
بسم الله الرحمن الرحیم

Personalized Medicine in Infertility Treatment

By:

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Evidence-based medicine

- Emphasizes the **relationship** between **clinical research** and **clinicians**.

But

- The **gap** between **clinicians** and **patients** **has not been filled**.

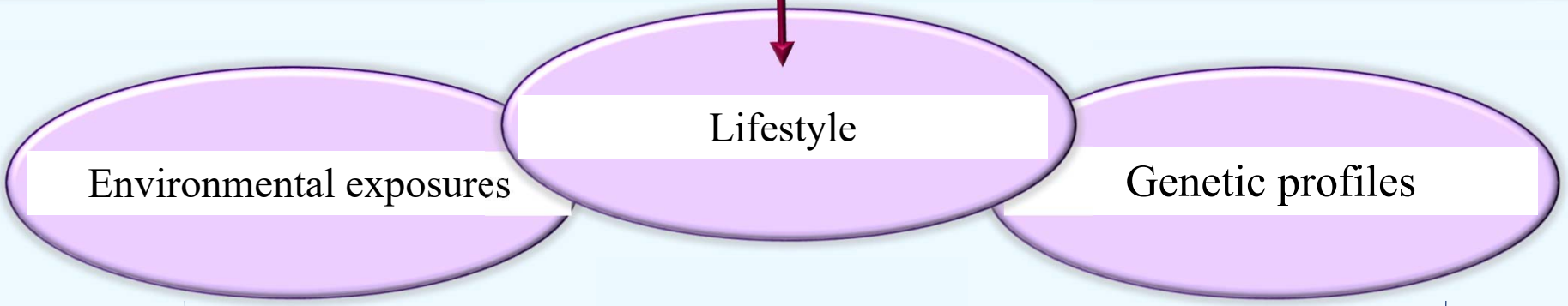
Precision medicine

- A new health care model that can **adapt** to **various patients**.
- This conception started from **oncology**, which refers to selecting chemotherapeutic agents based on **patients' genetic profiles** to **maximize** efficiency and safety.
- Currently, this conception has been employed in **many areas of medicine**, such as **psychiatry** and **cardiovascular** disease.
- **Genetic profiles** have been identified that predict the necessary therapeutic dose for opioid analgesia and the amount of heart disease risk reduction a patient will gain from statins.

What is precision medicine?

➢ According to the definition promulgated by the **National Institutes of Health** (**NIH**), **precision medicine** refers to:

A thorough understanding of the consequences of unique features of individual patients, such as



Requires

new individualized

treatments & prevention methods

Precision medicine in the realm of reproductive medicine

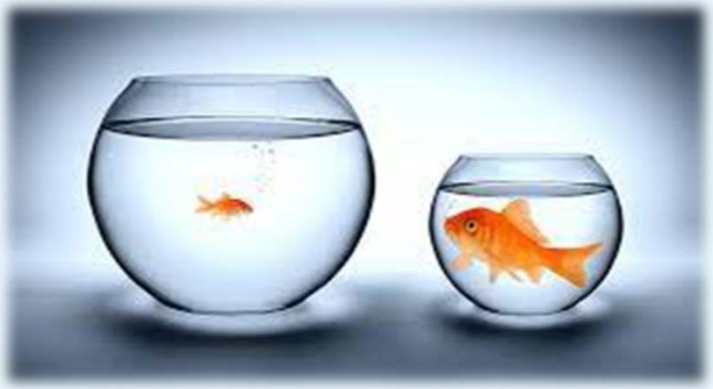
This conception refers to better information collection of

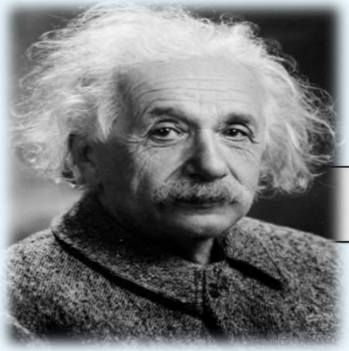
customized treatment protocols are made based on this information.

✓ Instead of treating all patients with the same procedures,
this type of personalized health model can **increase** the **efficacy** and **efficiency** of ART.

One size does not fit all!

Failed IVF cycles followed by repeating the same cycles for all coupes.





Albert Einstein: “If you want different results, don’t do the same.”

Personalized reproductive medicine

The focus of ART

- **Individualization of infertility treatment**
- **Tailoring the treatment according to the patient's conditions and requirements**
- **With the aim to increase the chance of achieving a live birth**

In this sense, **personalized reproductive medicine** is a good opportunity to

Improve

- **The efficiency of assisted reproduction treatments**
- **Cost effectiveness**

Decrease

- **The number of cycles needed**
- **The cost of treatment**
- **Patient's emotional burden**

What is precision medicine?

The goal of precision medicine

a better understanding and identification of patients who are at risk for particular diseases

requires

Refinement of the Screening guidelines

- **Start early intervention**
- **Prevent unwanted outcomes**
- **Urge potential patients to change their lifestyle and carefully consider overall life plans**

Different aspects of personalized medicine in ART

- Embryo Selection
- Endometrial receptivity
- Female infertility
- Male infertility

Personalized medicine in Embryo Selection

Embryo Selection

Morphology
(Grading based on the appearance)

Embryo culture to Day-5 (Blastocyst)
Versus
Day-3 (Cleavage stage)

Morphokinetic Assessment
(Time-lapse Imaging)

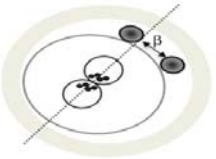
Preimplantation Genetic Testing (PGT)

Embryo Selection
Morphology-based grading

Traditionally,
morphology-based grading had been the **primary technique** used in IVF
to
evaluate and select the **most competent embryos for transfer**.

The **chromosomal complement** of an embryo
has the **greatest impact** on **embryo morphology** at the **blastocyst stage**.

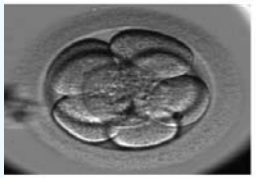
Key morphological features of human embryos with high viability



Ideal features shared by pronucleate oocytes with high viability:
(i) number of nucleolar precursor bodies (NPB) in both pronuclei never differed by more than 3
(ii) NPB are always polarized or not-polarized in both pronuclei but never polarized in one pronucleus and not in the other
(iii) angle β from the axis of the pronuclei and the furthest polar body is less than 50°

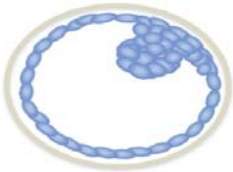
Ideal features shared by 2-cell embryos with high viability:
(i) Mononucleated blastomeres
(ii) Equal cell size
(iii) < 20% fragmentation

Ideal features shared by 4-cell embryos with high viability:
(i) Mononucleated blastomeres
(ii) Equal cell size
(iii) < 20% fragmentation



Ideal features shared by day 3 embryos with high viability:
(i) Mononucleated blastomeres
(ii) Equal cell size
(iii) < 20% fragmentation
(iv) At least 7 blastomeres

Ideal features shared by morulae with high viability:
(i) Visibly compacted cells denoted by the slight reduction in overall size of the embryo and increase in space between the embryo and zona pellucida
(ii) Lack of fragments



Ideal features shared by blastocysts with high viability:
(i) Expanded blastocoel cavity by day 5
(ii) Well formed ICM clearly composed of many cells
(iii) Cohesive epithelium made up from many cells in the TE
(iv) Signs of the zona pellucida thinning

Key morphological features of human embryos with medium to low viability

Sub-optimal features shared by pronucleate oocytes with medium/low viability:

(i) Number of nucleolar precursor bodies (NPB) differ by more than 3

(ii) Angle ß from the axis of the pronuclei and the furthest polar body is more than 50°

Sub-optimal features shared by pronucleate oocytes with medium/low viability:

(i) Number of nucleolar precursor bodies (NPB) differ by more than 3

(ii) Distribution of NPB being polarized in one pronuclei, whereas it's non-polarized in the other

Sub-optimal features shared by 2-cell embryos with medium/low viability:

(i) Multinucleated blastomeres

(ii) Unequal cell size

Sub-optimal features shared by 2-cell embryos with medium/low viability

(i) Unequal cell size

(ii) 20% fragmentation

Sub-optimal features shared by 4-cell embryos with medium/low viability:

(i) Multinucleated blastomeres

(ii) Unequal cell size

Sub-optimal features shared by 4-cell embryos with medium/low viability:

(i) Unequal cell size

Sub-optimal features shared by day 3 embryos with medium/low viability:

(i) Unequal cell size

(ii) 35% fragmentation

Sub-optimal features shared by morulae with medium/low viability:

(i) Non-participation of all cells in compaction

(ii) 20% fragmentation

Sub-optimal features shared by blastocysts with medium/low viability:

(i) Loosely formed ICM composed of few cells

(ii) Loosely formed epithelium made up from few cells in the TE

Key morphological features of human embryos during preimplantation development

18–19 h post-insemination/ICSI	**Score**
The fertilized embryo is examined for:	
(a) Equal size and symmetry of PN	10
(b) Alignment between the PN and polar bodies	5
(c) Lack of heterogeneity and granularity in cytoplasm	5
(d) Presence of PN with both polarized or both not-polarized NPB	10
(e) A difference of less than 3 in the number of NPB in the PN	10
(f) Polar bodies are not displaced from each other	10
25–26 h post-insemination/ICSI	
(a) Embryos that have already cleaved to form a 2-cell embryo with even blastomeres and no fragments	15
(b) Zygotes that have progressed to nuclear membrane breakdown	5
42–44 h post-insemination/ICSI	
(a) Number of blastomeres should be greater or equal to 4	10
(b) Fragmentation of less than 20%	10
(c) No multinucleated blastomeres	5
66–68 h post-insemination/ICSI	
(a) Number of blastomeres should be greater or equal to 8	10
(b) Fragmentation of less than 20%	10
(c) No multinucleated blastomeres	5
94–96 h post-insemination/ICSI	
(a) Compaction	10
(b) Signs of blastocoel formation	15
106–108 h post-insemination/ICSI	
(a) Full blastocoel cavity	10
(b) Inner cell mass with tightly packed numerous cells	10
(c) Trophectoderm with many cells forming epithelium	15

Morphological Selection

But..

Such differences in morphology are **not absolute.**

Due to

Subjectivity of analysis

Repeatability of such measures

Hence at present,
the **analysis of morphology** does **not represent**
an **alternative** to **biopsy** and **genetic analysis** for the **accurate assessment of ploidy**.

You can't always judge a book by its cover!

Rather than a future in which the morphology of the embryo is deemed irrelevant,
Some scientists propose that
key morphological features, such as **multinucleation**, **cell size** & **blastocyst differentiation** **should be included** in future iterations of **selection/deselection** algorithms.

