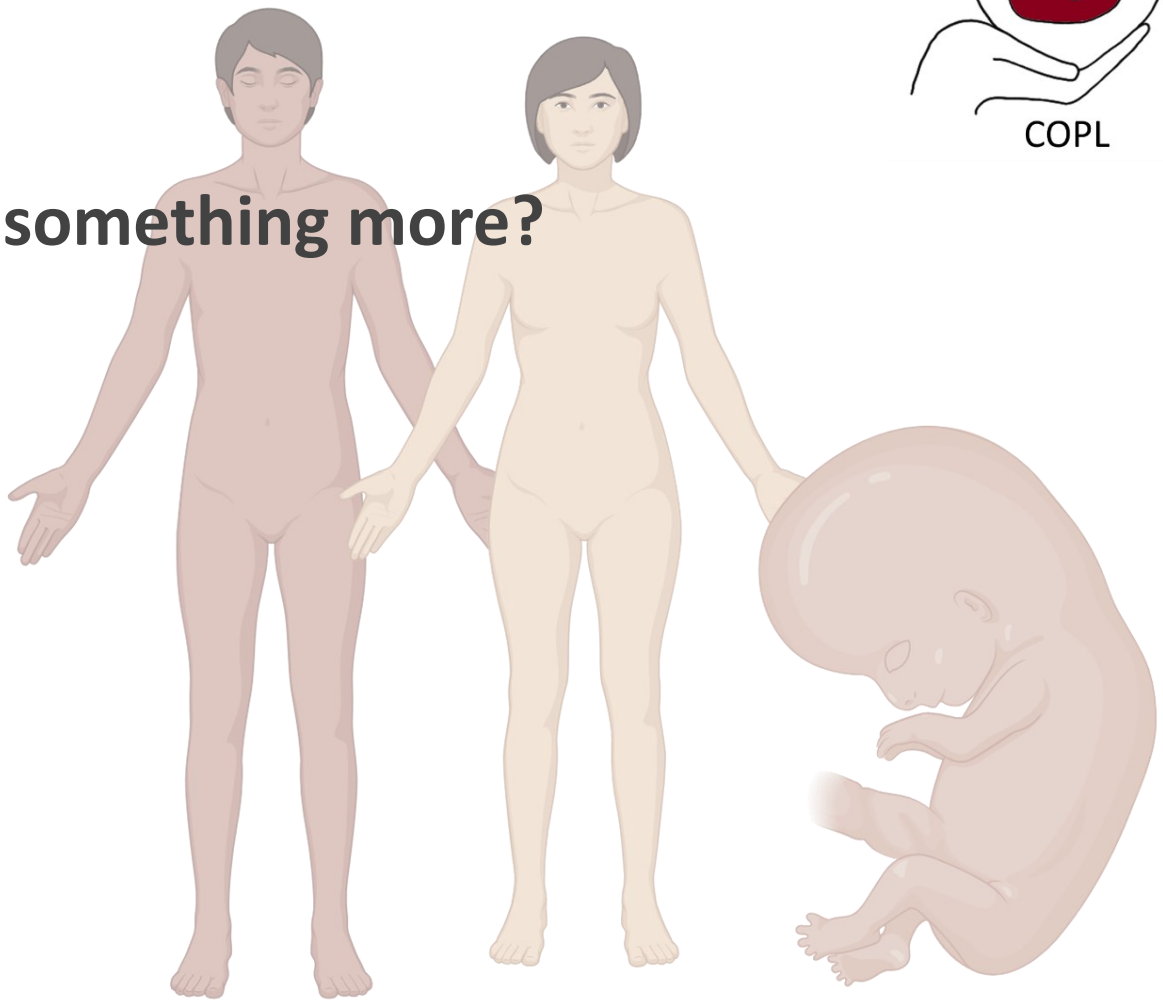
Pregnancy loss
– tender loving care or can we do something more?

COPL



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deCODE genetics

Conflicts of interest

- No personal conflicts of interest

- Copenhagen Pregnancy Loss (COPL) study is funded by BioInnovation Institute and Novo Nordisk Foundation

Pregnancy loss numbers

25%

of all pregnancies end as a loss (1)

>30M

pregnancy losses occur worldwide yearly (2)

No

investigations on why and no standard care (3)

Later

increased risk of multiple diseases such as CVD, diabetes and mental disease (4)

Risk

of another pregnancy loss increases for every loss(5)

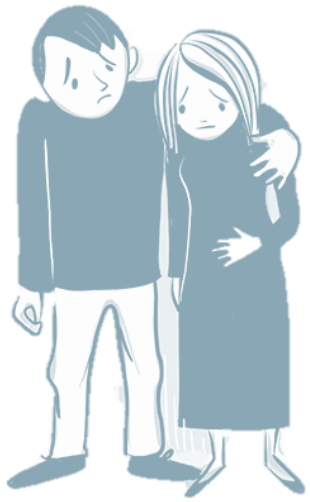
Lower

number of children in couples with previous PL (5)

(1) Macklon et al. 2005, (2) Siobhan et al., The Lancet, 2021, (3) ESHRE guidelines, (4) Westergaard et al., 2020, (5) Kolte et al., 2021.


How to prevent?
WHY?

Unmet need



"We wished for earlier access to diagnostic tests and treatment rather than the required three PLs"

"it was cruel to go through the emotional pain related to a third PL before being able to seek specialized care. ..precious time has been wasted waiting for a referral"

"we perceived insensitivity when hearing comments from physicians like 'it's common' or 'you can try again'."

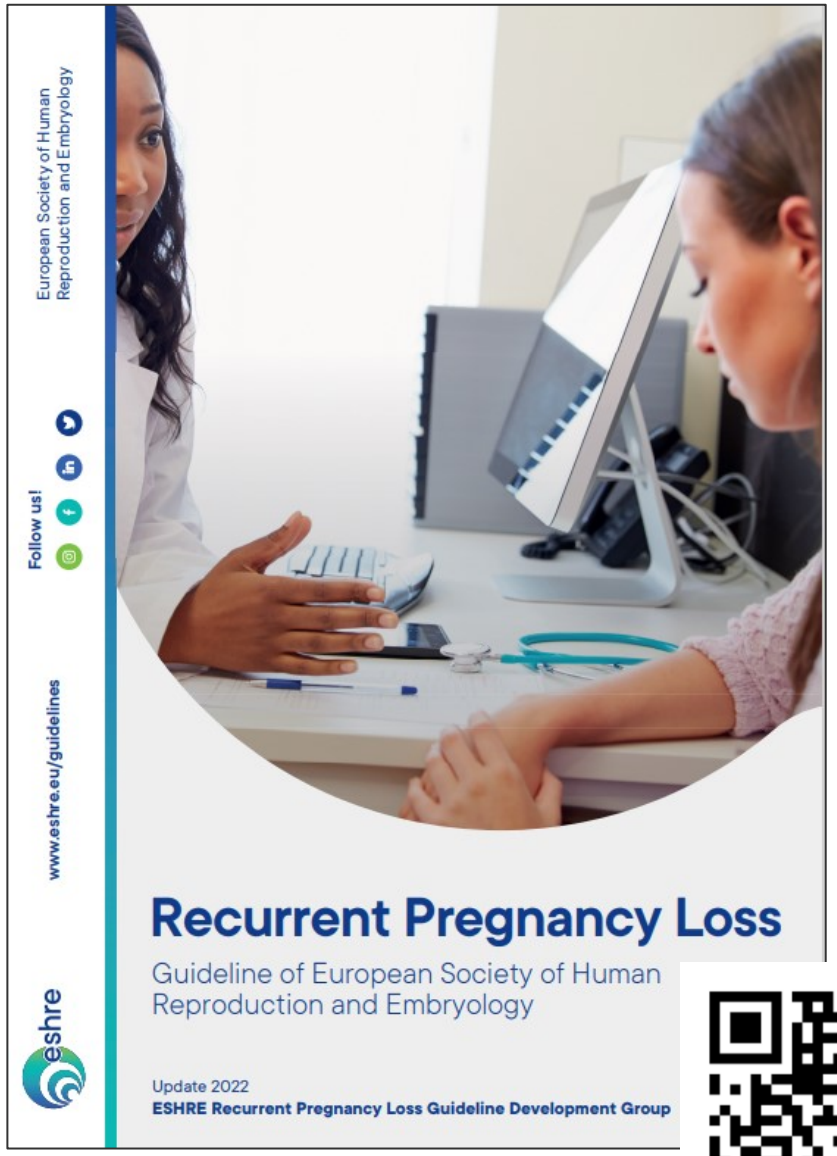
Koert et al. 2019. Human Reproduction

Recurrent pregnancy loss

Still no international consensus

- ESHRE's definition:
  - Two or more PL (confirmed by s/u-hcg)
  - Not necessarily consecutive
  - Mola and ectopic not included
- In Denmark:
  - Three consecutive or
  - Two second trimester losses

