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Cohort Profile: The Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

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ABSTRACT

Purpose The UppStART study is a prospectively recruited sample of couples undergoing assisted reproduction in Stockholm and Uppsala county in Sweden. The study was initiated to 1) investigate possible changes in the epigenetic profile of infants inferred through the ART procedures and their consequence; and 2) to assess the impact of lifestyle and health exposures on treatment outcome.

Participants Recruitment took place between September 2011 and December 2013, and IVF cycles initiated and pregnancies occurring during this time were followed until December 2014. The cohort includes 971 participants (n= 514 women; n= 457 men).

Findings to date Self-reported demographic, health, and lifestyle data was collected from a baseline questionnaire, and to assess changes to lifestyle a follow-up questionnaire was issued at the time of oocyte retrieval, and at subsequent IVF cycles. Questionnaire data was linked to data extracted from medical records. Biological samples were collected at baseline: blood for extraction of serum, plasma and DNA, morning and evening saliva samples for cortisol measurement; and at delivery including samples of maternal blood, placenta, and amniotic fluid, and cord blood for epigenetic analysis.

Future plans Through the unique identification number assigned to each Swedish citizen at birth or immigration, UppStART study participants will be linked to the to the Swedish population-based national and quality registers to provide data from prenatal, obstetrical, neonatal, and infant care, and subsequent updates will provide data on childhood health and educational outcomes. Collaboration and use of UppStART data is encouraged and more information about access can be found at www.ki.se/meb/uppstart.

Registration: N/A

Keywords: assisted reproductive techniques; in vitro fertilization; infertility; lifestyle; epigenetics.

Strengths and limitations of this study

- The UppStART dataset comprises a wide range of socio-demographic and lifestyle data, clinical data from medical records, biological samples (including blood, DNA, saliva, cord blood, placenta), epigenetic data.
- Questionnaires were completed at baseline, oocyte retrieval, and during subsequent IVF cycles so as to be able to assess health and lifestyle changes during and between cycles.
- The process of linking the participants to the Swedish national health registers has been initiated and will allow for long-term follow up of the couples and infants.
- Possible recall bias and volunteer bias are limitations. However, UppStART participants have characteristics typical of couples undergoing IVF treatment in Sweden. Further, individuals undergoing treatment are often aware of their lifestyle choices and consequently may be able to respond to the questionnaire more accurately.

INTRODUCTION

Assisted reproductive techniques (ART) include the use of hormones to down-regulate pituitary function and to stimulate multiple oocyte production, in vitro manipulation of the oocyte and sperm for in vitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI) procedures, and culturing of pre-implantation embryos in culture media and incubators before the embryo is transferred to the womb. Hence, ART manipulates many steps involved in natural conception and may therefore potentially alter biological processes in the foetus that may result in short- and long- term health consequences on the offspring. It has been well established that ART offspring have a higher risk for being born with a low birth weight or preterm.[1 2] The reasons behind these associations are unknown and some hypothesize that alterations in the epigenetic profile of the offspring are a contributing factor.[3-6]

Further, recent studies have indicated that a number of assisted reproduction procedures, including controlled ovarian stimulation, micromanipulation of gametes in addition to embryo culture, can cause epigenetic disruption.[7-9]

It is estimated that approximately 12-28% of couples trying to conceive are diagnosed with infertility.[10] Further, couples and especially women of today prioritize educational and career goals and securing economic stability, thereby delaying childbearing based.[11] The use of ART is increasing in Sweden, as well as elsewhere in Europe, with up to 5% of all conceptions being a result of some form of ART in the Nordic countries.[12] However, the pregnancy success rate per ART cycle is only 28%, and the take-home baby rate is barely 25%, which is not always known to the general public.[13] Little is known of how lifestyle factors can affect the outcomes of ART attempts (i.e. number of matured oocytes, number of embryos, pregnancy rate, miscarriage rate).

Therefore, the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study was initiated to: 1) investigate if epigenetic alterations exist in infants conceived via ART compared to those conceived spontaneously; 2) investigate if lifestyle factors (e.g. caffeine consumption, cortisol levels, folate, C-reactive protein, natural products and supplements, and prescribed medication use) are associated with time to pregnancy, pregnancy rates, subclinical and clinically recognized miscarriages, ART procedure specific outcomes (e.g. number and quality of oocytes and embryos, sperm characteristics), and obstetric and prenatal outcomes in ART cycles and pregnancies.

COHORT DESCRIPTION

Study population and setting

The UppStART study is a prospective cohort study of couples undergoing infertility treatment (specifically IVF or ICSI) in the greater Stockholm and Uppsala municipalities, which provide healthcare for approximately 3 million people. Participants were recruited from three of the four fertility and reproductive health clinics in Stockholm and one clinic in Uppsala, which also serves a large volume of patients from Stockholm. In Sweden, fertility treatments are provided within the tax-funded healthcare system. Treatments are individualized based on the identified causes of infertility.

At the time of planning their first IVF/ICSI treatment, couples were approached by the clinic´s nurse or midwife and asked to participate in the study. To facilitate the process of informed consent, the couples were provided with both verbal and written information approved by the regional ethical board about the purpose of the study, methods, the voluntary nature of participation, and the possible risks which included possible discomfort during blood sampling. Additionally, participants were informed

that they could withdraw from the study at any time with no impact on their medical care. The requirement for inclusion in the study was an understanding of the Swedish language and exclusion was the use of donor gametes. At the time of recruitment, Swedish law did not allow single women to receive ART treatment; hence no single women were recruited into the UppStART study. Amongst the couples, approximately 20% of male partners chose not to participate (n=105).

The signed consent forms were sent from the clinics to the UppStART research nurse at the host institution, the Karolinska Institute, who monitored recruitment and questionnaire responses, and reminded participants via email two weeks after signing the consent form to answer the baseline questionnaire if they had not done so. A second reminder by email was sent two weeks after the first, and if participants still had not completed the online questionnaire, the research nurse attempted to contact them by phone. No other attempts were made after the telephone call and participants were marked as non-responders. Once the ART treatment cycle began and the participants reached the stage of oocyte retrieval, they were asked to respond to a second online follow-up questionnaire.

Recruitment took place from September 2011 to December 2013. IVF treatment(s) of the participants were followed until December 2014 or drop-out/consent withdrawn (n=4), which ever came first. When the couples returned to the clinic for repeated ART attempts, clinic staff collected a blood sample, provided saliva collection tubes, and instructed the participants to respond to the online follow-up questionnaire.