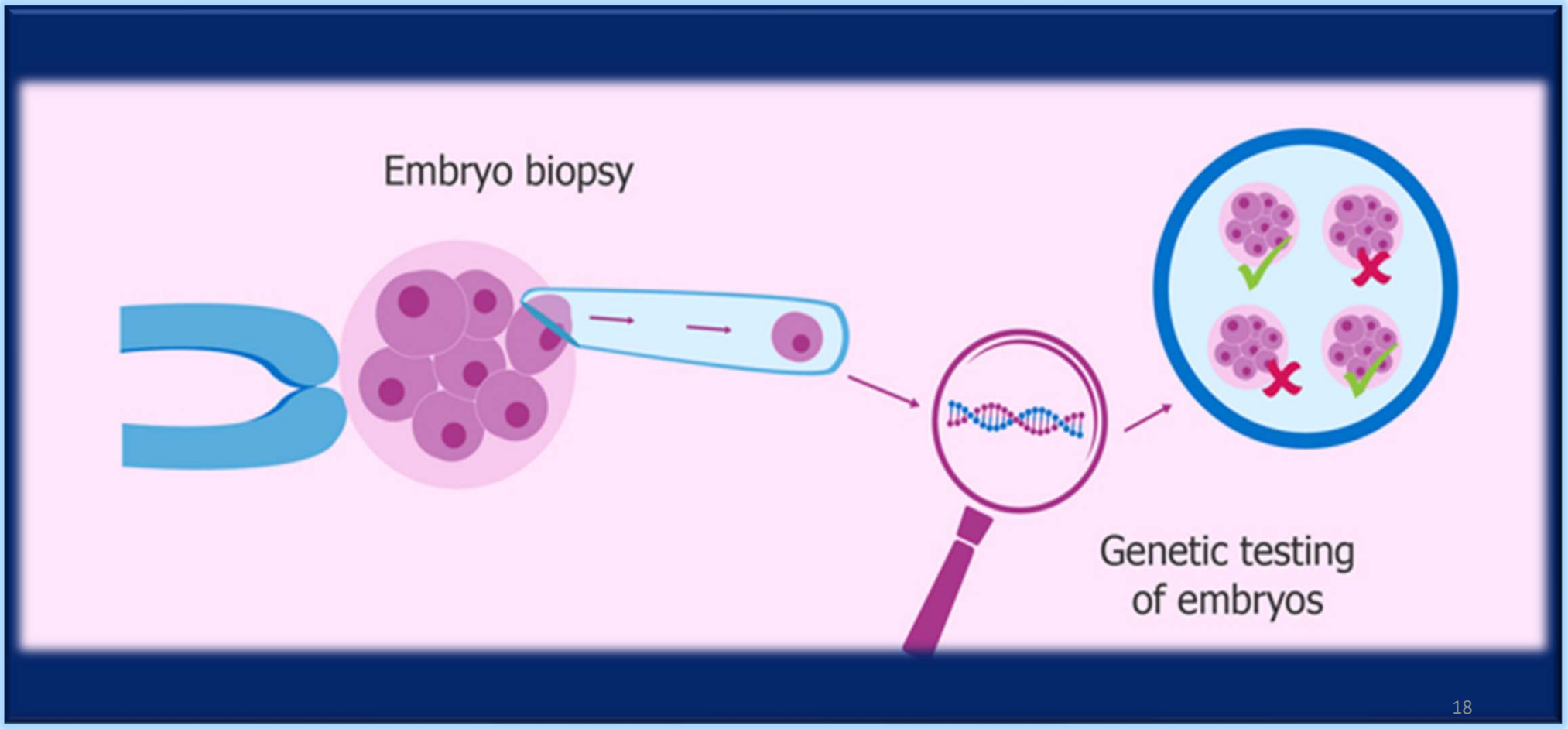
The **analysis** of **embryo physiology** through **non-invasive means** will be of value in the prospective selection of the most viable embryos within the cohort.

Key biomarkers will include:

- **Oxygen consumption**
- **Glucose utilization**,
- **Key amino acids**
- **Lactate**

The **technology** for **such works** are **not readily available** to date.

Embryo Selection
Preimplantation Genetic Testing



Preimplantation Genetic Testing (PGT)

PGT

- **PGT-A**
  (Aneuploidy)- Formerly known as **PGS** (Preimplantation Genetic Screening)
- **PGT-M**
  (Monogenic disease)
- **PGT-SR**
  (Structural Rearrangement)

Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)

- Used to test for a **specific genetic pathogenic variant** (mutation) associated with a **known diagnosis** or **known predisposition** within a family.

- It does **not** test for **all single gene disorders** at once and will **not detect de novo pathogenic variants**.

This technique examines **embryos** using either **cytogenetic** or **molecular techniques** for

1 **Single-gene disorders**

e.g.,
Huntington disease, cystic fibrosis, fragile X syndrome

including those that are **autosomal dominant** and **recessive** or **X-linked**

2 **Hereditary cancer syndromes**

e.g.,
hereditary breast and ovarian cancer, Lynch syndrome

Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)

Another application of PGT-M

It can be used to identify
human leukocyte antigen-compatible, **unaffected** **embryos** gestated with the goal of allowing **ill family members** to **receive** **compatible bone marrow transplants** or **cord blood transfusions**.

- Preimplantation genetic testing-monogenic uses **only a few cells** from the **early embryo**, usually at the **blastocyst stage**.
- **Misdiagnosis** is **possible** but **rare** with modern techniques.
- **Confirmation** of **PGT-M results** with **CVS** or **amniocentesis** should be offered.

Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR)

- Used to **test embryos** that are at risk for **chromosome gains** and **losses** related to **parental structural chromosomal abnormalities** (eg translocations, inversions, deletions, and insertions).
- **Genetic counseling** and discussion of possible preimplantation genetic testing should be offered when a structural rearrangement is discovered in a parent.
- At this time, it **cannot differentiate** between an embryo that has a **normal karyotype** and an embryo that **carries a balanced form of the familial chromosome rearrangement**.
- Individuals who carry a **balanced chromosome rearrangement** involving **imprinted genes** (eg, **13;14 robertsonian translocation**) are **at risk** for abnormalities related to **uniparental disomy**, which **cannot be excluded by all methods of PGT** analysis.
- Because of these **limitations**, and the fact that this testing method uses only a few trophectoderm cells, **confirmation** of PGT-SR results with **CVS** or **amniocentesis** should be offered.

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

Aneuploidy

➢ **A leading cause of :**

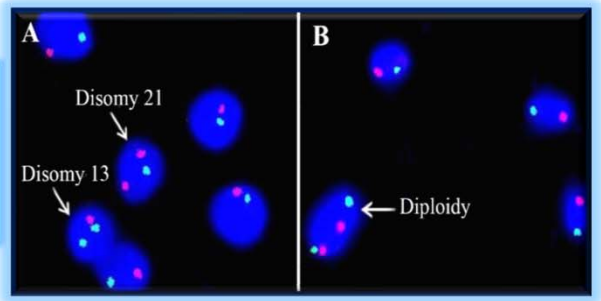
- **Implantation failure**
- **Miscarriage**
- **Congenital abnormalities**
- **A significant cause of ART failure**

Preclinical evidence of PGT-A indicates that
the **selection** and **transfer** of **euploid embryos** during ART should **improve clinical outcomes.**

Many women **failed** to **achieve pregnancy**
despite
transfer of **morphologically optimal** embryos

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

The original technique used **fluorescence in situ hybridization (FISH)**
But
was **limited** to just **a few chromosomes**.



The initial interest in **PGT-A** through **FISH** was tempered
by
the publication of **randomized studies** that **did not find improved IVF outcomes**.

At the time, **several major medical societies**
subsequently released opinions **discouraging routine use of PGT-A**.

Moreover, biopsy of the **early cleavage stage** embryo (day **3**) appears to
negatively affect implantation potential.

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

➢ In an effort to continue the quest toward **higher live birth** rates and **lower multiple gestation rates** in IVF, ongoing research pursued emerging techniques.

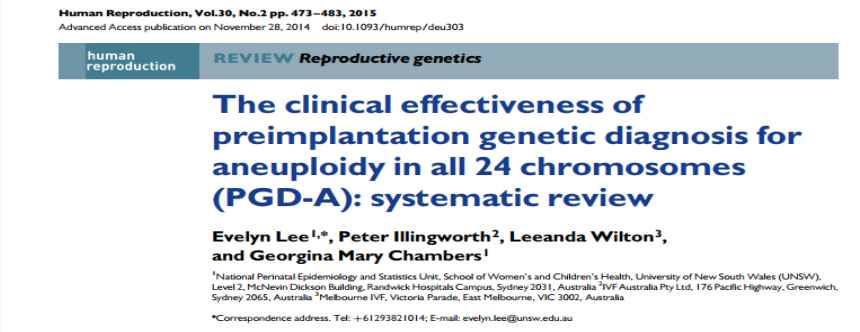
These involved..

- Biopsy of the **multiple cell trophectoderm** (future placenta) of the **blastocyst**.
- In addition, several **platforms** capable of **testing all chromosomes** have been developed.
- PGT-A now uses various techniques such as

array comparative genomic hybridization (aCGH) and **next generation sequencing (NGS)**.

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

A **systematic review** examined the **clinical effectiveness** of **PGT-A** and found **three randomized controlled trials** that reported **higher pregnancy rates** in **younger patients** with **no previous failed IVF attempts**;
however,
these were **small studies** with **substantial limitations**.



Human Reproduction, Vol.30, No.2 pp. 473–483, 2015
Advanced Access publication on November 28, 2014 doi:10.1093/humrep/deu303

human reproduction REVIEW *Reproductive genetics*

The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review

Evelyn Lee[1,*], Peter Illingworth[2], Leeanda Wilton[3], and Georgina Mary Chambers[1]

[1]National Perinatal Epidemiology and Statistics Unit, School of Women's and Children's Health, University of New South Wales (UNSW), Level 2, McNevin Dickson Building, Randwick Hospitals Campus, Sydney 2031, Australia [2]IVF Australia Pty Ltd, 176 Pacific Highway, Greenwich, Sydney 2065, Australia [3]Melbourne IVF, Victoria Parade, East Melbourne, VIC 3002, Australia

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- Another **randomized controlled trial** found that women **aged 38–41** had **significantly higher live birth rates** and **lower miscarriage rates** after PGT-A, as well as a **shorter time to pregnancy**.

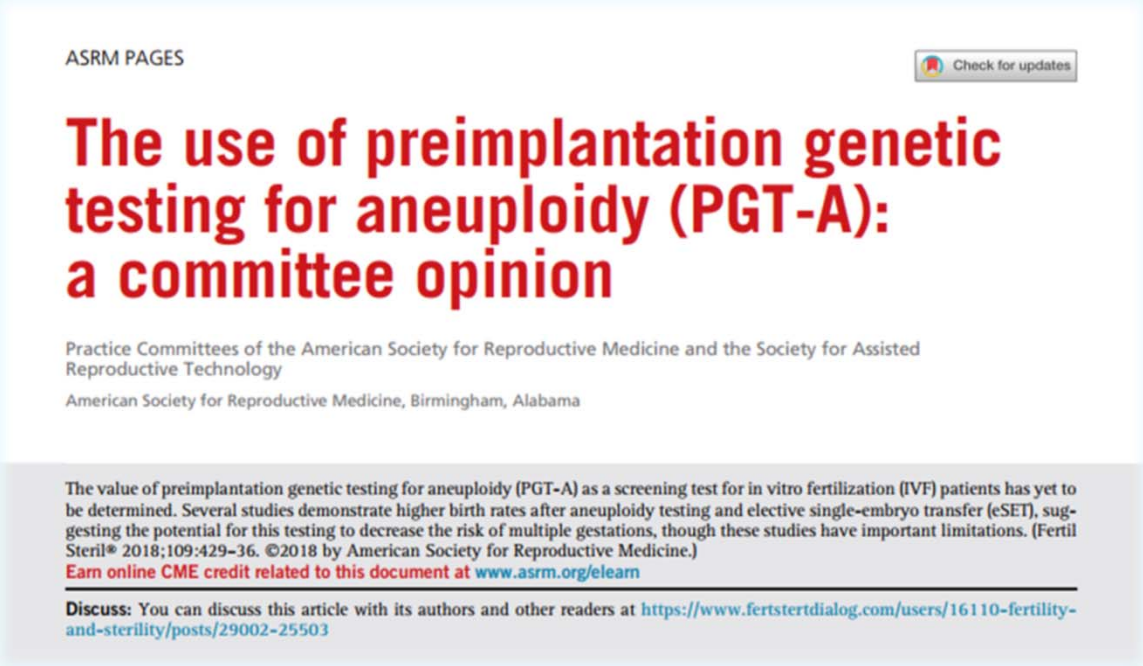
However,

the comparison is problematic because in the PGT-A group, **32% of patients** did **not** have **an embryo to transfer**.

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

- After a comprehensive review, **ASRM** published a **practice guideline** in **March of 2018** concluding that:

"There is insufficient evidence to recommend the routine use of PGT-Ain all infertile women."



ASRM PAGES

Check for updates

The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

The value of preimplantation genetic testing for aneuploidy (PGT-A) as a screening test for in vitro fertilization (IVF) patients has yet to be determined. Several studies demonstrate higher birth rates after aneuploidy testing and elective single-embryo transfer (eSET), suggesting the potential for this testing to decrease the risk of multiple gestations, though these studies have important limitations. (Fertil Steril® 2018;109:429–36. ©2018 by American Society for Reproductive Medicine.)