- Primary or secondary



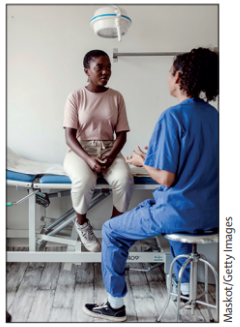
The Lancet series ‘Miscarriage matters’ (2021)

Editorial

Miscarriage: worldwide reform of care is needed

Globally, an estimated 23 million miscarriages occur every year. Despite the personal toll involved, many miscarriages—defined as the loss of pregnancy before viability—are managed in relative isolation. Private grief and misconceptions—eg, the belief that miscarriage can be caused by lifting heavy objects, or that there are no effective treatments—can lead to women and their partners feeling at fault or managing alone. Similarly, in the health-care system and broader society, the continuing conviction that miscarriages are unavoidable and the requirement, enshrined in many national guidelines, that women must have recurrent miscarriages before they are eligible for

Although most women who have a miscarriage will go on to carry a baby to term without complications, previous miscarriage is associated with a higher risk of preterm birth, fetal growth restriction, and other obstetric complications in subsequent pregnancies. Previous miscarriage is also associated with a higher risk of long-term health problems for women, including cardiovascular disease, venous thromboembolism, and mental health complications. These associations challenge the belief that miscarriage is a single event without wider repercussions, and the Series gives a more nuanced and graduated understanding of miscarriage, which is long overdue.



Maskot/Getty Images

Published Online
April 26, 2021
https://doi.org/10.1016/
S0140-6736(21)00954-5

The era of telling women to “just try again” is over.

Expert's recommendations from The Lancet's series

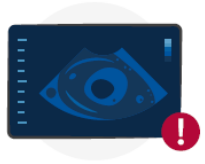
Diagnosing miscarriage

Accurate diagnosis of miscarriage relies on high-quality ultrasound scanning

The two key criteria for the diagnosis of a miscarriage are:

Absence of heart activity
When the crown rump length is ≥7mm with no fetal heart activity this is a sign of a miscarriage

Crown rump length (CRL) is the length of the embryo or fetus from the top of its head to bottom of the torso



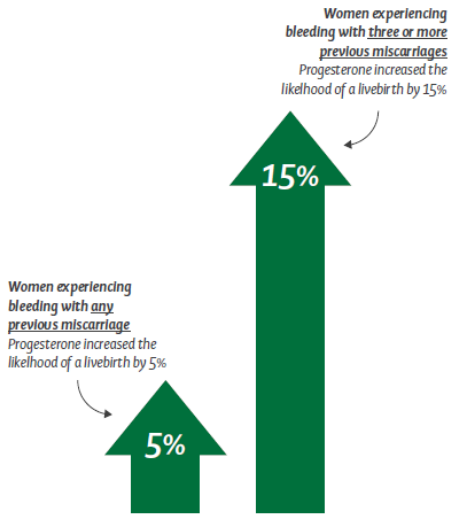
Absence of a fetal pole
When the gestational sac diameter is ≥25mm, but there is no fetal pole, this is a sign of a miscarriage

The fetal pole, or embryo, is the first direct imaging manifestation of a fetus and is seen as a thickening on the margin of the yolk sac

Preventing miscarriage

There is high-quality evidence that the use of progesterone can reduce the risk of miscarriage in women with early pregnancy bleeding and a history of miscarriage

Likelihood of livebirths with use of progesterone
versus placebo or no treatment



3

- Referral to a specialist led RPL clinic
- RPL investigations
- Screening and care for mental Health issues

2

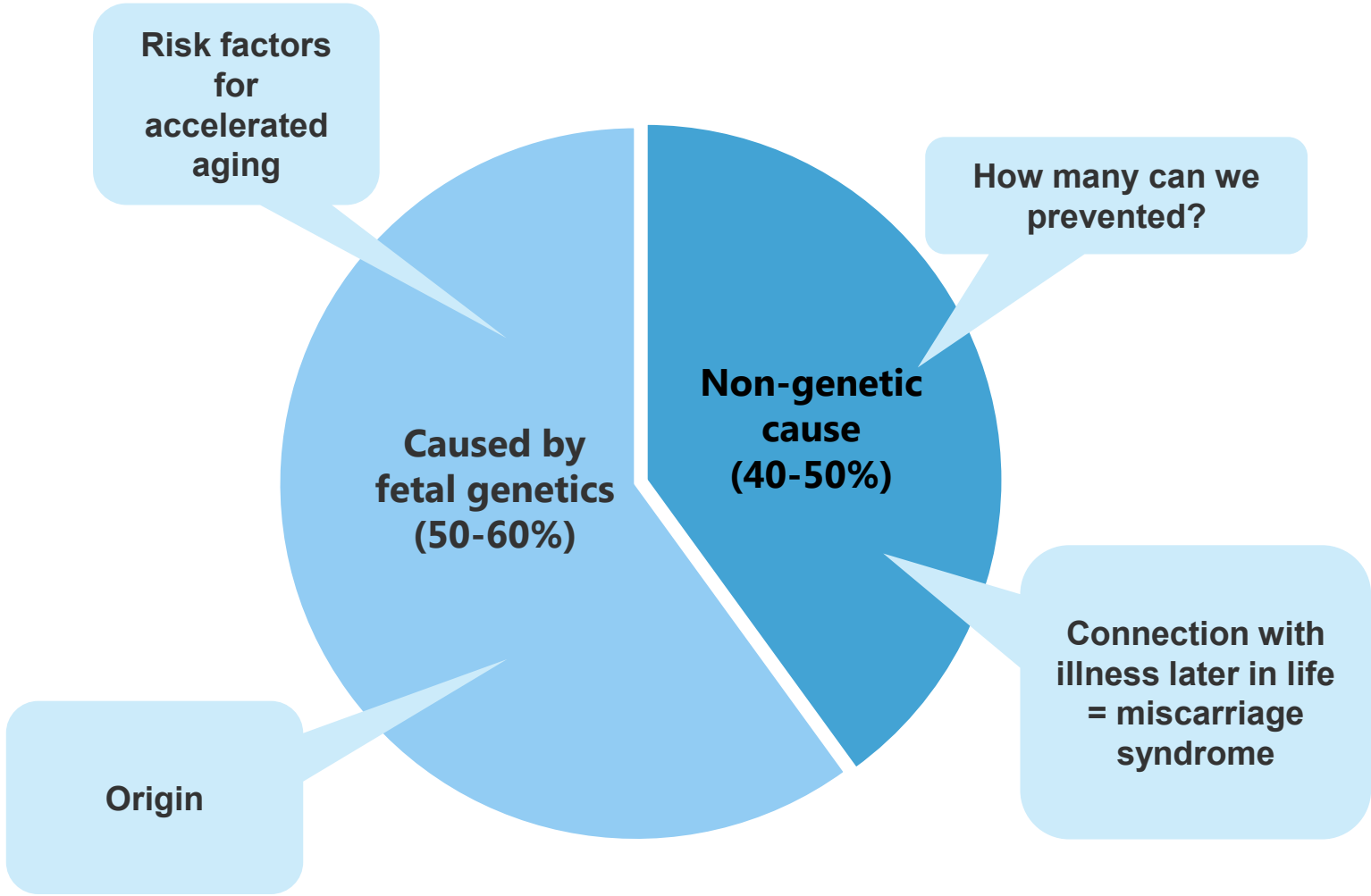
- Appointment with a miscarriage clinic
- Thyroid screening and full blood count and referral to a specialist if abnormal
- Access to reassurance scans in subsequent pregnancies

1

- Patient support groups
- Online self-help and information tools
- Referral if any chronic disease (Diabetes, hypertension ect)
- Mental health screening

Are all losses caused by fetal genetics?

