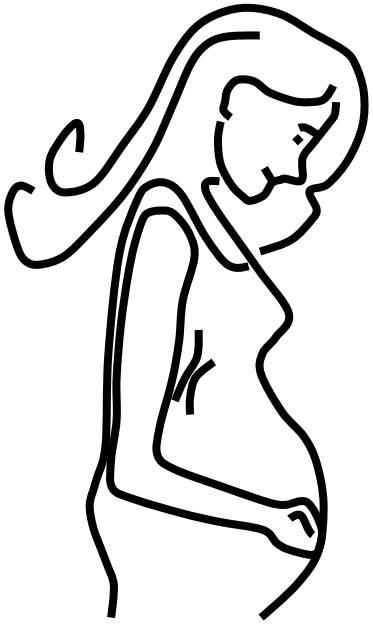
Competence Centre on
Health Technologies

EMBRYO AND FETAL GENOMIC STUDIES



ANDRES SALUMETS

SEARMET
ESTONIAN UNIVERSITY OF LIFE SCIENCES
3.12.2018

Andres Salumets – Short CV

- **1993** - Graduated from **University of Tartu, Estonia** as biochemist
- **2003** - Doctoral degree from **University of Helsinki, Finland**
- **2010** - ... Professor of reproductive medicine at **Women's Clinic and Institute of Bio- and Translational Medicine, University of Tartu**
- **2016** - **Visiting professor at University of Helsinki**
- **2010** - ... Head of **Competence Centre on Health Technologies (CCHT)**
- **2010** - ... President of **Baltic Fertility Society**
- **2013** - **2016** Member of the Executive Committee of **ESHRE (European Society of Human Reproduction and Embryology)**
- **Consultant to IVF clinics**

BALTIC FERTILITY SOCIETY

Competence Centre on Health Technologies

1792-1876

Fertilization and embryo development

Karl Ernst von Baer, Estonian-German embryologist

A. Discovered mammalian **oocyte**

B. Described preimplantation embryo development until **blastocyst**

C. Coined the term **spermatozoa**

3. Zygote has two pronuclei originated from oocyte and sperm cell

3. zygote

4. Zygote starts to devide, three cell divisions: 2, 4, 8 cells.

4.

5. Blastomeres start to adhere and form a morula

5.

Blastocyst

C. *„Spermatozoa“*

Fallopian tube

Uterus

Ovary

1.

2.

6.

B.

1. Ovulation happens in the middle of the ovarian cycle and oocyte moves to Fallopian tube

2. Fertilization takes place in Fallopian tube usually 24h after ovulation

6. By day 5 or 6 blastocyst arrives to the uterus where it implants and continiues development

Oocyte

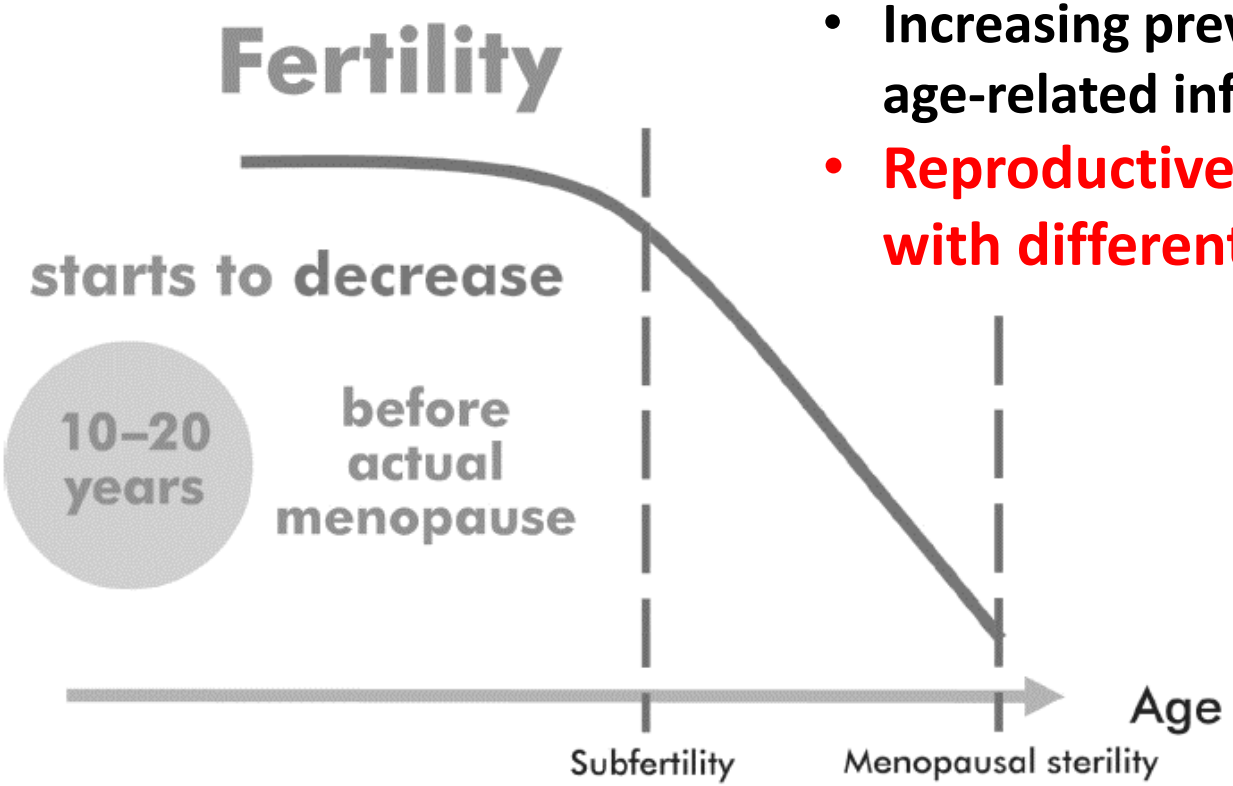
A.

***AGENDA*:**

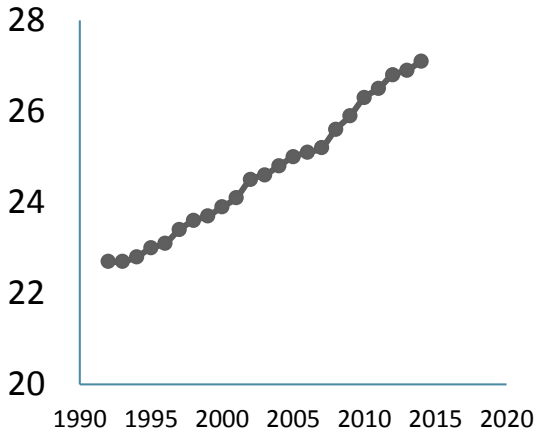
1. **Female reproductive aging** – applications in family planning
2. **Single sperm cell genomic testing** – applications in oncology & IVF
3. **Endometrial receptivity studies** – applications in infertility treatment
4. **Embryo genomic studies and mosaicism**
5. **Non-invasive prenatal testing** – applied for all pregnant women

Age and female fertility

- Aging – declined oocyte quality and quantity – oocyte aneuploidy
- Delayed motherhood – increased risk for infertility
- Increasing prevalence of female age-related infertility
- Reproductive aging is happening with different speed!



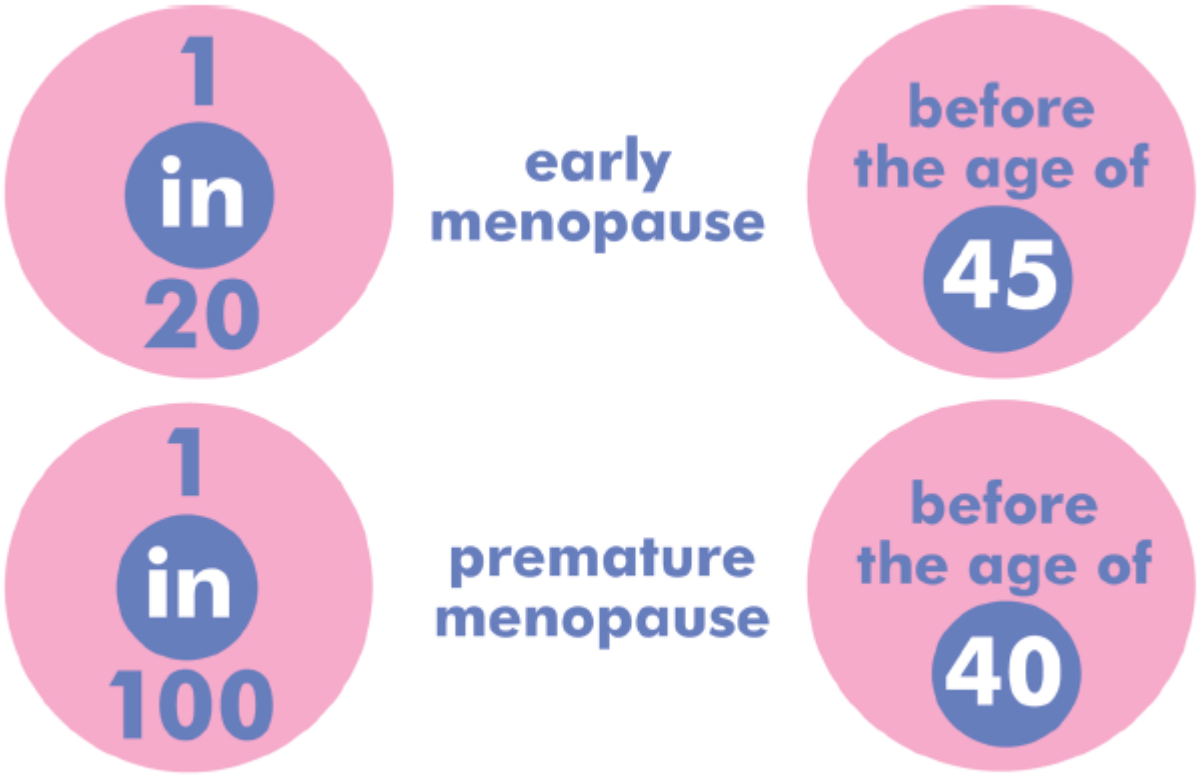
50 y



Women give birth to their first child later…

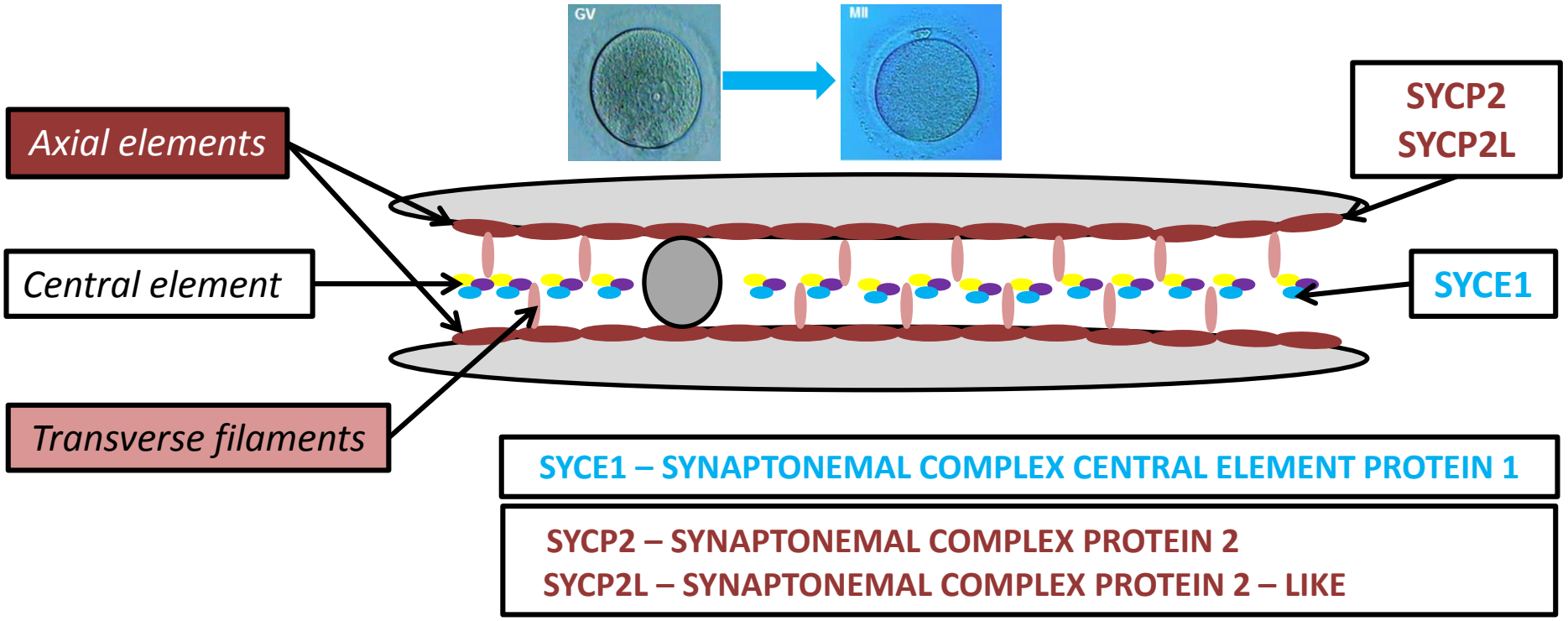
Age and female fertility

EARLY MENOPAUSE - EM



PREMATURE OVARIAN FAILURE - POF

Proteins of synaptonemal complex (SC) and menopause, ovarian failure and fertility



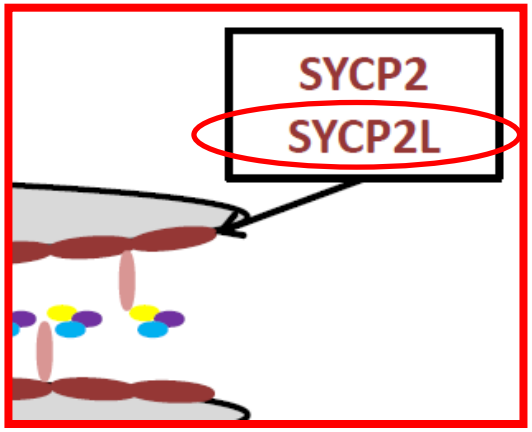
Synaptonemal complex is required:

- completion of meiosis
- homologous recombination
- correct segregation of chromosomes to avoid aneuploidies

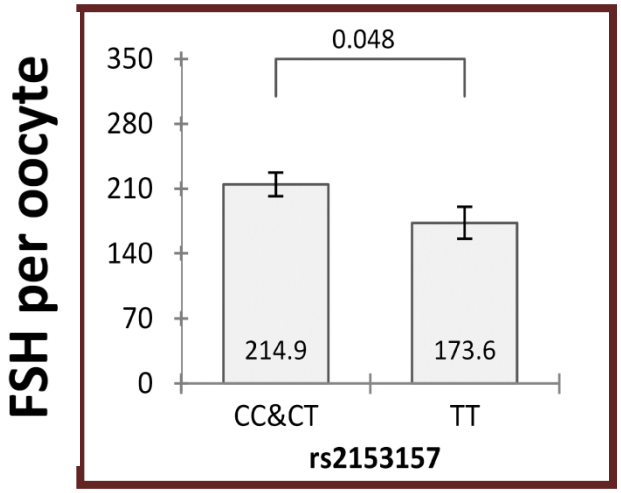
SC genes and menopause timing & IVF outcome

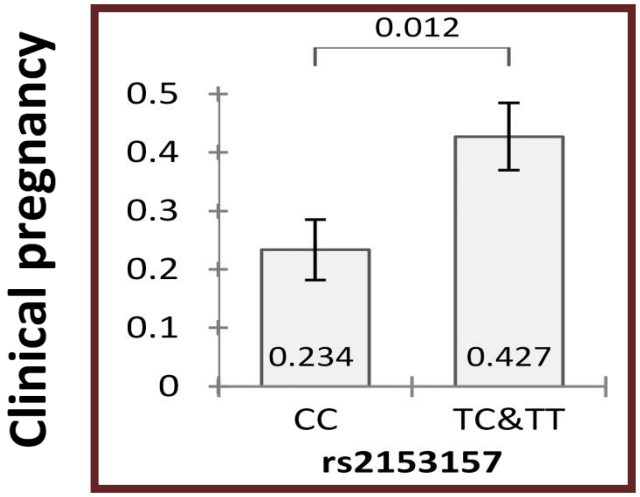
- *SYCP2L* gene (rs2153157), intron
- Minor T-allele increases average menopausal age by 2 months

