Herlev og Gentofte Hospital

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PROJECT

PRIOR: Pre-eclampsia Risk In Oocyte Recipients - Investigating matching, biomarkers and outcome

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It is hereby declared that the study will be performed according to the latest approved study protocol, the "Helsinki Declaration" and local regulatory requirements and legislation.

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Abbreviations

AFC = atrial follicle count

AMH = anti müllerian hormone

ART = assisted reproductive therapy

BMI = body mass index

BP = blood pressure

FSH = follicle-stimulating hormone

FET = frozen embryo transfer

HCG = human chorionic gonadotropin

Hgb = hemoglobin

HR = heart rate

ICSI = intracytoplasmic sperm injection

IVF = in vitro fertilization

ICSI = intracytoplasmic sperm injection

LH = luteinizing hormone

OD = oocyte donation

DGD = double gamete donation

Red Cap = research electronic data capture

SD = standard deviation

TSH = thyroid stimulation hormone

UA = umbillical artery

Background

Maternal age at conception has been continuously rising worldwide and the proportion of pregnant women over 35 years of age has increased up to eight times.[1] In 2021, the average maternal age at first birth in Danish women was 30 years compared to 24 years in 1970.[2]

As fecundity decreases with maternal age and rapidly after the age of 35 years, there is an increased need for assisted reproductive technology (ART). The success rate of ART with autologous oocytes decreases after the age of 40 years, and oocyte donation becomes a more relevant option for achieving pregnancy for these women.

The first oocyte donation was performed in 1984 to overcome infertility caused by premature ovarian failure (POI).[3] The indications for OD are however heterogenous and include Turner syndrome, prior surgical removal of ovaries, prior chemotherapy, or radiation therapy affecting reproductive ability are other diagnoses where oocyte donation might be indicated. Other causes of oocyte donation can be serious genetic disease in women where preimplantation diagnostic is not an option. With oocyte donation, these different groups of women, who were previously untreatable, now have a chance to become pregnant.

With increasing age and concomitant decreasing oocyte reserve and oocyte quality in women with inability to conceive spontaneously or after ART, the technique is nowadays used more widespread. We can now overcome natural peri- and postmenopausal infertility, thus theoretically prevail biology, making motherhood possible for women in their forties, fifties and even sixties. The success of oocyte donation relies more on the donor than the recipients age, however recent studies suggest that paternal age might also have an influence.[4,5] In Denmark, the age limit for the recipient is 46 years, but some women who have crossed this age limit go abroad to countries like Spain, Greece, Latvia, Ukraine, Poland, Cypress or Russia to have treatment for infertility. In total opposition to this, oocyte donation is prohibited in some countries such as Italy, Turkey, Germany, and Luxemburg.

In Denmark, oocyte donation has been legal since 2007 and double gamete donation has been legal since 2018. All healthy and fit women under the age of 36 can

donate their eggs either anonymously or non-anonymously, and the ideal age for donors is thought to be 23 to 30 years of age.[5,6]

The request for oocyte donors is steadily increasing. In 2011, 124 oocyte donations were performed nationally. In 2022, the number had increased more than 20-fold to 2782 oocyte donations of which approximately half of the donated eggs were given to Danish recipients. With a success rate of around 35%, this led to 756 deliveries after oocyte donation in Denmark in 2021.[7-8]

This increment in numbers of oocyte donations may partly be explained by growing request due to increasing age and decreasing fertility in women seeking ART, partly by an increase in the compensation for oocyte donation. Because double donation is now legal, ageing single women or same sex couples have a new option to obtain pregnancy. There is reason to believe that oocyte donation will only increase further over the next years, because we still see an increasing trend for maternal age.[2]

Few years after the first oocyte donation was performed, studies revealed that OD was associated with different obstetrical complications[9], and this has been documented over the past decades.[10–13]

Oocyte recipients have an increased risk of pre-eclampsia (PE), intrauterine growth restriction (IUGR), preterm birth, caesarean section, placental abruption, and postpartum hemorrhage.[10,14] In women who have received double gamete donation the risk of obstetrical adverse outcome may be further increased.[13] The explanation of this increase in obstetrical risk is unknown. Speculations are, that with the use of a sperm donor, the recipient's immune system is exposed to unknown paternal antigens which may activate immunological reactions that predispose to PE.

The increased risk of PE in oocyte pregnancies might be explained by an amplification of the pathophysiological changes seen in the development of preeclampsia in some spontaneously conceived pregnancies. Women undergoing fertility treatment with their own frozen gametes are also at increased risk of pre-eclampsia but not to the same extent as oocyte recipients.

Preeclampsia is a pregnancy-specific disease that complicates 3–8% of pregnancies.[15] It has been known for centuries and was previously referred to as toxaemia of the pregnancy. It is defined as hypertension (systolic blood pressure ≥ 140 mm Hg or

diastolic blood pressure ≥ 90 mm Hg) plus proteinuria (300 mg or more over 24 hours) and/or signs of organ dysfunction after the 20th week of gestation.[16]
Though the definition of PE is clear, a wide range of clinical manifestations makes PE more syndrome-like than a defined disease, which complicates the clinical diagnosis. These manifestations include headache, visual disturbances, chest pain, palpitations, upper abdominal pain, oedema, hyperreflexia, and thrombocytopenia.