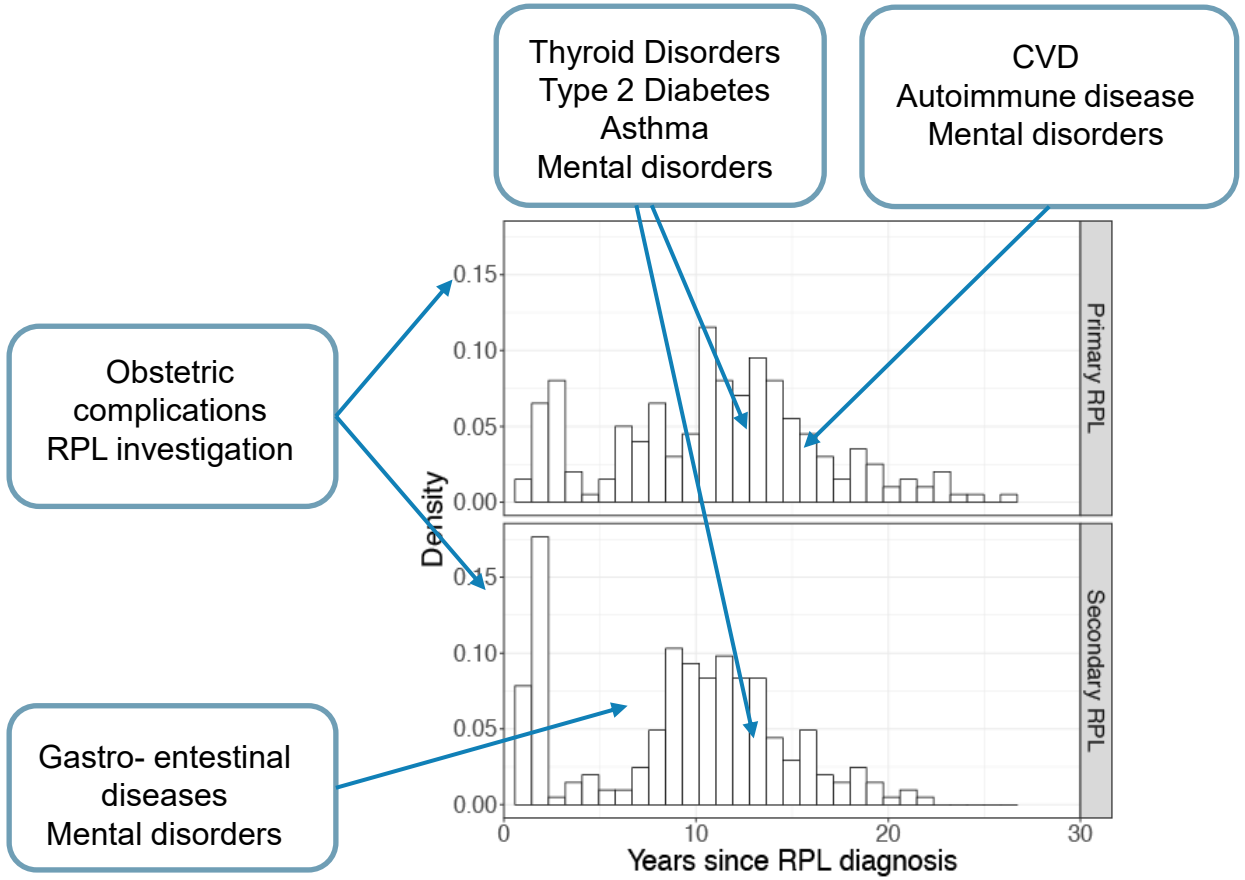
"Pregnancy loss is the body's quality control that ensures that only healthy fetuses survive"



Bortoletto P, Lucas ES, Melo P, et al. Miscarriage syndrome: linking early pregnancy loss to obstetric and age-related disorders. EBioMedicine 2022; 81: 104134.

PL associated with later disease – but what is the link?

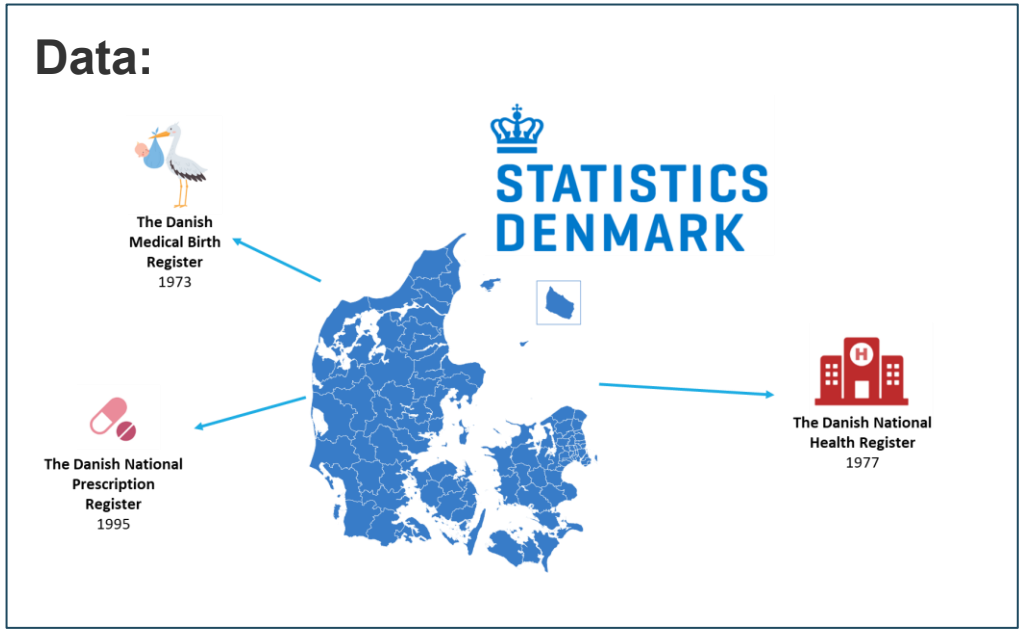
- Nation wide registry-based cohort of 1 370 896 ever pregnant women and 10 691 (0.77%) with RPL
- Women with RPL ≥ 3 PL divided into primary and secondary RPL
- Comparison group
  - Women without RPL
  - Matched 1:20 by year of birth, parity
- Followed until
  - Event occurred
  - Death
  - End of data



Westergaard D, et al. J Am Heart Assoc. 2020 Apr 21;9(8):e015069. PMID: 32299291.

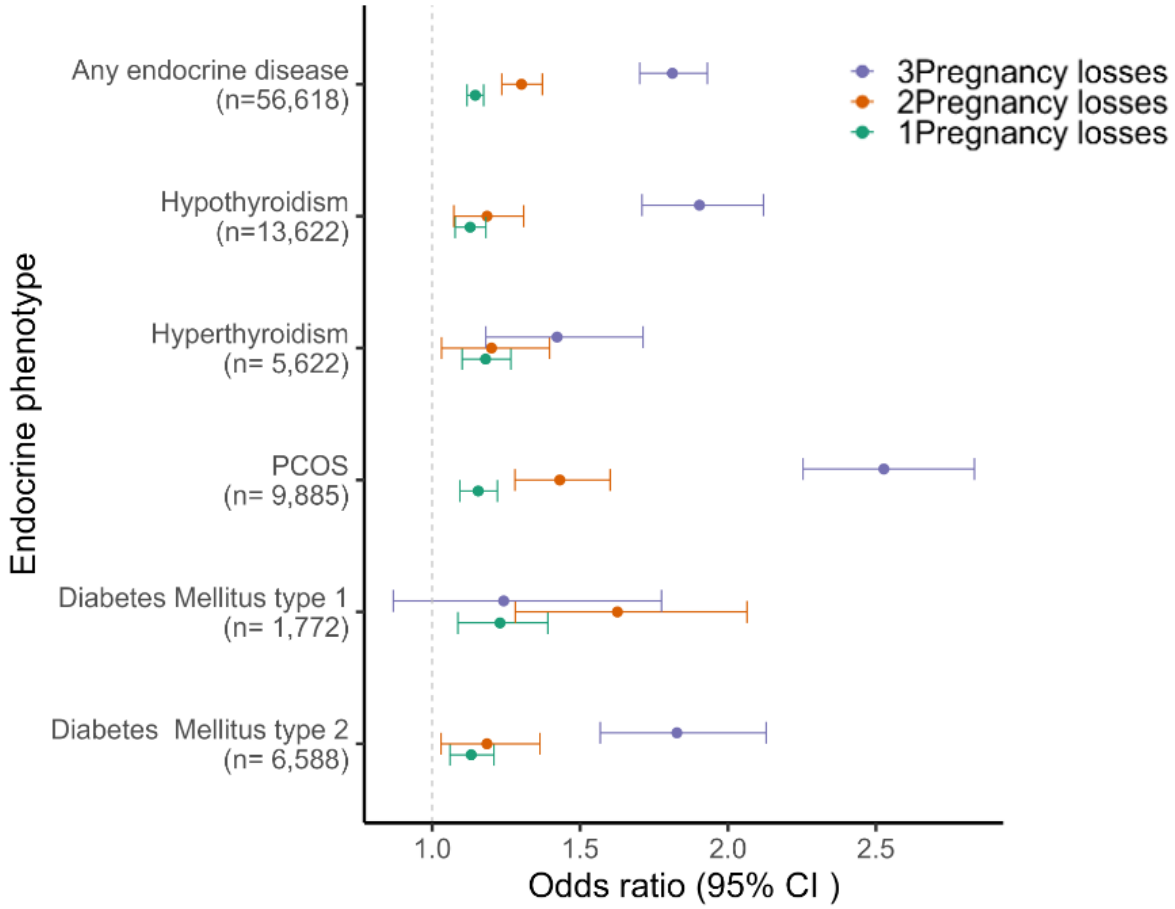
Association between PL and endocrine disease

