	850	(9.9)	367	(9.5)	157	(10.2)
3 years post high school	2627	(19.6)	1749	(20.5)	776	(20.2)	312	(20.4)
4 years post high school	1594	(11.9)	1042	(12.2)	440	(11.4)	161	(10.5)
≥5 years post high school	415	(3.1)	262	(3.1)	123	(3.2)	53	(3.5)
Not known	4219	(31.4)	2561	(29.9)	1185	(30.8)	470	(30.7)
Infertility cause								
Male factor	2666	(19.8)	1966	(23.0)	1011	(26.3)	460	(30.0)
Tubal factor	922	(6.9)	576	(6.7)	271	(7.1)	107	(7.0)
Endometriosis	1231	(9.2)	661	(7.7)	249	(6.5)	88	(5.7)
PCO	1920	(14.3)	1295	(15.1)	674	(17.5)	352	(23.0)
Unexplained	3783	(28.2)	2466	(28.8)	1055	(27.5)	506	(33.0)
Other female factor	6890	(51.3)	4381	(51.2)	1986	(51.7)	784	(51.1)
Mixed male/female	389	(2.9)	283	(3.3)	139	(3.6)	62	(4.0)
Involuntary childlessness (years)								
Mean (SD)	3.11	[2.0]	3.08	[1.8]	3.17	[1.9]	3.35	[2.0]
Not known	2756	(20.5)	1651	(19.3)	821	(21.4)	295	(19.2)
Maternal country of birth								
Sweden	10810	(80.5)	7109	(83.1)	3208	(83.5)	1280	(83.5)
Other Nordic	240	(1.8)	134	(1.6)	64	(1.7)	30	(2.0)
Other Europe	905	(6.7)	545	(6.4)	244	(6.3)	93	(6.1)
Africa	159	(1.2)	83	(1.0)	39	(1.0)	16	(1.0)
Asia	1037	(7.7)	548	(6.4)	232	(6.0)	86	(5.6)
Other	223	(1.7)	97	(1.1)	42	(1.1)	24	(1.6)
Country not known	41	(0.3)	27	(0.3)	10	(0.3)	3	(0.2)
Maternal disease								
Preexisting diabetes mellitus	99	(0.7)	66	(0.8)	21	(0.5)	9	(0.6)
Chronic hypertension	99	(0.7)	66	(0.8)	21	(0.5)	9	(0.6)
Renal disease	99	(0.7)	66	(0.8)	21	(0.5)	9	(0.6)
Treatment type								
IVF	7427	(55.3)	4564	(53.4)	1988	(51.7)	823	(53.7)
ICSI	6004	(44.7)	3988	(46.6)	1855	(48.3)	710	(46.3)
Number of oocytes ≤3	2008	(15.0)	0	(0.0)	0	(0.0)	0	(0.0)
Culture days								
1-3	12395	(92.3)	7165	(83.8)	3121	(81.2)	1200	(78.3)
4-7	1036	(7.7)	1387	(16.2)	722	(18.8)	333	(21.7)
Type of embryo transfer								
SET	9958	(74.1)	6636	(77.6)	3065	(79.8)	1251	(81.6)
DET	3470	(25.8)	1913	(22.4)	777	(20.2)	282	(18.4)
Number of gestational sacs								
Number of sacs = 1	12875	(95.9)	8234	(96.3)	3685	(95.9)	1472	(96.0)
Number of sacs ≥2	260	(1.9)	137	(1.6)	72	(1.9)	21	(1.4)
OHSS, severe	178	(1.3)	225	(2.6)	158	(4.1)	138	(9.0)

SET, single embryo transfer; DET, double embryo transfer; OHSS, ovarian hyperstimulation syndrome

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Table 7. Neonatal and obstetric outcome by number of oocytes retrieved