Figure 2 reports the number of consented participants and the number of participants who responded to baseline and follow-up questionnaires and provided samples. Table 1 shows baseline characteristics of participants and differences between responders and non-responders.

Table 1. Baseline characteristics of participants in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

	Women		Men	
Sociodemographic characteristics	**Responded to baseline questionnaire n = 432**	**Non-Responders n = 83**	**Responded to baseline questionnaire n = 348**	**Non-Responders n = 109**
Age, *mean* ± *sd* *	33.8 ± 4.12	33.9 ± 4.61	35.9 ± 5.44	37.2 ± 6.39
BMI, *mean* ± *sd*	23.5 ± 3.8	N/A	25.6 ± 3.0	N/A
Highest Education:**				
High school, n (%)	90 (21.2)	21 (26.3)	110 (32.2)	40 (37.4)
University, n (%)	334 (78.8)	59 (73.8)	232 (67.8)	66 (62.6)
Current smoker, n (%)	14 (3.3)	N/A	18 (5.3)	N/A

Data for non-responders provided by Statistics Sweden.
*Data missing for 10 non-responders.
**Data missing for 6 non-responders.
Abbreviations: BMI = body mass index; N/A = data not available; sd = standard deviation.

Data Collection and measures

Overview

Table 2 and Figure 1 provide an overview of data and sample collection in the UppStART study. After informed consent was given, the clinic staff withdrew blood samples and provided the participant with a kit for at-home saliva collection. The participants were asked to answer a web-based baseline questionnaire within a few days of their clinic visit and prior to their IVF/ICSI treatment start, which included an extensive list of questions on sociodemographic, anthropometric and life-style factors.

Table 2: Data and sample collection in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

Phase	Measurements
Baseline, collected prior to IVF cycle start	• Questionnaire: self-reported sociodemographic, health, lifestyle, and behaviour in the past 3 months or 1 year (listed in Table 2) • Blood samples collected: plasma, serum, extracted DNA stored at –80°C. • Saliva samples collected at 7 am and 9 pm on the same day, cortisol extracted and measured
At oocyte aspiration	• Questionnaire, follow-up: a shorter version of the baseline questionnaire to assess if the participant changed their lifestyle factors after cycle start

At delivery	• Maternal blood sample (PAX gene, EDTA) • Cord blood (PAX gene, EDTA) • Placenta tissue and amniotic fluid, stored at -80 C
Repeat IVF treatments	• Questionnaire, follow-up: a shorter version of the baseline questionnaire to assess if the participant changed their lifestyle factors between IVF cycles • Blood samples collected: plasma and serum stored at –80°C. • Saliva samples collected at 7 am and 9 pm on the same day

Questionnaires Data

The baseline questionnaire asked an extensive list of questions about sociodemographic, health, and life-style factors in the previous 3 months or the previous year (Summarized in Table 3). It has been reported that couples undergoing ART change to a healthier lifestyle or try a range of "natural" remedies in order to improve the outcome of their fertility treatment.[14] [15] Therefore, the follow-up questionnaire at oocyte retrieval and at subsequent ART procedures was a shortened version of the baseline questionnaire, designed to capture changes in lifestyle since the start of fertility treatment or since the previous cycle.

Table 3: Variables included in the baseline and follow-up questionnaire in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

Topic	Topic, *continued*
Socio-demography	**Lifestyle ***
General:	General health
Family / household	Diet:
Education	Meals
Occupation:	Beverages / caffeine intake
Work History	Alcohol intake
Working shift	Vitamins and supplements
Working nights	Physical Activity:
Working Environment:	Activity during occupational hours
Physiological discomfort / chemical exposures	Leisure time activities
Work / private life balance *	Sport activities
	Sleeping Habits
Smoking, Nicotine, and Alcohol use *	Mobile phone use
Smoking history	Hair colour use
Current smoking	Bath/sauna use
Current snus use	Tanning

Alcohol consumption	
Reproductive Health and Infertility Treatment	**Self-Care ***
Menstruation	Medication OTC
Contraceptive use	Complementary and alternative medicine
Pregnancy / giving birth	**Asthma and Allergy ***
Gynecological surgery	Asthma: Diagnosis, medication
Infertility / disease	Allergies: Diagnosis, medication (including hay fever, allergic rhinitis, pollen, fur, bee, wasp, contact allergy)
Sexarche	
Sexually Transmitted Disease	
Cause of infertility	
Previous infertility treatments	**Psychosocial ***
	General Stress:
Medical History *	Anxiety and Depression
Weight and gestational age at birth / prematurity	Perceived Stress Scale, 10 question version[16]
Current height and weight	Infertility-related psychosocial scales:[17]
Disease diagnosis:	Infertility related communication strategies
Year of diagnosis	Partner communication
Medication	Martial Benefit Measure
Painkiller use in the last 3 months	Fertility Problem Stress Scale
Cancer:	Attitudes to treatment
Year of diagnosis	Expectations of fertility treatment
Type of cancer and location	
Treatment and medication	

Baseline questionnaire questions were phrased to collect information on the participants' lifestyle and behaviour in the 3 months or 1 year prior to cycle start.
*These sections were included in the follow-up questionnaire issued at oocyte aspiration and at repeated IVF treatment cycles, where the questions were phrased to ask about behaviour/lifestyle since beginning IVF treatment.

Medical Records

The medical records of each participant were collected from the four participating clinics and included data on all IVF/ICSI cycles initiated between the date of study entry and the end of 2014. Clinical data included the medical and reproductive history of the participant, IVF/ICSI cycle protocol, indicators of oocyte and embryo quality, and IVF cycle outcome including pregnancy. Table 4 summarizes the available clinical data.

Table 4: Summary of available clinical data for UppStART participants

Treatment Data
• Ovarian stimulation procedure clinical data
• Type of treatment: IVF / ICSI
• Type of culture media
• Number of oocytes and embryos obtained
• Indicators of oocyte quality
• Indicators of embryo quality
• Sperm quality variables
Treatment Outcome Data
• Urine pregnancy test results (week 2/3 after embryo transfer)
• Ultrasound confirmed pregnancy (week 7 after embryo transfer)
• Miscarriage
• Live birth *to be supplemented by linkage to the Swedish Medical Birth Register

Biological samples: Baseline

Blood samples At the time of recruitment into the study, the clinic staff withdrew blood samples, which were then send to the Karolinska Biobank. Plasma, serum, and whole blood were aliquoted and stored at -80°C. DNA was extracted from whole blood and stored at -80°C.