Stolk ... Laisk ... et al, Nature Genetics, 2012



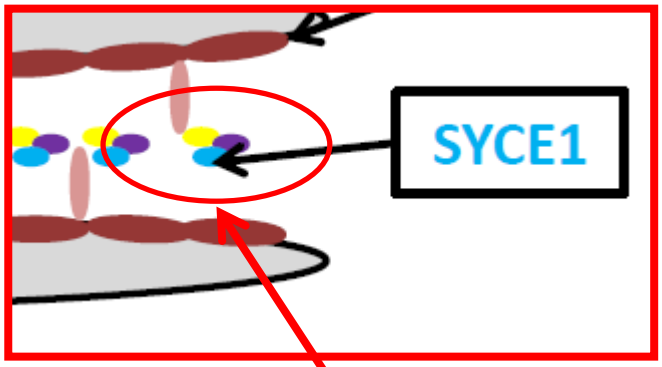
- *SYCP2L* gene (rs2153157) minor T-allele improves:
  - Ovarian stimulation outcome
  - Pregnancy outcome



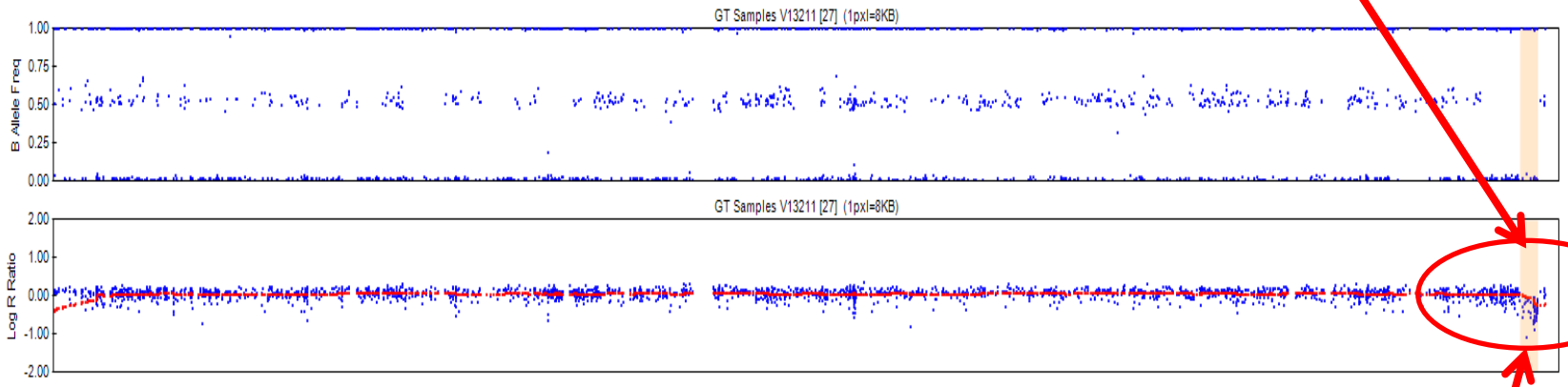


Laisk et al, 2015, RBM Online

SC genes and premature ovarian failure - POF



- Cessation of menstrual cycles <40y
- 1% of women
- 378 women with POF from Estonian Genome Centre



Tšuiko et al, 2016, Human Reproduction

2 women with DELETION OF 1 COPY OF **SYCE1** GENE AT 10q26.3

Female KO mice are infertile with meiosis arrested at prophase

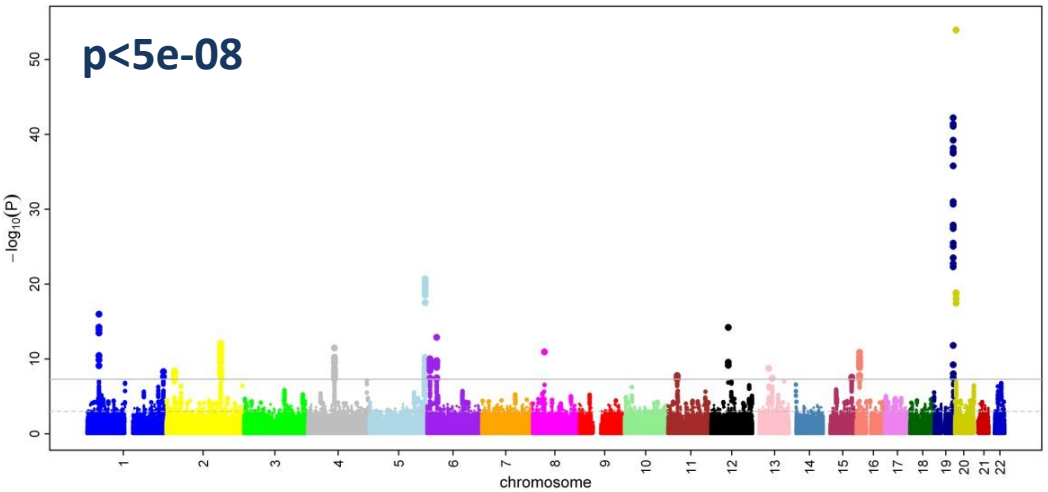
SYCE1 – SYNAPTONEMAL COMPLEX CENTRAL ELEMENT PROTEIN 1

Genome-wide association studies (GWAS) on menopausal age

- Menopause timing has a substantial impact on infertility
- GWAS of consortium of biobanks
- Including gene donors from **Estonian Genome Center**
- 44 gene regions related to menopausal age
- Associations with delayed puberty and DNA damage response (DDR) genes (including BRCA1 gene)

OVARIAN AGING = SOMATIC AGING

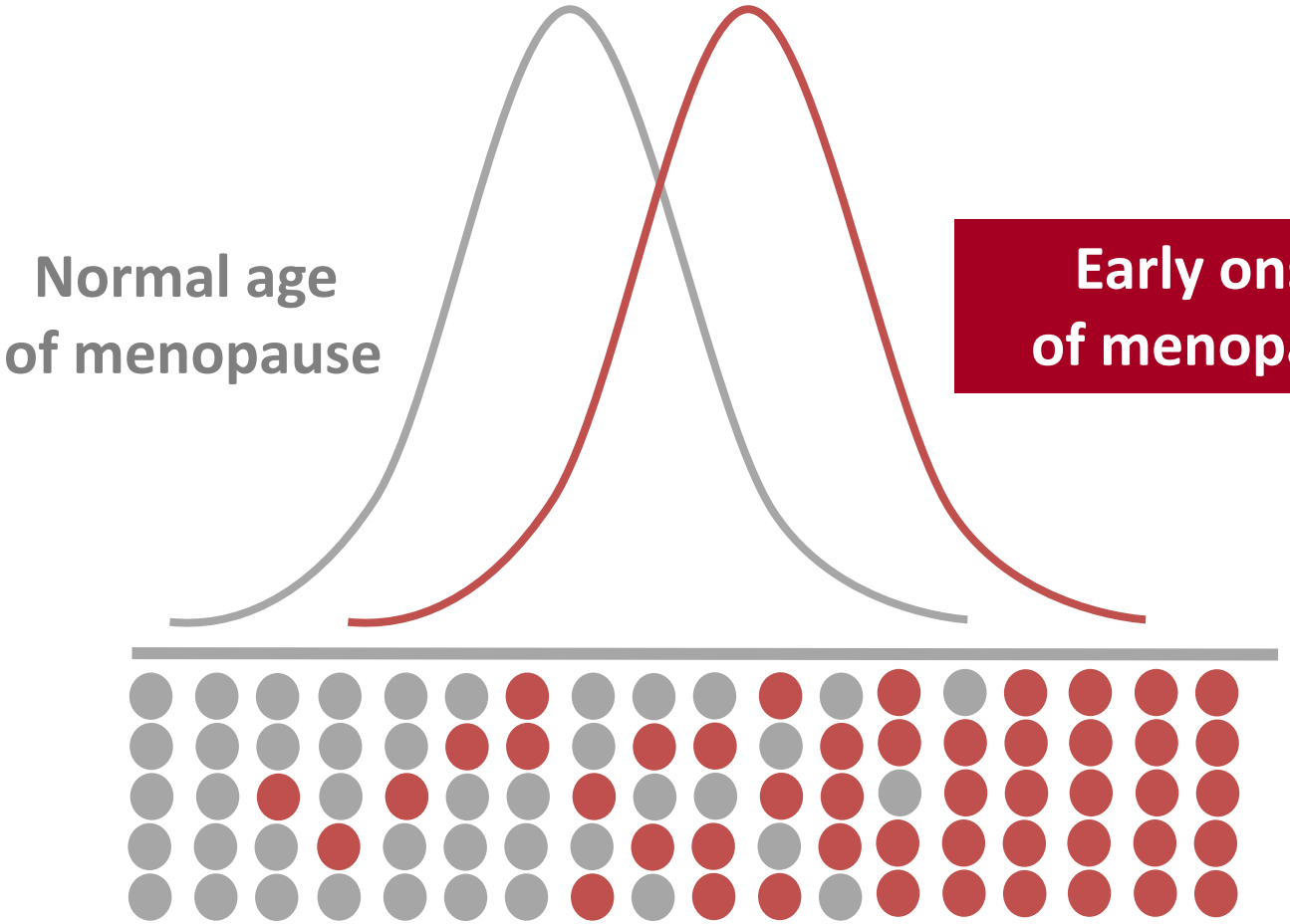
Day et al, Nature Genetics, 2015



Can we predict the speed of female reproductive aging?

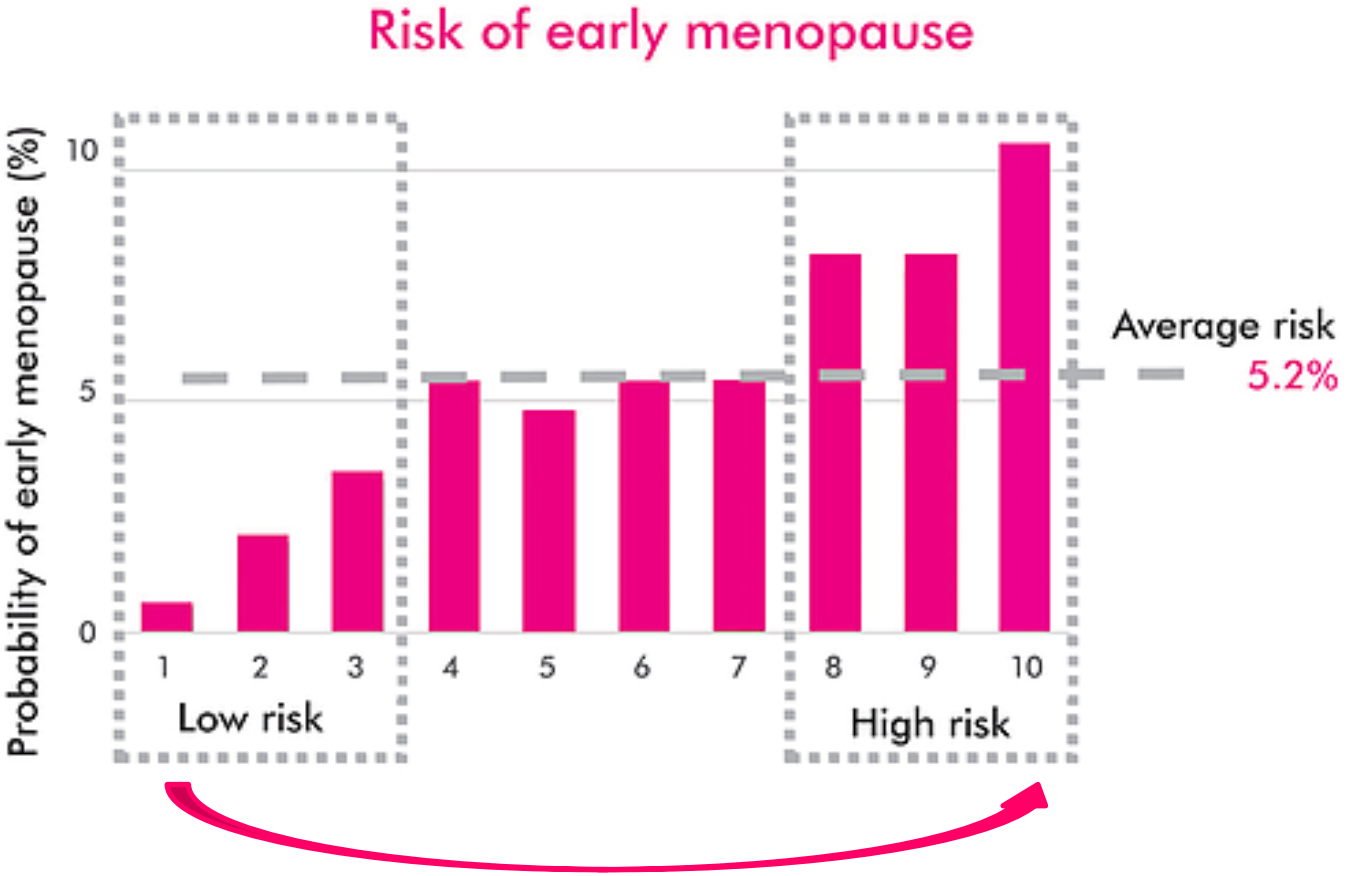
- **SNPs (single nucleotide polymorphisms)** in genome – may influence protein structure & level
- 750 SNPs – **'FERTIFY algorithm'** – prediction for early menopause and age-related infertility
- **Accumulation of SNPs with negative effects** – early onset of menopause
- **Polygenic risk scores** for early menopause
- **Verified in Estonian and UK biobanks**

Polygenic risk scores for early menopause



Negative genetic variants influencing the menopausal age

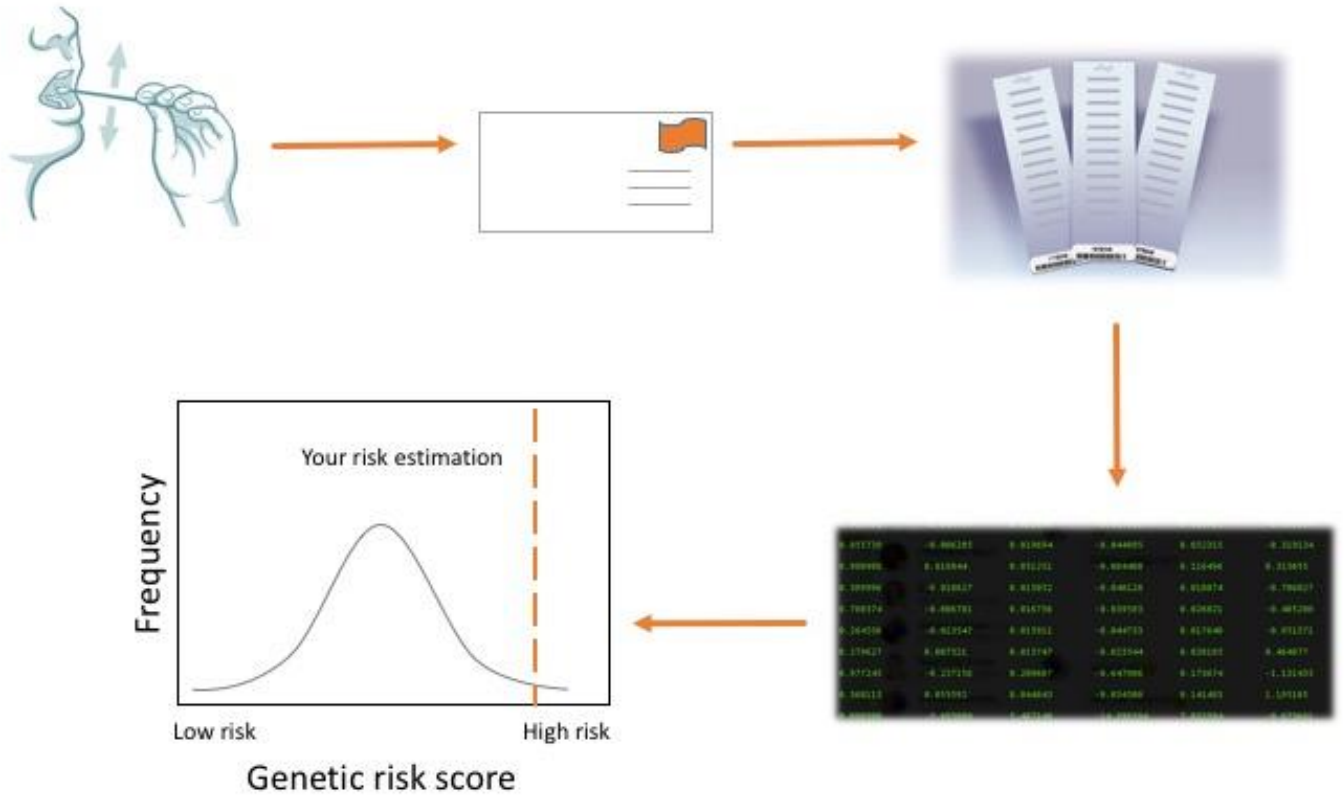
Polygenic risk scores for early menopause