	Number of oocytes retrieved							
	<10 N=13 431		10–14 N=8552		15–19 N=3843		≥20 N=1533	
	n	(%)	n	(%)	n	(%)	n	(%)
Gestational age								
Mean (SD), days	277	[15.0]	277	[15.3]	277	[14.8]	276	[16.6]
<37weeks	1023	(7.6)	625	(7.3)	288	(7.5)	127	(8.3)
<32weeks	178	(1.3)	120	(1.4)	44	(1.1)	29	(1.9)
≥42weeks	737	(5.5)	511	(6.0)	204	(5.3)	89	(5.8)
Not known	3	(0.0)	0	(0.0)	2	(0.1)	0	(0.0)
Birth weight								
Mean(SD), g	3423	[603]	3422	[605]	3428	[596]	3397	[633]
<2500g	723	(5.4)	470	(5.5)	211	(5.5)	106	(6.9)
<1500g	151	(1.1)	111	(1.3)	38	(1.0)	24	(1.6)
≥4500g	372	(2.8)	227	(2.7)	103	(2.7)	39	(2.5)
Not known	26	(0.2)	14	(0.2)	6	(0.2)	1	(0.1)
Birth weight								
SGA	669	(5.0)	402	(4.7)	174	(4.5)	80	(5.2)
LGA	512	(3.8)	313	(3.7)	132	(3.4)	53	(3.5)
Not known	30	(0.2)	15	(0.2)	10	(0.3)	1	(0.1)
Infant gender								
Males	6772	(50.4)	4432	(51.8)	1953	(50.8)	830	(54.1)
Females	6659	(49.6)	4120	(48.2)	1890	(49.2)	703	(45.9)
Apgar scores at 5 min								
<7	212	(1.6)	135	(1.6)	55	(1.4)	18	(1.2)
Not known	86	(0.6)	53	(0.6)	18	(0.5)	5	(0.3)
Peri/neonatal mortality								
Stillbirth	40	(0.3)	27	(0.3)	10	(0.3)	3	(0.2)
Neonatal death	23	(0.2)	21	(0.2)	4	(0.1)	2	(0.1)
Any significant malformation	565	(4.2)	389	(4.5)	176	(4.6)	79	(5.2)
Neonatal morbidity								
Preterm birth related*	138	(1.0)	93	(1.1)	44	(1.1)	29	(1.9)
Meconium aspiration	19	(0.1)	13	(0.2)	4	(0.1)	0	(0.0)
Septicaemia	136	(1.0)	94	(1.1)	45	(1.2)	20	(1.3)
HIE	10	(0.1)	6	(0.1)	3	(0.1)	2	(0.1)
PVL	4	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Pregnancy complications								
HPD	822	(6.1)	443	(5.2)	217	(5.6)	82	(5.3)
Gestational diabetes	319	(2.4)	175	(2.0)	84	(2.2)	16	(1.0)
PPROM	363	(2.7)	221	(2.6)	85	(2.2)	34	(2.2)
Placenta praevia	246	(1.8)	177	(2.1)	92	(2.4)	43	(2.8)
Placental abruption	98	(0.7)	58	(0.7)	31	(0.8)	10	(0.7)
Postpartum haemorrhage	849	(6.3)	544	(6.4)	248	(6.5)	109	(7.1)
Mode of delivery								
VE/forceps	1460	(10.9)	860	(10.1)	336	(8.7)	123	(8.0)
Cesarean section	3604	(26.8)	2115	(24.7)	946	(24.6)	344	(22.4)
Onset of delivery								
Spontaneous	8906	(66.3)	5882	(68.8)	2676	(69.6)	1067	(69.6)
Induction of labour	2734	(20.4)	1592	(18.6)	700	(18.2)	283	(18.5)
Cesarean section before contractions	1825	(13.6)	1095	(12.8)	472	(12.3)	184	(12.0)

*Neonatal morbidity related to preterm birth includes, retinopathy of prematurity [ROP], bronchopulmonary dysplasia [BPD], necrotizing enterocolitis (NEC) and intraventricular hemorrhage grade 3 [IVH].
SGA, small for gestational age; LGA, large for gestational age; HIE, hypoxic ischemic encephalopathy (HIE); PVL, periventricular leukomalacia; HDP hypertensive disorders of pregnancy; PPROM, preterm premature rupture of membranes; VE, vacuum extraction

Table 8. Association between the number of oocytes retrieved (continuous variable) and main perinatal and obstetric outcomes.

Perinatal outcome	OR	95 % CI	AOR*	95 % CI
PTB (<37 weeks)	1.004	0.996-1.013	1.002	0.994-1.011
VPTB (<32 weeks)	1.016	0.997-1.035	1.013	0.994-1.032
SGA (>2 SD)	0.999	0.988-1.009	0.998	0.988-1.009
Peri/neonatal death	0.992	0.960-1.025	1.008	0.975-1.043
Major birth defects	1.009	0.998-1.019	1.009	0.998-1.020
Obstetric outcome				
HDP	0.989	0.979-0.999	0.990	0.980-1.000
Placenta praevia	1.026	1.011-1.041	1.020	1.004-1.036

Adjustments were made for maternal age, maternal country of birth, parity, smoking, BMI, cause of infertility, maternal educational level, treatment period, embryo stage, fertilization method (IVF/ICSI), ovarian hyperstimulation syndrome (OHSS) and vanishing twin.
PTB, preterm birth; VPTB, very preterm birth; SGA, small for gestational age; HDP, hypertensive disorders of pregnancy
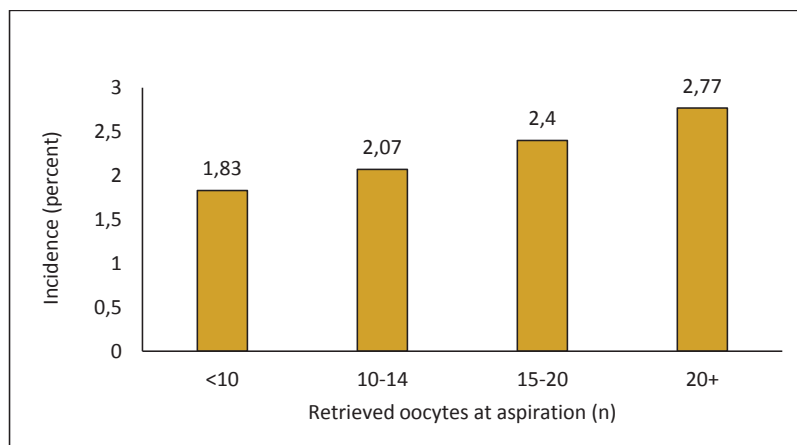
Figure 9 Incidence of placenta praevia in relation to the number of oocytes retrieved (categorized). p= 0.002 (Chi square for trend).