Saliva samples Participants were provided with two pre-labelled Salivette® (Sarstedt, UK) kits, a pre-paid envelope for the return of samples to the Karolinska Biobank, and saliva collection instructions: samples were to be collected at home at 7:00 am (upon awakening, before breakfast and teeth brushing) and at 9:00 pm the same day, with no eating, drinking, gum chewing, snus use, or smoking within 30 minutes prior to collection. Saliva samples were returned to the KI Biobank by the participant via post and the samples were stored at the KI Biobank at -80°C until analysis.

Saliva cortisol levels (nmol/L) were measured by radioimmunoassay (RIA; Orion Diagnostic, Finland) by the Study Center of Laboratory Medicine at the Karolinska University Laboratory. The detection limit of the RIA was < 1.0 nmol/L, and

the intra- and inter-assay coefficients of variance for this assay were 12% and 8% for the low control, and 7% and 5% for the high control, respectively.

Biological Samples: at Delivery

Staff at seven delivery units in Stockholm and Uppsala were recruited to assist in collecting samples from UppStART participants during delivery. Delivery clinics were provided with a sample collection kit including tubes for collection of maternal blood (EDTA, PAXgene), cord blood (EDTA, PAXgene), amniotic fluid, and placenta samples. Midwives were requested to sample one small piece of placenta (approx. 2x2 cm) from the centre of each quadrant, both on the maternal and fetal side, for a total of 8 samples. Samples were stored in -20°C freezers at the delivery units until they were collected by UppStART study staff and deposited into the KI Biobank.

Linkage to National Registers

Through the unique identification number assigned to each Swedish citizen at birth or immigration,[18] it is possible to link our study participants to the Swedish population-based national and quality registers. The process to link this cohort to the registers has been initiated and will include the Medical Birth Register, Perinatal Quality Register (for neonatal intensive care), Register of Malformations, Prescription Drug Register, Inpatient and Outpatient Registers, Cause of Death Register, and the Multigeneration Register.[19-21] This initial linkage will provide data from prenatal, obstetrical, neonatal, and infant care, and subsequent updates will provide data on childhood health and educational outcomes.

FINDINGS TO DATE

Stress

While it is well established that women undergoing fertility treatment experience high levels of stress and higher prevalence of symptoms of depression and anxiety, [22-24] it remains uncertain if and how psychological stress clinically affects the outcome of IVF treatments.[22 23] The first study completed using UppStART data investigated the association between self-reported perceived stress, infertility-related stress, and cortisol levels on IVF cycle outcomes including clinical pregnancy and indicators of oocyte and embryo quality. Overall, we found that stress measured prior to IVF cycle start was not associated with IVF cycle outcome, oocyte maturity, or embryo quality. The findings from this study and many other studies reporting no association between stress and IVF outcomes are potentially reassuring to women undergoing fertility treatment and the clinicians who may have concerns about the influence of stress levels on the success of their IVF treatment.[25]

Cumulative Impact of Lifestyle Factors on IVF treatment outcomes

The UppStART study has been used as a validation cohort in a study examining the cumulative impact of lifestyle factors on IVF treatment outcomes. An individual as well as a cumulative effect of smoking and BMI on the number of aspirated and mature oocytes in fresh IVF treatment cycles has been found, especially among women with low ovarian reserve. (*Manuscript submitted, currently under review*)

Epigenetics

In collaboration with researchers at Leiden University, the methylation profile of the cord blood of UppStART offspring has been measured (Illumina, San Diego, California, USA) and compared the methylation profile in the cord blood of spontaneously conceived offspring from the Swedish Born Into Life study.[26] The first aim is to identify potential differences in epigenetic marks between IVF- and spontaneously-conceived offspring. Further, comparisons between methylation

profiles from infants conceived via different assisted reproductive techniques will be conducted, such as between IVF and ICSI, fresh and frozen embryos, and Day 2 and blastocyst embryo transfer. Analyses are currently ongoing and the study is expected to be complete at the end of in 2018. Further, while not considered in this study the plethora of exposure information gathered from the questionnaires also provides unique possibilities to explore gene-environment interactions in the epigenetic analyses and the impact of specific environmental exposures on epigenetic marks.

STRENGTHS AND LIMITATIONS

The major strength of the UppStART study is the plethora of information from multiple sources, including an extensive questionnaire on a large number of lifestyle factors and behaviours, data from medical records, national registers, as well as biological specimens. Further, studies often only include women undergoing ART, however the UppStART study has collected data on the male partners as well. Additionally, the study has captured repeated ART cycles within the couple and monitored the change in lifestyle factors between cycles to determine if these changes are linked to ART outcomes of interest.

However, there are also some limitations and potential sources of bias. Recruitment of participants was slower than expected, especially at the beginning of the study for a number of reasons: one of the four IVF clinics in Stockholm declined to participate and one clinic halted recruitment for 8 months due to staff restructuring. To encourage the involvement of clinic staff, study personnel visited with clinic staff every six months and distributed a newsletter reporting recruitment statistics for each clinic. Additionally, during the last year of recruitment, ethical approval was granted to give participating couples movie tickets upon enrolment, thereby providing a small

compensation for their participation. While the goal of the study was to capture every treatment of the couples, the clinics often missed collecting blood samples and reminding the participants to complete the questionnaires at subsequent cycles. Lastly, due to the urgent and demanding nature of the labour and delivery units, the collection of samples at delivery of some infants was missed. Midwifes expressed that often there was not enough time to collect the number of samples requested while also fulfilling their clinical duties.

With any study including self-reported health and lifestyle information, such as this one, recall bias is an issue. The majority of the questions in the UppStART baseline questionnaire targeted specific lifestyle factors during the previous three months to a year. However, women and men experiencing infertility and planning to undergo infertility treatment often make changes in their lifestyle based on what they believe will maximize the chances of conception,[14] which will likely make the participants more aware of their lifestyle choices and consequently be able to respond to the questionnaire more accurately.

Further, volunteer bias may be another source of bias in the cohort. The UppStART study was presented to participants as a general study of lifestyle factors in IVF couples. Therefore, individuals with an interest in their lifestyle choices may choose to participate, and those with unfavorable lifestyle habits may have declined. However, the characteristics of the UppStART study are typical of the Swedish population undergoing IVF,[27] and a large degree of homogeneity exists in the demographic and lifestyle, including low rates of factors known to affect fertility and IVF outcome (e.g., high BMI and smoking). As with the known variables, we speculate that the study sample is also likely to be relatively homogeneous with respect

unmeasured variables which will reduce the influence of confounders on studies performed with data from this cohort.