10 X DIFFERENCE

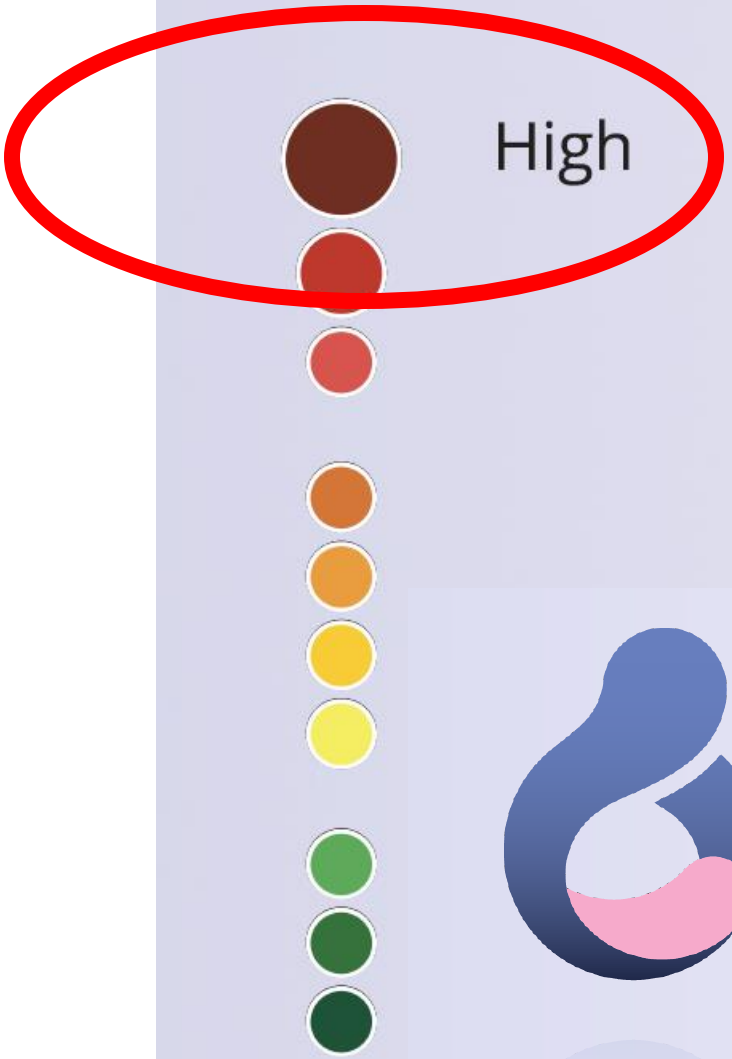
Laisk *et al.*, manuscript

How FERTIFY works?



- **Predicting fertility potential**
- **Combination with ultrasound & hormones**
- **Family planning recommendations (including oocyte freezing & IVF)**

RISK ASSESSMENT



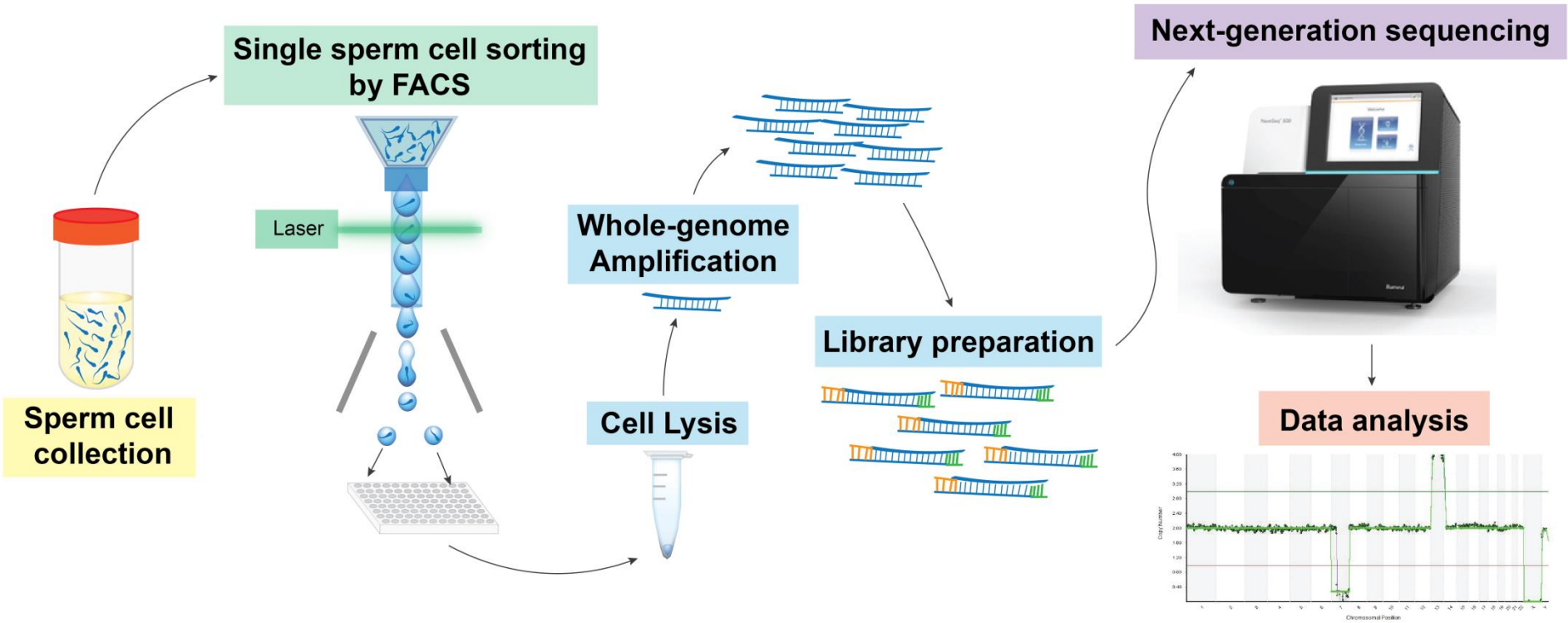
You have a 10.1% risk for early menopause and age-related infertility.

This means you have a high genetic risk for early menopause and associated earlier decline in fertility. Compared to women with low genetic risk, your risk for early menopause is **10 TIMES HIGHER**

Out of 1000 women with a similar genetic profile, 101 have early menopause.

Fertify report

SINGLE SPERM CELL CHROMOSOMAL ANALYSIS – TOOL FOR GENETICS, FERTILITY AND ONCOLOGY



Clinical applications - Personalised family planning for:

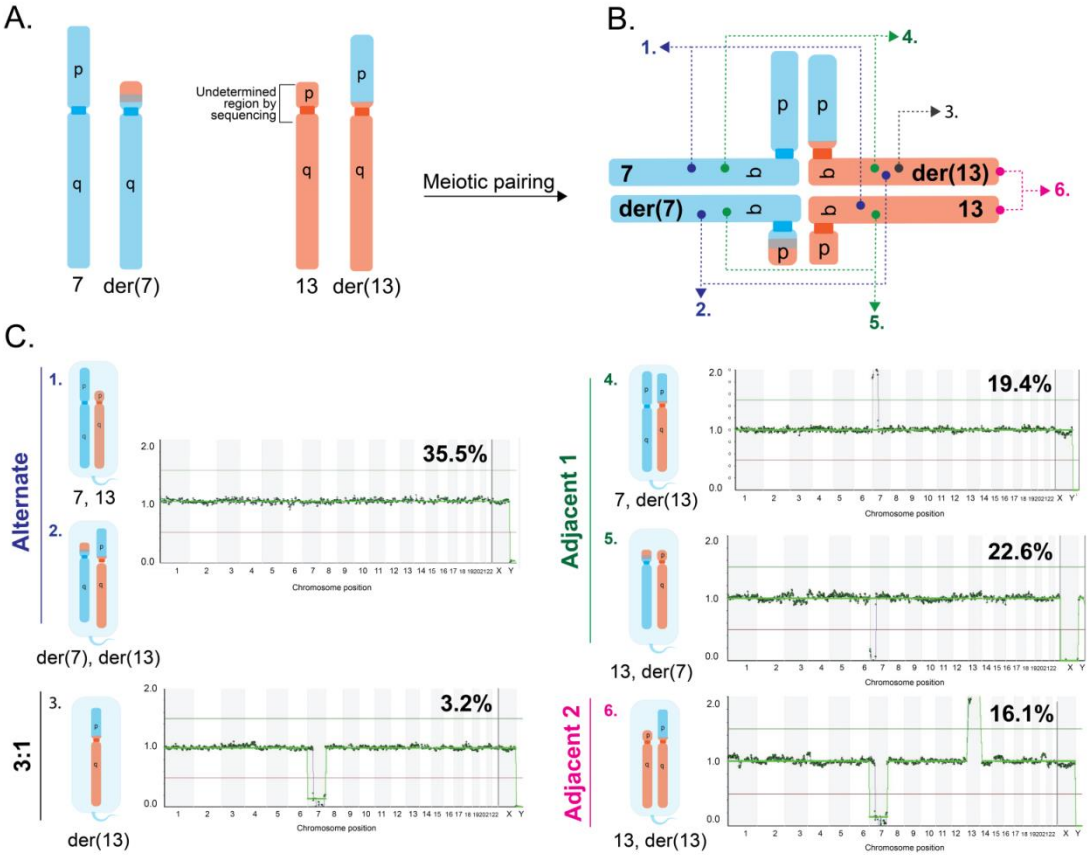
- Man with chromosomal aberrations
- Male infertility
- **Cancer treatment survivals**
- Recurrent miscarriages

SPERM CELLS
23 X or Y
<23 & >23 chromosomes

ANALYSIS IN PRACTICE ... QUESTIONS?

- Oncology treatment – increased chromosomal aberration in sperms – **when is the right time for fathering after oncology treatment?**
- Failed infertility treatment – **should I use donor semen?**
- Chromosomal aberrations – **which prenatal diagnosis method to use?**

Translocation between 7 and 13 chromosomes



Quoc Ty Tran et al. 2018, JARG

Receptive Endometrium – Advanced Diagnostic Yield

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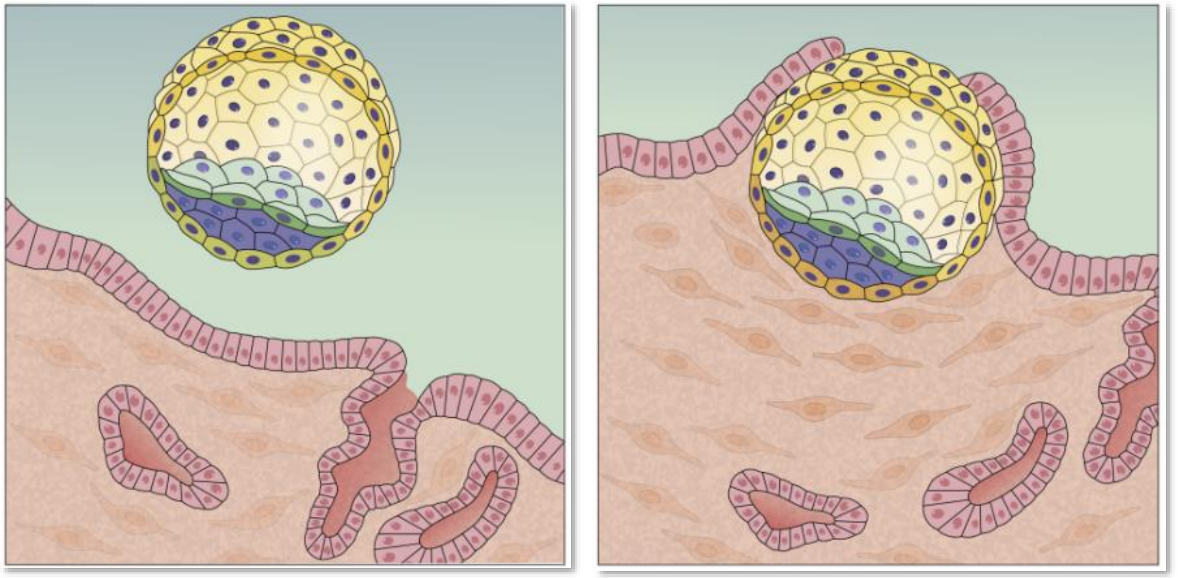
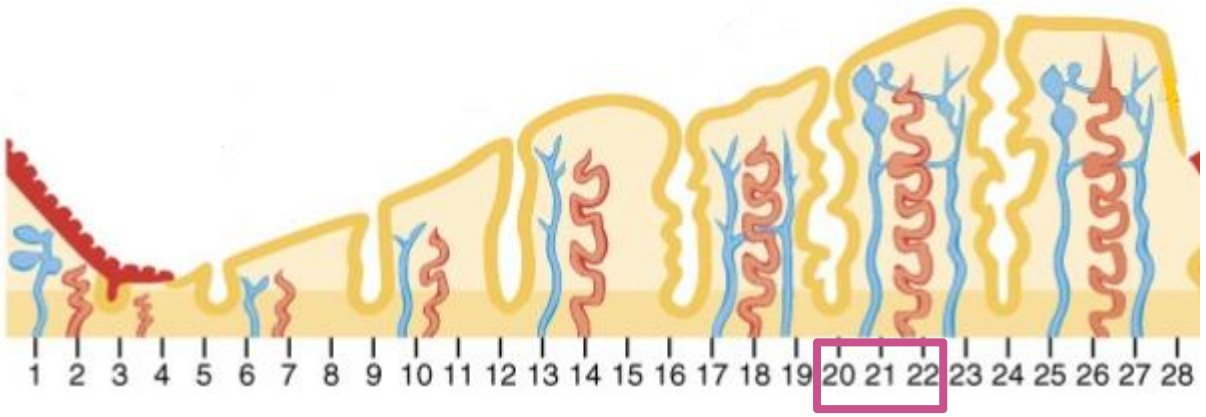
ENDOMETRIAL RECEPTIVITY TESTING





72h WINDOW OF IMPLANTATION

beREADY™
ENDOMETRIAL RECEPTIVITY TESTING

ADVANTAGES

☑ SELECTION OF GENES

Altmäe et al., Scientific Reports 2017
Koel et al., Human Reproduction, revised

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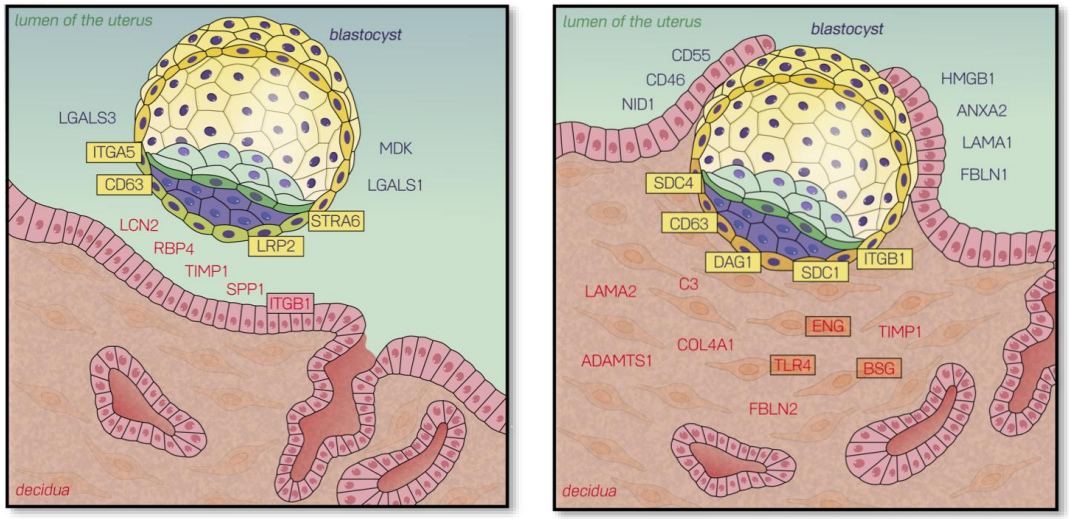
ENDOMETRIAL RECEPTIVITY TESTING