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Preimplantation Genetic Testing for Aneuploidy (PGT-A)

- In addition, the **ideal genetic testing platform** to analyze **all chromosomes** has not yet been established.
- **Worldwide randomized controlled trials** are needed to determine **which patient cohorts**, if any, may **benefit** from PGT-A.
- Traditional **diagnostic testing** or **screening** for **aneuploidy** should be offered to **all patients** who have had **PGT-A**, in accordance with recommendations for all pregnant patients.

Is PGT-A with analysis of all chromosomes during ART **clinically** and **cost-effective**?

The majority of published studies **comparing** a strategy of PGT-A with **morphologically** assessed embryos have reported a **higher implantation rate per embryo** using **PGT-A**,

But..

Insufficient data has been presented to evaluate the **clinical** and **cost-effectiveness** of PGT-A in the **clinical setting**.

PGT-A: Yes


Live Birth Rate
per transfer
Cumulative Live Birth Rate
per Retrieval

PGT-A: NO


Cumulative Live Birth Rate
per Retrieval
Live Birth Rate
per transfer

Does PGT-A Improve IVF Success Rate?

There is not always an easy answer..

It is a **selection tool** for embryos when we have the **luxury of choice**.

It may lead to:

- **Higher pregnancy rate per transfer**
- **Lower miscarriage rate in certain age groups**
- **Possibly lower cumulative pregnancy rates per retrieval**

We should consider:

- Time to pregnancy??
- Costs??

- There are pros & cons, and the relative advantages and disadvantages can vary based on **specific situations**.

Limitations of PGT-A

Biological Limitations

- Mosaicism

Technical Limitations

- Whole Genomic Amplification (WGA)
- Allelic Dropout and Uneven Amplification
- Resolution (Breadth of coverage)
- Fidelity (Depth of coverage)

Analytical Limitations

- Bioinformatics algorithms and variation in laboratory protocols – Artificial intelligence (AI)

Detrimental impact of Invasive embryo biopsy technique

- Blastomere biopsy (Cleavage stage – Day 3) versus trophectoderm biopsy (Blastocyst stage – Day 5, 6, 7)

Biological Limitations

Mosaicism

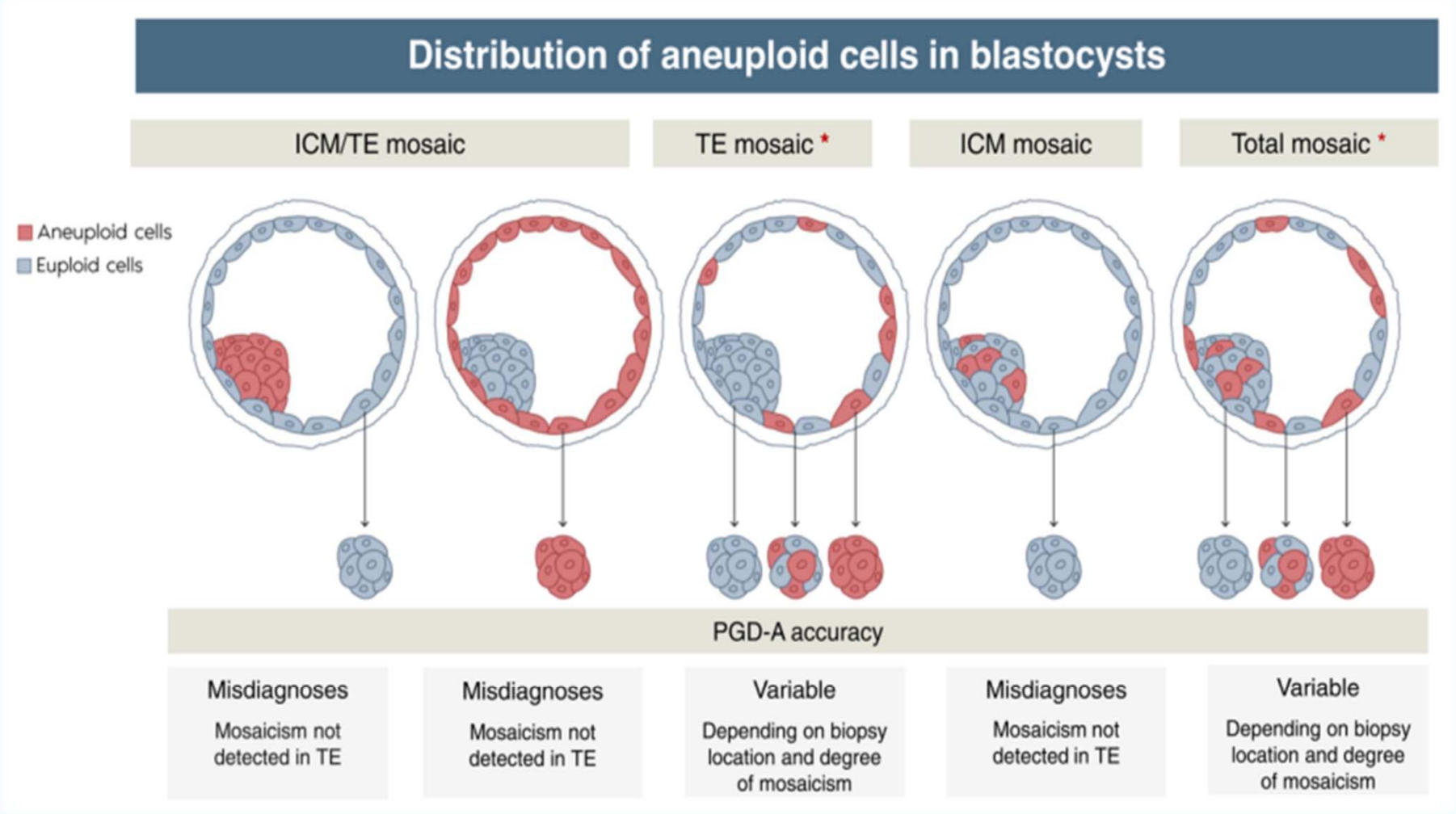
Embryonic mosaicism & Self-correction

Human IVF embryos **often** contain
cell lineages having **distinctly different chromosomal makeups**, termed **mosaicism**.

Unlike meiotic errors that are uniformly inherited by all embryonic cells,
mosaicism stems from **errors** in **post-fertilization mitotic cell divisions** that stochastically affect any chromosome and any cell within the embryo.

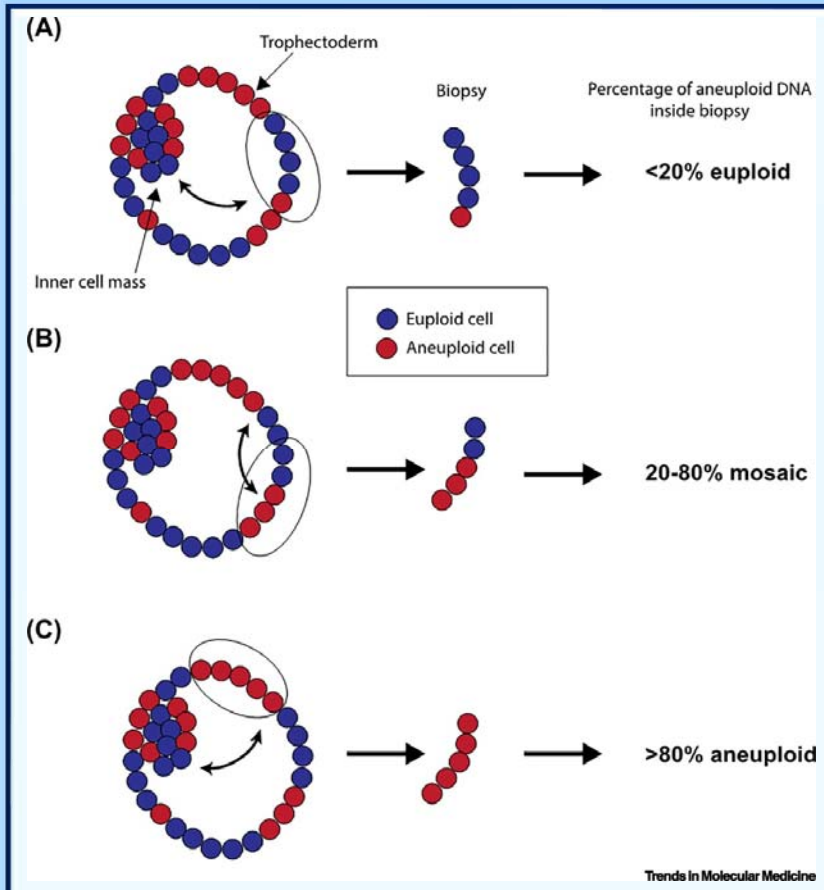
Studies in the mouse model show that
mosaic embryos '**self-correct**' through clonal depletion of abnormal cells to produce normal offspring.

This may also apply to **humans** since
transfer of mosaic embryos during IVF can produce **apparently normal offspring**,
although it remains unclear whether, or to what extent, mosaicism persists in neonates.



Chromosome abnormalities in human embryos, Rubio et al., **Reproduction**, **2020**, https://doi.org/10.1530/REP-20-0022

Embryonic mosaicism



The **inadequacy** of a **single 5–10 cell biopsy** is supported by theoretical **mathematical modelling**, which estimated that **at least 27 cells** would be needed for **reasonable predictive power**.

One **concern**, therefore, especially <u>for patients with few embryos</u>, is :
mistakenly discarding usable embryos
because
abnormalities identified in the TE are not replicated in the ICM.

Trends in Molecular Medicine **2021** 27731-742DOI: (10.1016/j.molmed.2020.11.009)

Embryonic mosaicism

- **Comprehensive chromosome screening** involves molecular genetic approaches including **aCGH** and **NGS**.
- Biopsied cells are **lysed together** in a composite sample for analysis. Importantly, therefore, the output from CCS represents the **mean of 5–10 cells**.
- CCS platforms have **differing abilities** for **detecting mosaicism**.

- **NGS** can detect **low levels** (**20%**) of mosaicism
- **aCGH** can detect mosaicism only if present at **higher levels** (>**40–50%**)

- The **two platforms** can therefore **generate different results** for **the same sample**,
- For instance, **37%** of stored TE biopsies initially diagnosed as **euploid** by **aCGH** were found to be **abnormal** when **re-analyzed using NGS**.