Risk factors for PE are previous pre-eclampsia, chronic renal disease, chronic hypertension, pre-existing diabetes mellitus, autoimmune disease, multifetal gestation, nulliparity, advanced age, high body mass index (albeit at differing cut-offs), inter-pregnancy interval of > 10 years and family history of preeclampsia.[17]

Pre-eclampsia is thought to be a spectrum of pregnancy complications that share a common pathophysiology centred upon disordered placentation. This spectrum of disorders of placentation also includes late spontaneous miscarriage, placental abruption, foetal growth restriction, pre-term rupture of the membranes and premature delivery.[18]

The exact etiology of pre-eclampsia remains unclear. However, several potential mechanisms related to the placentation including genes, immune response, insulin resistance, and maternal vascular disease are suggested to contribute to the development of the disease.[19] A recent Dutch study finds that the number of HLA mismatches between fetus and recipient is related to the risk of pre-eclampsia, and especially HLA-DR incompatibility has shown to be related to development of pre-eclampsia .[20,21]

We hypothesize that by studying oocyte recipients, whom we know are at increased risk of pre-eclampsia, we can get an insight to the mechanisms behind pre-eclampsia. The aim of the present study is thus to investigate pathophysiological mechanisms involved in development of pre-eclampsia and concomitant obstetrical adverse outcomes after oocyte and double gamete donation.

Objectives

Primary objectives

- Investigate the risk of pre-eclampsia in pregnancies obtained after single or double gamete donation compared with pregnancies achieved with autologous gametes.
- Investigate immunological, angiogenic biomarkers and HLA tissue type markers involved in the development of pre-eclampsia.

Secondary objectives

- Evaluation of obstetrical and neonatal complications such as pre-eclampsia, gestational diabetes, preterm birth, placental abruption, intrauterine growth retardation, asphyxia, neonatal morbidity, and mortality in women pregnant after oocyte or double donation in Denmark.
- To establish a database and biobank to enable future studies including follow-up of the children born after oocyte donation.

Study design

Patient population

Woman undergoing oocyte donation or double gamete donation (OD) and a matched (BMI and age) control group undergoing treatment with frozen thawed blastocysts after IVF treatment (IVF). Both groups can be included before conception (Pre-Preg Cohort) and after conception (Preg Cohort)

Recruitment

A sub cohort named Pre-Preg cohort will be included preconceptionally, when referred for oocyte or double donation at Herlev University Hospital. This is one of two public hospitals in the Central Region of Denmark, where oocyte donation is performed.

The rest of the participants named Preg cohort are followed from early pregnancy and can be recruited from two paths:

1) Most women will be recruited when they become pregnant and are seen for an early pregnancy scan around gestational week 7-8. The staff at the fertility clinic will shortly introduce the study verbally, and if the woman accepts, her information (name, cpr number, contact information) is given to the PI. She will then be contacted by the study group with more detailed information about the study. This is preferably done in person during a visit at the clinic (women at Herlev Hospital), where a printed copy of the patient information in handed out. For patients from other clinics (Rigshospitalet, Sellmer Fertility, Aleris Hamlet, and Trianglen), the patient information is given by telephone and written patient information is sent to the woman's secure e-mail (e-boks).
2) Others from the Preg cohort will be recruited when they are referred from the general practitioner to the obstetrical department in early pregnancy around gestational week 10. The visitation office will inform the PI about a possible study participant (name, cpr number, contact information). Written information about the study will be sent to her secure e-mail (e-boks) including information that she will receive a phone call within the following days. Here the woman will be informed about the study in more detail.

If the woman is still interested in participation after receiving oral and written information, an inclusion visit is scheduled. The woman is informed, that she can bring an assessor. At the inclusion visit, the patient information is repeated in person, possible questions are answered, and the patient is offered at least 24 hours to consider if she wants to sign the consents.

Inclusion criteria for the oocyte recipients

- Referred to (Pre-Preg cohort) or pregnant after (Preg cohort) oocyte or double gamete donation
- Age $\geq$ 18 years
- BMI $\leq$ 35 kg/m2
- Normal wet smear within the past three years

- Both nulli- and multiparous
- Singletons and multiple gestations

Exclusion criteria for oocyte recipients

These criteria correspond to the general criteria for treatment with oocyte donation in the fertility clinics:

- Age < 18 years
- BMI > 35 kg/m2
- HIV/ hepatitis
- Essential hypertension
- Chronic kidney disease
- Undiagnosed vaginal bleeding
- Uterine malformations
- Persisting ovarian cysts
- Tumors in hypothalamus, pituitary, thyroid, or adrenal glands.
- Previous breast cancer
- Known BRCA 1 or 2 gene
- Unregulated thyroid disease
- Cardiovascular disease
- Breast feeding
- Present or previous chemotherapy/radiation therapy
- Present or previous malignant disease
- Smoking
- Alcohol/drug abuse

Methods

Routine measures for study subjects (background):

- General physical examination: Height, weight, blood pressure, heart rate (at visit 1 for Pre-Preg cohort participants or visit 4 for Preg cohort participants).
- Questionnaire (Age, ethnicity, previous medical history, fertility history, alcohol, smoking ect.)