SCIENTIFIC REPORTS

Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers

Altmäe et al., 2017

☑ We selected 57 significant genes based on 9 articles



☑ First molecular interactions between embryo and endometrium

Koel et al., Human Reproduction, revised

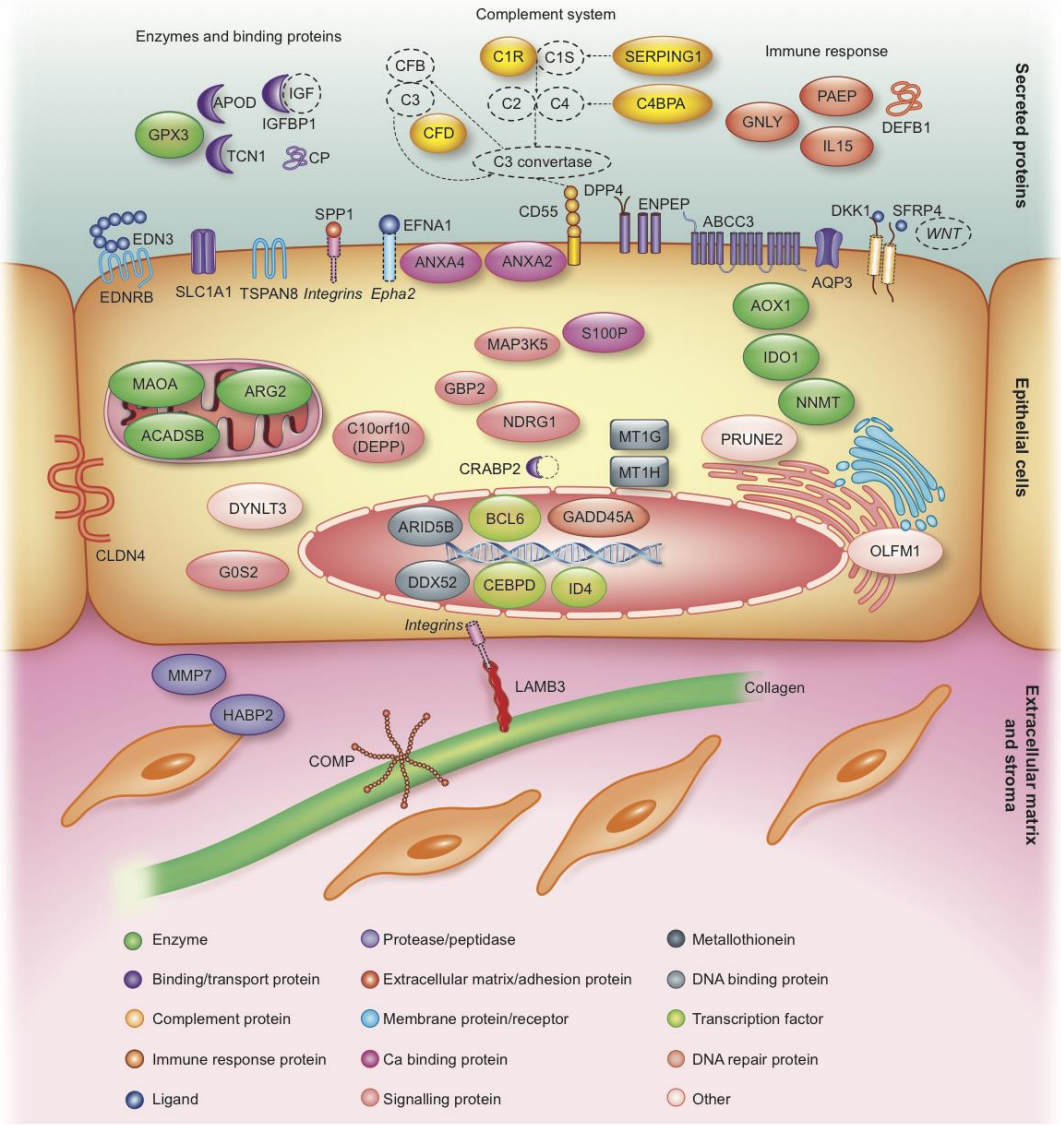
Meta-signature of endometrial receptivity genes

Transcriptome studies have shown little overlap!

- **57 mRNAs** in total

  52 ↑ & **5** ↓

- Involving: **complement cascade pathway** and **exosomal signalling** in mid-secretory endometrium



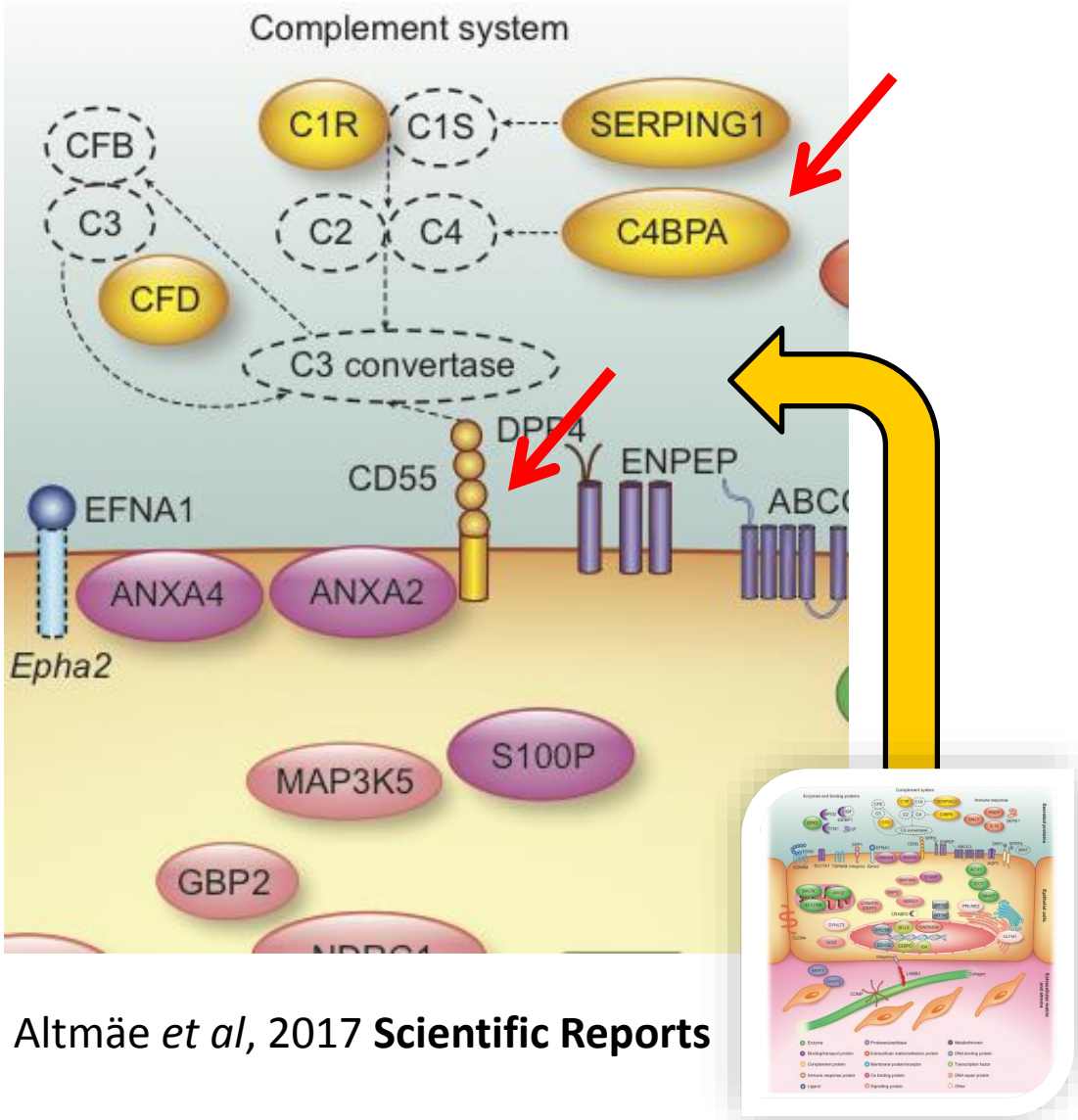
Altmäe *et al*, 2017 **Scientific Reports**

Endometrial receptivity genes and complement cascade of innate immunity

- C3 convertase triggers the complement cascade by cleaving **C3** into **C3b**

CD55 (DAF) ↑

- DAF suppresses C3 convertase and protects host (embryo/epithelial) cells from damage by autologous complement



Altmäe *et al*, 2017 **Scientific Reports**

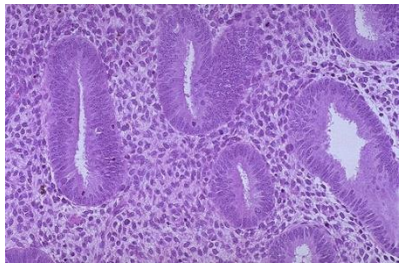
Hum. Reprod. Advance Access published February 13, 2016
Human Reproduction, Vol.0, No.0 pp. 1–10, 2016
doi:10.1093/humrep/dew008

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Single-cell transcriptome analysis of endometrial tissue

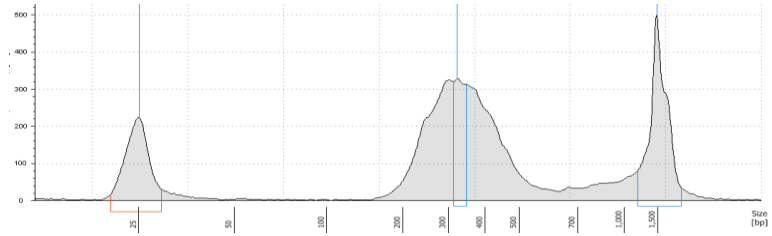
K. Krjutškov[1,2,†*], S. Katayama[2,†*], M. Saare[1,3], M. Vera-Rodriguez[4], D. Lubenets[5], K. Samuel[1], T. Laisk-Podar[1,3], H. Teder[1], E. Einarsdottir[2,6], A. Salumets[1,3,7], and J. Kere[2,6]

Single endometrial cell FACS-sorting and RNA-seq

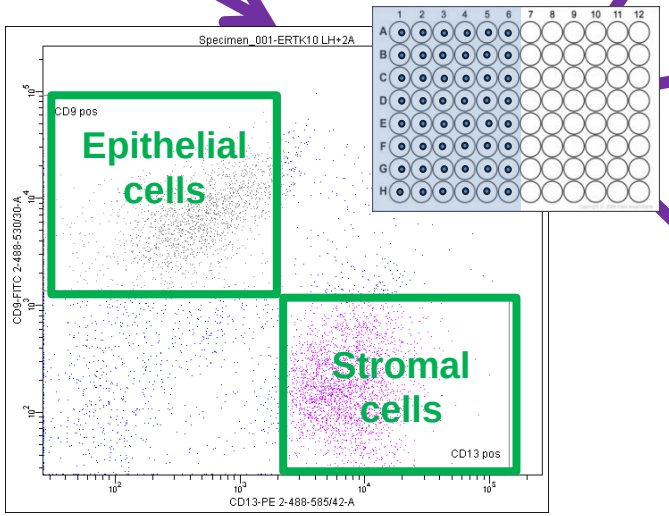


ENDOMETRIAL BIOPSY

CD45+ LEUKOCYOTES



SINGLE-CELL RNA-seq LIBRARY PREPARATION



CD9+ EPITHELIAL CELLS

CD13+ STROMAL CELLS

SINGLE-CELL FACS SORTING

SINGLE-CELL RNA-seq

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ENDOMETRIAL RECEPTIVITY TESTING

ADVANTAGES

☑ SELECTION OF GENES

Altmäe et al., Scientific Reports 2017

Koel et al., Human Reproduction, revised

ACCURATE ANALYSIS ☑

Suhhorutšenko et al., Human Reproduction, 2018

Human Reproduction, pp. 1–13, 2018
doi:10.1093/humrep/dey301

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Endometrial receptivity revisited: endometrial transcriptome adjusted for tissue cellular heterogeneity

Marina Suhorutshenko[1,2,†], Viktorija Kukushkina[3,†], Agne Velthut-Meikas[1,4], Signe Altmäe[1,5], Maire Peters[1,2], Reedik Mägi[3], Kaarel Krjutškov[1,6], Mariann Koel[1,7], Francisco M. Codoñer[8], Juan Fco. Martinez-Blanch[8], Felipe Vilella[9], Carlos Simón[9,10,11], Andres Salumets[1,2,12,13,*], and Triin Laisk[1,2,3]

[1]Competence Centre on Health Technologies, 50410 Tartu, Estonia [2]Department of Obstetrics and Gynecology, Institute of Clinical Medicine, University of Tartu, 50406 Tartu, Estonia [3]Estonian Genome Center, Institute of Genomics, University of Tartu, 51010 Tartu, Estonia [4]Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia [5]Department of Biochemistry and Molecular Biology, Faculty of Sciences, University of Granada, 18071 Granada, Spain [6]Research Program of Molecular Neurology, Research Programs Unit, University of Helsinki, FI-00014 Helsinki, Finland [7]Department of Cell Biology, Institute of Molecular and Cell Biology, University of Tartu, 51010 Tartu, Estonia [8]Lifesequencing SL., 46980 Valencia, Spain [9]Igenomix Foundation /INCLIVA, 46010 Valencia, Spain [10]Research Department, Igenomix SL., 46980 Valencia, Spain [11]Department of Pediatrics, Obstetrics and Gynecology, Valencia University, 46010 Valencia, Spain [12]Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, FI-00014 Helsinki, Finland [13]Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, 50412 Tartu, Estonia

*Correspondence address. Competence Centre on Health Technologies, 50410 Tartu, Estonia. E-mail: andres.salumets@ut.ee

Submitted on June 25, 2018; resubmitted on August 29, 2018; accepted on September 26, 2018

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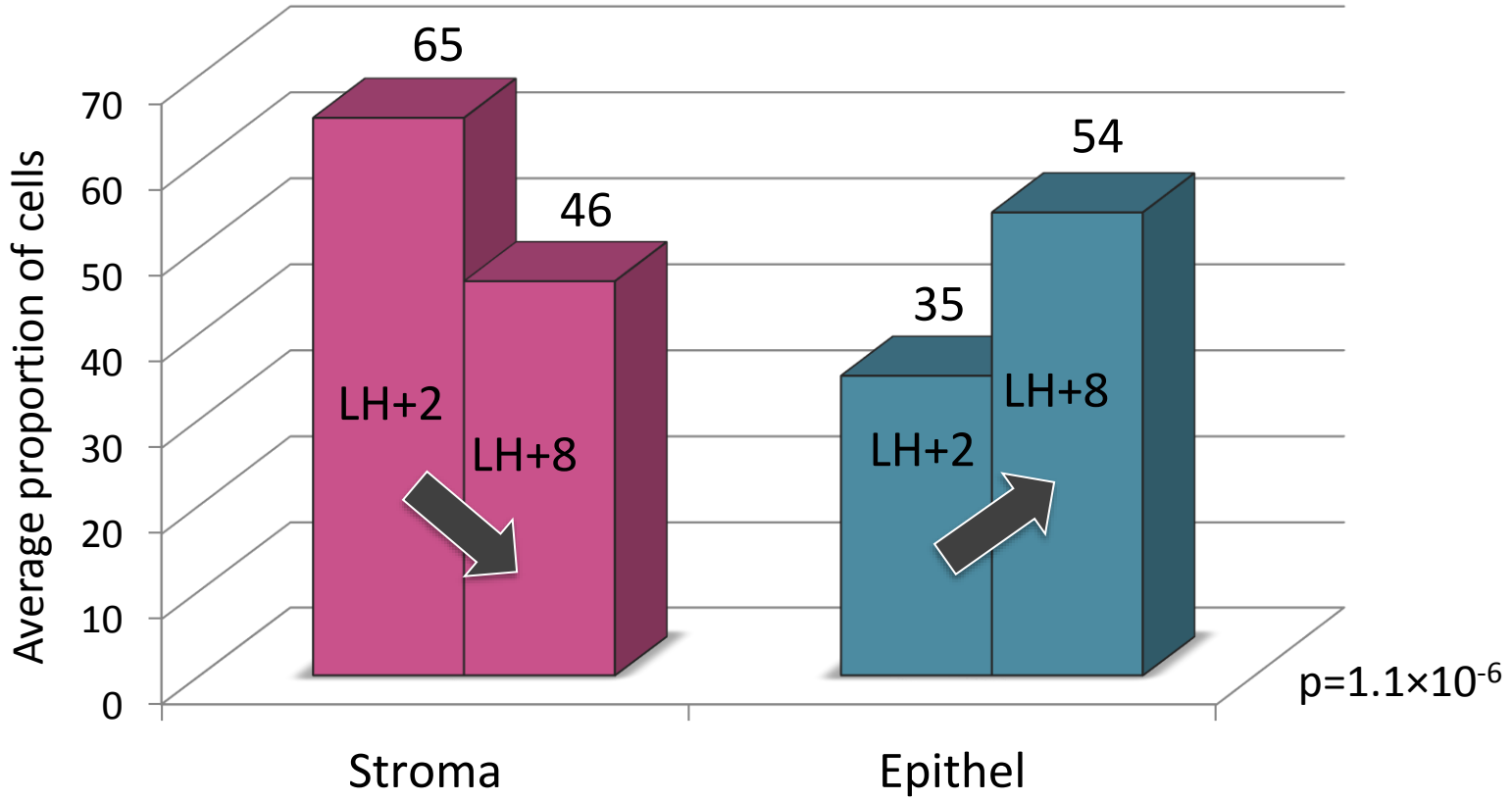
ENDOMETRIAL RECEPTIVITY TESTING