Collaboration: We encourage the use of UppStART data as a resource for epidemiological research. The data are available upon request and more information can be found on the UppStART webpage (www.ki.se/meb/UppStART). To access the data, a proposal should be submitted to the principal investigator by an applicant with a PhD or an equivalent degree. Proposals will be evaluated and granted according to principles of ethical and scientific soundness. Applicants based outside of Sweden are required to involve collaborator(s) based at a Swedish research institution. Applications can only be granted and data provided after approval by the Stockholm Ethical Review Board. Information and instructions for applicants are available on the website (https://ki.se/meb/uppstart) and from Carolyn Cesta at carolyn.cesta@ki.se.

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Data sharing statement: The data are available upon request and more information can be found on the UppStART webpage (www.ki.se/meb/UppStART). Information

and instructions for those interested in collaboration are available on the website and from CEC at carolyn.cesta@ki.se.

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Ethics approval: The UppStART study has been approved by the regional ethics review board at Karolinska Institutet (Dnr 2011/230-31/1, 2011/1427-32, 2012/131-32, 2012/792-32, 2013/1700-32, 2014/1956-32, 2015/1604-32).

Contributor statement: ANI, JP, SC, KARW, JIO, JH, HW, MW, and ASÖ were responsible for the study design, protocol development, and data collection. CEC was responsible for the data analysis. CEC was responsible for writing the initial draft of the manuscript, and subsequent drafts were reviewed by all authors listed. All authors had input on reporting of the findings. All authors provided approval for the submitted version of this manuscript.

Conflicts of Interest: None to declare.

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Figure Legends

Figure 1: Timeline of participant recruitment and data collection in the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

Figure 2. UppStART study: Number of participants and samples collected

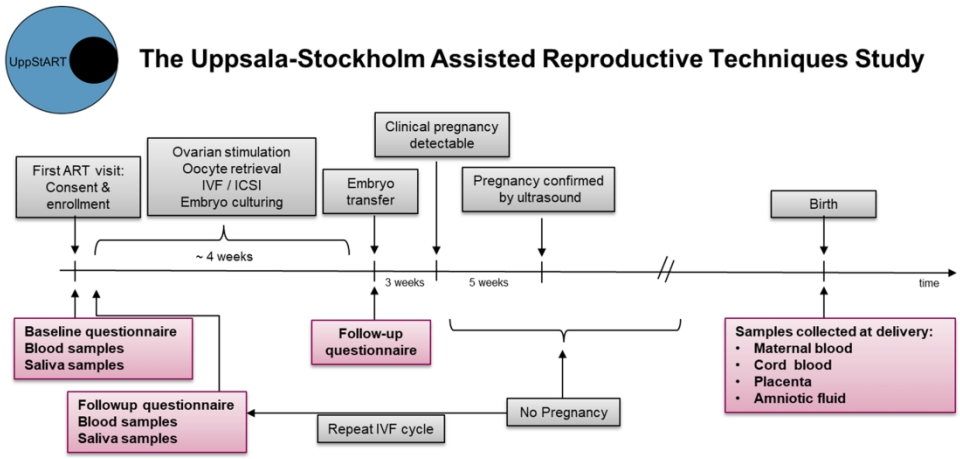

Figure 1: Timeline of participant recruitment and data collection in the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

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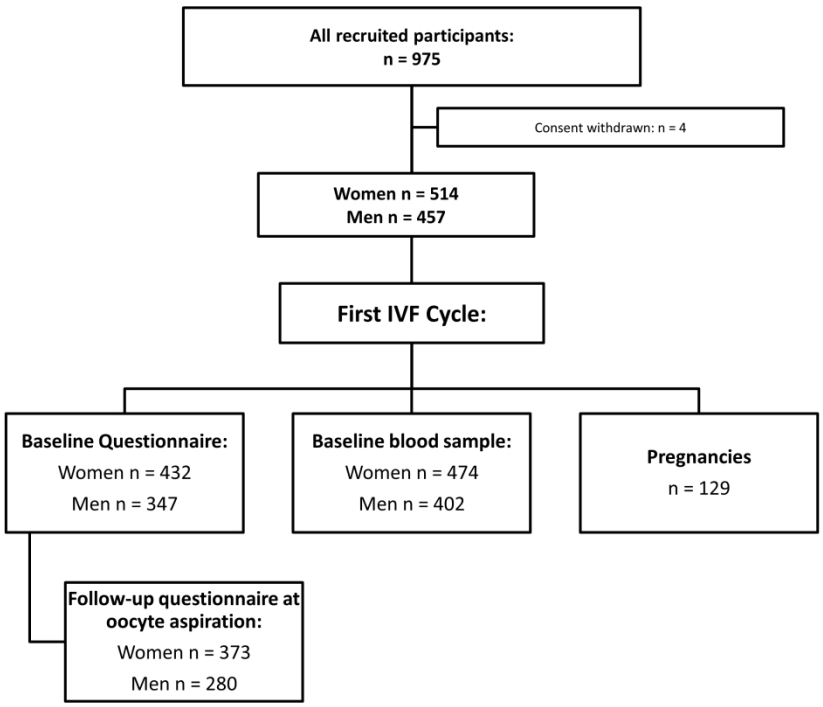

Figure 2. UppStART study: Number of participants and samples collected

254x190mm (300 x 300 DPI)

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Cohort Profile: The Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

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Cohort Profile: The Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

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ABSTRACT

Purpose The UppStART study is a prospectively recruited sample of couples undergoing assisted reproduction in Stockholm and Uppsala county in Sweden. The study was initiated to 1) investigate possible changes in the epigenetic profile of infants inferred through the ART procedures and their consequence; and 2) to assess the impact of lifestyle and health exposures on treatment outcome.

Participants Recruitment took place between September 2011 and December 2013, and IVF cycles initiated and pregnancies occurring during this time were followed until December 2014. The cohort includes 971 participants (n= 514 women; n= 457 men), and 129 pregnancies were achieved from the first IVF cycle included in the study.

Findings to date Self-reported demographic, health, and lifestyle data was collected from a baseline questionnaire, and to assess changes to lifestyle a follow-up questionnaire was issued at the time of oocyte retrieval, and at subsequent IVF cycles. Questionnaire data was linked to data extracted from medical records. Biological samples were collected at baseline: blood for extraction of serum, plasma and DNA, morning and evening saliva samples for cortisol measurement; and at delivery including samples of maternal blood, placenta, and amniotic fluid, and cord blood for epigenetic analysis.