- Transvaginal ultrasound at inclusion (At visit 1 for Pre-Preg cohort participants to examine the endometrium. At visit 4 for the Preg cohort, to confirm a viable pregnancy)
- Check that routine blood-tests are already taken: p-thyroid stimulation hormone (p-TSH), p-thyroid peroxidase antibody (p-TPO), p-anti-Mullerian hormone (p-AMH), Rubella, Syphilis, Hepatitis, and HIV.

Blood tests

In the section below, the following group of blood tests will be referred to as **pre-eclampsia markers**:

- Growth markers involved in early pregnancy (PAPP-A, IGF, PlGF, PGF and VEGFR)
- Pro- and anti-inflammatory cytokines (Interleukins, TNF-α and interferon-γ)
- Metabolic markers (Adiponectin, Leptin and Resistin)
- Complement and thrombocyte activation markers (Selectin and BF-4)
- Markers for coagulation and fibrinolysis (Thrombin/antithrombin complexes and ex vivo fibrinogenesis/fibrinolysis)
- miRNA markers (Broad array including previously described PE-associated mi-RNAs in the Danish population. That is miR-181b-5p, miR-323a-3p, miR-518b, miR-363-3p, miR-20a-5p, miR-291-3p and miR-142-3p)

An estimated 37 ml of blood will be collected in total from study participants per visit. To specify, this includes two 9 mL EDTA tube (purple color), two 6 mL serum tube (red color), and two 3,5 mL sodium citrate glass (blue color). Blood will be separated and stored according to specific requirements for each analyte, as per procedures available at Statens Serum Institut and Depts. of Clinical Biochemistry at Herlev Hospital, Glostrup/Rigshospitalet and Aarhus University Hospital

In addition, HLA type Pre-Preg cohort participants will be tested with one extra blood test (6 mL EDTA tube) taken post-partum.

A total of 376 mL of blood will be drawn from the Pre-Preg cohort participants whereas 222 mL of blood will be drawn from the Preg cohort participants.

Urine dip stix is performed at some visits to check for proteinuria as a sign of pre-eclampsia.

The intention is to gather placentas from the 50 Pre-Preg participants to send for pathology. Pathologists will look for pathoanatomical signs of pre-eclampsia such as infarcts, increased syncytial knots, hypovascularity of the villi, cytotrophoblastic proliferation, thickening of the trophoblastic membrane, obliterative enlarged endothelial cells in the fetal capillaries, and atherosis of the spiral arteries.

Study visits

According to the national guideline, all oocyte recipients in Denmark are recommended to take 150 mg of acetylsalicylic acid from approximately week 10-12 in order reduce the risk of pre-eclampsia.[22]

Later in pregnancy, the control-program for women pregnant after oocyte donation differs among the hospitals in the Capital Region of Denmark. In three out of four hospitals, women pregnant after oocyte donation are not per se offered extra ante-natal care compared to women who have become spontaneously pregnant. Unless any problem evolves during pregnancy, they are only seen for routine visits with nucleal translucency scan (GA 12), malformation scan (GA 18-20) and routine midwife consultations. This is at Rigshospitalet, Hvidovre Hospital, and Hillerød Hospital.

At Herlev Hospital, women pregnant after oocyte donation are routinely offered a control program. Initially they also have nucheal translucency scan (GA 12) and malformation scan (GA 18-20). Later they are monitored with blood pressure measurements sent to the hospital using telemedicine every second week from GA 24 and physical visits at the hospital with ultrasound and doctors' appointments (GA 28, GA 34, and GA 38). In Denmark we recommend induction of labor at week 40+0 for oocyte recipients.

To standardize, we gained permission to offer all pregnant oocyte recipients, to be transferred to Herlev Hospital, in case of pregnancy. If transferred to Herlev Hospital, they would be followed according to the above-mentioned routine control program. This model was accepted by the visitation office for deliveries in the Capital Region of Denmark ("Central Visitation for Fødsler").

In case the woman declines transfer to Herlev Hospital during her pregnancy,

she can still be included in the study. However, she would then have to visit Herlev Hospital three times during her pregnancy to have an introduction to telemedicine (GA 28) and two times for ultrasound examinations (GA 34 + 38). During these visits, blood to test for pre-eclampsia markers will be drawn.

In the Pre-Preg cohort, the placenta is sent for pathology examination and a post-partum study visit with their newborn will be arranged.

For controls who are pregnant after IVF-treatment, study participation will be similar. Of course, they are still seen pre-pregnancy when they undergo fertility treatment either at Herlev Hospital (Pre-Preg and Preg cohorts) or if they have fertility treatment elsewhere (Preg cohort). Some will be included before the fertility treatment starts (Pre-Preg cohort, study visit 1-3) and others in their early pregnancy (Preg cohort, study visit 4). Women pregnant after IVF will have the routine nuchal translucency scan in week 12 and malformation scan in week 18-20. In relation to these already scheduled visits, study participants will also have blood drawn for this study (named study visit 5 and 6). Unless something in their pregnancy rises concern, they would not have extra ultrasound examinations or doctor's appointments. Thus visit 1 to 3 (see below) which are in relation to fertility treatment, are already routine for IVF-pregnancies. However, the pre-eclampsia marker blood tests taken in relation to routine visits, the inclusion visit in early pregnancy and visit 7-8 are extra for women pregnant after IVF. Additionally, they will report urine dip stix and blood pressure measured at home every second week from gestational age 28 until delivery. Instructions on the use of telemedicine will be given at a visit in week 28 by study personal. These IVF women will be recommended induction of labor at gestational age 41+5, which is standard treatment for all pregnant women in Denmark.