☑ Results adjusted to proportions of cell types in endometrium

Suhhorutšenko et al., Human Reproduction, 2018

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ENDOMETRIAL RECEPTIVITY TESTING

ADVANTAGES

☑ SELECTION OF GENES

Altmäe et al., Scientific Reports 2017
Koel et al., Human Reproduction, revised

ACCURATE ANALYSIS ☑

Suhhorutšenko et al., Human Reproduction, 2018

☑ TECHNOLOGY

International patent application
Teder et al., njp Genomic Medicine, In Press

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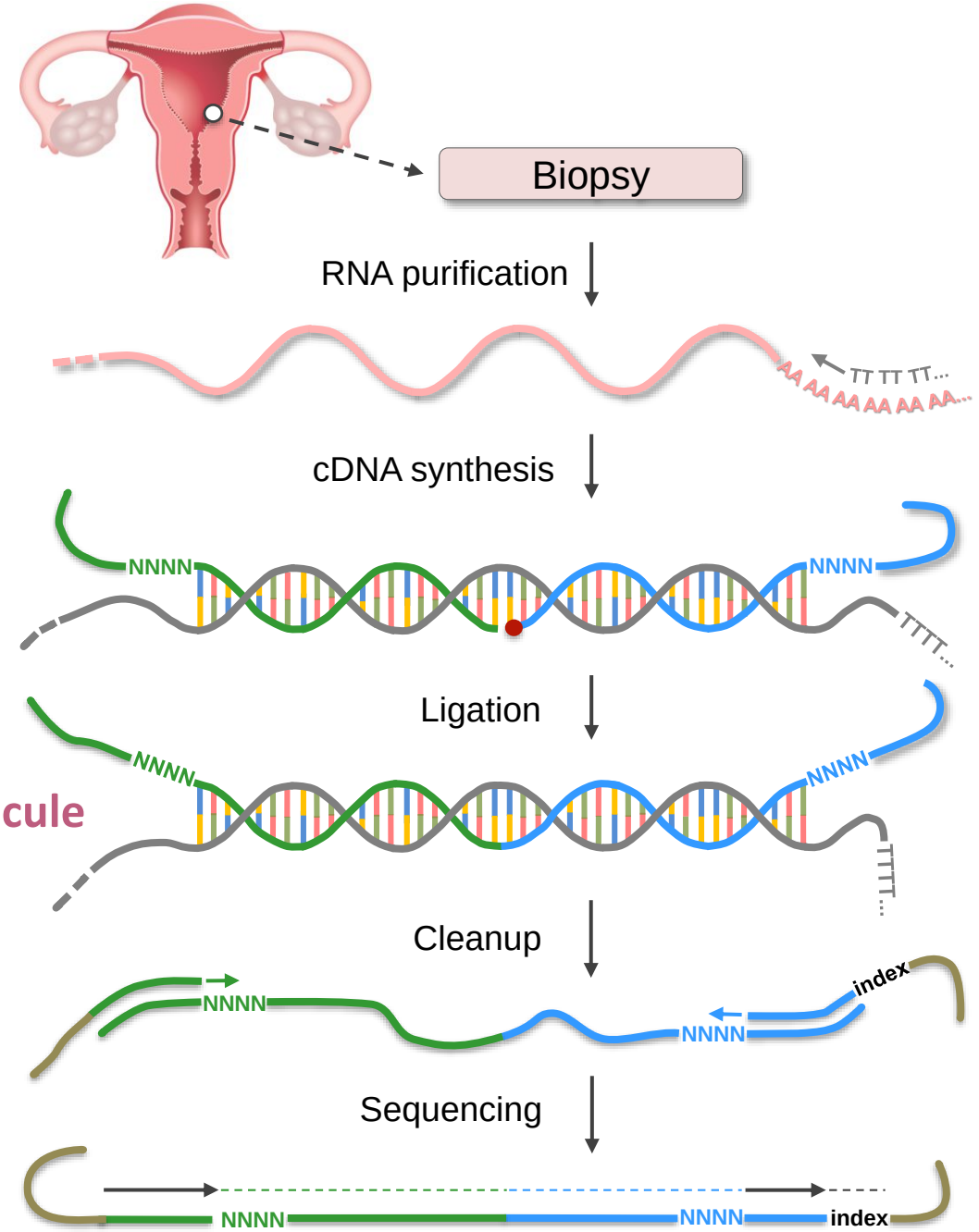
ENDOMETRIAL RECEPTIVITY TESTING

TAC-seq technology

(Targeted Allele Counting by Sequencing)

- ☑ **Selected genes**
- ☑ **Accuracy down to single molecule**
- ☑ **Cost-effective**



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ENDOMETRIAL RECEPTIVITY TESTING

WHO NEEDS IT?

☑ **EVERYONE in the beginning of infertility treatment**

☑ **In case of repeated IVF failure**

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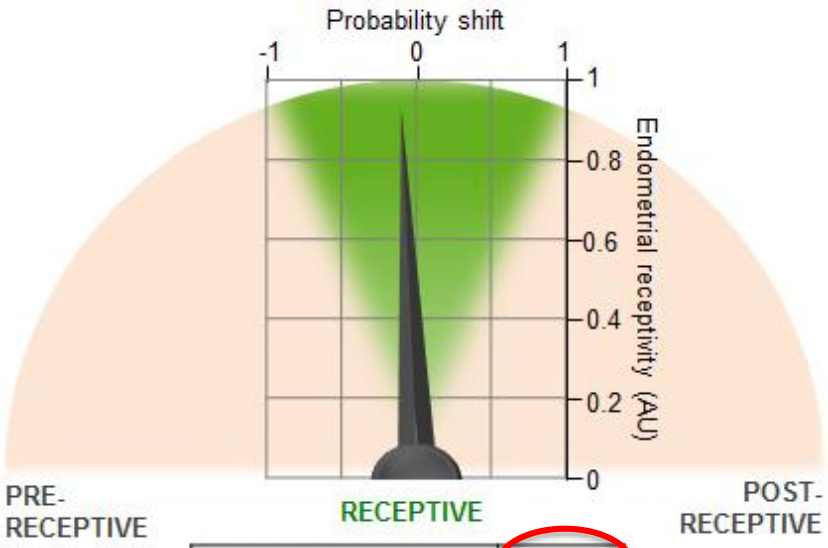
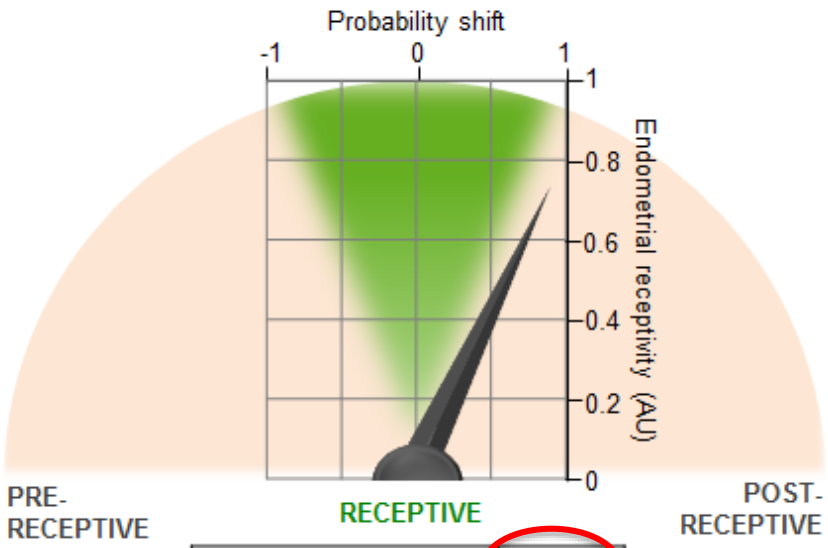
RECEPTOMETER

Example 1 – receptive



Receptive probability	0.922
Pre-receptive probability	0.047
Post-receptive probability	0.031

Example 2 – already post-receptive



Receptive probability	0.782
Pre-receptive probability	0.048
Post-receptive probability	0.170

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ANALYSIS REPORT

Patient information

Laboratory code: 05R
Name:
Date of Birth: March 01 1974

Clinic information

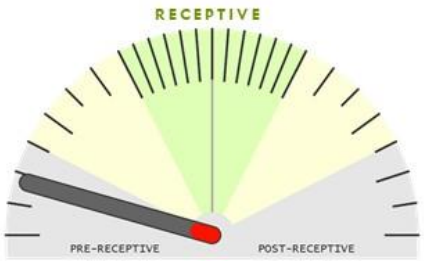
Clinic:
Doctor:
Biopsy: 1

Sample information:

Sample collection date: Oct 03 2018
Date received: Oct 05 2018
Biopsy quality: High
Date reported: Oct 19 2018
Cycle type: HCG+6

PATIENT

PRE-RECEPTIVE | RECEPTIVE | POST-RECEPTIVE



Analysis:

General outcome: **Pre-receptive**
Receptivity score (0-100): NA

Recommendations:

Analyzed gene expression profile is concordant with pre-receptive endometrium. Based on the analysis, receptivity is potentially achieved at least after 48h (HCG+8). In her case we recommend to take new biopsy at HCG+8.