Discussion

The main findings from this thesis are:

➤ A dosage algorithm including AMH was not superior to an algorithm without AMH in predicting the targeted number of oocytes.

➤ The correlation between AMH assays differs depending on actual AMH levels.

➤ The optimal number of oocytes retrieved for the highest cumulative LBR was between 18 and 20.

➤ The incidence of OHSS and thromboembolic events increased rapidly if more than 20 oocytes were retrieved.

➤ No association was found between the number of oocytes retrieved and main perinatal outcomes. However an association was found between the number of oocytes retrieved and placenta praevia.

The overall aim in the field of ART is to provide fertility treatment in order to optimize the chance of the birth of a healthy baby. However, IVF involves the use of potent drugs for controlled ovarian hyperstimulation, which causes a risk of excessive ovarian response and serious side effects. Hence the challenge for the clinician is to find the treatment balancing the best chance of pregnancy and live birth with the lowest risk of complications.

The aim of this thesis was to address some important aspects of treatment efficacy and safety.

How to achieve the optimal ovarian response?

Several studies have shown that there is an optimal interval in ovarian response, expressed as between 5 and 15 oocytes retrieved, for the best chance of live birth after a fresh IVF cycle (van der Gaast *et al.,* 2006; Hamoda *et al.,* 2010; Sunkara *et al*., 2011; Fatemi *et al.,* 2013; Ji *et al.,* 2013; Stanger *et al.,* 2013; Steward *et al.,* 2014). Hence the number of oocytes retrieved has become a commonly used surrogate outcome variable for LBR and cumulative LBR.

However the optimal ovarian response must also be considered from a safety perspective, keeping the risk of serious adverse events in mind.

Hormone dosage is an important step in achieving the optimal number of oocytes and requires knowledge of predictive factors for ovarian response. Well-known predictive factors traditionally used for dose decision are age and BMI. In addition, different biomarkers such as FSH, inhibin B, AMH and AFC are highly correlated to ovarian response and of these, AMH and AFC are considered to have the highest sensitivity and specificity.

Methodological issues considering biomarkers

To be useful in clinical practice, prediction models developed for individual dosage should be superior to standard dosage. Furthermore they should be standardized and reproducible and thereby able to use in any infertility unit. Hence, biomarkers used as part of dosage strategies based on prediction models should be well defined.

AFC
All women starting IVF treatment are subject to a thorough medical and physical examination. A vaginal sonography is always performed including assessment of ovarian morphology and AFC. However AFC varies during and between menstrual cycles though these variations have not been considered as significant in evaluating AFC for prediction of ovarian response (Hansen *et al.,* 2003; Deb *et al.,* 2009). Inter observer agreement has also been found to be fairly good (Hansen *et al.,* 2003; Deb *et al.,* 2009). The accuracy of sonography equipment is improving steadily and the previous limits defined for high response ($\geq$12 follicles) and poor response ($\leq$3 follicles) are now being questioned. A recent review publication from the Androgen Excess and Polycystic Ovary Syndrome Society suggests a threshold for an increased number of antral follicles to be $\geq$25 (Dewailly *et al.,* 2014 b). A study investigating AFC in oocyte donors (Melo *et al.,* 2009) found a higher rate of cancelled cycles if AFC was <10, hence questioning the threshold for poor response. Consequently future classification of patients according to presumed ovarian response might be re-evaluated. However, with the use of high quality sonography equipment, experienced investigators and a standard procedure (Broekmanns *et al.,* 2010) it is possible to achieve high precision in AFC assessment.

In the randomized controlled trial presented in Paper I, the control group had their starting dose decided by using AFC as a single biomarker, together with BMI and age. In order to optimize the precision of AFC assessment, this procedure was limited to two physicians using the standardized method described by Broekmanns *et al*. A Wilcoxon Signed Rank test showed very good interobserver agreement (ICC=0.94, p=0.17).

AMH and ovarian response
In recent years AMH has become widely used for fertility assessment and the prediction of ovarian response.

Several observational studies (Nelson *et al*., 2007; Nelson *et al*., 2009; Brodin *et al*., 2015; Nelson *et al*., 2015) and systematic reviews (La Marca *et al*., 2010; Broer *et al*., 2013; La Marca *et al*., 2014; Ilidromiti *et al*., 2015) have stated that both AMH and AFC are good and comparable predictors of ovarian response and that the two biomarkers are highly correlated (Brodin *et al*., 2015).

A few studies have suggested AMH to be superior to AFC in the prediction of ovarian response (Arce *et al.,* 2013; Nelson *et al.,* 2015). However, the conclusion was based on post hoc analyses of AMH and AFC from two previous large randomized multicentre studies (Nyboe Andersen *et al.,* 2006; Devroey *et al.,* 2012) and compared AMH samples analyzed in a central

laboratory for all study sites with AFC assessments performed locally.