Technical Limitations

Allelic Dropout and Uneven Amplification

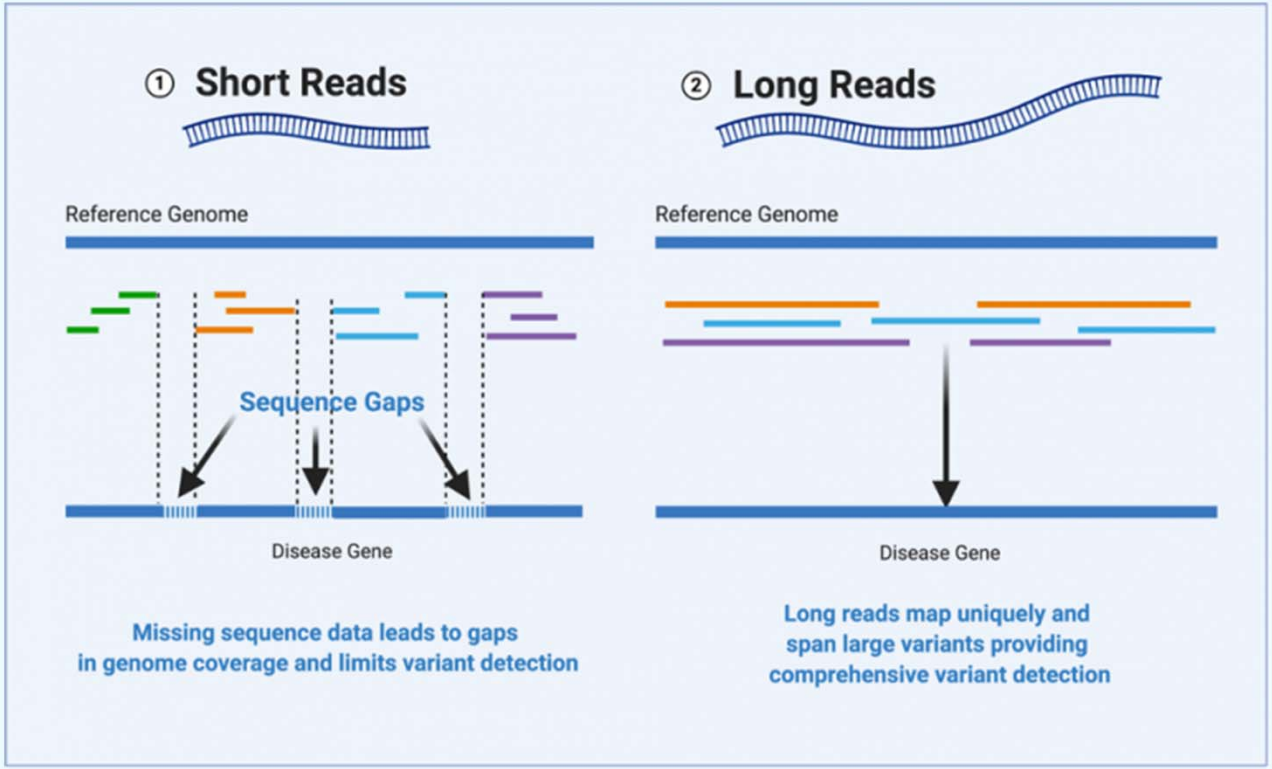
One of the **major drawbacks** of WGA in human PGT → **Incomplete genomic coverage** named as **allelic** or **locus dropout**

What is ADO?

- Once the **template DNA concentration** falls **below** a **certain input level**, the **probability** of obtaining a **complete template genome**, especially with the expectation of **uniform amplification**, **decreases** dramatically.
- At very **low initial DNA concentrations**, **random** and difficult to predict (stochastic) effects dictate whether a **particular genomic region** will be **amplified** or **not**.
- **The so-called 'Monte Carlo effect' states that "the lower the abundance of any template, the** less likely its **true abundance** will be reflected in the amplified product".
- Targeted amplification of **WGA-amplified material** demonstrated that **ADO rates** almost **double** in comparison to **direct PCR without WGA**.

Resolution (Breadth of coverage)

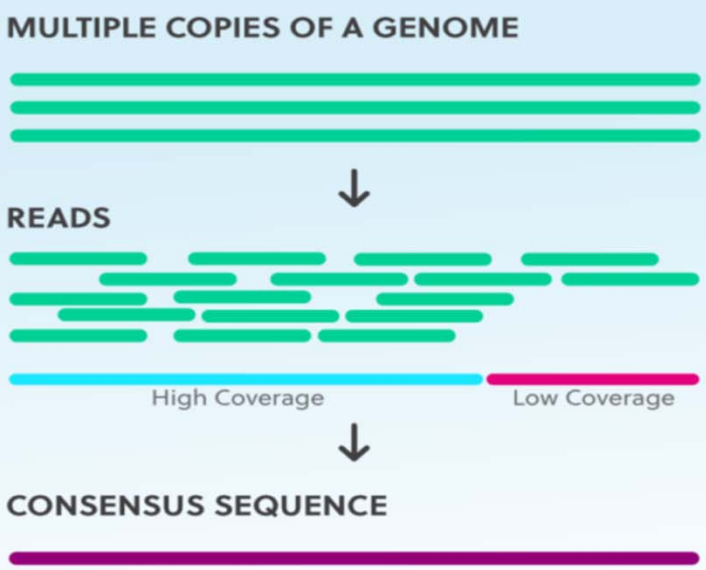
Segmental aneuploidies (CNVs), currently **smaller than 5 Mb**, will be missed.



https://www.hudsonalpha.org/hudsonalpha-researchers-use-highly-accurate-long-read-sequencing-technology-to-help-diagnose-rare-disease/

Fidelity (Depth of coverage)

Lower cost = Lower accuracy

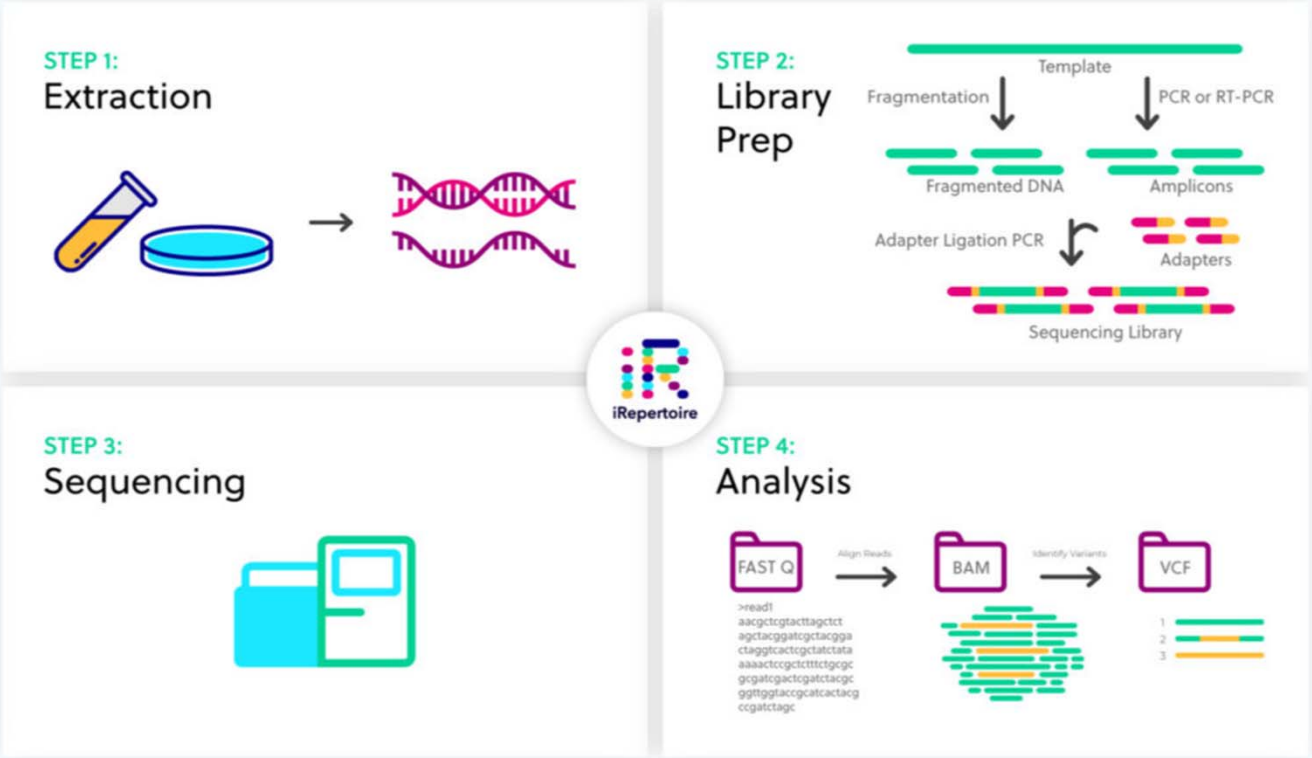


https://irepertoire.com/ngs-considerations-coverage-read-length-multiplexing/

Analytical Limitations

Bioinformatics algorithms and variation in laboratory protocols – Artificial intelligence (AI)

The same raw data can be interpreted differently in different protocols.



https://irepertoire.com/ngs-overview-from-sample-to-sequencer-to-results/

Detrimental impact of Invasive embryo biopsy technique

Embryo biopsy

➢ Currently, PGT relies on **invasive approaches** to obtain DNA from embryos generated by IVF.

➢ Embryo biopsy may be carried out

- at the **cleavage stage**, i.e. **removal** of **one** to **two blastomeres**

or

- at the **blastocyst stage**, i.e. **removal** of **five to 10 trophectodermal cells**

➢ Both techniques present **technical advantages** and **disadvantages**.

Non-invasive PGT (niPGT)

Recently, the **detection** of **cell-free nucleic acids (cf-DNA)** in **biological fluids** has opened new perspectives for the development of **non-invasive tests** in reproductive medicine.

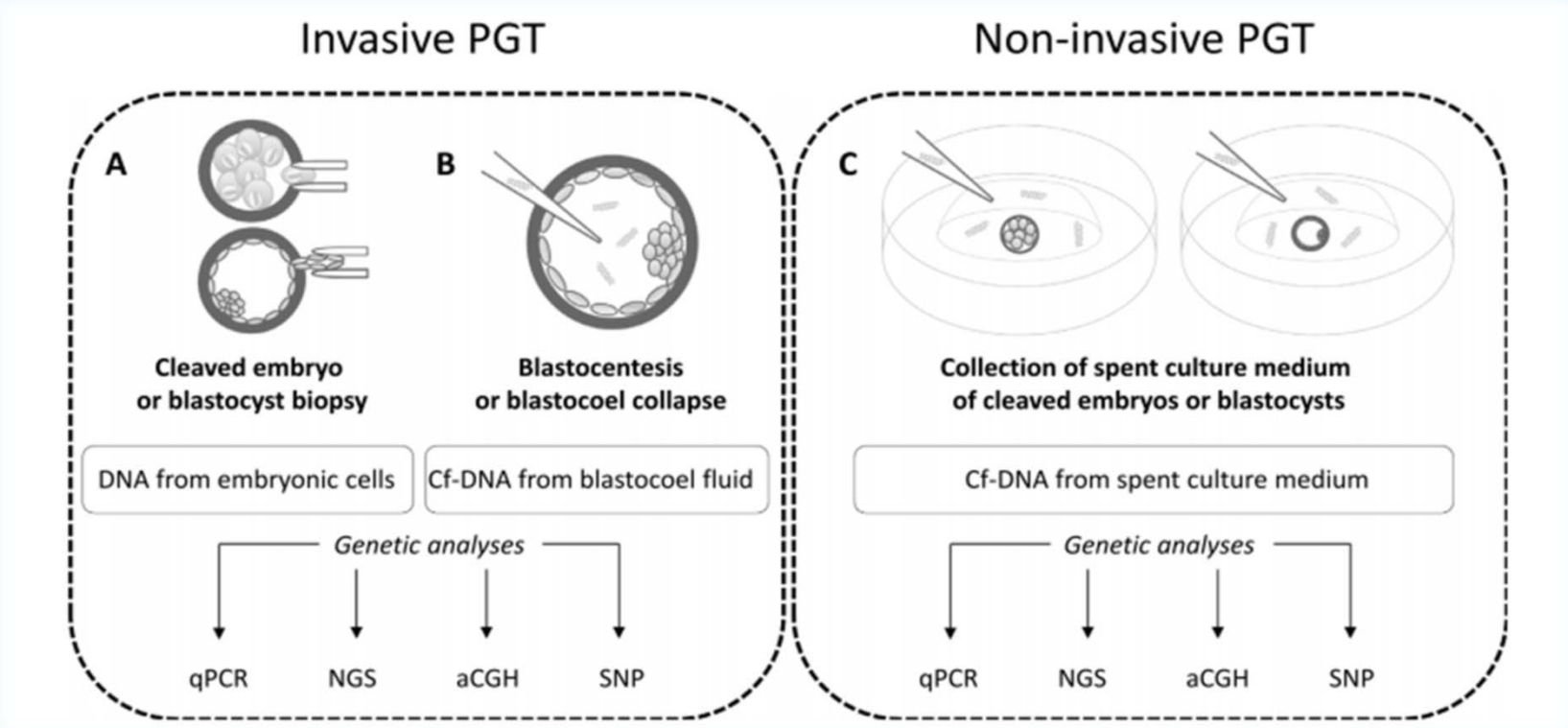
cf-DNA has been detected in and in:

Blastocoel fluid (BF)
Blastocentesis

spent culture medium (SCM)
of
developing embryos generated by IVF

Potential risk of
compromising embryo development, **long-term consequences** and associated **high costs** with the biopsy procedure have encouraged scientists to investigate options for a niPGT correlating genetic material found in BF and SBM to the one in TE following the biopsy.