Data:



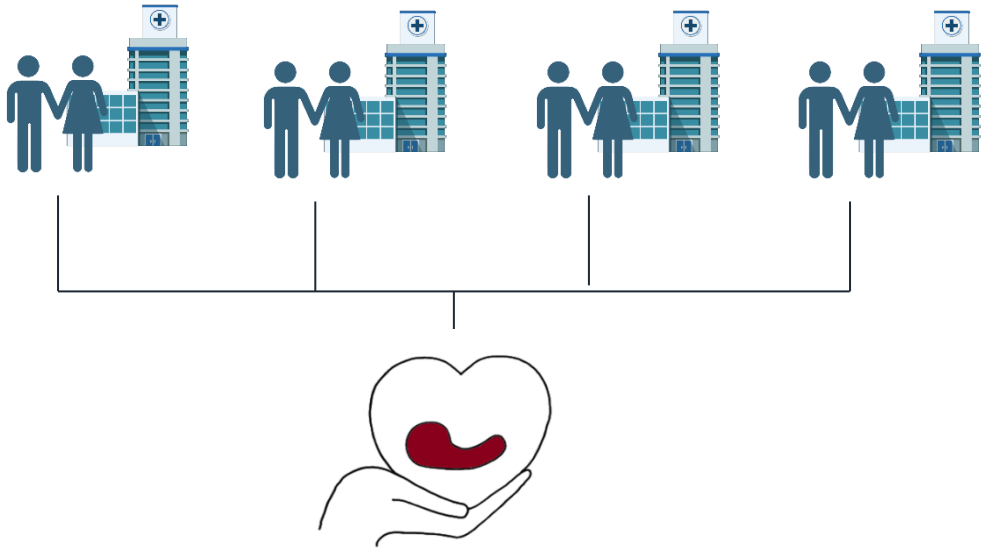
Women without endocrine disease:
309,921 (85%) women

Women with endocrine disease:
56,618 (15%) women



Unpublished results, manuscript in preparation

Copenhagen Pregnancy Loss study



75% participation rate

N= 2500 (continuing to 3000)

Inclusion criteria:

- ✓ Age above 18 years
- ✓ Referred to a gynaecological dept/clinic with a confirmed PL (missed miscarriage, ongoing spontanous, anembryonic)
- ✓ Willing to collect the pregnancy tissue
- ✓ Able to give an informed concent

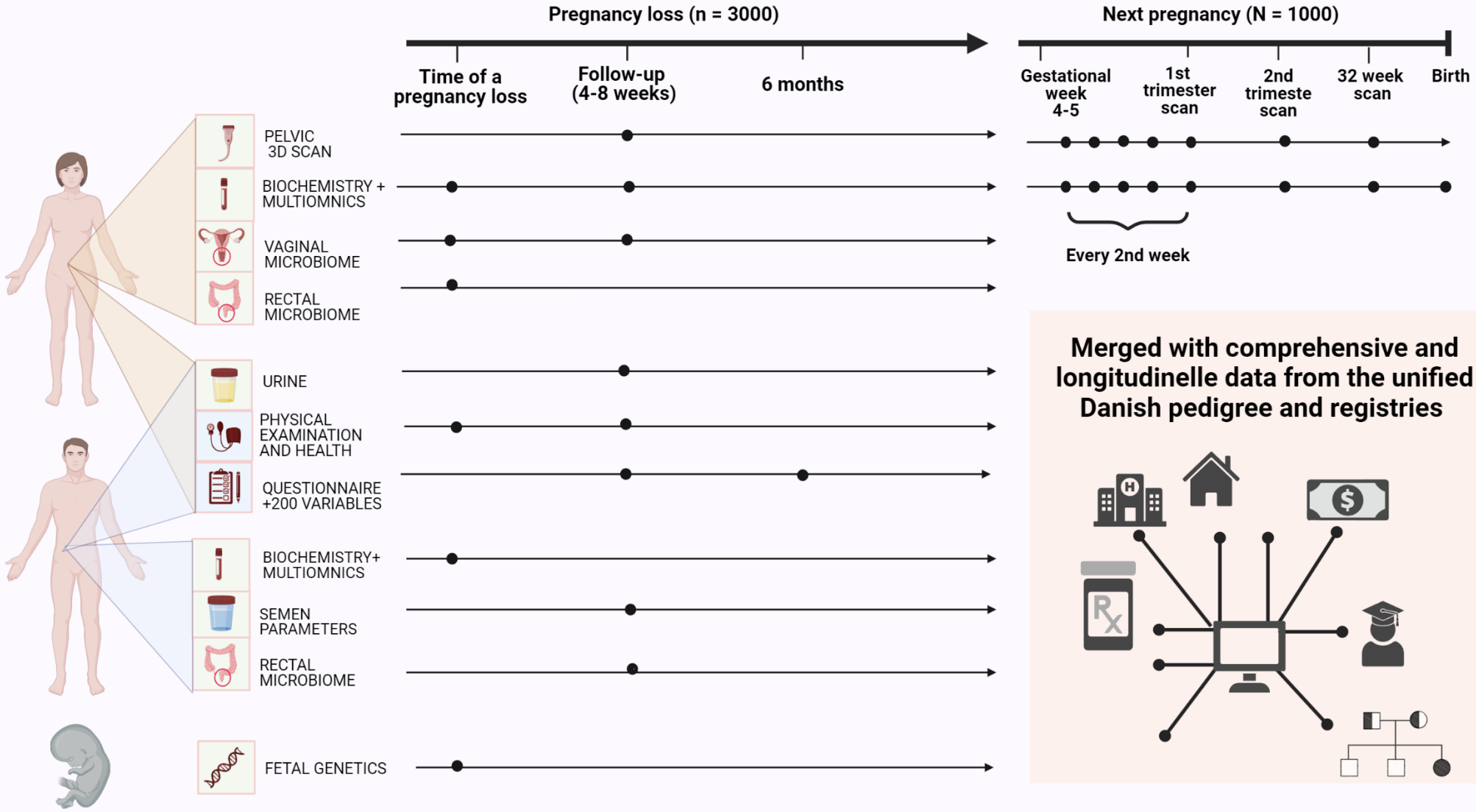
Not included:

- Ectopic, mola or pregnancy of unknown location

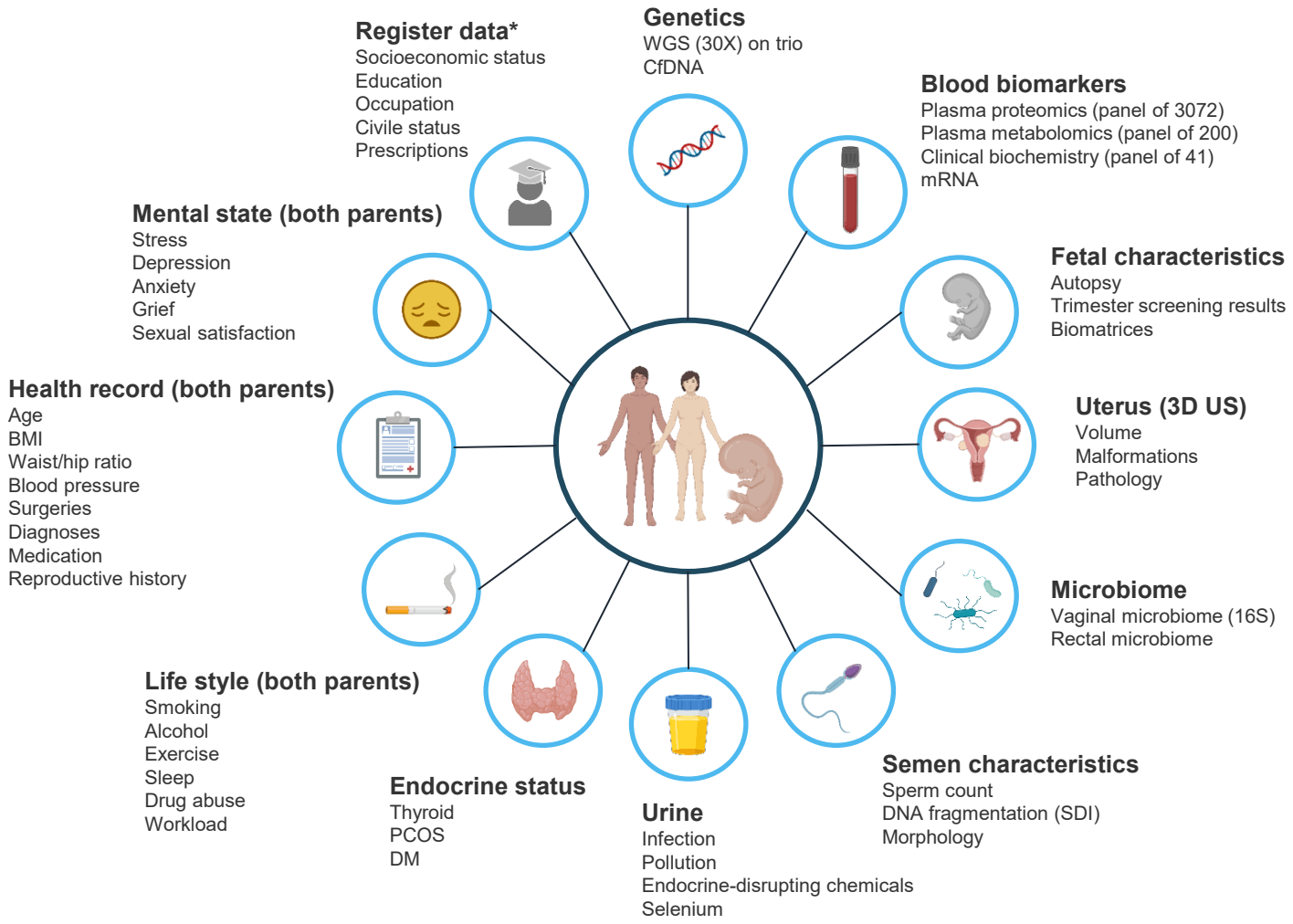
The aim of COPL:

To gain knowledge on causes for and the impact of pregnancy loss

To improve diagnosis, treatment and prevention of pregnancy loss


Pregnancy loss (n = 3000)
Time of a pregnancy loss
Follow-up (4-8 weeks)
6 months
Next pregnancy (N = 1000)
Gestational week 4-5
1st trimester scan
2nd trimeste scan
32 week scan
Birth
Every 2nd week
PELVIC 3D SCAN
BIOCHEMISTRY + MULTIOMNICS
VAGINAL MICROBIOME
RECTAL MICROBIOME
URINE
PHYSICAL EXAMINATION AND HEALTH
QUESTIONNAIRE +200 VARIABLES
BIOCHEMISTRY+ MULTIOMNICS
SEMEN PARAMETERS
RECTAL MICROBIOME
FETAL GENETICS
Merged with comprehensive and longitudinelle data from the unified Danish pedigree and registries

Data modalities in COPL



* From 1977 until now

SUB-STUDY COPL-EM

- 20% of COPL participants are ART treated
- Collection of arrested embryos and GV oocytes in subsequent cycles
- 234 embryos/ GVs collected from 44 cycles
- Single cell sequencing to explore genetic status and similarities with previous loss

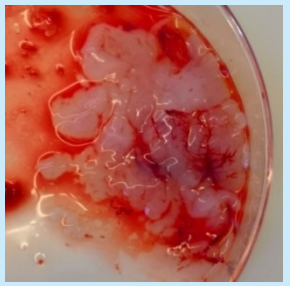
Deep phenotyping and genotyping of fetuses

Unknown tissue (28%)

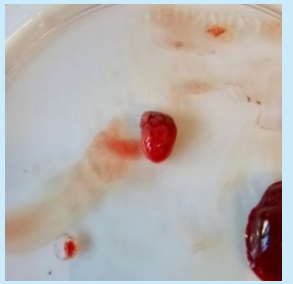
Fetus (20%)

Chorionic villi (52 %)