One single randomized controlled study has compared an AMH based dosage algorithm with one based on AFC and found no difference in the desired ovarian response (Lan *et al.,* 2013).

AMH is generally considered to be cycle-independent and is considered to be an attractive biomarker possible to analyse from a simple blood test on any cycle day. Furthermore, it is supposed to lack the inter observer agreement associated with AFC assessment. However there are also considerable and clinically important methodological issues connected to this biomarker.

AMH has been shown to have considerable variations in the menstrual cycle (Overbeek *et al.,* 2012; Hadlow et a*l.,* 2013; Gnoth *et al.,* 2015) and a recent study showed intra cyclic variations up to 20%, indicating that almost 30% of patients classified for presumed ovarian response might be misclassified depending on the day of serum sampling (Hadlow *et al.,* 2016).

One might expect that AMH values from different laboratories using the same assay should be comparable. However, considerable inter-laboratory variations were recently described in a study comparing AMH values from 10 different laboratories, all using the Beckman Coulter Gen II assay, analysing the same serum samples, (Zuvela *et al*., 2013).

There are three main automatized AMH assays available on the market today, the Ultra-Sensitive AMH/MIS ELISA kit

(Ansh Labs), the automated Access AMH assay (Beckman-Coulter) and the Elecsys® AMH Immunoassay (Roche). The correlation between the assays has been tested and found good although the AMH levels differ between the assays (Li *et al.,* 2016). Hence classification of ovarian response cannot be done using the same reference values. Iliodromiti *et al*., addressed this issue in a recent publication. The study, comparing two of the most frequently used automatized assays today (Elecsys® and Access), found that 28% of patients having had their starting dose decided based on serum AMH levels might have been misclassified if values from the two assays had used the same reference intervals (Iliodromiti *et al.,* 2017).

In 2007 Nelson *et al.,* presented a prediction model for the classification of fertility patients into presumed low, normal and high responders, according to serum AMH levels (Nelson *et al.,* 2007). The prediction model, developed with the DSL enzyme-linked immunosorbent (ELIZA) AMH assay was found to be useful for deciding on starting dose, increasing the rate of patients with an appropriate ovarian response and reducing the rate of excessive and poor response (Nelson *et al.,* 2009).

The first generation Diagnostic Systems Lab (DSL) and Immunotech (IOT) assays were eventually abandoned and in 2010 replaced by the Beckman Coulter Gen II AMH assay. The correlation between these assays was investigated and considered to be good, although the AMH values measured by the Gen II assay were found to be approximately 40% higher than the DSL assay (Wallace *et al*., 2011). Consequently a conversion factor had to be

used for translation between the assays, and a method for this procedure was described by Nelson and La Marca (Nelson and La Marca 2011). This classification for presumed ovarian response and the Gen II assay was initially used in the study presented in Paper I.

In 2013 Beckman Coulter reported instability in their assay due to complement interference, and analyses of serum samples after storage in room temperature were shown to result in AMH values of between 20 and 40% lower in the Gen II assay than in the DSL assay (Rustamov *et al*., 2012). Beckman Coulter modified the Gen II original assay with a pre-diluting step before analysis to eliminate complement interference and the Modified AMH assay (Premix method) was released and considered stable. However serum AMH analysed with the Premix model showed higher levels than the Gen II assay and again a conversion factor was used. The majority of the patients in our study were randomized after 2013, hence AMH values analysed with the Premix method were used for dose decision in these patients.

However, in a post hoc analysis, we decided to analyse all serum samples in a parallel setting using both methods in order to describe the correlation between the methods. The methodological problems connected to the AMH assays are described in Paper II.

A study comparing the Gen II original assay with the Premix method in a population of women in early pregnancy described the Premix method as giving considerably higher values than the original assay (Bonifacio *et al.,* 2015).

The findings from the study by Bonifacio were confirmed by our study. Furthermore, when comparing serum samples from our study using the original Gen II assay and the Premix method in a parallel setting, an unexpected and important finding was revealed. The difference between the assays depended on the actual AMH levels, resulting in overall 18% higher values with the Premix method. However for AMH in the lower range (up to 4,5 μg/L) the difference was 40%.

This might partly explain the surprisingly high rate of patients with poor response (<5 oocytes retrieved) found in the group of patients randomized to the AMH algorithm. Some of these patients were probably incorrectly classified as high responders and consequently received a starting dose that was too low.

Furthermore this finding indicates that conversion factors between different assays may not be valid for the the whole range of serum AMH values.

AMH and ovarian reserve
The use of AMH in fertility counselling is especially troublesome given the lack of international standards on reference intervals and conversion factors. The possibility of misclassifying presumed ovarian response depending on the assay used (Iliodromiti *et al.,* 2017) almost certainly also applies to assessing ovarian reserve. Hence there is a considerable risk of incorrect information being provided to women who want to take decisions on

advancing or postponing pregnancy or even having oocyte retrieval and vitrification for fertility preservation. Even though there is a correlation between ovarian reserve, expressed as the number of antral follicles in the ovary, and serum-AMH (Hansen *et al.,* 2011), several studies have shown the lack of association between low AMH values and fecundity (Hagen *et al.,* 2012; Somigliani *et al.,* 2015; or time to pregnancy (Depmann *et al.,* 2017; Hvidman *et al.,* 2017; Korsholm *et al.,* 2018).