Invasive and non-invasive preimplantation diagnostic testing (PGT)



Is cell-free DNA in spent embryo culture medium an alternative to embryo biopsy for preimplantation genetic testing? A systematic review, Brouillet et al., **RBMO**, **2020,** https://doi.org/10.1016/j.

cf-DNA from SCM seems to be the best option for noninvasive PGT.

Specific marker detection

Many studies that analyzed the presence of the **soluble human leukocyte antigen G** (**sHLA-G**) concluded that the standardized sHLA-G assay has the potential to identify **the most competent embryos for implantation**.

✓ An **increased secretion** of this biomarker was found to be associated with a **successful pregnancy**.

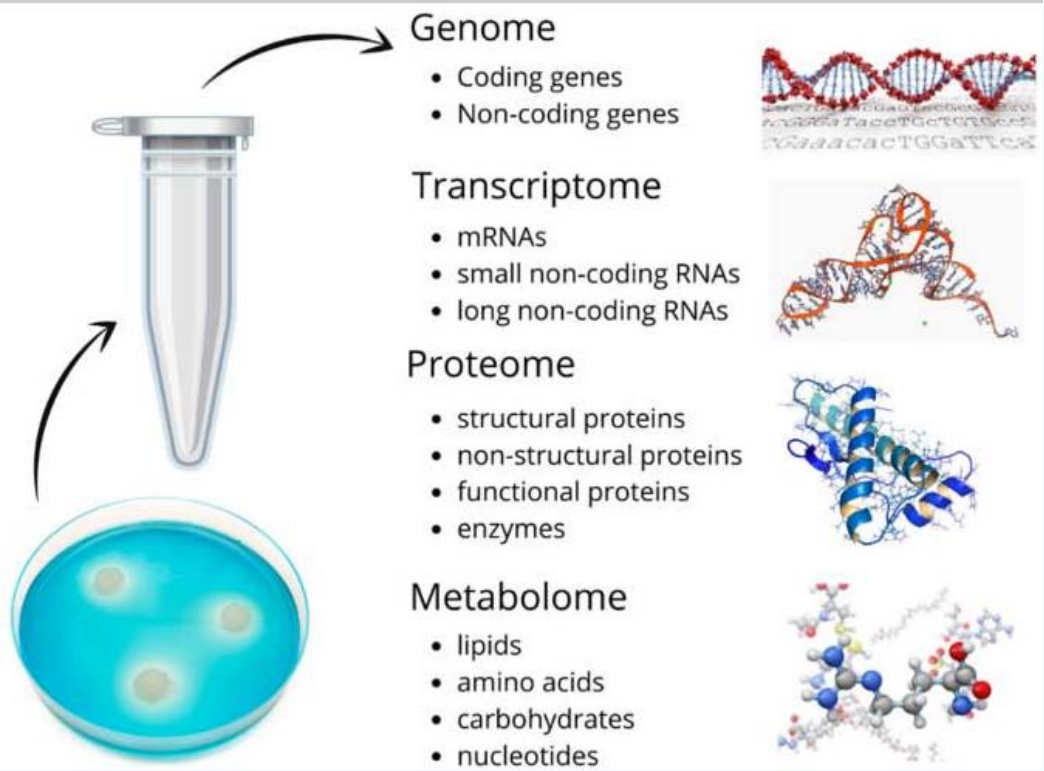
The secretion of **sCD146**, as measured by ELISA, was shown to be significantly ($p = 0.00624$) associated with a **lower implantation potential**.

An miRNA (***miR-142-3p***) to be significantly correlated with **implantation failure**; thus, embryos producing this miRNA could be **excluded from implantation**.

Proteomics and Metabolomics of Spent Blastocyst Media

- **High-throughput proteomics** and **metabolomics** technologies are valuable tools to **study** the **molecular components of biological systems**.
- The **biggest advantage** of such technology is that **it can differentiate between embryos that appear morphologically identical**.
- It also has the **potential** to identify the **ploidy status noninvasively** prior to transfer or verification for a later frozen cycle.
- However, one of the issues with metabolomics and proteomic **mass spectrometric profiling** is the **scale** and **amount** of **generated data** from a single sample.
- It requires cutting-edge data processing pipelines to effectively extract the meaning from the generated data and to build meaningful algorithms optimized for the desired outcome.
- Another important factor to consider when analyzing SBM is **environmental factors** such as **temperature**, **humidity**, and **air quality**. They have been shown to **affect epigenetics** and, subsequently, **embryo morphology**, **developmental kinetics**, **physiology** and **metabolism**.

Schematic of materials found in SBM that pose the diagnostic potential for embryo quality and ploidy status



Current Advancements in Noninvasive Profiling of the Embryo Culture Media Secretome, Zmuidinaite etal., **International journal of Molecular Science**, **2021**, doi: 10.3390/ijms22052513

Personalized medicine in endometrial receptivity

While **embryology** and **embryo transfer technologies** have **improved** considerably over the past 30 years, the **efficacy of IVF** remains **low** worldwide, with current **live birth rates** of **25–30%** per started cycle.

Since the inception of this field, the **oocyte/embryo** has remained the **central focus**.

In contrast, the **maternal endometrium** was considered a **passive part of the reproductive** process: a 'good embryo' (or four or five) was all that mattered.

Despite its many advances and achievements, reproductive medicine has **long neglected the endometrial factor**.

Successful implantation requires:

- Receptive endometrium
- A functional embryo at the blastocyst developmental stage
- A synchronized dialog between maternal and embryonic tissues

Window of implantation (WOI)

is **not constant** in all women

It's rather personalized

Even **displaced** in **one** **out of** **four** **recurrent implantation failure** (**RIF**) patients.

Endometrium Receptivity

The **endometrium** is a **highly dynamic** tissue.

The **embryo** is **unable** to **adhere** to **endometrium** through **most of the menstrual cycle** in humans, except during a **short**, **self-limited period** in which the endometrial tissue acquires a **functional** and **transient receptive status** that permits **blastocyst adhesion**.

Endometrium undergoes **physiological changes** in response to:

- **Steroid hormones**
- **Genetic factors**

Undergoes specific **structural**, **functional**, and **morphological** **changes** to create **a receptive status** in a **synchronized manner** with the **arrival** of the **implanting blastocyst** during the **window of implantation** (WOI)

What is Endometrial receptivity?

Endometrial receptivity is the **endometrium's capability** to **let** an **embryo implant**.

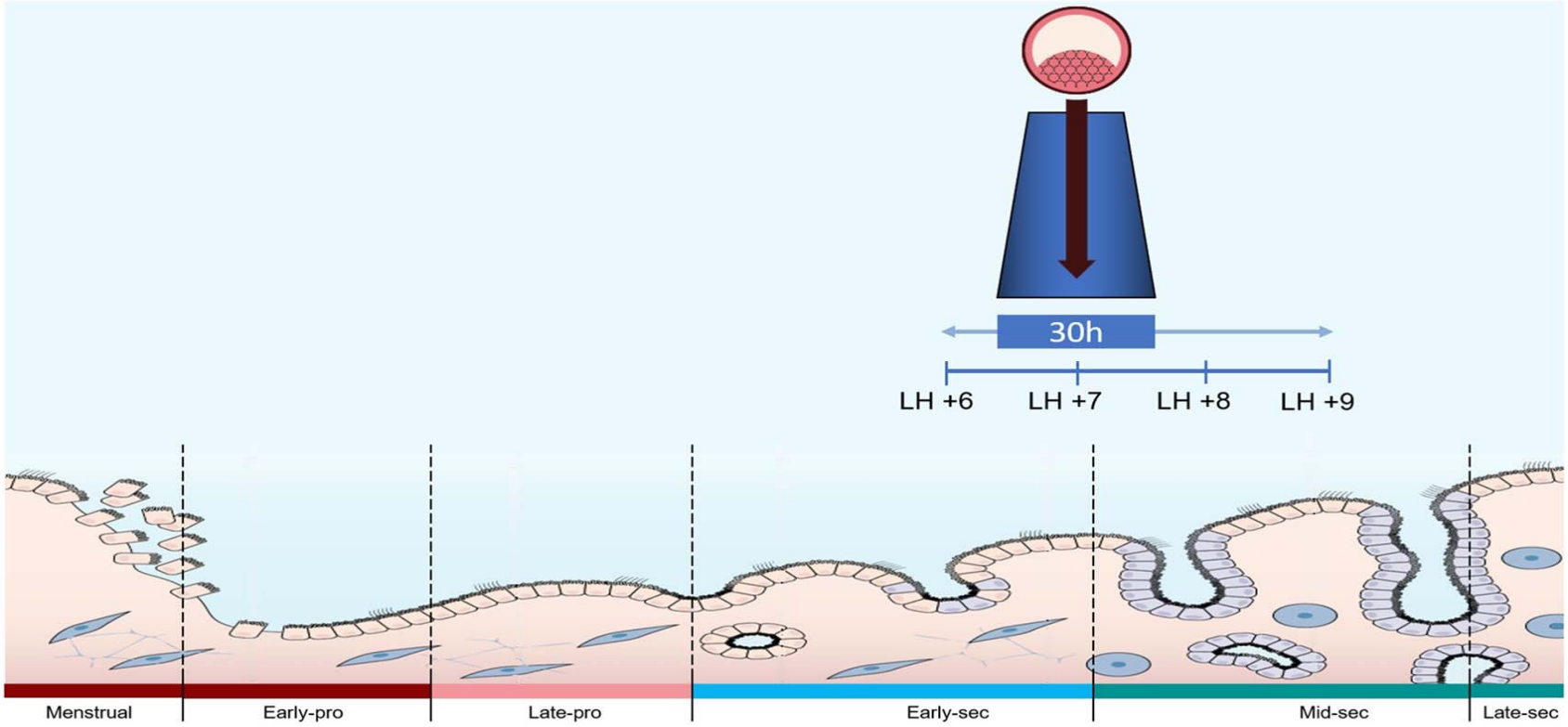
Endometrium allows the **embryo** to **adhere** to itself only during a specific phase in the menstrual cycle, termed as the **receptive phase** or the **window of implantation**.

In a **normal** menstrual cycle, the endometrium reaches the **receptive state** around **6 to 9 days after** the **LH peak**.

This period of **5 days** spans day **19** to day **23** of the **menstrual cycle** in humans.

The WOI lasts **30–36 hours** and, depending on the patient, occurs between **LH + 6 to LH + 9** in **natural cycles** or from **P + 4 to P + 7** in **hormonal replacement therapy** (HRT) **cycles**.

Diagram representing duration and timing of the window of implantation (WOI)



Hum Reprod Open, Volume 2021, Issue 2, **2021**, hoab011, https://doi.org/10.1093/hropen/hoab011

Endometrium Receptivity

The purpose of the tight regulation of endometrial receptivity can be partly explained by the fact that the **endometrium during the receptive phase** is also **able to assess embryo quality**.

It therefore serves not only as the **receiver and growing ground** for embryos but can also **expel embryos unlikely to succeed**.

This has been shown **in vitro** where **endometrial stromal cells** could be shown to **migrate** towards **healthy embryos**
whereas
migration was inhibited by low quality embryos.

In **normal** IVF/ICSI cycles

Embryos are **transferred** on the **same day** (day 2, 3, or 5) each time considering a **fixed WOI** in **every patient**.

But..

When **good quality** **embryos fail to implant** **repeatedly**,
endometrial receptivity should be checked as the chances of it being **displaced** are high in such patients.

Endometrium Receptivity

Histological, **biochemical**, and **ultrasound markers** of endometrial receptivity are:

- **Subjective**
- **Lack of accuracy and a predictive value**

Receptive phase is marked by **several molecular events** which **prepare** the **endometrium** to **host** the **embryo**.

Endometrial transformation at **structural** and **functional** levels is brought out by **alterations** in the **expression of specific genes**.