brain

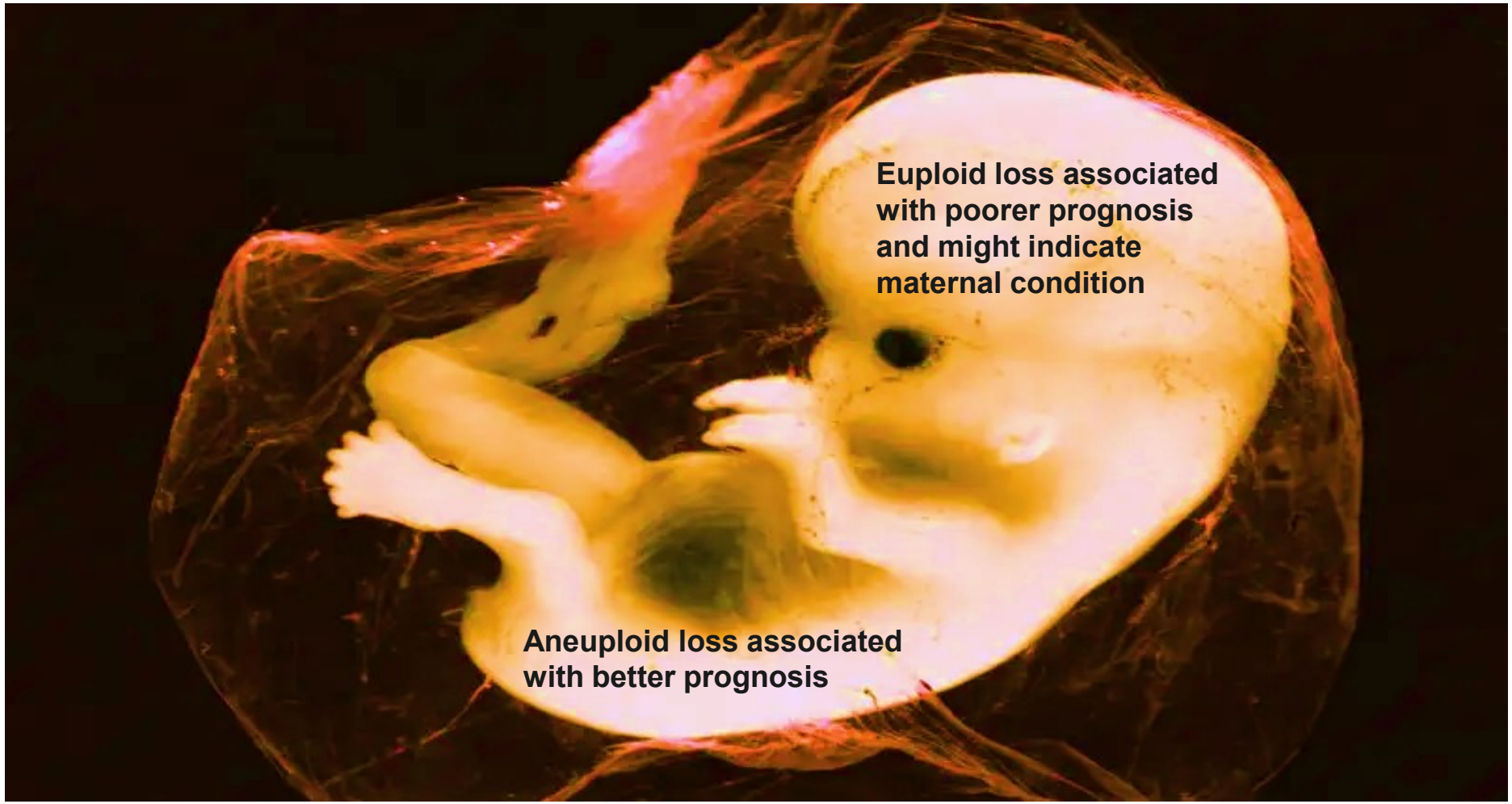


heart



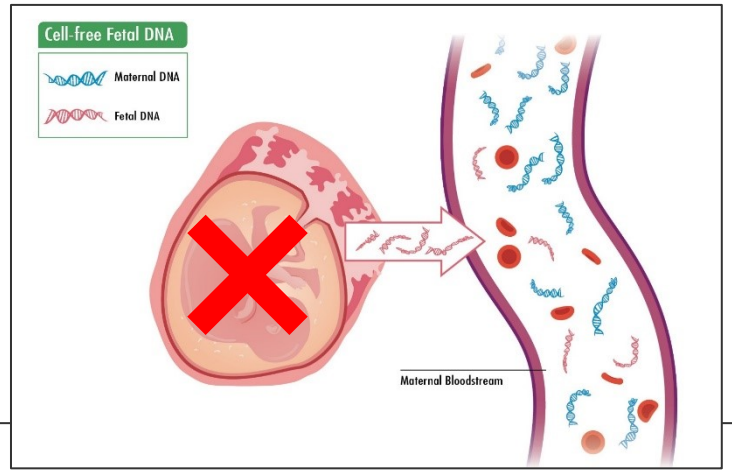
liver

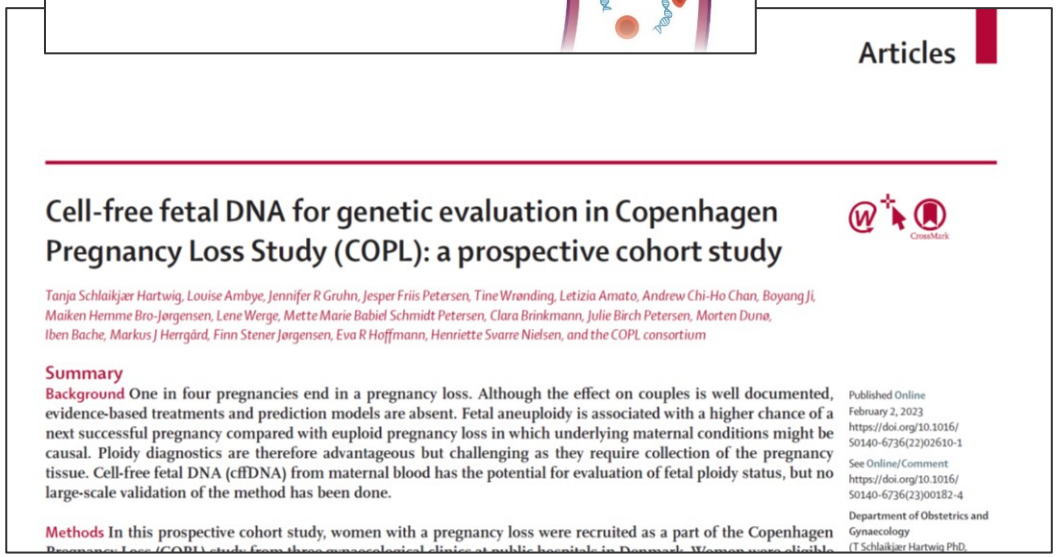
- Ectoderm (brain)
- Mesoderm (heart)
- Endoderm (liver)



Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages.Fertil Steril 2000; 73: 300–04.

CffDNA-based fetal assessment in PL





Articles

Cell-free fetal DNA for genetic evaluation in Copenhagen Pregnancy Loss Study (COPL): a prospective cohort study

Tanja Schlaikjær Hartwig, Louise Ambye, Jennifer R Gruhn, Jesper Friis Petersen, Tine Wrønding, Letizia Amato, Andrew Chi-Ho Chan, Boyang Ji, Maiken Hemme Bro-Jørgensen, Lene Werge, Mette Marie Babiel Schmidt Petersen, Clara Brinkmann, Julie Birch Petersen, Morten Duno, Iben Bache, Markus J Herrgård, Finn Stener Jørgensen, Eva R Hoffmann, Henriette Svarre Nielsen, and the COPL consortium

Summary

Background One in four pregnancies end in a pregnancy loss. Although the effect on couples is well documented, evidence-based treatments and prediction models are absent. Fetal aneuploidy is associated with a higher chance of a next successful pregnancy compared with euploid pregnancy loss in which underlying maternal conditions might be causal. Ploidy diagnostics are therefore advantageous but challenging as they require collection of the pregnancy tissue. Cell-free fetal DNA (cffDNA) from maternal blood has the potential for evaluation of fetal ploidy status, but no large-scale validation of the method has been done.

Methods In this prospective cohort study, women with a pregnancy loss were recruited as a part of the Copenhagen

Published Online February 2, 2023 https://doi.org/10.1016/S0140-6736(22)02610-1

See Online/Comment https://doi.org/10.1016/S0140-6736(23)00182-4

Department of Obstetrics and Gynaecology (T Schlaikjær Hartwig PhD,

Conclusions

- CffDNA based testing using an adjusted bioinformatic pipeline is performing well in PL
- 11% No-call rate in PL between GA 5 and 22 weeks
- Gives clarification for couples
- Enables early identification of women losing euploid pregnancies -> focus on euploid losses (clinical and research
- Different from NIPT in ongoing pregnancies as it is not having a direct therapeutic consequence

Schlaikjær Hartwig T., Lancet. 2023 Mar 4;401(10378):762-771. doi: 10.1016/S0140-6736(22)02610-1. Epub 2023 Feb 2. PMID: 36739882.

Mental impact on both men and women

Human Reproduction, Vol.34, No.2 pp. 291–296, 2019
Advanced Access publication on December 15, 2018 doi:10.1093/humrep/dey362

human reproduction ORIGINAL ARTICLE *Psychology and counselling*

Recurrent pregnancy loss: couples' perspectives on their need for treatment, support and follow up

E. Koert[1,*], G.M.H. Malling[2], R. Sylvest[3], M.C. Krog[1], A.M. Kolte[1], L. Schmidt[2], and H.S. Nielsen[1]

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OXFORD hro

Human Reproduction Open, 2023, **2023(3)**, hoad032
https://doi.org/10.1093/hropen/hoad032
Advance Access Publication Date: August 1, 2023
Original article

'You're never pregnant in the same way again': prior early pregnancy loss influences need for health care and support in subsequent pregnancy

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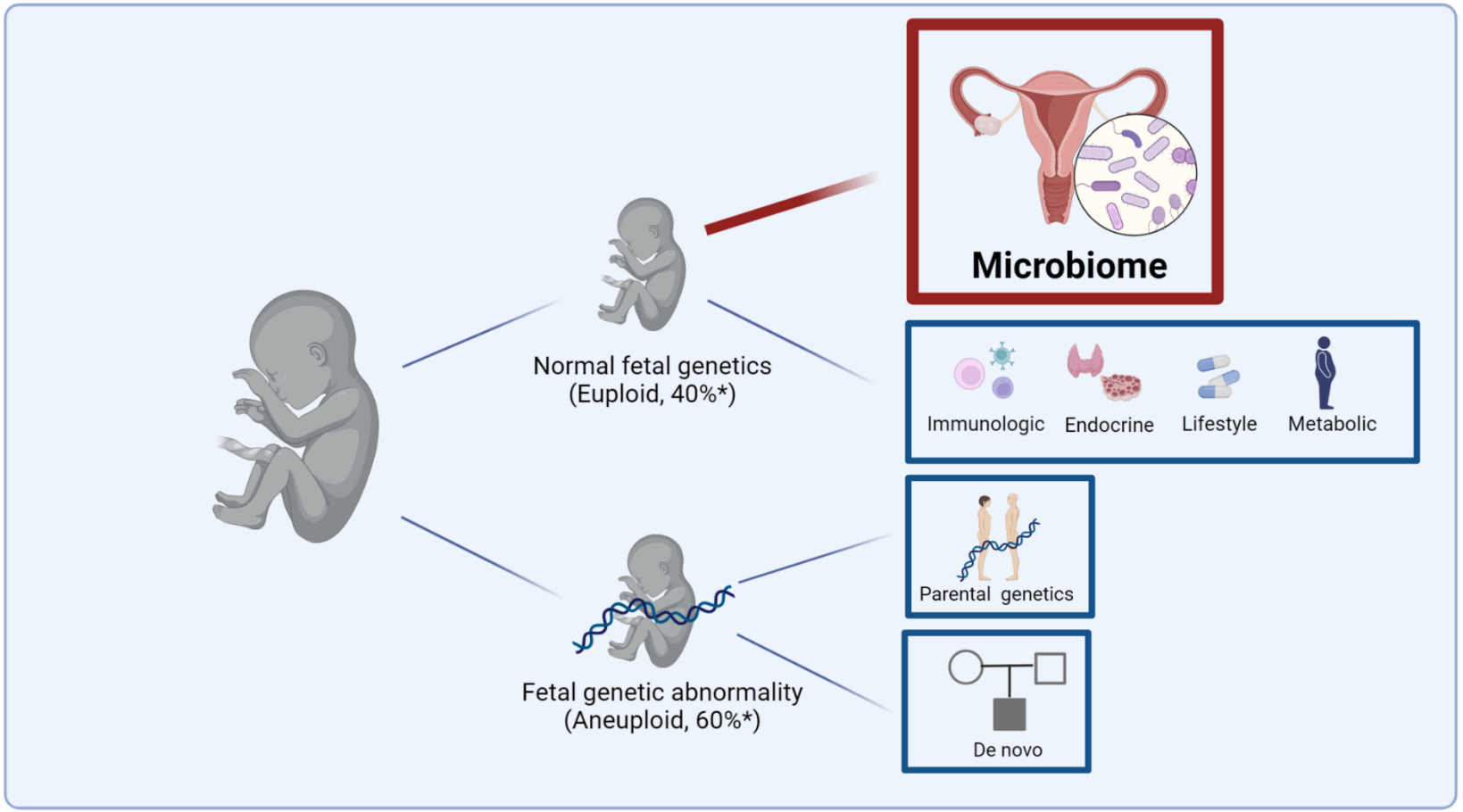
- Cumulative effect of RPL with an increase in pressure and exhaustion in men and women
- Important to include partner in consultations and treatment
- Men felt pressured to remain positive and support their partners despite their own feelings of loss
- Both men and women describe worry and anxiety in the next pregnancy