Low AMH values have also been described in combined contraceptive pill users (Bentzen *et al.,* 2012) and low AMH values in women with hypothalamic amenorrhea has not been found to indicate an inability to respond to ovarian stimulation (Billington and Corenblum 2016; Bry-Gauillard *et al.,* 2017), which is important to bear in mind in fertility counselling.

 AMH and IVF outcomes
Several studies have suggested AMH to be a predictor of embryo quality (Brodin *et al.,* 2013) and LBR after IVF (Yates *et al.,* 2011; La Marca *et al.,* 2011; Brodin *et al.,* 2013; Arce *et al.,* 2013; Nelson *et al.,* 2015). However, the association with embryo quality seems mainly to have to do with the larger number of oocytes retrieved and embryos created in patients with a high AMH, and hence more embryos being available for selection (Nelson *et al.,* 2012; Scheffer *et al.,* 2017). In systematic reviews (Broer *et al.,* 2013; Iliodromiti *et al.,* 2014) and a recently published large cohort study (Tal *et al.,* 2018) AMH was found to be a poor predictor of live birth after IVF.

Individual or standard dosage?

Even though individually tailored stimulation protocols have become more frequently used in recent years, only a few RCT´s have shown an advantage in predicting ovarian response when compared to standard dosage. (Popovic-Todorovic *et al.,* 2003; Nyboe-Andersen *et al.,* 2017; Allegra *et al.,* 2017). In two recently published RCT´s from the Netherlands randomization was based on AFC (vanTilborg *et al.,* 2017; Oudshoorn *et al.,* 2017). Presumtive poor responders and high responders were randomized to an individual or standard dosage, while presumtive normoresponders received the standard dose.

In the study by van Tilborg *et al.,* on poor responders, similar LBR was found in the individual and the standard dosage groups. However a higher number of cancelled cycles, mainly due to poor response, was observed in the standard dosage group (20.3% vs 9.6%) and a significantly higher number of cycles with excessive response was observed in the individual dosage group (8.6% vs 3.4%). There was no difference in the rate of severe OHSS between the groups. Treatment costs were significantly higher in the individual dosage group.

In the study by Oudshoorn *et al.,* on high responders there was no difference in LBR between the two groups. There was however a significantly higher rate of excessive response in the standard dosage group (30.4 vs 10.5%) and a significantly higher rate of poor response in the

individual dosage group (29.0 vs 14.3%). No significant difference in the rate of severe OHSS was found.

A recently published Cochrane analysis (Lensen *et al.,* 2018) concludes that the evidence for the advantage of an individually tailored dosage compared with standard dosage is insufficient when applied to a general infertile population (Figure 10) though individual dosage for presumtive high responders might lower the risk of excessive ovarian response (Figure 11).
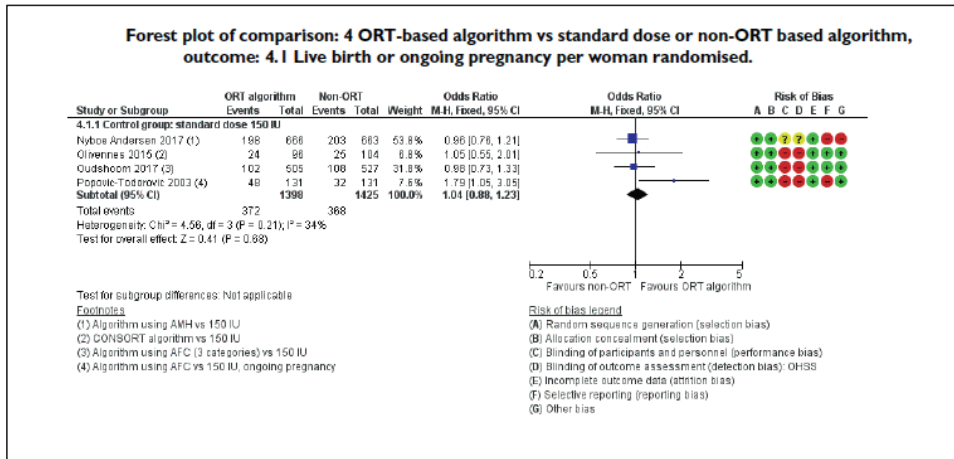