Merli-Saare PhD
Laboratory Manager
beREADY-test laboratory

Kaarel Krjutskov PhD
Head of Laboratory
beREADY-test laboratory

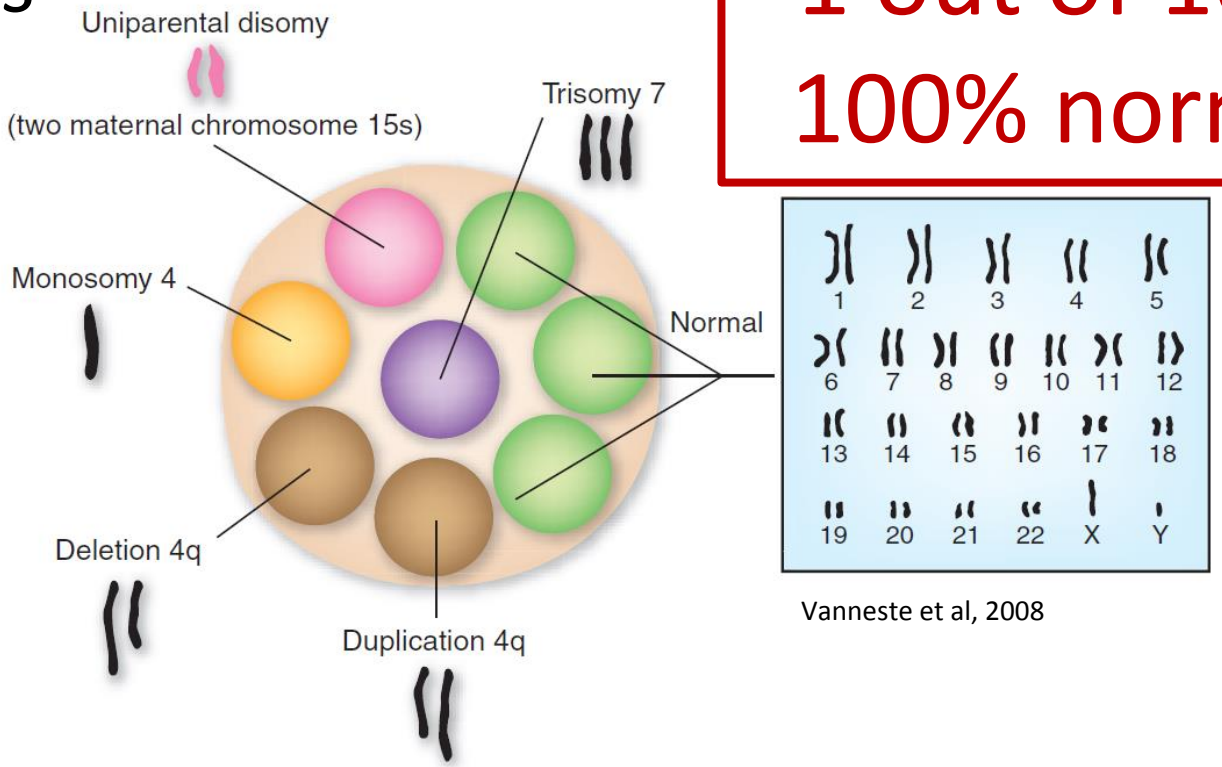
REPORT

SECOND TEST AFTER TIME-ADJUSTMENT IS RECOMMENDED

Chaos in human IVF embryos

8-cell IVF embryos from young couples analysed for chromosomal aberrations

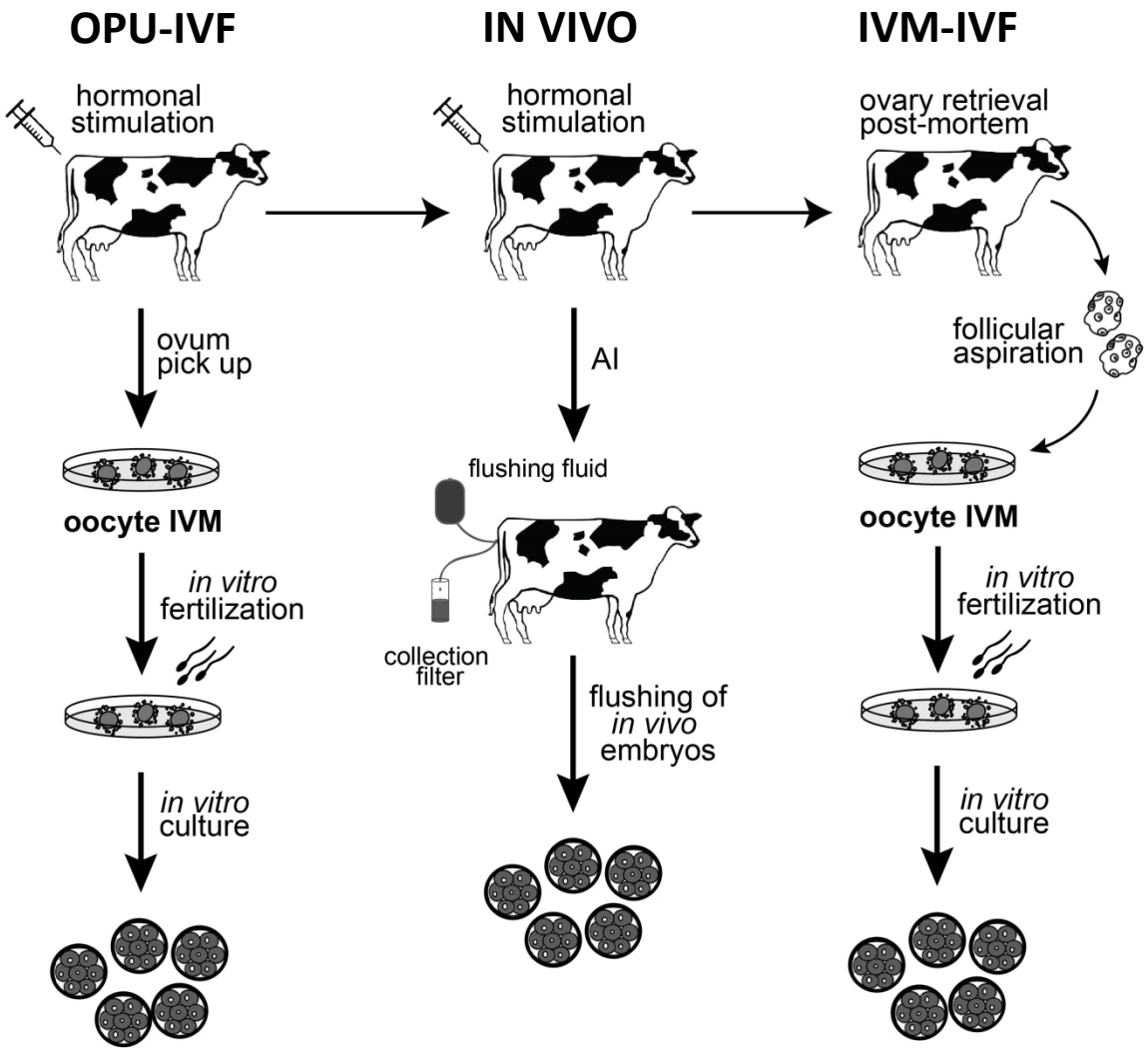
1 out of 10 is 100% normal



Vanneste et al, 2008

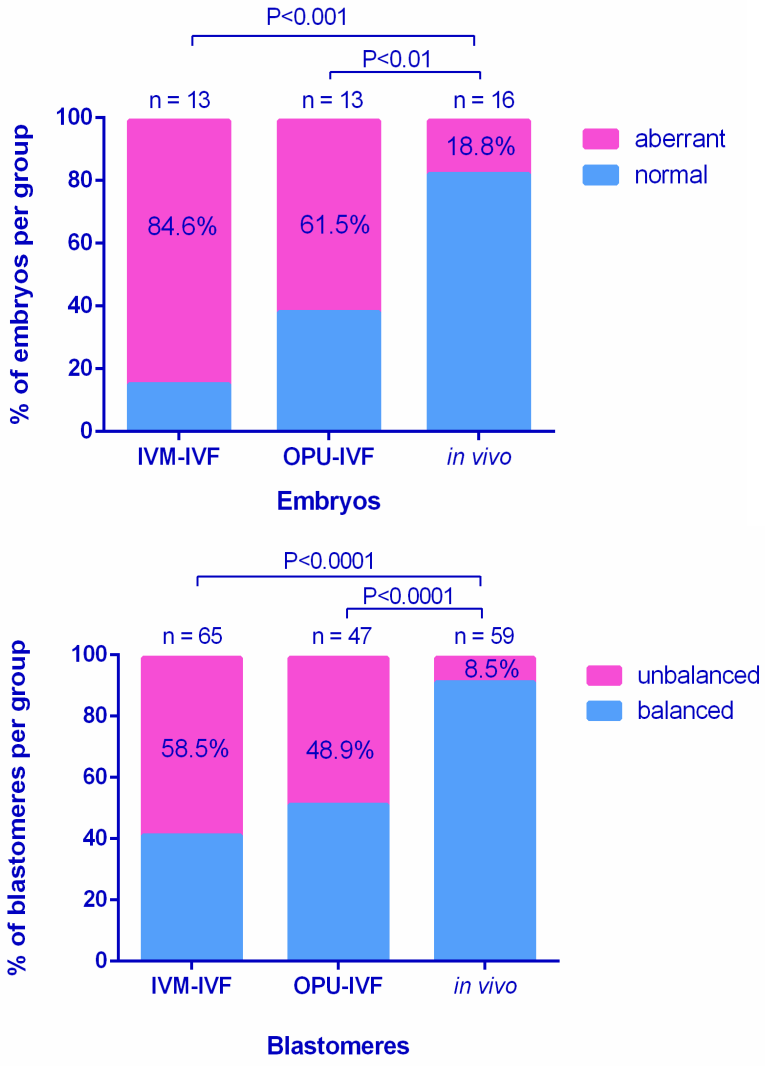
CIN – Chromosomal instability

CIN: *in vivo* vs. *in vitro* embryos



Tšuiko et al, Human Reproduction, 2017, KU Leuven + Ghent University + Tartu University

CIN: *in vivo* vs. *in vitro* embryos



In vitro **maturation, fertilization and culture increase chromosomal instability in early cleavage stage embryos**

Tšuiko et al, 2017, Human Reproduction

HOW TO SELECT THE BEST EMBRYO FOR TRANFER?
PGS – Preimplantation Genetic Screening

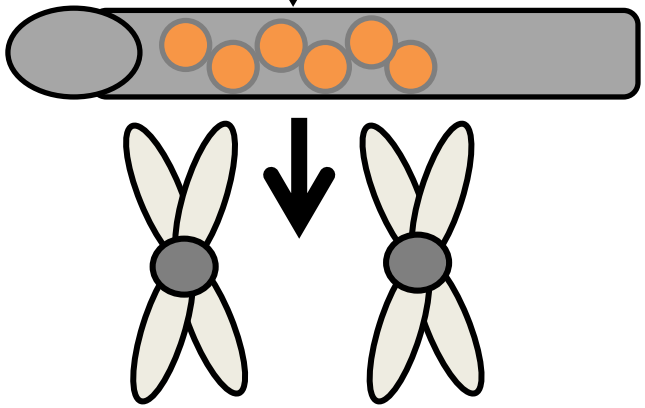
Trophectodermal cells, future placental cells, biopsy 5-6 cells

Laser

Total 200 cells

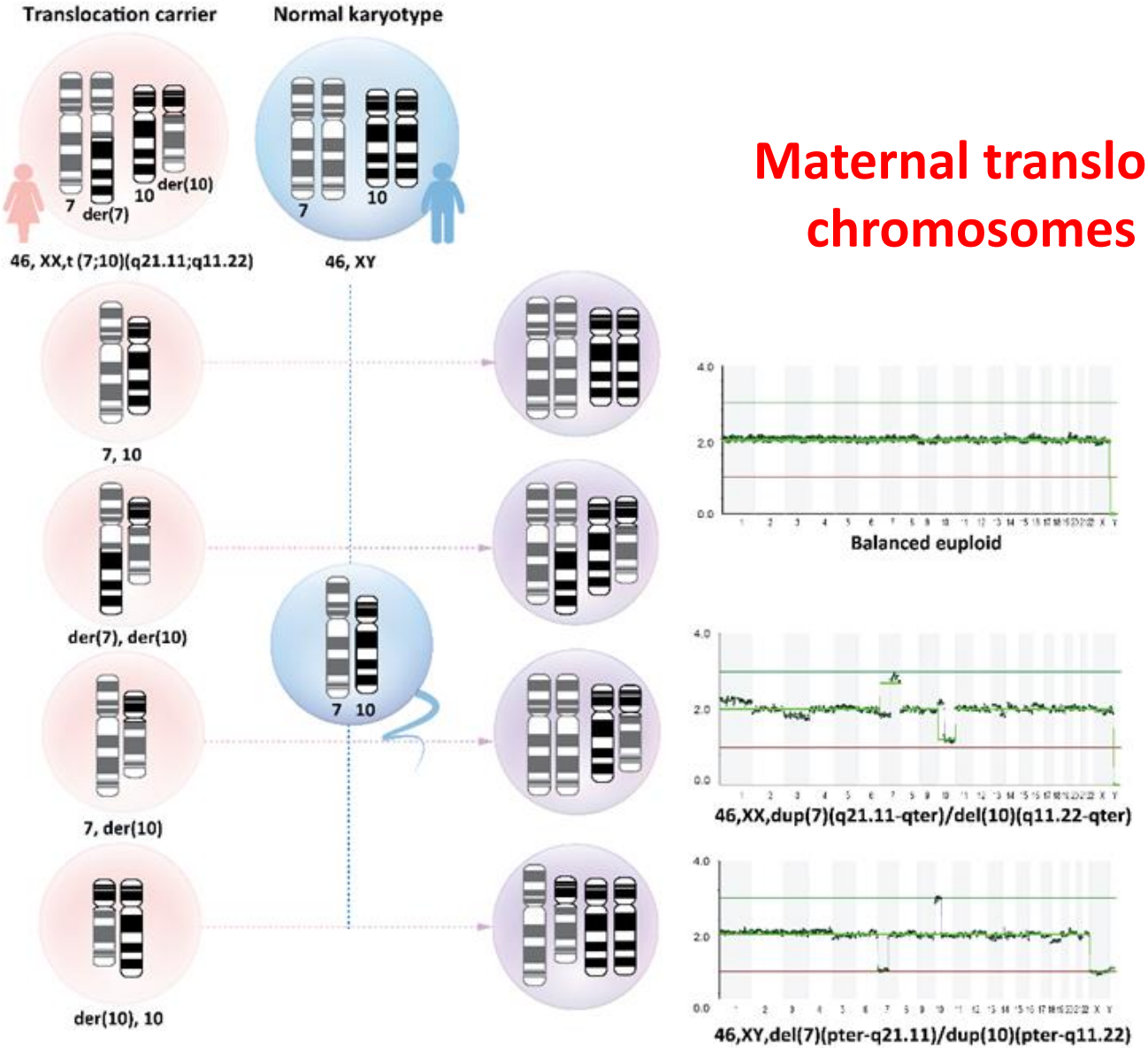
Blastocoel fluid

Inner Cell Mass cells, future fetus



ONLY chromosomally normal embryos are transferred

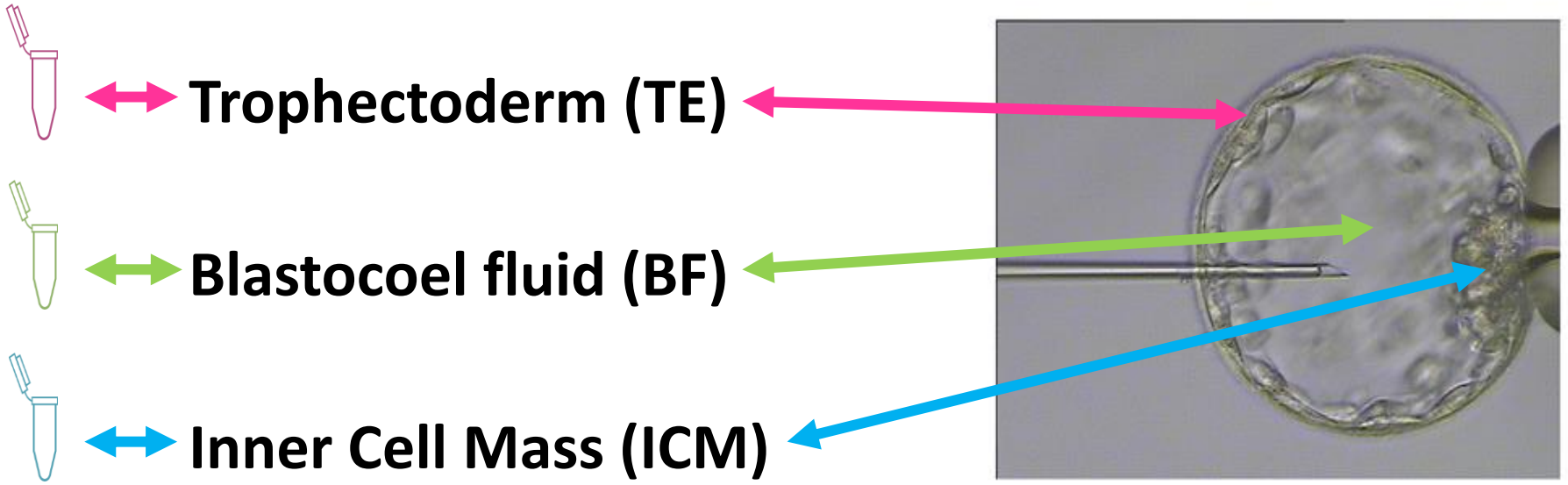
PGS reveals (hidden) chromosomal aberrations of parents



Maternal translocation of chromosomes 7 & 10

Tšuiko *et al*, manuscript

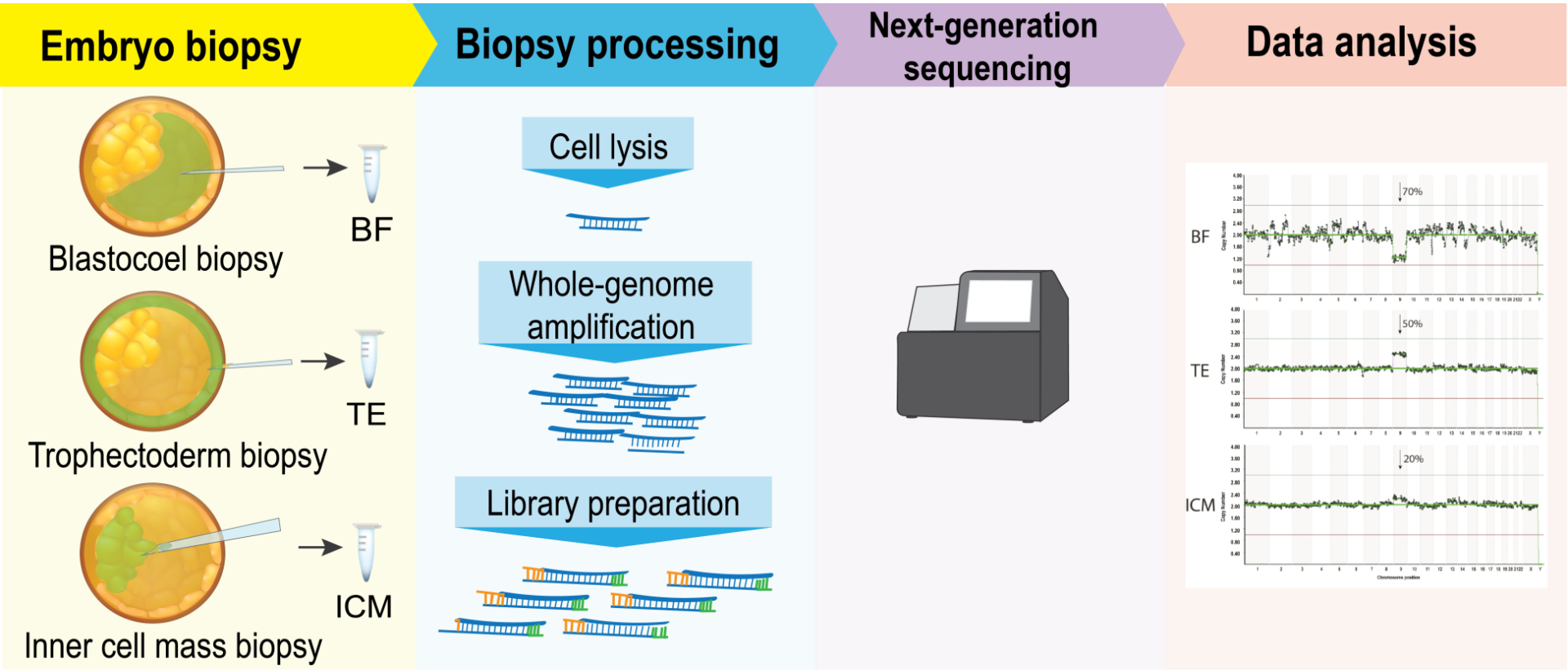
Blastocentesis: study design

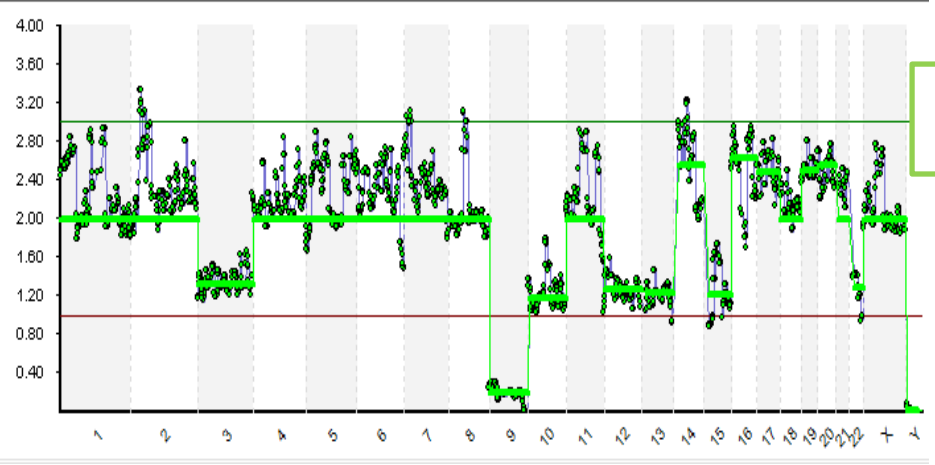


- Next Generation Sequencing (Illumina VeriSeq platform)
- In total 12 embryos analyzed