	GW		Herlev				Other hosp.
			OD rec. (PrePreg)	IVF (PrePreg)	OD rec. (Preg)	IVF (Preg)	OD rec. (Preg)
Pre-Preg Cohort	Pre-conc.	*Pre-conception inclusion of Pre-Preg (**Study visit 1**)*	Extra	Extra			
	Pre-conc.	*Transfer of blastocyst (**Study visit 2**)*	Standard	Standard			
	7	*Early pregnancy ultrasound (**Study visit 3**)*	Standard	Standard			
Preg Cohort	10-12	*Inclusion of Preg cohort (**Study visit 4**)*			Extra	Extra	Extra
	12	*Nuchal Translucency ultrasound (**Study visit 5**)*	Standard	Standard	Standard	Standard	Standard
	18-20	*Malformation ultrasound (**Study visit 6**)*	Standard	Standard	Standard	Standard	Standard
	24	*Introduction to telemedicine*	Standard		Standard		
	26	*Telemedicine*	Standard		Standard		
	28	*Introduction to telemedicine or*		Extra		Extra	Extra
		*Ultrasound and osbstetric check-up (**Study visit 7**)*	Standard		Standard		
	28	*Telemedicine*	Standard	Extra	Standard	Extra	Extra
	30	*Telemedicine*	Standard	Extra	Standard	Extra	Extra
	32	*Telemedicine*	Standard	Extra	Standard	Extra	Extra
	34	*Ultrasound and obstetric check-up (**Study visit 8**)*	Standard	Extra	Standard	Extra	Extra
	36	*Telemedicine*	Standard	Extra	Strandard	Extra	Extra
	38	*Ultrasound and obstetric check-up **(Study visit 9)***	Standard	Extra	Standard	Extra	Extra
	40	*Telemedicine*	Standard	Extra	Standard	Extra	Extra
	Delivery	*Delivery, placenta and cord samples (**Study visit 10***)	Extra	Extra			
	4 w PP	*Four weeks post-partum (**Study visit 11**)*	Extra	Extra			

*All **study-visits** will include blood tests for pre-eclampsia markers, which are all extra bloodtests compared to standard control programme.*

Contacts with oocyte recipients

For **Pre-Preg cohort** participants, a woman who calls the fertility clinic to start fertility treatment will have a brief introduction to the study by the doctor/nurse in charge of their treatment. After acceptance, her information (CPR number, name, type of treatment, contact information) is given to the PI. She will then be contacted by telephone with more detailed information about the study and written information in her secure e-mail (e-boks). The woman in this Pre-Preg cohort needs to be thoroughly informed about the study before her visit to the clinic, as inclusion and the first blood tests have to be on the day she visits the clinic, as the first blood tests must be drawn before she starts her fertility medication later the same day.

The **Preg cohort** participant is also briefly introduced to the study by the doctor in charge of their treatment during one of the physical visits in the one of the fertility clinics. After acceptance, her information (name, CPR number, contact information) is given to the PI. For a woman who becomes pregnant at Herlev Hospital, detailed information about the study is given in person during a physical visit in the clinic and a printed copy of the patient information is handed out. For a woman who becomes pregnant at the other clinics (Rigshospitalet, Sellmer Fertility, Aleris Fertility, and Trianglen) this information is given by telephone and written patient information is sent to her secure e-mail (e-boks).

If the patient is still interested after oral and written information is given as described above, an inclusion visit is arranged.

During the inclusion visit, the patient information will be repeated as needed. The woman is offered at least 24 hours to consider participation. After signed consent, the woman can be included as described below. Information about the age of the donor will be retracted from patient files in "Formatex"

Pre-conception – *inclusion of Pre-Preg cohort participants* (**Study visit 1**)

- Informed consent obtained by primary investigator, or trained study nurse. Study subjects are given the opportunity for another consideration period if needed before signing the consents.

- Assessment of study in- and exclusion criteria.
- Questionnarie
- Demography (age, ethnicity, family status), medical history (history of concomitant disease, hereditary diseases, and fertility history).
- Concomitant medication.
- Smoking and alcohol habits.
- Physical examination
- Height and weight.
- Ultrasound of the ovaries.
- Blood pressure and heart rate sitting position.
- Check for routine measures in the patient file
- Smear, TSH, TPO, AMH, HIV, hepatitis, syphilis, and rubella (These are standard tests for all fertility patients)
- Blood test for the study
- Blood samples for measuring pre-eclampsia markers.

Ovulatory women will be instructed to sign up for treatment on the second to third day of their menstrual cycle. Anovulatory women with an endometrial thickness > 4 mm measured by transvaginal ultrasound will be given Gestagen tablets 10 mg daily for 10 days. They are instructed to sign up for treatment on the second day of the withdrawal bleeding.

During the beginning of stimulation, women will have a transvaginal ultrasound with measurement of endometrial thickness (≤ 4 mm) and evaluation of ovarian morphology. They will be instructed to administer estradiol with 6 tablets a day.

At stimulation day 10, women will have repeated transvaginal ultrasound with measurement of endometrial thickness (≥ 7 mm) and evaluation of ovarian morphology. Embryo transfer will be planned and instructions on progesterone administration with 1200 mg daily (400 mg vaginally + 400 mg vaginally + 400 mg rectally) 4 days before embryo transfer.