Figure 10 (With permisson from Cochrane Library)
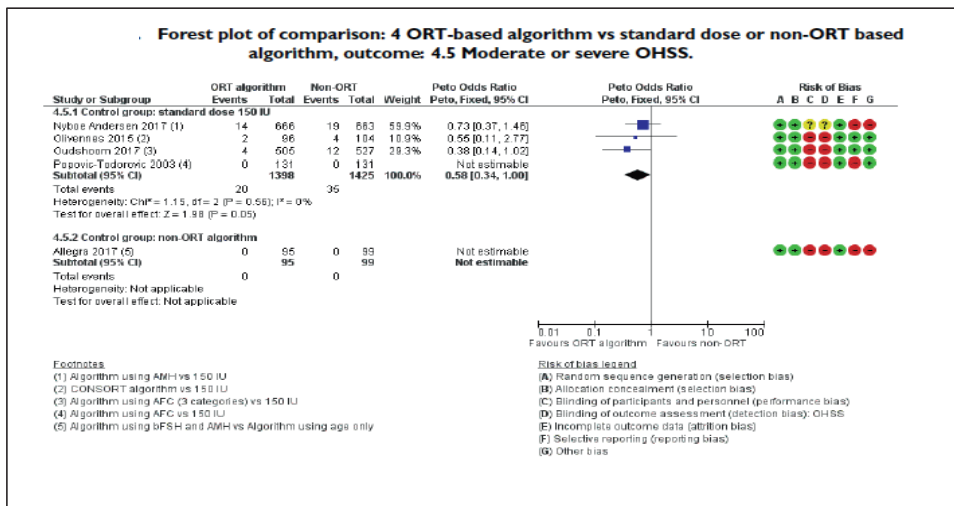
Figure 11. (With permisson from Cochrane Library)

How to evaluate treatment efficacy?

The most important efficacy variable is the rate of live birth resulting from a given treatment. As previously described the optimal number of oocytes retrieved for the highest chance of live birth after fresh cycle is approximately between 5 and 15. However, in recent years the techniques for embryo culturing and cryopreservation have improved rapidly and the chance of a live birth after transfer of a frozen/thawed blastocyst is now comparable to fresh embryo transfer (Shi *et al.,* 2018; Vuong *et al.,* 2018) or even higher (Chen *et al.,* 2016). As the number of FET cycles performed increases and now constitutes one third of all IVF cycles in Sweden (Q-ivf 2015), the cumulative LBR resulting from one oocyte aspiration and its subsequent fresh and all frozen embryo transfer has become a more relevant efficacy variable.

A few studies have investigated cumulative live birth rate in relation to the number of oocytes retrieved. An Australian study including almost 4,000 oocyte aspirations found that FET cycles contributed to increasing live birth rates in line with the increasing number of oocytes retrieved (Stanger and Yovich 2013). However, with high oocyte yield the proportion of embryos suitable for transfer or cryopreservation decreased. The authors concluded that a high number of oocytes might have a negative impact on embryo viability.

Two, recent, large cohort studies presented at ESHRE 2017 (Polyzos *et al.,* 2017; Malchau *et al.,* 2017) reported increasing cumulative live birth rates by the number of oocytes retrieved. However, in the study by Malchau *et al.*, representing a study period from 2002 to 2011, a plateau in the cumulative live birth rate was observed at 15 oocytes retrieved, while no plateau was reported in the study conducted by Polyzos *et al.* from 2009 to 2014. The reason for the different results of the two studies might be the different study periods, reflecting better cryopreserving techniques in the later study. Neither of these two cohort studies reported on complications or adverse events connected to stimulation.

How to evaluate treatment safety?

Only a few studies have simultaneously investigated both efficacy and safety in relation to ovarian response. A small Chinese single center study reported increasing cumulative live birth rates with an increasing number of oocytes retrieved. However, there was also an increased risk of moderate/severe OHSS (Ji *et al.,* 2013). A large American registry study (Steward *et al.,* 2014) including more than 250,000 fresh IVF cycles found that at approximately 15 oocytes retrieved, the live birth rate after fresh cycles evened out while the rate of OHSS increased. The study did not however evaluate cumulative live birth rate.

In Paper III we present the results of a large population based registry study including all fresh cycles with own gametes performed in Sweden in the period 2007 to 2013, and the majority of FET cycles generated from these fresh cycles performed in the period between 2007 and

2014. LBR per fresh cycle, cumulative LBR as well as the serious complications OHSS and thromboembolic events in relation to the number of oocytes retrieved, were all investigated.

The condition of OHSS is not clearly defined and one single ICD 10 code, N98.1 represents a spectrum from mild discomfort to a serious condition requiring intensive care. Since the aim of the study was to evaluate serious adverse reactions, we decided to include only hospitalized OHSS cases.

The main results of our study indicate a shift at 18 to 20 oocytes to a point where cumulative live birth evens out and the incidence of serious complications begins to increase rapidly. Compared to the studies by Ji *et al.,* and Steward *et al.,* the overall incidence of moderate/severe OHSS was low in our study, reaching 2.5% when >25 oocytes were retrieved. In the study by Ji *et al.*, defining moderate/severe OHSS according to Golan et al (Golan *et al.,* 1989), the overall number of oocytes retrieved was higher and the incidence of moderate/severe OHSS was 7% if >16 oocytes were retrieved. In the study by Steward *et al.*, although OHSS was not clearly defined, the incidence of OHSS was 6.3 % if > 25 oocytes were retrieved. These different incidences of OHSS could be real but might also reflect different ways of diagnosing OHSS.