Interest in **identity** and **functions** of these **genes** has prompted several investigations in the recent years.

Gene expression signatures

✓**LIF**, **HOXA10**, **MUC1** and **integrin αvβ3** are all examples of **individual markers** that have been **investigated** in the context of **endometrial receptivity**.

Studies into **individual markers** are important to elucidate their specific role and function,
but
there is no single factor that can **explain the full picture** of **endometrial receptivity**.

With the development of **global gene expression studies and bioinformatics**,
more comprehensive overviews of the molecular events governing endometrial receptivity can now be made.

Precise Identification of Endometrium Receptivity

A **genomic tool** named the **endometrial receptivity array** (**ERA**), based on a **customized microarray**, was developed.

Also, a specially trained **bioinformatic prediction computer algorithm** was created.

Identify the **WOI timing** of the endometrium when it is specifically **receptive** to **blastocyst adhesion**.

Endometrial receptivity array (ERA)

ERA consists of a **customized array**
containing **238 genes** **expressed at the** **different stages of the endometrial cycle**
and is coupled to a **computational predictor.**

That is able to **identify the receptivity status of an endometrial sample**
and
diagnose the personalized WOI (pWOI) of a given patient.

The **accuracy** of **ERA** is **much higher** compared to other methods in **predicting a receptive endometrium**.

Sensitivity: 0.99758

Specificity: 0.8857

The **results** are **reproducible** in the same patients **29–40 months** after the first test.

Endometrial receptivity array (ERA)

ERA requires **endometrial biopsy** from a woman during her **natural cycle** or **hormone replacement therapy** (HRT) **cycle** at a specific time.

Natural cycle biopsy

On luteinizing hormone (LH) surge + day 7 (**LH + 7**)

HRT cycle biopsy

After five full days of progesterone (**P + 5**)

The endometrial pipelle sampling procedure

Pipelle

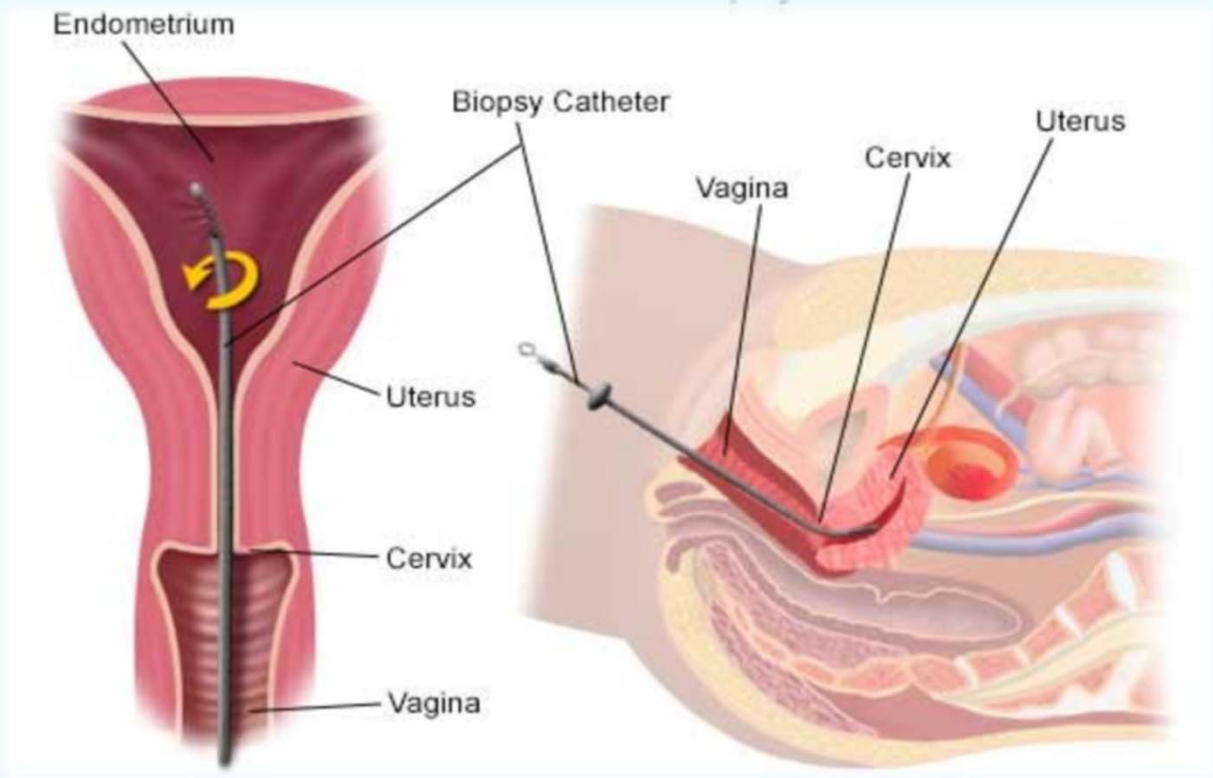


Image kindly supplied by Krames StayWell 780 Township Line Road, Yardley, PA 19067 267-685-2500

The status of peak endometrial receptivity is diagnosed by the ERA computational predictor.

➢ The **ERA test result** is given as **Receptive** (**R**) or **Non-Receptive** (**NR**).

NR test results are usually reported as
Pre-receptive or **Post-receptive**
and a recommendation is also to be given for a putative pWOI.

In some cases, to **validate this pWOI**,
a **second endometrial biopsy** and **ERA analysis** was performed after the recommendation of the ERA classifier.

➢ The **second endometrial biopsy**
should be performed with a **similar protocol** and the biopsy is taken **on the recommended day**.

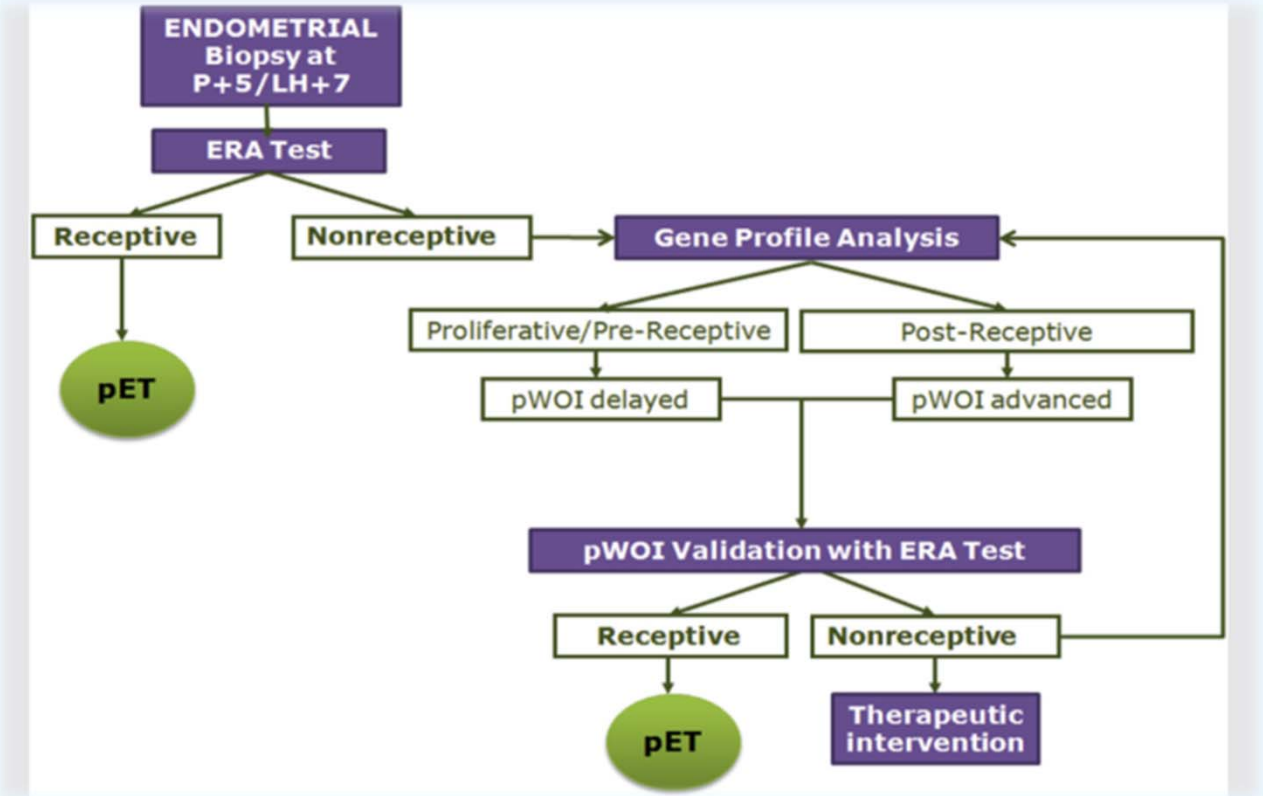
Pre-receptive

The biopsy is to be taken on the recommended day **after the previous biopsy day**.

Post-receptive

The biopsy is to be taken on the recommended day **before the previous biopsy day**.

Clinical algorithm for personalized embryo transfer (pET)



The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure, Ruiz-Alonso et al, **Fertility and Sterility, 2013**

ERA is convenient and usually well tolerated by patients except for:

- **Temporary mild abdominal pain**
- There is a **theoretical risk** for **infection** while taking an endometrial biopsy, but in clinics with best practices, it is seldom reported.
- In addition to the **cost of the first ERA** test, the patient has to bear the **cost of the subsequent ERA** test when the first test shows **NR pattern** along with the recommendation of a second ERA test.
- The patient has to **wait for her embryo transfer till the ERA results**, which usually come **after 3 weeks of biopsy**.
- The subsequent embryo transfer should be done in a similar protocol as used during the receptive ERA cycle, which in fresh self-IVF cycles involves an **extra cost of freezing and thawing of embryos**.

Gene expression signatures
(Number of genes tested)

The **number of global gene expression studies** relating to endometrial receptivity

quite **vast**

Produce
numerous candidates
for **gene expression signatures** and **markers**

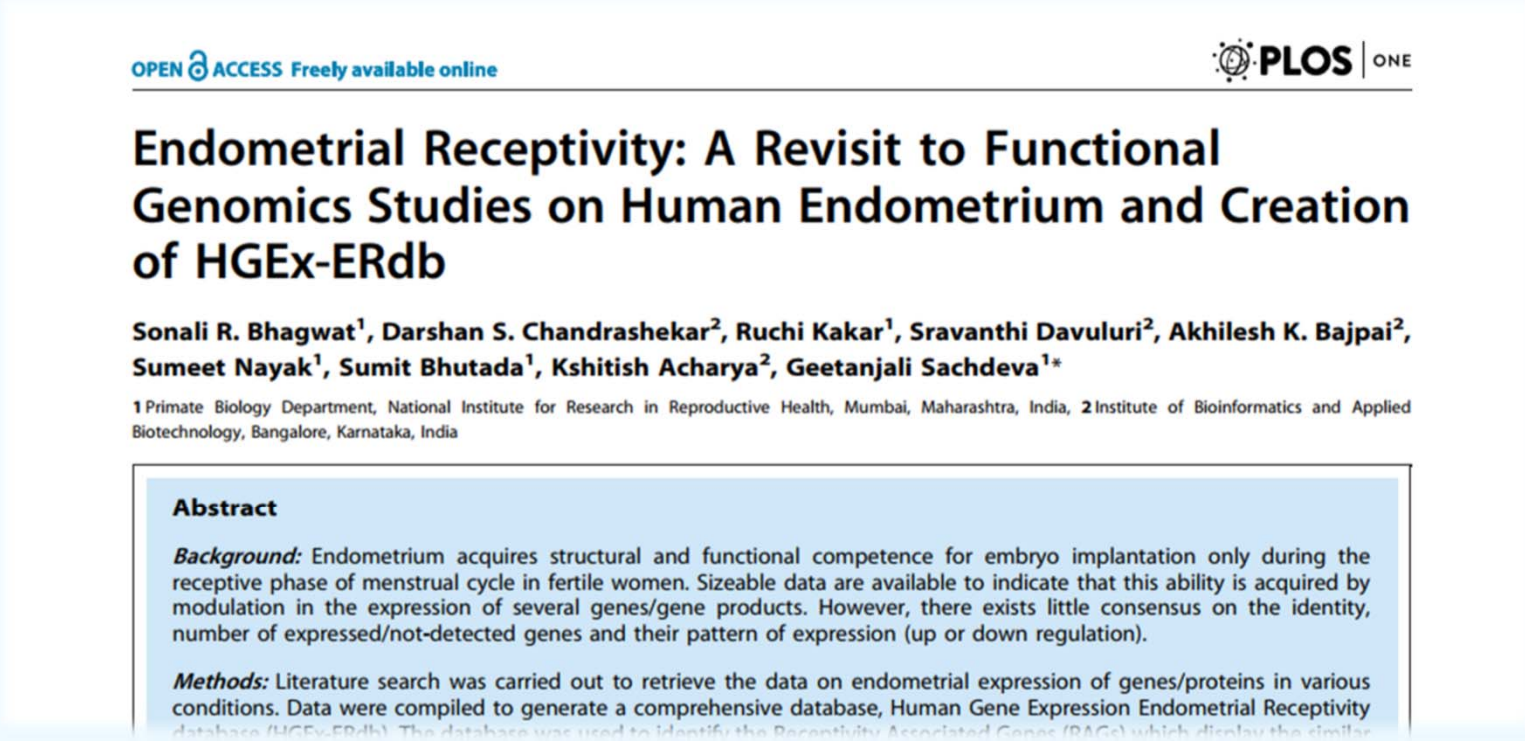
But

The **overlap between studies have been small**

This has been much attributed to **heterogeneity in study designs between studies** concerning such factors as **patient/ healthy volunteer characteristics**, **sample collection** and **technological platforms**, as well as **bioinformatic tools for data analysis**.