Transfer of blastocyst - *only PrePreg cohort* (**Study visit 2**)

- Transfer of a frozen thawed blastocyst.
- Blood samples for measuring pre-eclampsia markers.

Women will have an HCG blood test approximately 10 days after the transfer to test for pregnancy. If the test is positive, an early pregnancy ultrasound is planned at the fertility clinic.

Gestational week (GW) 7-8, test for pregnancy - *only Pre-Preg cohort* (**Study visit 3**)

- Early pregnancy ultrasound to confirm a viable pregnancy.
- Blood samples for measuring pre-eclampsia markers.

Part of the Preg cohort will be included directly from the fertility clinics (Herlev Hospital, Rigshospitalet, Sellmer Fertility, Trianglen, Aleris Fertility). Recruitment will happen when the woman becomes pregnant and is at the fertility clinic for an early pregnancy scan in approximately gestational week 7 to confirm a viable pregnancy. The doctor/nurse at the clinic will briefly inform the patient about the study and ask for acceptance for the study group to contact the patient. If the patient wants to participate after both oral (preferably in person or else by telephone) and written information (preferably handed out or else in e-boks), the above-mentioned inclusion visit will be arranged. The woman is informed, that she can bring an assessor. At the inclusion visit, the patient information will be repeated.

Lastly, we will include women in the Preg cohort who have become pregnant after oocyte donation performed in other Danish fertility clinics or outside Denmark. These women will be identified for possible inclusion, when referred from the general practitioner to Dept. of Obstetrics and Gynecology in early pregnancy around gestational week 6-10. The possible participant will receive a secure e-mail (e-boks) with written information about the study and information that she will receive a phone call within the following days. The mail also tells her how to write or call in case she does not want any further information. The possible participant is given a few days to read the material, after which she will receive a phone call with oral information about the study. If she is still interested, an inclusion visit is arranged. Here the patient information will be repeated, and she is offered time (at least 24 hours) to consider

participation.

GW10 – inclusion – *Preg cohort only (*Study visit 4)

- Informed consent obtained by primary investigator, or trained study nurse.
- Assessment of study in- and exclusion criteria.
- Questionnarie
- Demography (age, ethnicity, family status), medical history (history of concomitant disease, hereditary diseases, and fertility history).
- Concomitant medication.
- Smoking and alcohol habits.
- Physical examination
- Height and weight.
- Ultrasound of the ovaries.
- Blood pressure and heart rate sitting position.
- Check for routine measures in the patient file
- Smear, TSH, TPO, AMH, HIV, hepatitis, syphilis, and rubella (These are standard tests for all fertility patients)
- Blood test for the study
- Blood samples for measuring pre-eclampsia markers.

GeW12, Nucheal translucency scan – *Pre-Preg+Preg cohorts* (**Study visit 5**)

- Nuchal translucency scan
- Blood samples for measuring pre-eclampsia markers

GW18-20, Malformation scan – *Pre-Preg+Preg cohorts* (**Study visit 6**)

- Malformation scan
- Blood samples for measuring pre-eclampsia markers.

GW24 - *Pre-Preg + Preg cohorts*

- OD (Herlev): Introduction to telemedicine.

GW26 – *Pre-Preg + Preg cohorts*

- OD (Herlev): Telemedicine.

GW28 – *Pre-Preg* + *Preg cohorts* (**Study visit 7**)

- OD (Herlev): Telemedicine, ultrasound, obstetrical check-up and pre-eclampsia markers.
- IVF + OD (other hospitals): Introduction to telemedicine and pre-eclampsia markers.

GW30 – *Pre-Preg* + *Preg cohorts*

- All participants: Telemedicine.

GW32 – *Pre-Preg* + *Preg cohorts*

- All participants: Telemedicine

GW34 – *Pre-Preg* + *Preg cohorts* **(Study visit 8)**

- All participants: Telemedicine, ultrasound, obstetrical check-up and pre-eclampsia markers.

GW36 – *Pre-Preg* + *Preg cohorts*

- All participants: Telemedicine

GW38 – *Pre-Preg* + *Preg cohorts* (**Study visit 9)**

- All participants: Telemedicine, ultrasound, obstetrical check-up, and pre-eclampsia markers.

GW40 – *Pre-Preg* + *Preg cohorts*

- OD (Herlev): Telemedicine and induction of labor, if not delivered yet.
- IVF + OD (other hospitals): Telemedicine.

Delivery – *Pre-Preg cohort only* (**Study visit 10**)

- Delivery blood samples with pre-eclampsia markers and including umbilical cord samples.
- Placenta pathological examination.

Gestational week 41+3-5

- OD (Herlev) + OD (other hospitals): Hopefully already delivered.
- IVF: Induction of labor, if not delivered yet.

Four weeks post-partum – *Pre-Preg cohort only (***Study visit 11**)

- Blood test to identify HLA tissue type
- Buccal swap on new-borns to identify HLA tissue type

Blood samples for measuring pre-eclampsia markers.