Thromboembolic events associated with IVF have previously been described as mainly associated with OHSS and an incidence of 1.7% was found in a large population-based study from Sweden of women giving birth after IVF (Rova *et al.*,

2012). In our study, comprising both women becoming pregnant or not pregnant after the IVF treatment, thromboembolic cases were rare, reaching an incidence of 0.08% if >20 oocytes were retrieved. Previous studies have not established the incidence of thromboembolic events in a general infertile population having IVF treatment. However, one reason for the low incidence observed in this study might be a higher consciousness in recent years of the risks and an increased use of low molecular heparin. In 2013 the Swedish Society of Obstetrics and Gynaecology (SFOG) published evidence-based national guidelines concerning low molecular heparin treatment in connection with IVF (www.sfog.se). The guidelines include a scoring system for risk evaluation in relation to conditions existing prior to IVF, such as chronic or hereditary diseases and previous thromboembolic events, as well as treatment recommendations in cases of excessive ovarian response and impending OHSS.

An evaluation of safety variables should also include assessment of perinatal and obstetric outcomes. It is well known that pregnancies resulting from IVF have a higher risk of adverse perinatal and obstetric outcomes. Even though a majority of the adverse outcomes are associated with multiple pregnancies, the risk of adverse perinatal outcomes such as PTB, LBW, SGA and obstetric complications such as PIH, gestational diabetes, placenta complications and peripartal hemorrhage are still increased in singleton pregnancies after IVF compared to singletons from spontaneous pregnancies (Wennerholm *et al.*, 2013; Qin *et al.,* 2016; Luke *et al.,* 2017).

Furthermore, ART singletons have a higher risk of congenital malformations (AOR between 1.3 and 1.6) (Källén *et al.,* 2010; Pandey *et al.,* 2012; Hansen *et al.,* 2013) Studies investigating the impact of embryo stage at fresh transfer on pregnancy outcome have presented conflicting results. Blastocyst transfer has, in some studies, been reported to be associated with a higher risk of PTB (Kalra *et al.,* 2012; Maheshwari *et el.,* 2018; Alviggi *et al.,* 2018) while a recently published large registry study showed no difference in the risk of PTB depending on embryo stage (Chambers *et al.,* 2015). The association between embryo stage at transfer and low birth weight seems to be less pronounced. Several studies report no difference in the risk of SGA and LBW (Källén *et al.*, 2010; Kalra *et al.,* 2012;), a lower risk of SGA and LBW (Maheshwari *et al.,* 2018; Ginström *et al.,* 2016) and even a higher risk of LGA after blastocyst transfer compared to cleavage stage embryo transfer (Martins *et al.,* 2016). However an increased risk of perinatal death and placenta complications has been reported after blastocyst transfer compared to fresh cleavage stage embryo transfer (Ginström *et al.,* 2016).

The reason for adverse perinatal and obstetric outcome in singletons born after IVF is not clear. Adverse outcomes have been found to be associated with subfertility *per* se (Källén *et al.,* 2010) but treatment parameters such as embryo culturing and cryopreservation have also been considered (Pinborg *et al.,* 2013). Sibling studies have shown adverse outcomes in IVF children compared to siblings born after spontaneous conception (Henningsen *et al.,* 2011).

A few studies have investigated the association between the ovarian response, perinatal outcome and obstetric complications. A large British cohort study found a higher rate of PTB and VPTB in line with the number of oocytes retrieved (Sunkara *et al.,* 2015). Outcomes were adjusted for treatment period, maternal age and cause of infertility though data on important confounders such as BMI, parity, previous delivery and smoking were missing. Another study, though smaller, adjusting for these confounders could not confirm the association between the number of oocytes retrieved and VPTB and SGA, nor placental complications (Sazonova *et al.,* 2011). The association between high peak E2 and perinatal outcome has also been studied. A significant association has been found between LBW and peak E2 above 3096 pg/mL after adjustment for woman´s age, embryo stage and gestational age at birth (Pereira *et al.,* 2015).

In Paper IV we extend the concept from Paper III by evaluating perinatal outcome and obstetric complications in relation to the number of oocytes retrieved. Ovarian response can only be assessed in fresh cycles; hence only singleton births after fresh cycles were included. The study includes all singletons born after ovarian stimulation and IVF in Sweden between 2002 and 2015 and outcomes were adjusted for several confounders concerning demographic data, infertility, medical conditions and socioeconomic factors.

We found no significant association between the number of oocytes retrieved and the main perinatal outcomes PTB, VPTB, SGA, major birth defects or

peri/neonatal death. However we found a weak although significant association between more than 20 oocytes retrieved and placenta praevia, while no association was found with preeclampsia.

Balancing efficacy and safety

The results from Papers III and IV suggest a shift at approximately 18 to 20 oocytes where cumulative live birth rate evens out and serious complications become more common. Cancelling the cycle or freezing all embryos might be considered if the ovarian response exceeds this level.

However, these results are based on one study and will need confirmation from future large registry studies.

Pregnancy rates from FET cycles, particularly after blastocyst transfer, have improved rapidly in recent years and LBR are now comparable to those observed after fresh embryo transfer. The new approach to ovarian stimulation, using an antagonist protocol with agonist trigger and cryopreservation of all embryos, has become a new option and recent randomized controlled studies show reassuring results regarding both pregnancy and safety , almost eliminating the risk of OHSS (Chen *et al.,* 2016; Vuong *et al.,* 2018). However, even though the transfer of frozen/thawed blastocyst results in a lower risk of PTB, SGA and LBW compared to fresh transfer, an increased risk of LGA, macrosomia and perinatal death has been observed (Sazonova *et al.,* 2012; Wennerholm e*t al.,* 2013) as well as HDP (Maheshwari *et al.,* 2018; *Sha et al*., 2018; Opdahl *et al*., 2015) and postpartum hemorrhage (Sha *et al.,* 2018), and the "freeze all" concept cannot be recommended as a routine procedure. Knowledge of dosage strategies for balancing efficacy and safety in order to achieve a fresh embryo transfer is still the gold standard.