- Efforts have despite this been made to **pool the available data** in order to **decipher** the **bigger patterns**.

In Bhagwat et al.'s Human Gene Expression Endometrial Receptivity database (HGEx-ERdb)
179 genes were identified as **related to endometrial receptivity**
with
151 up-regulated and **28 down-regulated**.



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PLOS ONE

Endometrial Receptivity: A Revisit to Functional Genomics Studies on Human Endometrium and Creation of HGEx-ERdb

Sonali R. Bhagwat[1], Darshan S. Chandrashekar[2], Ruchi Kakar[1], Sravanthi Davuluri[2], Akhilesh K. Bajpai[2], Sumeet Nayak[1], Sumit Bhutada[1], Kshitish Acharya[2], Geetanjali Sachdeva[1]*

1 Primate Biology Department, National Institute for Research in Reproductive Health, Mumbai, Maharashtra, India, 2 Institute of Bioinformatics and Applied Biotechnology, Bangalore, Karnataka, India

Abstract

Background: Endometrium acquires structural and functional competence for embryo implantation only during the receptive phase of menstrual cycle in fertile women. Sizeable data are available to indicate that this ability is acquired by modulation in the expression of several genes/gene products. However, there exists little consensus on the identity, number of expressed/not-detected genes and their pattern of expression (up or down regulation).

Methods: Literature search was carried out to retrieve the data on endometrial expression of genes/proteins in various conditions. Data were compiled to generate a comprehensive database, Human Gene Expression Endometrial Receptivity

Two other data mining studies suggested
148 and **61** genes
as **potential biomarkers** for **endometrial receptivity**.

40 up-regulated and **21 down-regulated**

Tapia *et al. Reproductive Biology and Endocrinology* 2011, **9**:14
http://www.rbej.com/content/9/1/14

REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

Bioinformatic detection of E47, E2F1 and SREBP1 transcription factors as potential regulators of genes associated to acquisition of endometrial receptivity

Alejandro Tapia[1*], Cristian Vilos[2], Juan Carlos Marín[3], Horacio B Croxatto[2,4], Luigi Devoto[1,5]

Abstract

Background: The endometrium is a dynamic tissue whose changes are driven by the ovarian steroidal hormones. Its main function is to provide an adequate substrate for embryo implantation. Using microarray technology, several reports have provided the gene expression patterns of human endometrial tissue during the window of implantation. However it is required that biological connections be made across these genomic datasets to take full advantage of them. The objective of this work was to perform a research synthesis of available gene expression profiles related to acquisition of endometrial receptivity for embryo implantation, in order to gain insights into its molecular basis and regulation.

73 up-regulated and **75 down-regulated**

Reproductive Sciences
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DOI: 10.1177/1933719112442248
http://rs.sagepub.com
SAGE

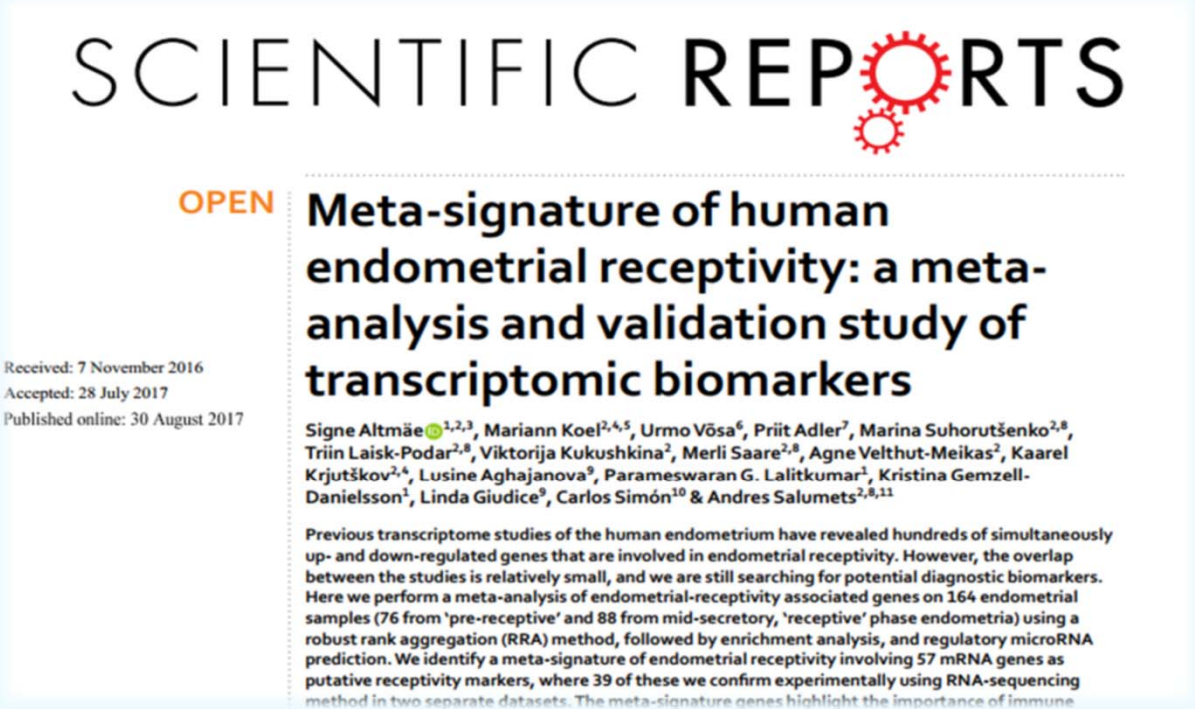
Data Mining of Spatial–Temporal Expression of Genes in the Human Endometrium During the Window of Implantation

Dan Zhang, MD[1], Cuixiang Sun, MD[2], Chengbin Ma, MD[3], Haiyan Dai, MD[4], and Wei Zhang, PhD[1]

Abstract

Previous studies of microarrays have produced mass data that are far from fully applied. To make full use of the available mass data and to avoid redundancy and unnecessary waste, we employed bioinformatics tools GeneSifter and Ingenuity Pathway Analysis (IPA) to mine and annotate 45 microarrays related to endometrium receptivity from GEO (Gene Expression Omnibus) database. In total, 1543 gene sets were found to express differentially, of which 148 highly regulated genes were listed as potential biomarkers of the receptive endometrium. The function and pathway analysis identified the differentially expressed genes primarily involved in immune response and cell cycle. Two networks related to the cardiovascular system and cancers were generated within the genes which changed more than 10-fold. Nine genes were validated by real-time polymerase chain reaction. It was a meaningful exploration of the existing data to acquire useful and reliable information, and our results undoubtedly provided valuable clues for further studies.

Altmae et al. also used a **data mining approach** to create an updated meta-signature of **57 endometrial receptivity genes**.



SCIENTIFIC REPORTS

OPEN

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

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Accepted: 28 July 2017
Published online: 30 August 2017

Signe Altmäe[1,2,3], Mariann Koel[2,4,5], Urmo Võsa[6], Priit Adler[7], Marina Suhorutšenko[2,8], Triin Laisk-Podar[2,8], Viktorija Kukushkina[2], Merli Saare[2,8], Agne Velthut-Meikas[2], Kaarel Krjutškov[2,4], Lusine Aghajanova[9], Parameswaran G. Lalitkumar[1], Kristina Gemzell-Danielsson[1], Linda Giudice[9], Carlos Simón[10] & Andres Salumets[2,8,11]

Previous transcriptome studies of the human endometrium have revealed hundreds of simultaneously up- and down-regulated genes that are involved in endometrial receptivity. However, the overlap between the studies is relatively small, and we are still searching for potential diagnostic biomarkers. Here we perform a meta-analysis of endometrial-receptivity associated genes on 164 endometrial samples (76 from 'pre-receptive' and 88 from mid-secretory, 'receptive' phase endometria) using a robust rank aggregation (RRA) method, followed by enrichment analysis, and regulatory microRNA prediction. We identify a meta-signature of endometrial receptivity involving 57 mRNA genes as putative receptivity markers, where 39 of these we confirm experimentally using RNA-sequencing method in two separate datasets. The meta-signature genes highlight the importance of immune

Though **the number of genes differ** between studies and **the overlap is not complete**,

these efforts are important

in

creating consensus regarding key players and networks in endometrial receptivity.

miRNA

- MicroRNA (miRNA) are **small non-coding RNA** molecules and a fairly recent discovery in RNA biology.
- They are about **21-25 nucleotides** long and are involved in **post-transcriptional gene expression regulation** through **mRNA silencing** and **degradation**.
- They are **expressed in the endometrium** and their role in endometrial receptivity have started to be investigated.
- In the **mid-secretory phase**, miRNAs have been found to be involved in **cell cycle regulation** in **epithelial cells** in a manner that suggests **suppression of cell proliferation**.

miRNA

Comparing miRNA profiles between **biopsies** from day 2 after the LH peak (**LH+2**) and **LH+7**, **differential expression analysis** has further indicated **specific miRNA regulation** in the **receptive phase**.

- **Differences** in **miRNA expression** have also been shown in **endometrial tissue** from healthy women compared to women with recurrent implantation failure (RIF).
- The discovery of miRNA-containing microvesicles/ exosomes in secretions from endometrial cells has further implied a role for miRNA not only in endometrial receptivity regulation but in **embryo-endometrial interaction**.
- Further characterization of miRNA content in endometrial secretions/ uterine fluid could shed light on how secreted miRNA might also function in a **paracrine way** to **regulate endometrial receptivity and implantation**.