Scheme of study visits

	Pre-Preg cohort	**Preg cohort**
Pre-conception - inclusion Pre-Preg cohorte (Study visit 1)		
Information about the study and signed consent from Pre-Preg cohort	X	
Questionaire about previous medical history	X	
Physical examination with height, weight, and blood pressure	X	
Check routine blood tests (*TSH, TPO, AMH)*	X	
Check routine blood tests (Syphilis, Rubella, HIV, and Hepatitis)	X	
Transvaginal ultrasound	X	
Blood tests (*PE-markers*)	X	
Transfer of blastocyst (Study Visit 2)		
Ultrasound examination *(Transfer of blastocyst)*	X	
Blood tests (*PE-markers*)	X	
Early pregnancy GW 7-8 (Study Visit 3)		
Ultrasound examination *(Viable pregnancy?)*	X	
Blood tests (*PE-markers*)	X	
Inclusion of Preg cohorte (GW 8-11) (Study Visit 4)		
Information about the study and signed consent from Pre-Preg cohort		X
Questionaire about previous medical history		X
Physical examination with height, weight and blood pressure		X
Check routine blood tests (*TSH, TPO, AMH)*		X
Check routine blood tests *(Syphilis, Rubella, HIV, and Hepatitis)*		X
Transvaginal ultrasound to check for a viable pregnancy		X
Blood tests (*PE-markers*)		X
Nucheal Translucency Scan (GW12) (Study visit 5)		
Ultrasound examination *(Nuchal translucency scan)*	X	X
Blood tests (*PE-markers*)	X	X

Malformation scan (GW 18-20) (Study Visit 6)		
Ultrasound examination *(Malformation scan)*	X	X
Blood tests (*PE-markers*)	X	X
GW 24		
Introduction to telemedicine (Only OD) (*Blood pressure, urine dip stix)*	X	X
GW 26		
Telemedicine (Only OD) (*Blood pressure, urine dip stix)*	X	X
GW 28 (Study visit 7)		
Fetal growth scan (Only OD)	X	X
Obstetrical check-up (Only OD)	X	X
Introduction to telemedicine (Only IVF) (*Blood pressure, urine dip stix)*	X	X
Blood tests (*PE-markers)*	X	X
GW 30		
Telemedicine (*Blood pressure, urine dip stix*)	X	X
GW 32		
Telemedicine (*Blood pressure, urine dip stix*)	X	X
GW 34 (Study Visit 8)		
Fetal growth scan	X	X
Obstetrical check-up	X	X
Telemedicine (*Blood pressure, urine dip stix)*	X	X
Blood tests (*PE-markers)*	X	X
GW 36		
Telemedicine (*Blood pressure, urine dip stix*)	X	X
GW 38 (Study Visit 9)		
Fetal growth scan	X	X
Obstetrical check-up	X	X
Telemedicine (*Blood pressure, urine dip stix)*	X	X
Blood tests (*PE-markers)*	X	X
GW 40		
Telemedicine (*Blood pressure, urine dip stix)*	X	X
Induction of labor at 40+0 (Only OD)	X	X
Delivery (Study visit 10)		
Placental tests	X	
Umbillical cord samles	X	
4 weeks post-partum (Study visit 11)		
HLA-type on newborn (*buccal swap*)	X	
Blood tests (*PE-markers + HLA type on mother*)	X	

Definition of pre-elampsia

In this study, pre-eclampsia is defined as:

- Hypertension after GA 20 weeks with SBT >= 140 and/or DBT >=90.

and either one or both of

- Proteinuria >= 0,3 g / 24 hours
- Signs of organ dysfunction (*thrombocytopenia, affection of kidney or liver function, lung oedema, neurological complications, utero-placental dysfunction*)

Data management

Data collection and processing

Source data will be recorded in the patient record or on specific worksheets. Data will be stored in Redcap. A Case Report Form (CRF) will be constructed for data capture. Data will be stored in coded form for 15 years according to recommendations from" Videnscenter for dataanmeldelser", Rigshospitalet. Afterwards it will be transmitted to "Rigsarkivet."

Data on which women have undergone oocyte donation (cases) will be retrieved from the visitation papers from the general practitioner to hospital department in early pregnancy (Preg cohort), to identify possible study participants. This information about CPR number, contact details and mode of conception will be passed on to principal investigator before consent from the patient in accordance with the Danish Health Act paragraph 46. The possible study participant will receive a secure e-mail (e-boks) with written information about the study and that study personal will call her within a few days and how to call or write in case she does not want additional information. Otherwise, she will receive a phone call with information about the study. If still interested, she can either accept participation or have a consideration period after which she will be contacted again a few days later. If the interest persists, an initial visit will be arranged. Here, the woman will again receive detailed information about the study and sign consents. After signed consents, prior hospitalizations, chronic disease, and medication will be obtained from patient

files “Sundhedsplatformen” as well as the age of the patient’s oocyte donor from the patient’s journal in “Formatex”. Later in the study, information on gestational age at delivery, mode of delivery, birth weight, fetal malformations, obstetrical complications (preeclampsia, gestational diabetes, intrauterine growth retardation, post-partum haemorrhage) will be obtained from patient files “Sundhedsplatformen”.

Signed consent allows for principal investigator, supervisors, and other study staff to access the information from the patient and children’s files. This consent is crucial to monitor, control and complete the study.