Tšuiko et al, Fertility & Sterility, 2018

Genomic comparison of BF, TE and ICM

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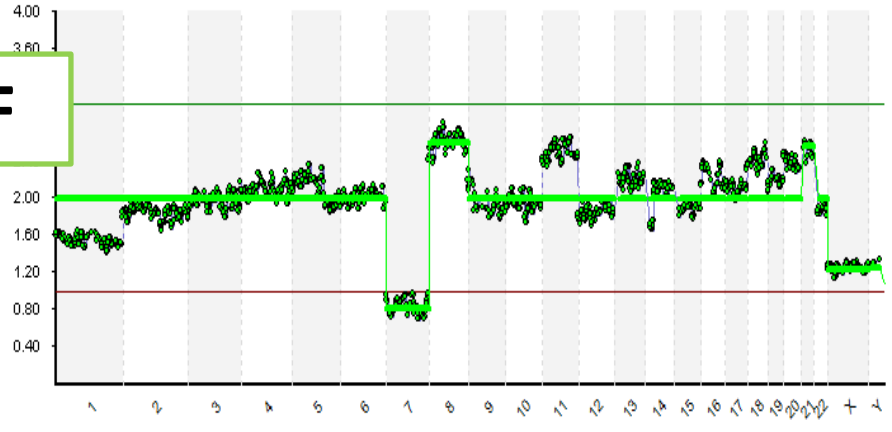
BF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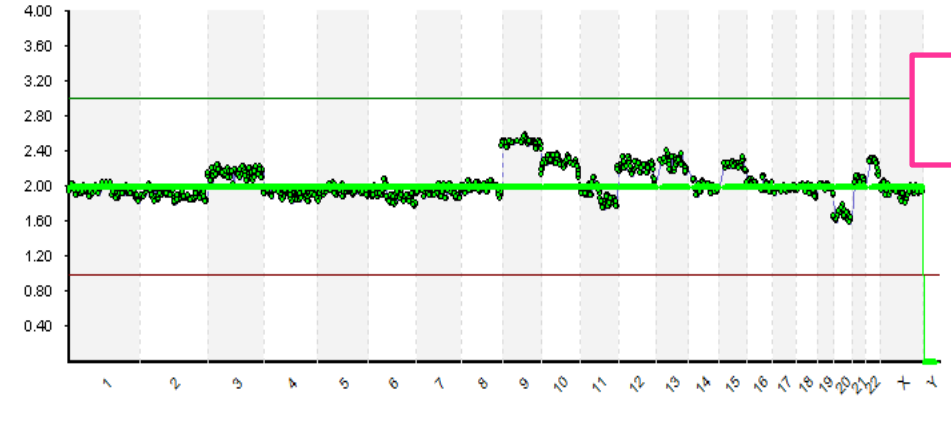
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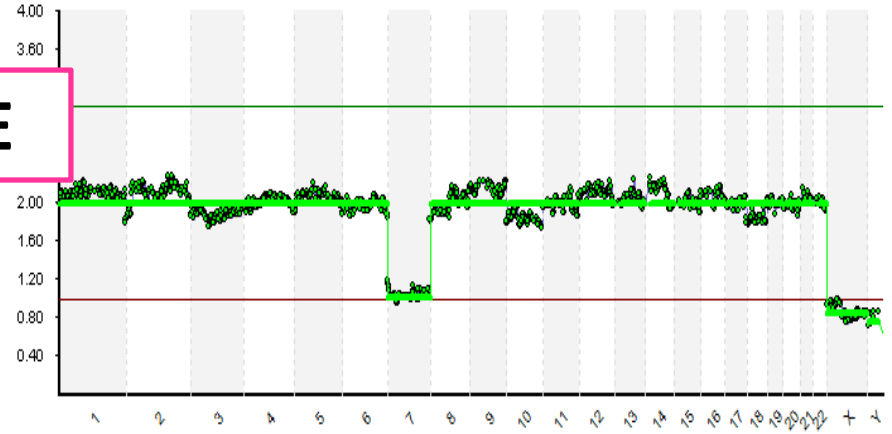
TE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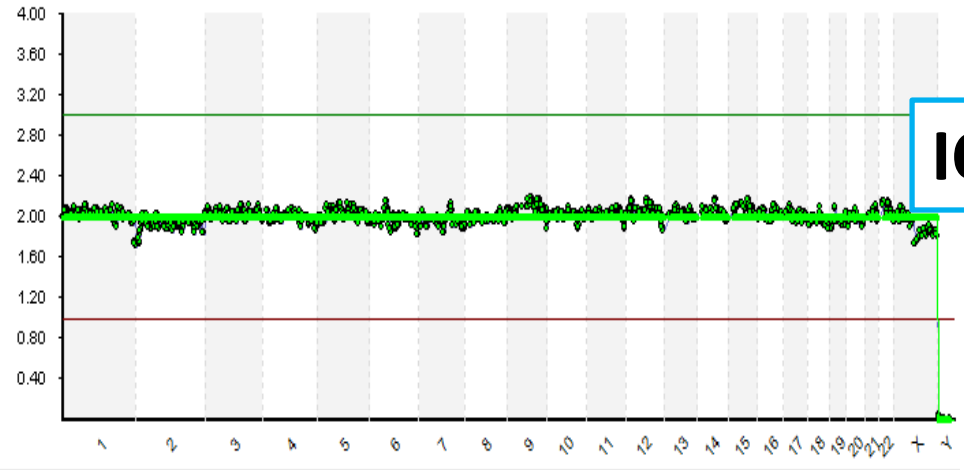
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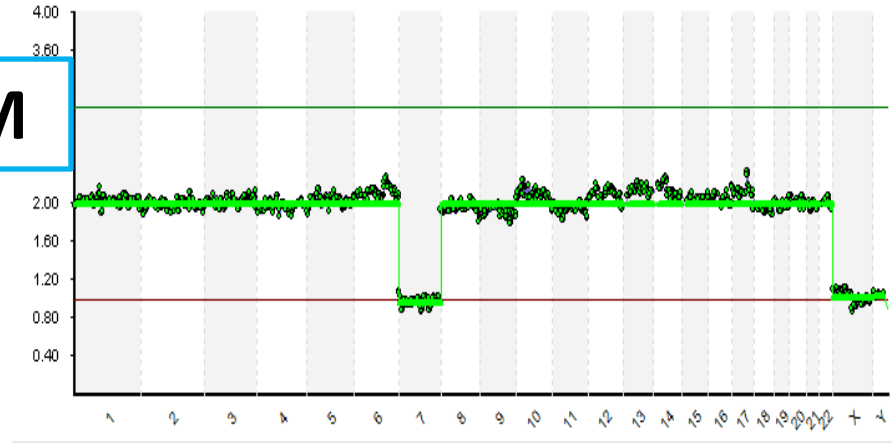
ICM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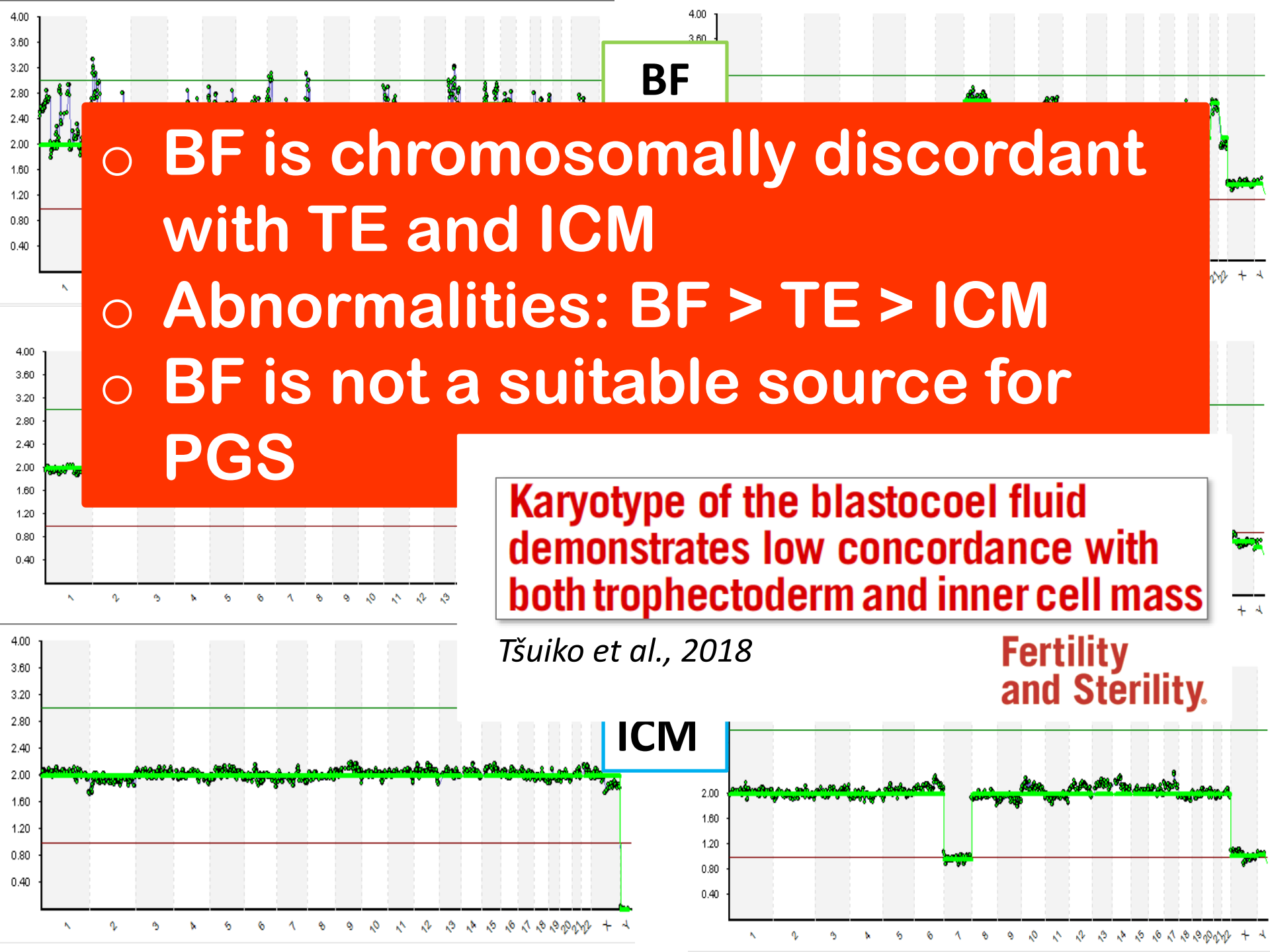


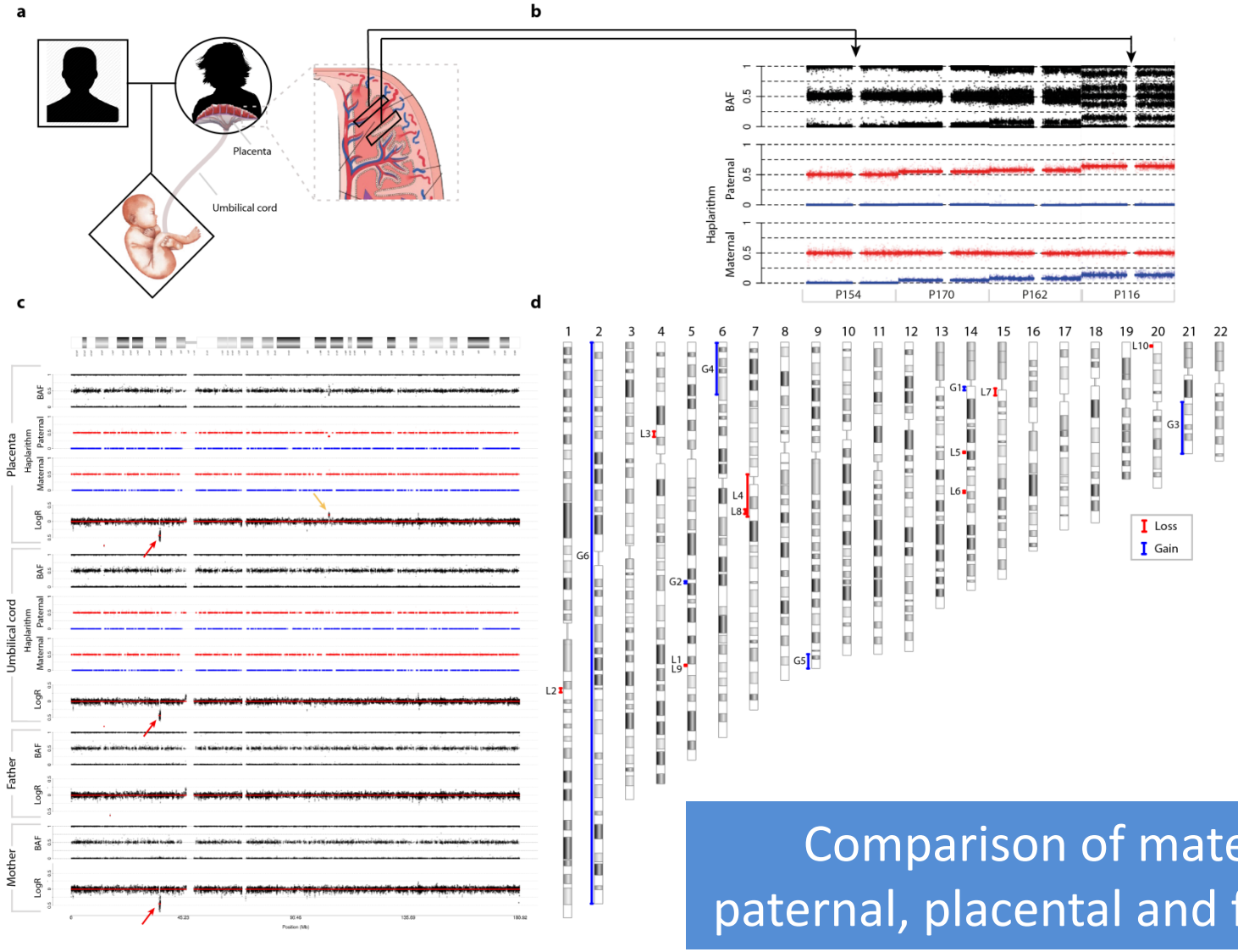
- BF is chromosomally discordant with TE and ICM
- Abnormalities: BF > TE > ICM
- BF is not a suitable source for PGS

Karyotype of the blastocoel fluid demonstrates low concordance with both trophectoderm and inner cell mass