Future plans Through the unique identification number assigned to each Swedish citizen at birth or immigration, UppStART study participants will be linked to the to the Swedish population-based national and quality registers to provide data from prenatal, obstetrical, neonatal, and infant care, and subsequent updates will provide data on childhood health and educational outcomes. Collaboration and use of UppStART data is encouraged and more information about access can be found at www.ki.se/meb/uppstart.

Registration: N/A

Keywords: assisted reproductive techniques; in vitro fertilization; infertility; lifestyle; epigenetics.

Strengths and limitations of this study

- The UppStART dataset comprises a wide range of socio-demographic and lifestyle data, clinical data from medical records, biological samples (including blood, DNA, saliva, cord blood, placenta), epigenetic data.
- Questionnaires were completed at baseline, oocyte retrieval, and during subsequent IVF cycles so as to be able to assess health and lifestyle changes during and between cycles.
- The process of linking the participants to the Swedish national health registers has been initiated and will allow for long-term follow up of the couples and infants.
- Possible recall bias and volunteer bias are limitations,however UppStART participants have characteristics typical of couples undergoing IVF treatment in Sweden.

INTRODUCTION

Assisted reproductive techniques (ART) include the use of hormones to down-regulate pituitary function and to stimulate multiple oocyte production, in vitro manipulation of the oocyte and sperm for in vitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI) procedures, and culturing of pre-implantation embryos in culture media and incubators before the embryo is transferred to the womb. Hence, ART manipulates many steps involved in natural conception and may therefore potentially alter biological processes in the foetus that may result in short- and long- term health consequences on the offspring. It has been well established that ART offspring have a higher risk for being born with a low birth weight or preterm.(1; 2) The reasons behind these associations are unknown and some hypothesize that alterations in the epigenetic profile of the offspring are a contributing factor.(3-6)

Further, recent studies have indicated that a number of assisted reproduction procedures, including controlled ovarian stimulation, micromanipulation of gametes in addition to embryo culture, can cause epigenetic disruption.(7-9)

It is estimated that approximately 12-28% of couples trying to conceive are diagnosed with infertility.(10) Further, couples and especially women of today prioritize educational and career goals and securing economic stability, thereby delaying childbearing.(11) The use of ART is increasing in Sweden, as well as elsewhere in Europe, with up to 5% of all conceptions being a result of some form of ART in the Nordic countries.(12) However, the pregnancy success rate per ART cycle is only 28%, and the take-home baby rate is barely 25%, which is not always known to the general public.(13) Little is known of how lifestyle factors can affect the outcomes of ART attempts (i.e. number of matured oocytes, number of embryos, pregnancy rate, miscarriage rate).

Therefore, the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study was initiated to: 1) investigate if epigenetic alterations exist in infants conceived via ART compared to those conceived spontaneously; 2) investigate if lifestyle factors (e.g. caffeine consumption, cortisol levels, folate, C-reactive protein, natural products and supplements, and prescribed medication use) are associated with time to pregnancy, pregnancy rates, subclinical and clinically recognized miscarriages, ART procedure specific outcomes (e.g. number and quality of oocytes and embryos, sperm characteristics), and obstetric and prenatal outcomes in ART cycles and pregnancies.

COHORT DESCRIPTION

Study population and setting

The UppStART study is a prospective cohort study of couples undergoing infertility treatment (specifically IVF or ICSI) in the greater Stockholm and Uppsala municipalities, which provide healthcare for approximately 3 million people. Participants were recruited from three of the four fertility and reproductive health clinics in Stockholm and one clinic in Uppsala, which also serves a large volume of patients from Stockholm. In Sweden, fertility treatments are provided within the tax-funded healthcare system. Treatments are individualized based on the identified causes of infertility.

At the time of planning their first IVF/ICSI treatment, couples were approached by the clinic´s nurse or midwife and asked to participate in the study. To facilitate the process of informed consent, the couples were provided with both verbal and written information approved by the regional ethical board about the purpose of the study, methods, the voluntary nature of participation, and the possible risks which included possible discomfort during blood sampling. Additionally, participants were informed

that they could withdraw from the study at any time with no impact on their medical care. The requirement for inclusion in the study was an understanding of the Swedish language and exclusion was the use of donor gametes. At the time of recruitment, Swedish law did not allow single women to receive ART treatment; hence no single women were recruited into the UppStART study. Amongst the couples, approximately 20% of male partners chose not to participate (n=105).

The signed consent forms were sent from the clinics to the UppStART research nurse at the host institution, the Karolinska Institute, who monitored recruitment and questionnaire responses, and reminded participants via email two weeks after signing the consent form to answer the baseline questionnaire if they had not done so. A second reminder by email was sent two weeks after the first, and if participants still had not completed the online questionnaire, the research nurse attempted to contact them by phone. No other attempts were made after the telephone call and participants were marked as non-responders. Once the ART treatment cycle began and the participants reached the stage of oocyte retrieval, they were asked to respond to a second online follow-up questionnaire (Figure 1).

Recruitment took place from September 2011 to December 2013. IVF treatment(s) of the participants were followed until December 2014 or drop-out/consent withdrawn (n=4), which ever came first. When the couples returned to the clinic for repeated ART attempts, clinic staff collected a blood sample, provided saliva collection tubes, and instructed the participants to respond to the online follow-up questionnaire. Figure 2 reports the number of consented participants and the number of participants who responded to baseline and follow-up questionnaires and provided samples. The cohort includes 971 participants (n= 514 women; n= 457 men). Eighty percent of the cycles progressed to an embryo transfer, of which 57.6% of the embryos were created

via standard IVF, and 42.4% via ICSI. From the first IVF cycle included in the study, 129 pregnancies were achieved. Only pregnancies and children conceived via IVF procedures are included in this cohort.

Table 1 shows baseline characteristics of participants and differences between responders and non-responders.