Bio bank

Initial blood tests with hormones and infection markers are always taken routinely as part of fertility treatment and analyzed immediately, as they are needed in relation to fertility treatment. Urine dip stix will be analyzed immediately and destroyed afterwards. The entire placenta will be sent for pathology just after delivery from Pre-Preg cohorte to check for maternal hypofusion (e.g. placental weight, thickness of umibillical cord, decidual vasculopathy of the spiral arteries, infarcts, hypermature villi). A small proportion of the placenta (a cotyledon) will be stored in the biobank for possible future research. Our buccal swap tests and blood samples (pre-eclampsia markers) will be gathered and frozen consecutively over a long inclusion-period, as they need to be analyzed at several different places and over a longer time span.

Most blood samples at a total of 28 mL will be stored in a research bio bank and analyzed for the present study and additional 9 mL will be drawn and stored in a biobank for possible future studies. In case there is excess blood out of the 28 mL drawn for the present study, they will also be stored in the same research biobank for possible future studies. Blood samples from the bio bank for future studies will be stored for 30 years provided patient acceptance with signed consent now. Estimated date for termination of the biobank for future studies is first of June 2054. Termination of the biobank for the present study is estimated to first of June 2029.

The data protection rules are still complied with in accordance with The Data Protection regulation and the law of data protection. After 30 years, the material will be destroyed. This will open to the possibility of future studies including studies on the later outcomes of children born after oocyte donation.

Additional or future analyses will only be performed after renewed approval from the

Ethics Committee and the material will not be transported out of the country. Later genetic data will be handled according to ethics and data regulation at the time of use of the samples.

The patients can participate in the study without having biologic material stored in the bio bank. If a patient does not want blood to be stored for future research, this will be registered in RedCap and the consent for this will not be signed. Blood samples that are not stored for future research, will be destroyed immediately after analysis.

Collaborations

We collaborate with stakeholders that specialize in different fields that are all relevant to this subject. Thus, blood tests will be analyzed at four different locations:

1) *"Statens Serum Institut" (SSI)*, which is a government public health and research institution under the Danish Ministry of Health. We collaborate with Michael Christiansen, Professor (adj) and chief physician and Dr. Paula Hedley, as well as biochemists and genetics, "Department for Congenital Disorders", SSI. They have previously been involved in research with pre-eclampsia markers and have a special interest in miRNA's and to establish a biobank for possible future "-omics"-studies.
2) *Department of Clinical Biochemistry, Rigshospitalet/Glostrup.* We collaborate with Line Rode, M.D., Ph.D., who dedicated her work-life to screening in early pregnancy, including screening for possible biomarkers.
3) *Department of Clinical Biochemistry, Aarhus University Hospital*. This is in collaboration with Julie Brogaard Larsen, M.D., Ph.D. Her previous research area has been medical conditions with altered thrombosis or complement-activation, such as pre-eclampsia.
4) *Department of Clinical Immunology, Rigshospitalet.* We collaborate with Anne Werner Hauge, M.D., Ph.D.. The department specializes in tissue type HLA and HLA antibodies, primarily used in transplantation, transfusions, and diagnostics.

Data analysis

The per protocol population will consist of all patients who completed the study without any major protocol violations.

Normally distributed variables will be presented as mean ±SD, non-parametric statistics and appropriate log-transformation will be performed if assumption of normality is not met. After log transformation the parameter will be further tested for normality distribution as indicated. A two-tailed p value of 0.05 or less is considered statistically significant. Comparisons between treatment groups will be performed by an unpaired two-sample t-test, Mann-Whitney test, or Chi-squared test as appropriate. Analysis for non-linear relations will be performed using ML methods, e.g. symbolic regression in the QLattice formulation.

Additional analysis due to loss of follow-up:
Data from the Intention to Treat Population will be analyzed to determine the validity of the conclusions of the Per Protocol population. Analysis will include duration in study and reason for discontinuation as co-variables.

Power analysis

A statistical power analysis was performed for sample size estimation, based on data from previous studies regarding oocyte donation and risk of preeclampsia. Previous studies showed odds ratios for pre-eclampsia among oocyte recipients ranging from 2,11 to 3,99.[10,23] We used the more conservative estimate with an aOR of 2,11.
Using the clinically relevant effect size of 20% absolute increase in risk of PE among oocyte recipients with a two-sided significance level of 0.05 and with
80% power, the projected sample size needed is N = 250 women. To allow for an estimated drop-out or major protocol deviations rate of 5 % we will need to include263 patients in each arm of this study. Dropouts will be replaced by new patients.

Quality control and assurance

The study will be carried out in accordance with the Helsinki Declaration, after approval by the Regional Scientific Ethics Committee Agency and the “Videnscenter

for dataanmeldelse", Rigshospitalet. It is Sponsors responsibility to ensure that data protection rules are complied with in accordance with The Data Protection regulation and the law of data protection.

The study will be registered on www.clinicaltrials.gov

Ethical considerations

Patient discomfort and risks

Participation in the study involves more visits in pregnancy than routinely. This specifically applies for IVF-controls and OD cases from other hospitals than Herlev, who would not by default routinely be examined by a doctor nor have extra ultrasound examinations in their pregnancy. This also applies for women, who agree to be transferred to Herlev Hospital from one of the other three hospitals. Some women will find these visits to be a safety, whereas other might think of it as a burden.