Ref: Koert et al., Human Reproduction, Vol.34, No.2 pp. 291–296, 2019 and Human Reproduction Open, 2023, 2023(3), hoad032

Is the vaginal microbiome associated with PL?



Vaginal microbiota transplantation (VMT)

28. jun 2023, kl. 07:36

Artiklen er mere end 30 dage gammel

Pernille mistede tre børn – behandlingen er banebrydende

Hun følte skam og skyld hver gang, hun fødte et barn, der var dødt eller døde kort efter fødslen.

Pernille Burgdorf har tre gange mistet et barn. Nu kan forskere forklare hvorfor.

Articles

Antibiotic-free vaginal microbiota transplant with donor engraftment, dysbiosis resolution and live birth after recurrent pregnancy loss: a proof of concept case study

Tine Wrønding,[a] Kilian Vomstein,[a] Elleke F. Bosma,[b] Brynjulf Mortensen,[b] Henrik Westh,[c,k] Julie Elm Heintz,[c] Sarah Mollerup,[c] Andreas Munk Petersen,[c,d,k] Laura M. Ensign,[e,f,g,h,i,j] Kevin DeLong,[b] Johan E. T. van Hylckama Vlieg,[b] Anne Bloch Thomsen,[b] and Henriette Svarre Nielsen[a,k,*]

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eClinicalMedicine 2023;61: 102070

Published Online 26 June 2023

https://doi.org/10.1016/j.eclinm.2023.102070

Summary

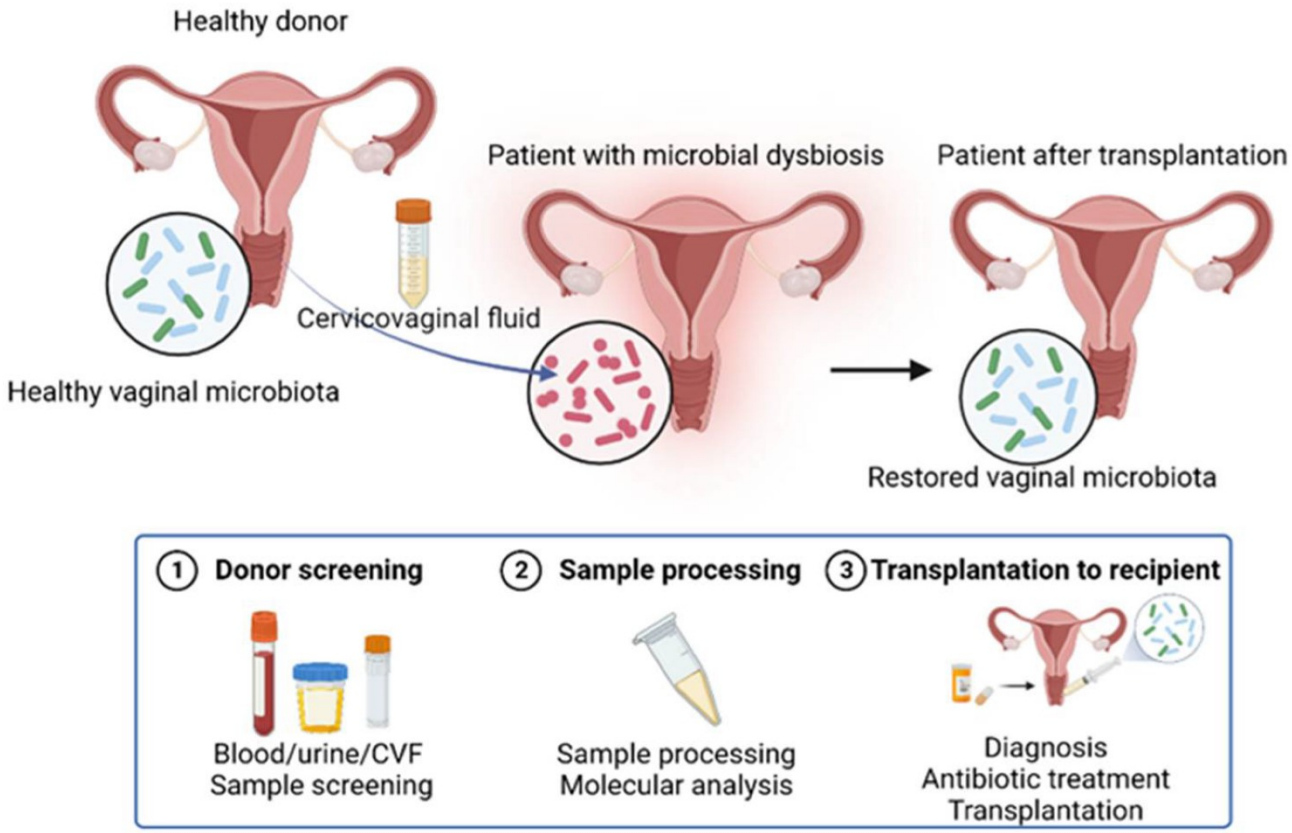
Background Vaginal dysbiosis covers imbalances in the vaginal microbiota, defined by altered composition of bacteria, viruses, and fungi and is associated with euploid pregnancy losses, premature birth, infertility, or bacterial vaginosis. A large proportion of women who have vaginal dysbiosis do not experience any symptoms. Antibiotics are the traditional treatment, recently combined with local probiotics in some cases. Vaginal Microbiota Transplantation (VMT) with eubiotic vaginal bacterial microbiota after antibiotic eradication of pathogens has successfully been performed in a case study with five patients, but no VMT has been performed without the use of antibiotics.

Methods This is a proof of concept case study. The patient was found to have vaginal dysbiosis at the RPL clinic at Copenhagen University Hospital Hvidovre, Denmark on the 23rd of June 2021. She was offered and accepted to receive experimental treatment in the form of a VMT as a compassionate use case. VMT is the transfer of cervico-vaginal secretions (CVS) from a healthy donor with a *Lactobacillus*-dominant vaginal microbiome to a recipient with a dysbiotic vaginal microbiome. CVS is a mixture of e.g., mucus, bacteria, metabolites present in the vaginal canal.

Wrønding T, et al. EClinicalMedicine. 2023 Jun 26;61:102070. doi: 10.1016/j.eclinm.2023.102070. PMID: 37528843