Table 1. Baseline characteristics of participants in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

	Women		Men	
Sociodemographic characteristics	**Responded to baseline questionnaire n = 432**	**Non-Responders n = 83**	**Responded to baseline questionnaire n = 348**	**Non-Responders n = 109**
Age, *mean* ± *sd* *	33.8 ± 4.12	33.9 ± 4.61	35.9 ± 5.44	37.2 ± 6.39
BMI, *mean* ± *sd*	23.5 ± 3.8	N/A	25.6 ± 3.0	N/A
Highest Education:**				
High school, n (%)	90 (21.2)	21 (26.3)	110 (32.2)	40 (37.4)
University, n (%)	334 (78.8)	59 (73.8)	232 (67.8)	66 (62.6)
Current smoker, n (%)	14 (3.3)	N/A	18 (5.3)	N/A

Data for non-responders provided by Statistics Sweden.
*Data missing for 10 non-responders.
**Data missing for 6 non-responders.
Abbreviations: BMI = body mass index; N/A = data not available; sd = standard deviation.

Data Collection and measures

Overview

Table 2 and Figure 1 provide an overview of data and sample collection in the UppStART study. After informed consent was given, the clinic staff withdrew blood samples and provided the participant with a kit for at-home saliva collection. The participants were asked to answer a web-based baseline questionnaire within a few days of their clinic visit and prior to their IVF/ICSI treatment start, which included an extensive list of questions on sociodemographic, anthropometric and life-style factors.

Table 2: Data and sample collection in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

Phase	Measurements
Baseline, collected prior to IVF cycle start	• Questionnaire: self-reported sociodemographic, health, lifestyle, and behaviour in the past 3 months or 1 year (listed in Table 2) • Blood samples collected: plasma, serum, extracted DNA stored at –80°C. • Saliva samples collected at 7 am and 9 pm on the same day, cortisol extracted and measured
At oocyte aspiration	• Questionnaire, follow-up: a shorter version of the baseline questionnaire to assess if the participant changed their lifestyle factors after cycle start
At delivery	• Maternal blood sample (PAX gene, EDTA) • Cord blood (PAX gene, EDTA) • Placenta tissue and amniotic fluid, stored at -80 C
Repeat IVF treatments	• Questionnaire, follow-up: a shorter version of the baseline questionnaire to assess if the participant changed their lifestyle factors between IVF cycles • Blood samples collected: plasma and serum stored at –80°C. • Saliva samples collected at 7 am and 9 pm on the same day

Questionnaires Data

The baseline questionnaire asked an extensive list of questions about sociodemographic, health, and life-style factors in the previous 3 months or the previous year (Summarized in Table 3). It has been reported that couples undergoing ART change to a healthier lifestyle or try a range of “natural” remedies in order to improve the outcome of their fertility treatment.(14; 15) Therefore, the follow-up questionnaire at oocyte retrieval and at subsequent ART procedures was a shortened version of the baseline questionnaire, designed to capture changes in lifestyle since the start of fertility treatment or since the previous cycle.

Table 3: Variables included in the baseline and follow-up questionnaire in the Uppsala Stockholm Assisted Reproductive Techniques (UppStART) study

Topic	Topic, *continued*
Socio-demography	**Lifestyle ***
General:	General health
Family / household	Diet:
Education	Meals
Occupation:	Beverages / caffeine intake
Work History	Alcohol intake
Working shift	Vitamins and supplements
Working nights	Physical Activity:
Working Environment:	Activity during occupational hours
Physiological discomfort / chemical exposures	Leisure time activities
Work / private life balance *	Sport activities
	Sleeping Habits
Smoking, Nicotine, and Alcohol use *	Mobile phone use
Smoking history	Hair colour use
Current smoking	Bath/sauna use
Current snus use*	Tanning
Alcohol consumption	
	Self-Care *
Reproductive Health and Infertility Treatment	Medication OTC
Menstruation	Complementary and alternative medicine
Contraceptive use	
Pregnancy / giving birth	**Asthma and Allergy ***
Gynecological surgery	Asthma: Diagnosis, medication
Infertility / disease	Allergies: Diagnosis, medication (including hay fever, allergic rhinitis, pollen, fur, bee, wasp, contact allergy)
Sexarche	
Sexually Transmitted Disease	
Cause of infertility	
Previous infertility treatments	**Psychosocial ***
	General Stress:
Medical History **	Anxiety and Depression
Weight and gestational age at birth / prematurity	Perceived Stress Scale, 10 question version(16)
Current height and weight	Infertility-related psychosocial scales:(17)
Disease diagnosis:	Infertility related communication strategies
Year of diagnosis	Partner communication
Medication	Marital Benefit Measure
Painkiller use in the last 3 months	Fertility Problem Stress Scale
Cancer:	Attitudes to treatment
Year of diagnosis	Expectations of fertility treatment
Type of cancer and location	
Treatment and medication	

Baseline questionnaire questions were phrased to collect information on the participants' lifestyle and behaviour in the 3 months or 1 year prior to cycle start.
*a moist form of smokeless tobacco used in Sweden which is usually placed under the upper lip
**These sections were included in the follow-up questionnaire issued at oocyte aspiration and at repeated IVF treatment cycles, where the questions were phrased to ask about behaviour/lifestyle since beginning IVF treatment.

Medical Records

The medical records of each participant were collected from the four participating clinics and included data on all IVF/ICSI cycles initiated between the date of study entry and the end of 2014. Clinical data included the medical and reproductive history of the participant, IVF/ICSI cycle protocol, indicators of oocyte and embryo quality, and IVF cycle outcome including pregnancy. Table 4 summarizes the available clinical data.

Table 4: Summary of available clinical data for UppStART participants

Treatment Data
- Ovarian stimulation procedure clinical data
- Type of treatment: IVF / ICSI
- Type of culture media
- Number of oocytes and embryos obtained
- Indicators of oocyte quality
- Indicators of embryo quality
- Sperm quality variables

Treatment Outcome Data
- Urine pregnancy test results (week 2/3 after embryo transfer)
- Ultrasound confirmed pregnancy (week 7 after embryo transfer)
- Miscarriage
- Live birth *to be supplemented by linkage to the Swedish Medical Birth Register

Biological samples: Baseline

Blood samples At the time of recruitment into the study, the clinic staff withdrew blood samples, which were then send to the Karolinska Biobank. Plasma, serum, and whole blood were aliquoted and stored at -80°C. DNA was extracted from whole blood and stored at -80°C.

Saliva samples Participants were provided with two pre-labelled Salivette® (Sarstedt, UK) kits, a pre-paid envelope for the return of samples to the Karolinska Biobank, and saliva collection instructions: samples were to be collected at home at

7:00 am (upon awakening, before breakfast and teeth brushing) and at 9:00 pm the same day, with no eating, drinking, gum chewing, snus use, or smoking within 30 minutes prior to collection. Saliva samples were returned to the KI Biobank by the participant via post and the samples were stored at the KI Biobank at -80°C until analysis.