Overall, we find that participation in this study poses the women at minimal risk compared to the information that this study might give us. Information about the pathophysiological mechanisms behind pre-eclampsia might not only benefit women pregnant after oocyte donation but possibly benefit all the 3-8% of women who develop pre-eclampsia during pregnancy. Using telemedicine, we will most probably at an earlier stage identify some IVF controls and OD cases from the other hospitals, who develop pre-eclampsia, because they will be screened for continuously from gestational week 28.

Transvaginal and abdominal ultrasound are non-invasive procedures without any known side effect from the sound waves used. The procedure can be associated with minor discomfort in some women and there is a minimal risk of allergic reaction to the examination gel.

Since this study is an evaluation of standard procedures for OD recipients belonging to Herlev Hospital, additional discomfort for these oocyte recipients is limited to extra blood sampling during pregnancy and buccal swap on newborn.

An estimated 37 ml of blood will be collected in total from study participants per visit. This amount of blood drawn is not thought to affect the participants, though we are aware that blood will be drawn several times, but over a period of months. Blood

will be separated and stored according to specific requirements for each analyte, as per procedures available at both dept. of Clinical Biochemistry (Aarhus and Rigshospitalet/Glostrup) and dept. of Clinical Immunology, Rigshospitalet

Blood sampling can have side effects such as hematoma, superficial thrombophlebitis, and infection, but the risk is generally considered low.

To identify fetal HLA tissue type on Pre-Preg cohort participants' newborn, a buccal swap will be performed 4 weeks post-partum. This is done by sweeping two cotton swaps on the mucous membranes on the inside of the newborn's cheeks for 20 seconds. This may be associated with a bit of discomfort for the newborn. The method is already used in newborn in the case of paternity tests. HLA type for oocyte recipient in Pre-Preg cohort will be tested with an extra blood test (6 mL EDTA tube) taken during the same visit four weeks post-partum. Considerations of inclusion of newborn have been made, as these are incompetent to consent to participation, so consent must be given from both parents in case of shared custody. However, the buccal swap must evidently be taken on their own offspring to look at the possible HLA mismatch, which can contribute to induction of pre-eclampsia. HLA tissue type does however not change over years, so in theory, it could be taken once the child gets older. However, this would delay the study and the results for years. We find it crucial to investigate this relation now, as the usage of oocyte donation is rapidly increasing these years and possible adverse side effects, or long-term effects of the treatment need to be examined.

Informed consent

Subjects recruited will attend an outpatient clinic at the study center. The investigator will ensure, that the subject is adequately informed about the study background and design, verbally and in writing.

Pre-Preg cohort participants will initially be informed briefly about the study in relation to their treatment at the fertility clinics. They will also more thorough information by telephone and written information in their secure e-mail (e-boks). Patient information will be repeated during the inclusion visit.

For Preg cohort participants, oral information will be given either in person or by telephone. The written patient information will be handed out during a visit to the fertility clinic or sent by e-mail along with the brochure “Forsøgspersoners rettigheder I et biomedicinsk forskningsprojekt” and “Før du beslutter dig” (Your rights as a participant in biomedical research).

For interested subjects (both Pre-Preg and Preg cohort), a subsequent inclusion will be scheduled. This is called visit 1 for Pre-Preg cohort participants and visit 4 for Preg cohort participants (even though it is their first visit). Verbal information will be given be repeated as needed by the principal investigator or a specially trained study nurse in a quiet environment. The subject will have the opportunity to ask questions and bring a companion to the interview.

Before signing the consent form, the subject will be offered to reflect at least 24 hours. Should the patient need further time, a follow-up will be scheduled. In case of participation, the informed consent is signed prior to inclusion in the study.

Parents cannot consent on behalf of the child before birth. Thus, consent for buccal swap on newborn must be signed at the study visit 4 weeks post-partum, where the buccal swap is performed on newborn (only Pre-Preg cohort participants).

The subjects are informed that they may, at any time, withdraw their informed consent to participate in the study without it having consequences for subsequent care.
No study-related examinations will be conducted until after the informed consent has been obtained.

Termination of the entire study before time could happen in case legislation in the field of oocyte donation is changed.

Insurance

Patients are covered under “Patienterstatningen” (The patient compensation association).

Financial Remuneration

The study subjects will receive no financial remuneration for participation in the study, as all the treatments investigated are standard treatments.

Initiative to the study was taken by the sponsor Pernille Fog Svendsen.

We received funding from the private sector with a research grant of 600.000 DKR from Ferring and a research grant of 200.000 DKR from Gedeon Richter. All members of the study group do not have any affiliations with neither Ferring nor Gedeon Richter. Financial support is paid out to the Department of Obstetrics and Gynaecology, Herlev Hospital, who will administer the funding.

Neither principal investigators nor supervisors have any financial interest in the project.

Budget

See appendix 1.

Publication plan

Positive, negative, and inconclusive study results will be published in international peer reviewed scientific journals and made publicly available at www.clinicaltrials.gov.

The Ph.D. student, Mette Schou Hedegaard, will be first author and Pernille F. Svendsen last author.

Timetable

Procedure	**Timeframe**
Approval from the Ethics Committee and the Danish Data Protection Agency	July 2024
Preparation of study sites	August 2024
PhD enrollment	January 2024
Start inclusion (First patient's first visit)	October 2024
Completion of study (Last patient's last visit)	September 2026
Specialized analysis / Pre-eclampsia markers	October 2026
Data analysis	October to December 2026
Publications	January to august 2027

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