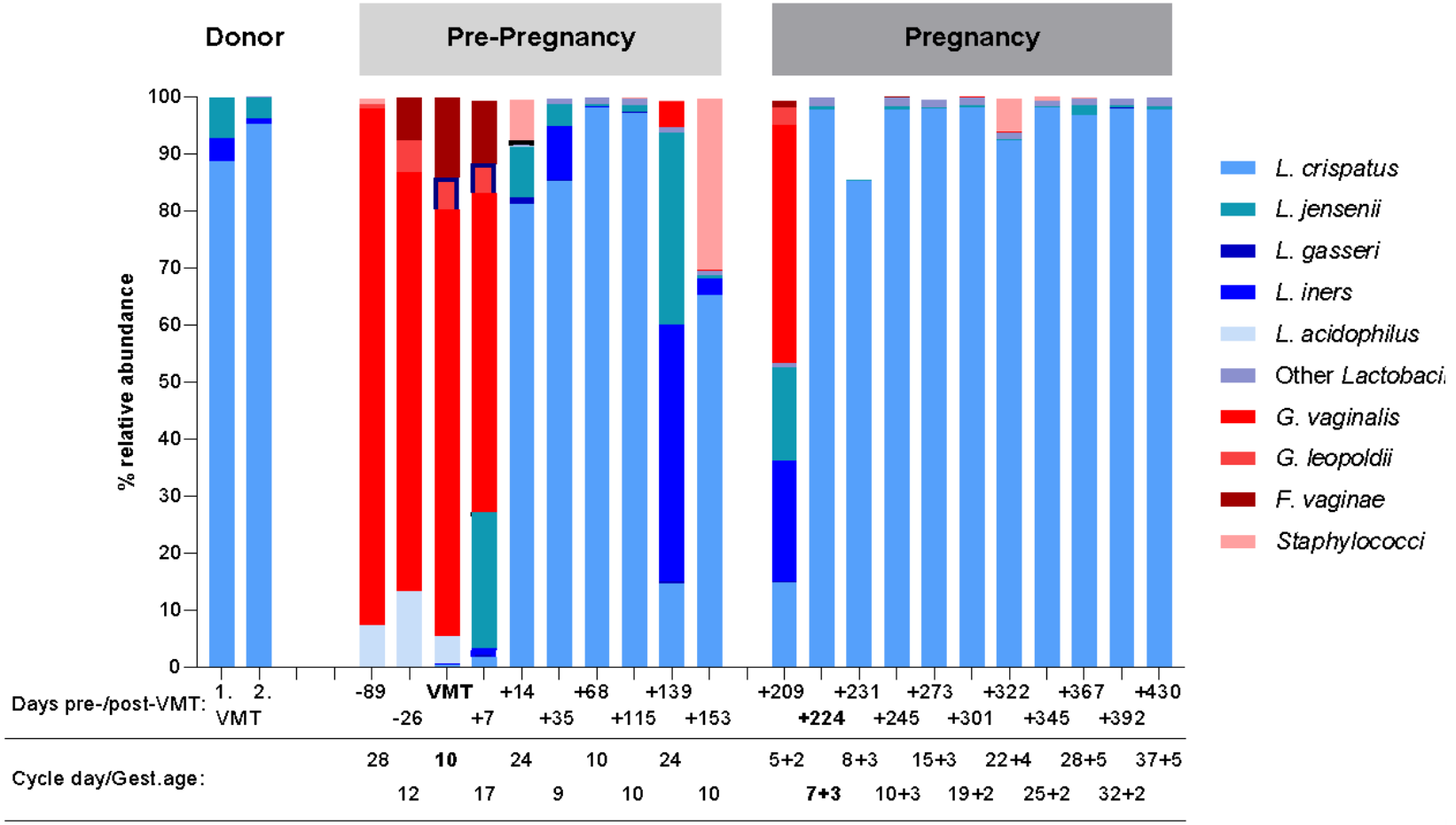
What is VMT?

- Eubiotic Cervico Vaginal fluid is extracted as the entire microbiome
- Contains bacteriophages, cytokines and metabolites.
- Processed and screened for infections
- Transplanted to dysbiotic recipient in the vagina



Xiumiao Zhao, Potential Role of Vaginal Microbiota in Ovarian Cancer Carcinogenesis, Progression and Treatment, Pharmaceutics 2023.

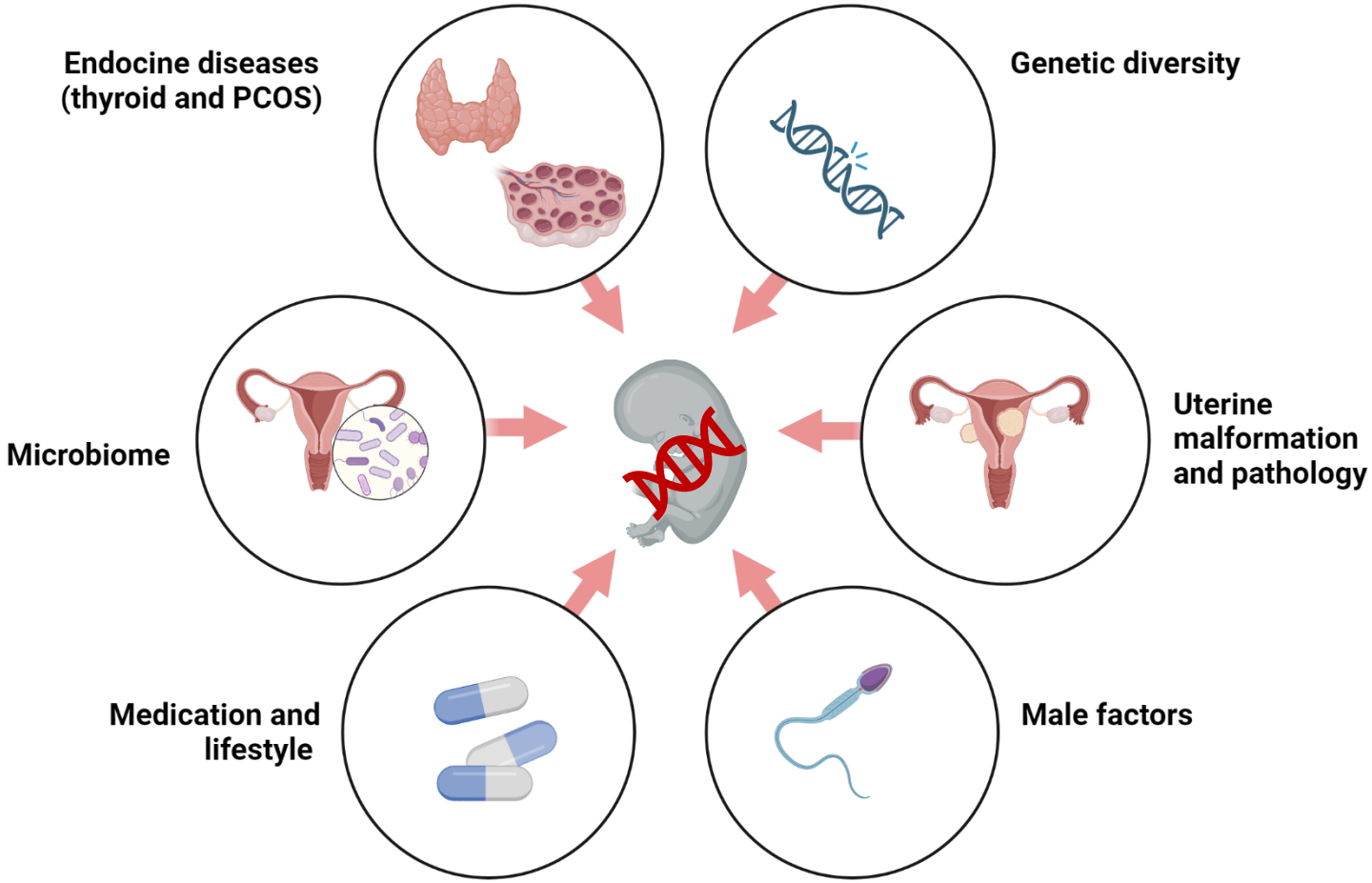
Sequencing results of patient 0



Remember the partner

Where to go from now

?



Summary – yes, we can do more

- Pregnancy loss is complex and can not always be explained by fetal genetics
- Many misconceptions about PL exist – we need to break the taboo and keep exploring!
- Couples desire acknowledgement, earlier assessment, and inclusion of partners
- Important to differentiate between genetic and non-genetic causes to get the full picture and identify “miscarriage syndrome”
- Fetal genetic status in PL can be assessed down to GA 5 by cffDNA testing
- Be aware of endocrine disease as it is highly associated with PL
- Indications that dysbiosis is associated with PL
- Progesterone in next pregnancy in case of bleeding
- Couples experiencing pregnancy loss are motivated and committed to research – even in the acute phase

Thank you!

THANK YOU!

Clinical Team COPL (Copenhagen Pregnancy Loss Cohort)

Henriette Svarre Nielsen, David Westergaard, Sofie Bliddal, Signe B Blom, Ida Behrendt-Møller, Mette MBS Petersen, Ida M Søndergaard, Sarah Dandanell, Clara Brinkmann, Yasmin IE Sammaa-Aru, Emma S Juul, Emilie Dahlberg, Emilie P Madsen, Jesper Friis Petersen, Tine Wrønding, Nana, Aiza, Linea, Nina lC Freiesleben, Anne Zedeler , Emily Koert

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deCODE Genetics

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BII

Markus Herrgård, Trine Bartholdy, Louise Clemmensen, Shivani Joshi, Boyang Ji, Jonathan Robinson, Heike Ross, Maria Henriques De Jesus