Saliva cortisol levels (nmol/L) were measured by radioimmunoassay (RIA; Orion Diagnostic, Finland) by the Study Center of Laboratory Medicine at the Karolinska University Laboratory. The detection limit of the RIA was < 1.0 nmol/L, and the intra- and inter-assay coefficients of variance for this assay were 12% and 8% for the low control, and 7% and 5% for the high control, respectively.

Biological Samples: at Delivery

Staff at seven delivery units in Stockholm and Uppsala were recruited to assist in collecting samples from UppStART participants during delivery. Delivery clinics were provided with a sample collection kit including tubes for collection of maternal blood (EDTA, PAXgene), cord blood (EDTA, PAXgene), amniotic fluid, and placenta samples. Step-by-step instructions with pictures, to ensure consistent sampling technique, were provided to the midwives attending the delivery for sampling of one small piece of placenta (approx. 2x2 cm) from the centre of each quadrant, both on the maternal and fetal side, for a total of 8 samples. Samples were stored in -20°C freezers at the delivery units until they were collected by UppStART study staff and deposited into the KI Biobank.

Linkage to National Registers

Through the unique identification number assigned to each Swedish citizen at birth or immigration,(18) it is possible to link our study participants to the Swedish

population-based national and quality registers. While no further direct participation by the UppStART participants is currently planned, all participants consented to their study data being linked to the national population registers for long term follow-up of themselves and of the children they conceived while being part of the study. The process to link this cohort to the registers has been initiated and will include the Medical Birth Register, Perinatal Quality Register (for neonatal intensive care), Register of Malformations, Prescription Drug Register, Inpatient and Outpatient Registers, Cause of Death Register, and the Multigeneration Register.(19-21) This initial linkage will provide data from prenatal, obstetrical, neonatal, and infant care, and subsequent updates will provide data on childhood health and educational outcomes.

Patient and Public Involvement

Throughout the study's inception, design, and execution, the Principal Investigator of the UppStART study (ANI) was herself a patient, undergoing IVF treatments. Therefore, the patient perspective was taken into account from start to end. The study was designed by ANI, in collaboration with the clinicians from the participating clinics. ANI also made regular visits to the participating clinics to discuss with clinicians about the patient reactions to the study, how they could optimize recruitment while ensuring that the delicate circumstances of the couples struggling to become pregnant were respected. Further, the questionnaires answered by the participants included a section on how they felt about answering the survey, including a free text option for them to express any concerns related to their participation in the study. These responses were monitored by ANI and the study staff as they were submitted.

FINDINGS TO DATE

Stress

While it is well established that women undergoing fertility treatment experience high levels of stress and higher prevalence of symptoms of depression and anxiety, it remains uncertain if and how psychological stress clinically affects the outcome of IVF treatments.(22-24) The first study completed using UppStART data investigated the association between self-reported perceived stress, infertility-related stress, and cortisol levels on IVF cycle outcomes including clinical pregnancy and indicators of oocyte and embryo quality. Overall, we found that stress measured prior to IVF cycle start was not associated with IVF cycle outcome, oocyte maturity, or embryo quality. The findings from this study and many other studies reporting no association between stress and IVF outcomes are potentially reassuring to women undergoing fertility treatment and the clinicians who may have concerns about the influence of stress levels on the success of their IVF treatment.(25)

Cumulative Impact of Lifestyle Factors on IVF treatment outcomes

The UppStART study has been used as a validation cohort in a study examining the cumulative impact of lifestyle factors on IVF treatment outcomes. An individual as well as a cumulative effect of smoking and BMI on the number of aspirated and mature oocytes in fresh IVF treatment cycles has been found, especially among women with low ovarian reserve. (*Manuscript submitted, currently under review*)

Epigenetics

In collaboration with researchers at Leiden University, the methylation profile of the cord blood of UppStART offspring has been measured and compared the methylation profile in the cord blood of spontaneously conceived offspring from the Swedish Born Into Life study. Details on the Born into Life study are described at length

in Smew et al., (2018)(26) The UppStART study and the Born into Life study were run in parallel from the same department, with the protocols for sample collection coordinated. Genome-wide DNA methylation data were generated using the Illumina Infinium Human Methylation 450K BeadChip (450k array). A total of 500ng of genomic DNA isolated from cord blood was bisulfite treated using the EZ-96 DNA methylation kit (Zymo Research, Orange County, USA). The first aim was to identify potential differences in epigenetic marks between IVF- and spontaneously-conceived offspring. Further, comparisons between methylation profiles from infants conceived via different assisted reproductive techniques will be conducted, such as between IVF and ICSI, fresh and frozen embryos, and Day 2 and blastocyst embryo transfer. The study results are currently being written up for submission for publication.

Further, while not considered in this study the plethora of exposure information gathered from the questionnaires also provides unique possibilities to explore gene-environment interactions in the epigenetic analyses and the impact of specific environmental exposures on epigenetic marks.

STRENGTHS AND LIMITATIONS

The major strength of the UppStART study is the plethora of information from multiple sources, including an extensive questionnaire on a large number of lifestyle factors and behaviours, data from medical records, national registers, as well as biological specimens. Further, studies often only include women undergoing ART, however the UppStART study has collected data on the male partners as well. Additionally, the study has captured repeated ART cycles within the couple and monitored the change in lifestyle factors between cycles to determine if these changes are linked to ART outcomes of interest.

However, there are also some limitations and potential sources of bias. Recruitment of participants was slower than expected, especially at the beginning of the study for a number of reasons: one of the four IVF clinics in Stockholm declined to participate and one clinic halted recruitment for 8 months due to staff restructuring. To encourage the involvement of clinic staff, study personnel visited with clinic staff every six months and distributed a newsletter reporting recruitment statistics for each clinic. Additionally, during the last year of recruitment, ethical approval was granted to give participating couples movie tickets upon enrolment, thereby providing a small compensation for their participation. While the goal of the study was to capture every treatment of the couples, the clinics often missed collecting blood samples and reminding the participants to complete the questionnaires at subsequent cycles. Lastly, due to the urgent and demanding nature of the labour and delivery units, the collection of samples at delivery of some infants was missed. Midwifes expressed that often there was not enough time to collect the number of samples requested while also fulfilling their clinical duties.