Tšuiko et al., 2018

Fertility and Sterility

MOSAICISM in IVF and naturally conceived children



Comparison of maternal, paternal, placental and fetal DNA

Zamani Esteki 2017, manuscript

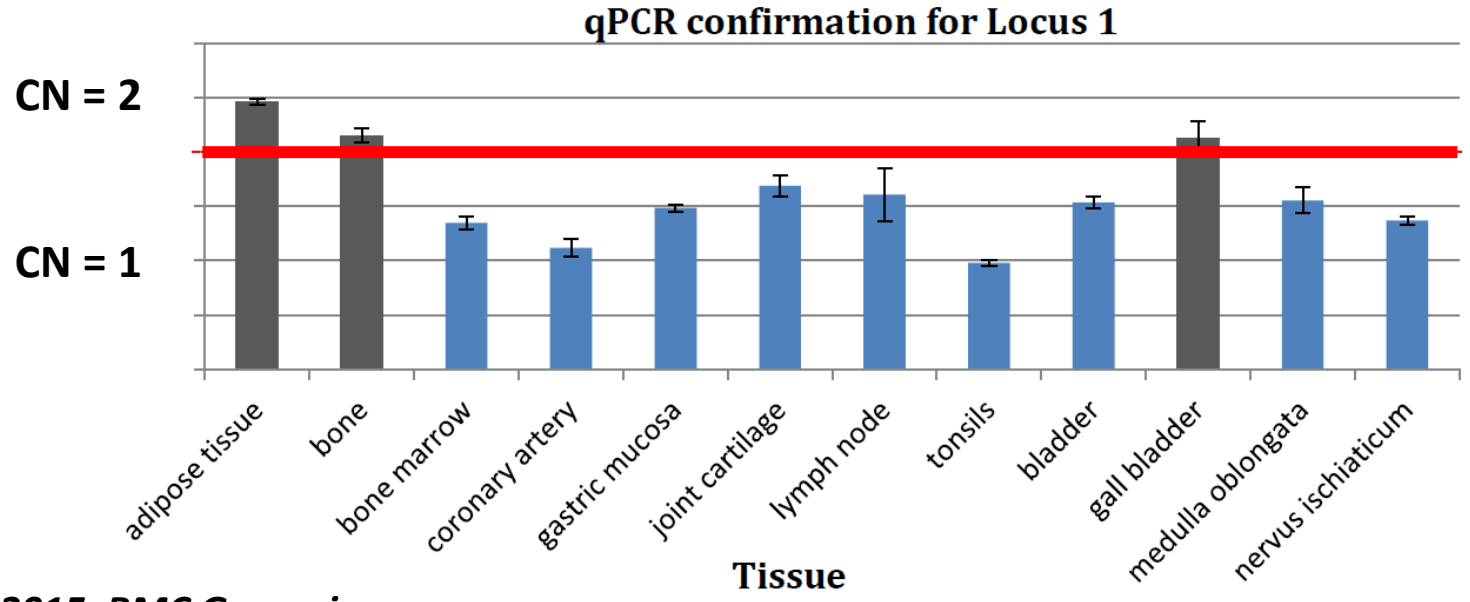
MOSAICISM in IVF and naturally conceived children

- **Similar rate of ca 15% *de novo* mosaicism in IVF and naturally conceived children**
- **Mosaicism: 5-70% and 100K-full chromosome**
- **Located randomly**
- **11% only placental mosaicism**
- **4% placental and child mosaicism!**

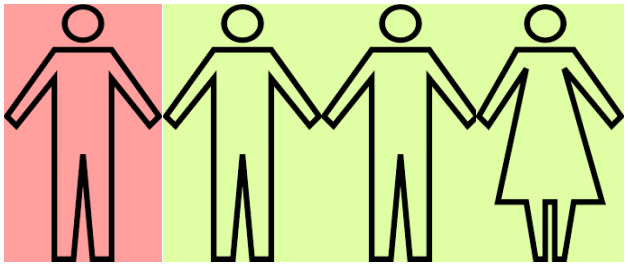
Zamani Esteki 2017, manuscript

Embryo mosaicism – consequences?

- Autopsy patients; 3 men and 1 woman
- 11-12 tissues from the same individual!
- 1 out of 4 individuals had 3 tissue-specific CNVs



Žilina, et al, 2015, BMC Genomics

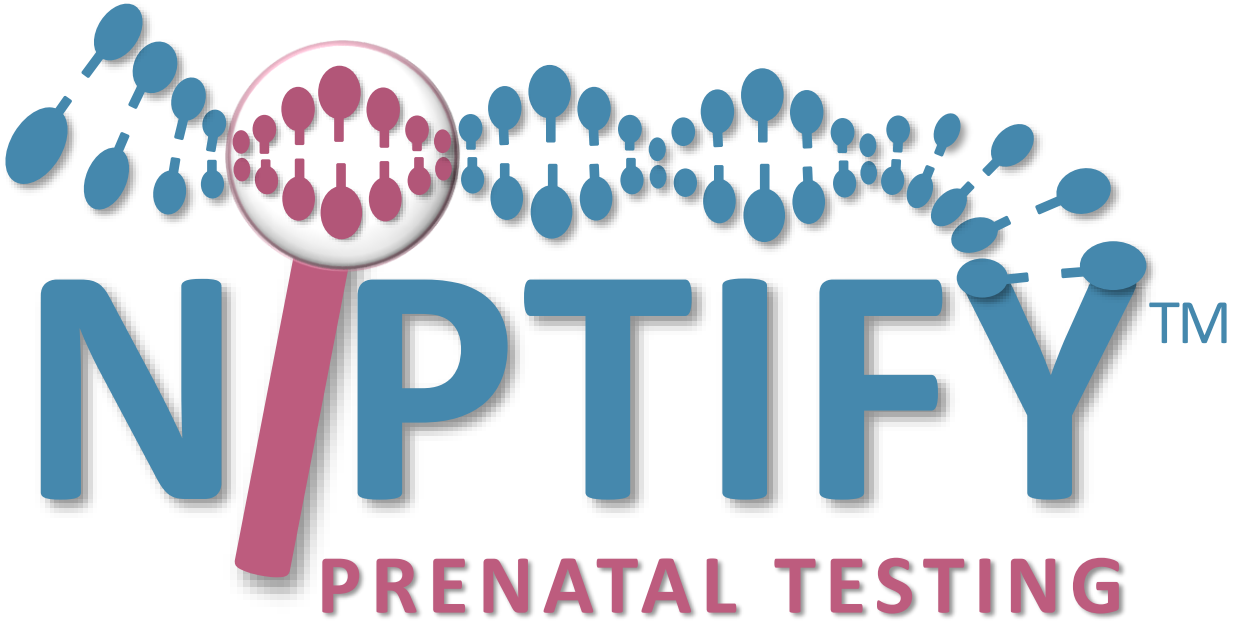


SOMATIC MOSAICISM, LINKS TO:

- CANCERS

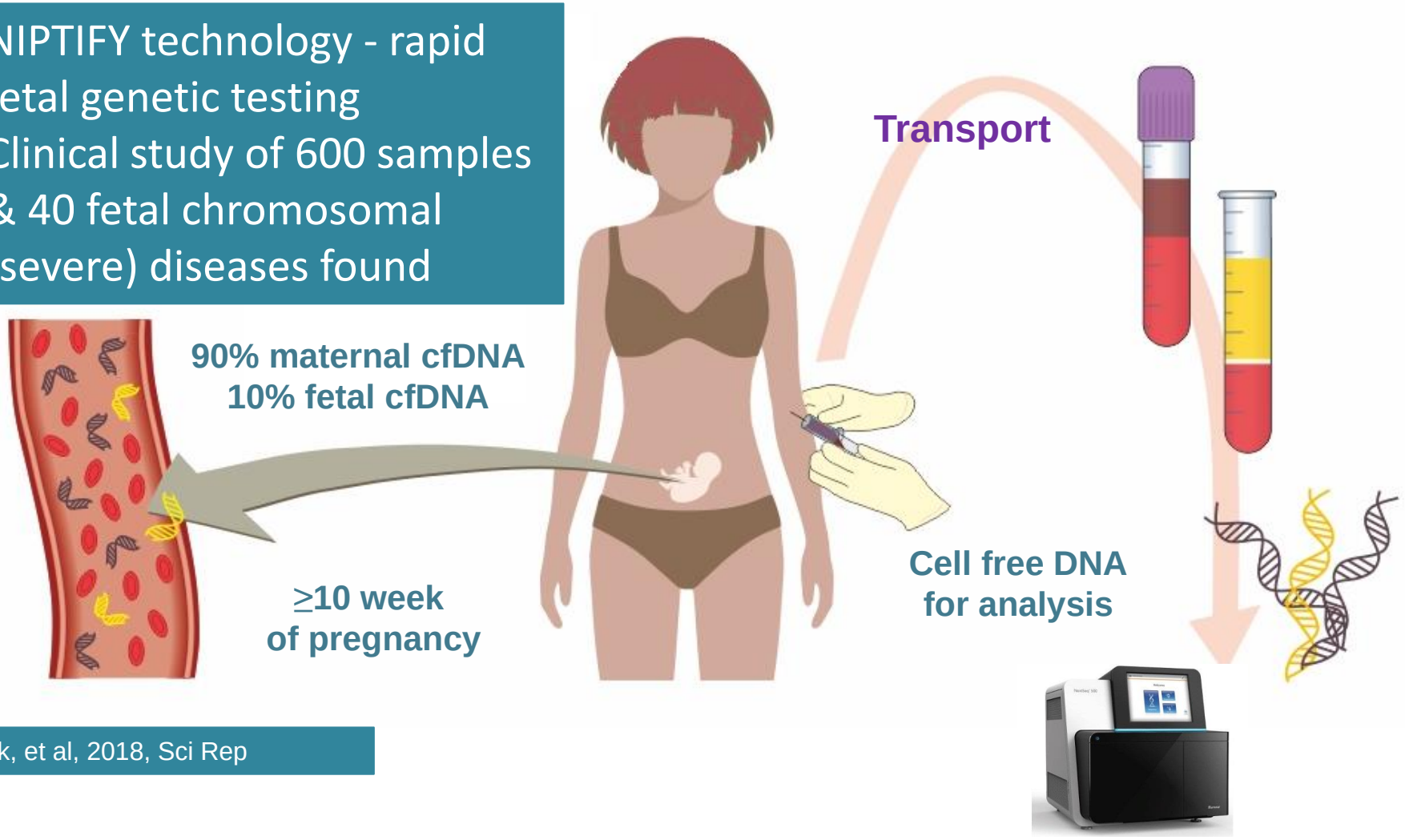
- TISSUE-SPECIFIC DISEASES

?


NIPTIFY™
PRENATAL TESTING

NIPTIFY technology – Estonian proprietary NIPT technology

- NIPTIFY technology - rapid fetal genetic testing
- Clinical study of 600 samples & 40 fetal chromosomal (severe) diseases found



Sauk, et al, 2018, Sci Rep

NIPTIFY™ PRENATAL TESTING

ADVANTAGES

☑ SOFTWARE

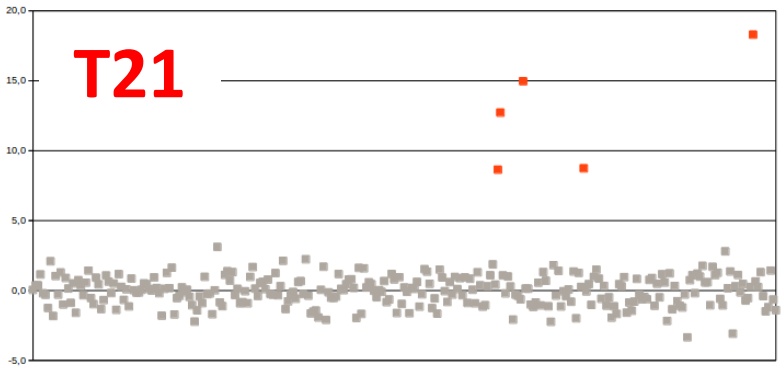
Martin Sauk et al., Scientific Reports 2018

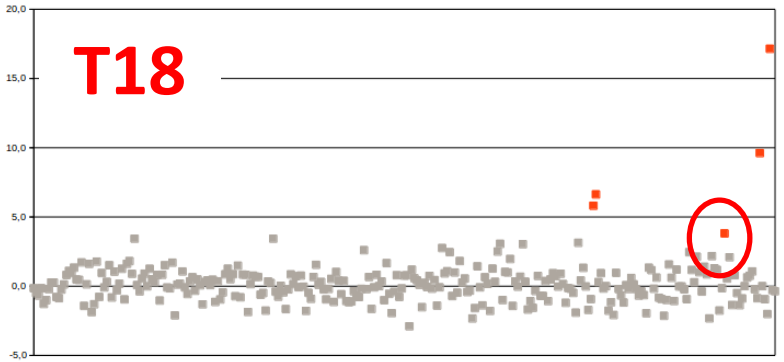
VALIDATION ☑

Olga Fjodorova et al., Prenatal Diagnosis,
submission in process

! Fetal/placental mosaicism and NIPT !

EST





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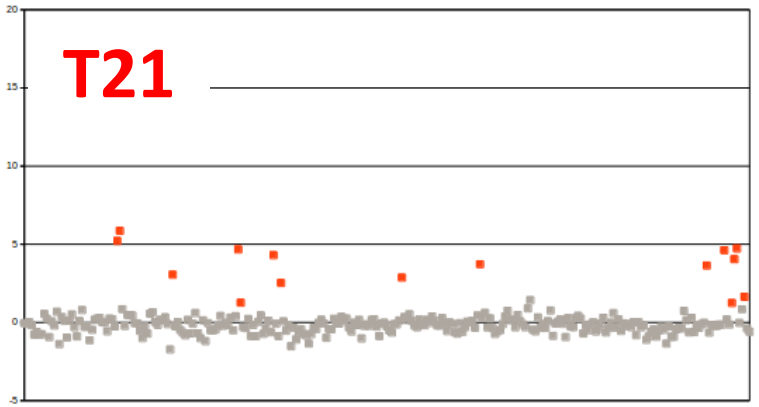

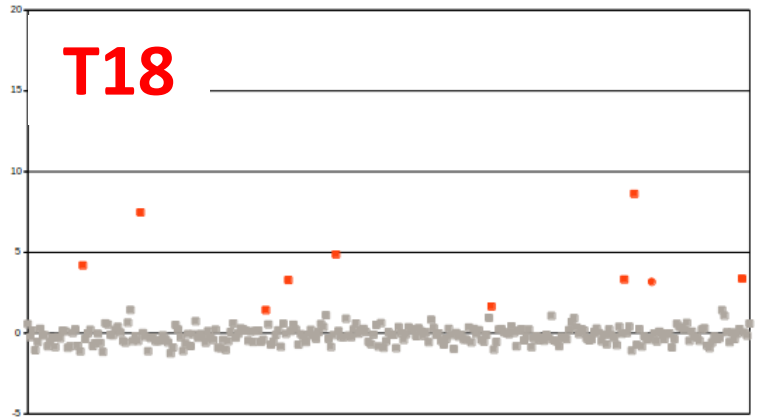

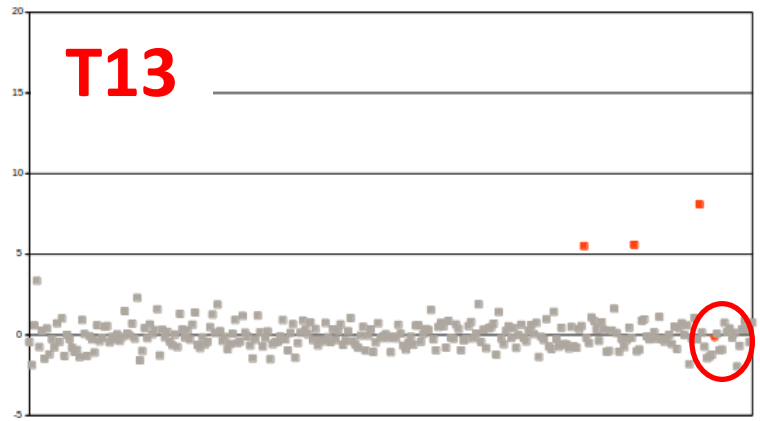

Ca 300 NIPT from Tartu University Hospital
Identified: 5xT21; 4xT18;
borderline results for mosaic T18

Ca 300 NIPT from KU Leuven
Identified: 15xT21; 10xT18 and 3xT13;
undetected mosaic T13

Sauk et al, Scientific Reports, 2018

CONCLUSIONS

- Human reproduction is genetically vulnerable
- Oocytes demonstrate often age-related aneuploidy
- POF may be related to genes of meiotic division
- Human (bovine) early embryos are often mosaic
- Adverse *in vitro* conditions on embryo genome
- Abnormal cells are eliminated during pregnancy
- Challenge for NIPT
- IVF children are not at risk
- Remaining adult tissue mosaicism may originate from early embryo

Competence Centre on
Health Technologies

THANK YOU!

Andres.Salumets@ccht.ee

Eesti Maaülikool
EMU Estonian University of Life Sciences

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A.D. 1804

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UNIVERSITY OF HELSINKI

UNIVERSITY OF OULU