Strengths and limitations

One strength of the thesis is the large size of the two registry studies. Furthermore the population-based design which means that all IVF cycles (Paper III) and singleton babies born (Paper IV) in the study periods are included, which gives the studies high generalizability.

In Sweden, all IVF treatments are linked to the woman's personal social security number, giving a unique possibility to crosslink IVF data to many different registries. This gives information on deliveries, diseases, cause of death, pharmaceutical treatments and socio-economic data.

However, registry studies do have limitations. Confounders are factors having an impact on both independent and dependent variables and might result in false conclusions concerning the association between exposure and outcome.

There are several known factors affecting the outcome after IVF such as age, smoking, BMI, infertility diagnosis, previous delivery, the number of oocytes retrieved, the number of good quality embryos achieved, the number of embryos transferred and previous failed IVF cycles (Templeton *et al.,* 1996; Arvis *et al.,* 2012). Even though the outcomes in the two registry studies were adjusted for these confounders, unknown confounding factors may still affect the results. The unexpected association between more than 20 oocytes retrieved and a higher rate of males observed in the post hoc analysis of the data presented in Paper IV might be due to unknown confounders.

The output from registries is also affected by the quality of the input data. There might be missing data that has to be accounted for. Data might also have been registered incorrectly and the data set should be checked for unreasonable values and outliers.

A weakness of the Q-IVF registry is the current lack of data on infertility diagnoses and embryo data.

The thesis also includes a randomized, controlled, double blind trial, including a general infertile population planned for standard IVF. No patient category except for oocyte donation cycles was excluded. Hence the study population mirrors the fertility patients seen in daily clinical practice. The study was cited in a recently published Cochrane meta-analysis (Lensen *et al.,* 2018) and was assessed as having a minimal risk of bias.

However, in the randomized controlled trial there were some important issues of concern. Classifying patients for predicted ovarian response was based on an AMH assay with some analysis problems and a change in AMH assays took place during the study period. A conversion of AMH values became necessary during the ongoing study. Paper II illustrates the fact that the conversion of values from one assay to another might be associated with considerable problems.

Conclusions from the thesis

➢ A dosage algorithm including age, AFC and BMI performs well in predicting the desired ovarian response. Adding AMH to the dosage algorithm did not improve the predictive performance.

➢ Conversion of AMH values between assays may result in misclassification of presumed ovarian response and lead to incorrect dosage. Furthermore, the difference between assays might depend on the actual AMH level.

➢ The highest cumulative LBR was observed when 18 to 20 oocytes were retrieved. Ovarian stimulation resulting in a higher number of oocytes increased the risk of severe OHSS and thromboembolic events.

➢ The balance between efficacy and safety during IVF in relation to the number of oocytes retrieved is a delicate issue. This study suggests that around 18 oocytes would be optimal from a cumulative live birth perspective, keeping severe complications at a reasonable level and also taking perinatal and obstetric outcome into account.

Future perspectives

Even though individual dosage does not seem to have any advantages in poor or normoresponders, it might be useful in high responders, especially in preventing excessive response.

An important issue for future research is the challenges associated with PCOS patients. Ovarian stimulation in this patient group is a delicate matter, balancing excessive response and unexpectedly poor response. Individual dosage, based on current predictors, might prevent excessive response and OHSS but at the cost of an increased risk of poor response.

A post hoc analysis of the study population in our RCT indicated that high BMI could be part of the explanation for poor response. However a recent study comparing ovarian response in PCOS patients with an AMH above or below 4.6 ng/mL indicate that other factors might influence the ovarian response. Patients with AMH levels above 4.6 ng/mL showed a higher threshold for response in a step up dosage protocol resulting in higher total dose and longer stimulation compared to patients with a lower AMH. Both groups had a mean BMI of approximately 30 with no difference between the groups (Kamel *et al.,* 2018).

In many randomized trials, concerning stimulation regimes, PCOS is an exclusion criteria because of the unpredictible ovarian response. However, more knowledge on how to stimulate these women is needed. AMH and AFC seem to have less predictive accuracy for ovarian response in PCOS and future research could investigate if other factors such as endocrine and metabolic status might be additional predictive factors for ovarian response in these patients.

AMH has the potential to become a useful tool in the prediction of ovarian respose and dose decisions though the issue of finding international robust standards of reference values and conversion factors between AMH assays has to be adressed.

Quality registries are important tools in the follow up of efficacy and safety and further large registry studies should be encouraged for a continuous improvement of fertility treatment. The increasingly widespread practice of blastocyst culture and "freeze all" strategy appears to be safe, but further follow up of perinatal and obstetric outcomes in large registry studies is needed.

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