With any study including self-reported health and lifestyle information, such as this one, recall bias is an issue. The majority of the questions in the UppStART baseline questionnaire targeted specific lifestyle factors during the previous three months to a year. However, women and men experiencing infertility and planning to undergo infertility treatment often make changes in their lifestyle based on what they believe will maximize the chances of conception,(14) which will likely make the participants more aware of their lifestyle choices and consequently be able to respond to the questionnaire more accurately.

Further, volunteer bias may be another source of bias in the cohort. The UppStART study was presented to participants as a general study of lifestyle factors

in IVF couples. Therefore, individuals with an interest in their lifestyle choices may choose to participate, and those with unfavorable lifestyle habits may have declined. However, the characteristics of the UppStART study are typical of the Swedish population undergoing IVF,(27) and a large degree of homogeneity exists in the demographic and lifestyle, including low rates of factors known to affect fertility and IVF outcome (e.g., high BMI and smoking). As with the known variables, we speculate that the study sample is also likely to be relatively homogeneous with respect unmeasured variables which will reduce the influence of confounders on studies performed with data from this cohort.

Collaboration: We encourage the use of UppStART data as a resource for epidemiological research. The data are available upon request and more information can be found on the UppStART webpage (www.ki.se/meb/UppStART). To access the data, a proposal should be submitted to the principal investigator by an applicant with a PhD or an equivalent degree. Proposals will be evaluated and granted according to principles of ethical and scientific soundness. Applicants based outside of Sweden are required to involve collaborator(s) based at a Swedish research institution. Applications can only be granted and data provided after approval by the Stockholm Ethical Review Board. Information and instructions for applicants are available on the website (https://ki.se/meb/uppstart) and from Carolyn Cesta at carolyn.cesta@ki.se.

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Fertilitetscentrum Kungsholmen (previously IVF-kliniken Stockholm, S:t Görans Sjukhus), Stockholm. We thank Bozenna Iliadou, Mariam Lashkariani, and Mikael Bröms for assistance with the database management.

Data sharing statement: The data are available upon request and more information can be found on the UppStART webpage (www.ki.se/meb/UppStART). Information and instructions for those interested in collaboration are available on the website and from CEC at carolyn.cesta@ki.se.

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Ethics approval: The UppStART study has been approved by the regional ethics review board at Karolinska Institutet (Dnr 2011/230-31/1, 2011/1427-32, 2012/131-32, 2012/792-32, 2013/1700-32, 2014/1956-32, 2015/1604-32).

Contributor statement: ANI, JP, SC, KARW, JIO, JH, HW, MW, and ASÖ were responsible for the study design, protocol development, and data collection. CEC was responsible for the data analysis. CEC was responsible for writing the initial draft of the manuscript, and subsequent drafts were reviewed by all authors listed. All authors had input on reporting of the findings. All authors provided approval for the submitted version of this manuscript.

Conflicts of Interest: None to declare.

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Figure Legends

Figure 1: Timeline of participant recruitment and data collection in the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

Figure 2. UppStART study: Number of participants and samples collected

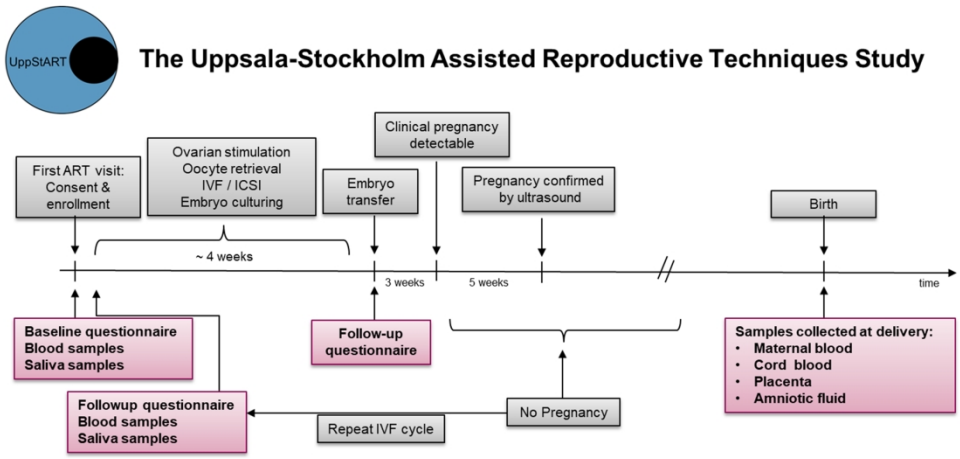

Figure 1: Timeline of participant recruitment and data collection in the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study

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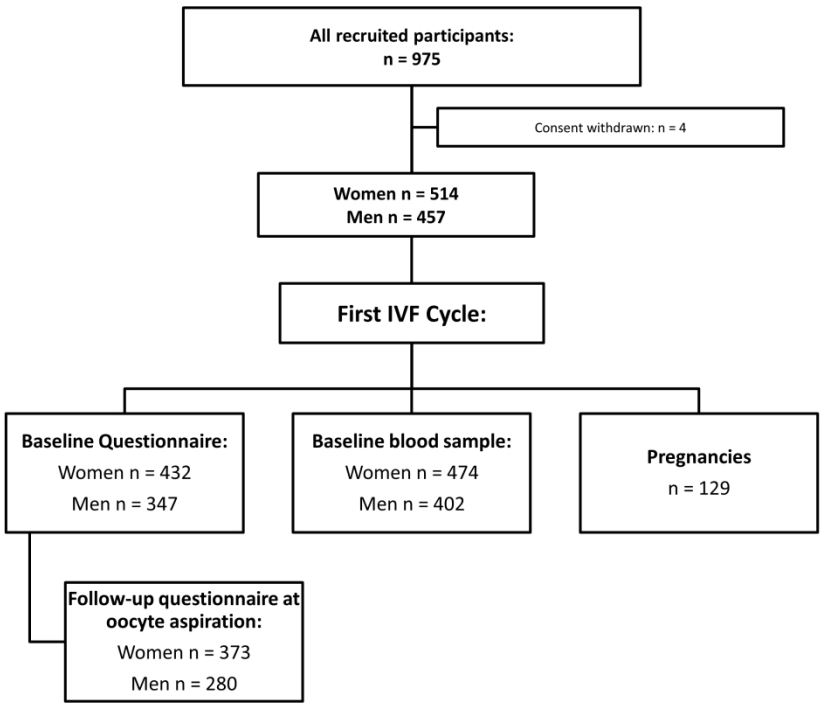

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