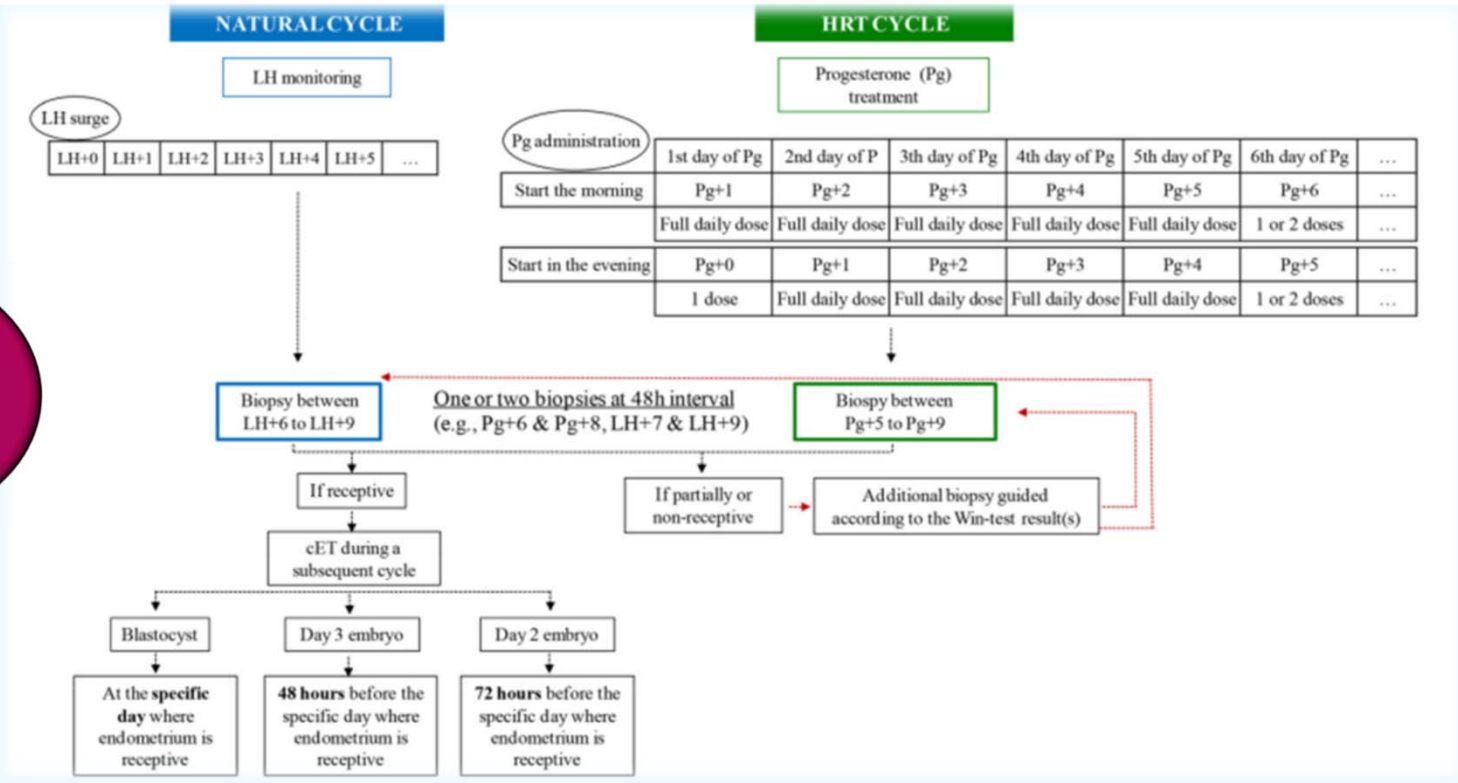
Since the publication of the seminal paper identifying the transcriptomic signature of endometrial receptivity (Díaz-Gimeno et al., 2011),

6 different companies have launched **commercial endometrial transcriptomic tests** under different acronyms with different evidence.

1. **WinTest** from **INSERM** (www.inserm.fr/en) is based on **11 genes** detected using **RT-qPCR**, with **four publications** demonstrating transcriptomic and clinical consistency. (Haouzi et al., 2009; Haouzi, 2015; Bissonnette et al., 2016; Haouzi et al., 2021)
2. **ERPeak** from **Cooper Surgical** (USA) (https://fertility.coopersurgical.com/genomics/erpeak-endometrial-receptivity-test/)
3. **ERMap** from **IGLS** (Spain) (https://www.igls.net/es/services/mapa-de-receptividad-endometrial/) both use **40 genes** with **RT-qPCR** supported by the same paper (Enciso et al., 2018).
4. ERT based on **100 genes** is commercially available from **Yikon** (China) (www.yikongenomics.com) but has **not been reported in a peer-reviewed publication**.
5. **BeREADY** from **Competence Centre on Health Technologies Ltd** (Estonia) (https://beready.ccht.ee/) is based on **67 genes** supported by one publication in collaboration with our group (Altmäe et al., 2017).
6. **BioER** from **Bioarray** (Spain) (https://bioarray.es/es/info/BioEr-TEST-DE-RECEPTIVIDAD-ENDOMETRIAL-60) is based on **72 genes** but has not been supported by a peer-reviewed report or proof-of-concept study.

Using a set of **11 genes**
(*BCL2L10, CD68, TRPC4, SORCS1, FST, KRT18, LAMB3, MFAP5, ANGPTL1, PROK1, and C2CD4B*)
that are **overexpressed** in the **endometrium** during the **implantation window** and that seem to be relevant **candidate biomarkers** of **human endometrial receptivity**

Win-Test



Haouzi, Reproductive Sciences (**2021**) 28:69–78, https://doi.org/10.1007/s43032-020-00252-0

Array versus sequencing

- Technology is rapidly evolving, and critics should update their knowledge at the same pace.
- **Microarray** and **PCR-based clinical tests** are being **replaced** by **NGS technology**.
- In **January 2017**, the **ERA test** was **moved from microarray-based** to **NGS-based technology**.
- **Results of ERA** in the RCT that began in October 2013 and ended in November 2017 were **reconfirmed** **by NGS** technology.
- Thus, transitioning to new platforms as technology advances is a viable option.

Single-cell RNA sequencing (scRNA-seq)

- An important point noted by the **opponent** is that **bulk tissue analysis obtained from a "blind" endometrial biopsy may not be accurate enough** to perform the ERA test.
- The **best possible technology** currently available to challenge the ERA test in bulk endometrial tissue in any part of the uterine cavity is **single-cell RNA sequencing** (scRNA-seq).
- scRNA-seq can promote understanding of how an organ or tissue is arranged **at the single-cell level** by blending biology and genetics with mathematics, new computational tools and pragmatism.
- **Cells** are **isolated** using **microfluidic circuits** and **nanodroplets**, and the mRNA of every cell is sequenced separately.
- The spatial distribution of RNA or translated proteins can then also be mapped within a tissue or organ (https://data.humancellatlas.org).
- This technology was chosen as the 2018 breakthrough of the year by *Science*, and its application in the human endometrium is no exception (Wang *et al.*, 2020).

Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle

➢ In **2020**, the **characterization** of the **human endometrial transcriptome** at a **single-cell level** was reported, revealing **cell-specific expression signatures** across the **menstrual cycle**.

(Wang *et al.*, 2020)

ARTICLES
https://doi.org/10.1038/s41591-020-1040-z

nature medicine

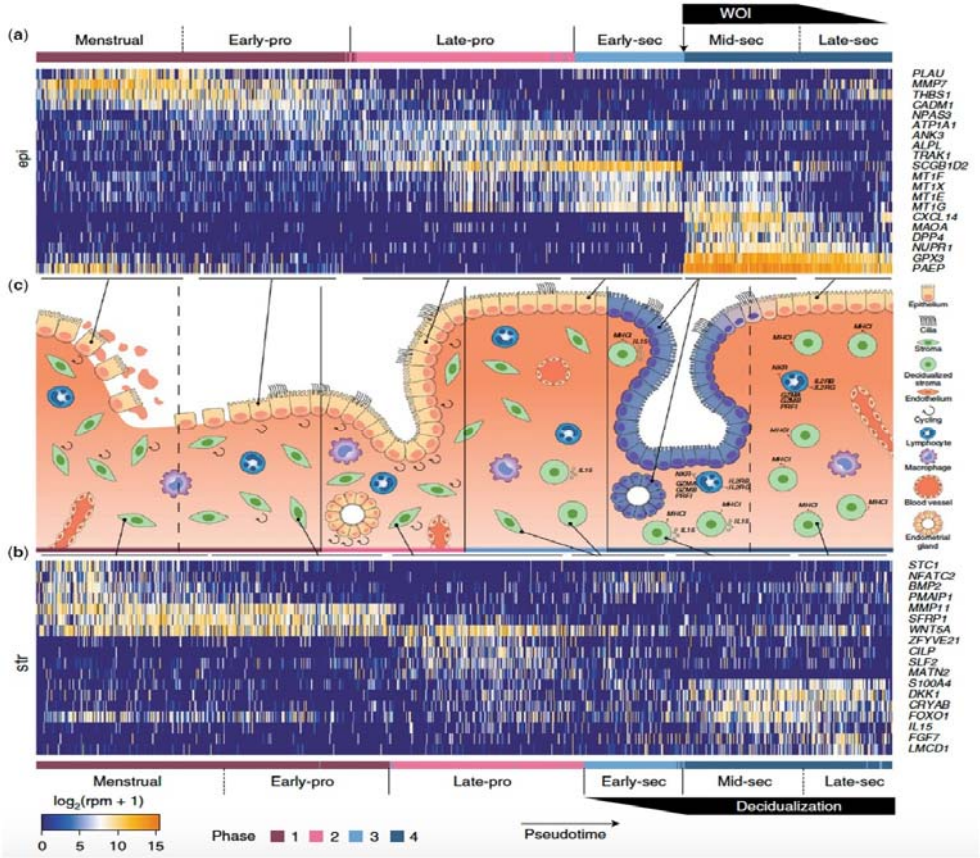
Check for updates

Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle

Wanxin Wang[1,8], Felipe Vilella[2,3,8], Pilar Alama[4], Inmaculada Moreno[2,3], Marco Mignardi[5], Alina Isakova[1], Wenying Pan[1], Carlos Simon[2,3,6] ✉ and Stephen R. Quake[1,5,7] ✉

Employing **canonical markers** and **highly differentially expressed genes**, they identified **six endometrial cell types**:
epithelial and **endothelial cells**, **stromal fibroblasts**, **macrophages**, **lymphocytes** and a **novel ciliated epithelial** cell type.

- Further, the signatures revealed that the **human WOI** involves **transcriptomic activation** in the **epithelia** that is both **abrupt** and **discontinuous** (Wang *et al.*, 2020).
- These **cellular-resolution findings** confirmed the previous identification from **bulk tissue** of a unique endometrial receptivity transcriptomic signature (Díaz-Gimeno *et al.* 2011).



Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle, Wang *et al.*, **Nature medicine, 2020**

A decade of ERA clinical application

The **initial ERA proof** of concept in **Caucasian patients with recurrent implantation failure** (RIF) was published in **2013** in a prospective multicentre interventional clinical trial.

The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

Maria Ruiz-Alonso, M.Sc.,[b] David Blesa, Ph.D.,[a,b] Patricia Díaz-Gimeno, Ph.D.,[a,c] Eva Gómez, M.Sc.,[a] Manuel Fernández-Sánchez, M.D.,[d] Francisco Carranza, M.D.,[d] Joan Carrera, M.D.,[e] Felip Vilella, Ph.D.,[a] Antonio Pellicer, M.D., Ph.D.,[a,b] and Carlos Simón, M.D., Ph.D.[a,b]

[a] Fundación Instituto Valenciano de Infertilidad, and Instituto Universitario IVIVincliva, Valencia University, Valencia; [b] Iviomics, Paterna; [c] Computational Medicine Institute, Centro de Investigación Príncipe Felipe, Valencia; [d] Instituto Valenciano de Infertilidad Sevilla, Seville; and [e] Clínica Girona Unidad de Reproducción Humana, Girona, Spain

Objective: To demonstrate the clinical value of the endometrial receptivity array (ERA) in patients with repeated implantation failure (RIF), for guiding their personalized embryo transfer (pET) as a novel therapeutic strategy.
Design: Prospective interventional multicenter clinical trial.
Setting: University-affiliated infertility and private clinics.
Patient(s): Eighty-five RIF patients and 25 comparison patients.
Intervention(s): Endometrial sampling and pET guided by ERA.
Main Outcome Measure(s): A receptive (R) or nonreceptive (NR) endometrial status according to ERA. Pregnancy (PR) and implantation (IR) rates after pET.
Result(s): The ERA test gave an R result of 74.1% in RIF patients versus 88% in control subjects. Clinical follow-up was possible in 29 RIF patients, in whom pET was performed, resulting in 51.7% PR and 33.9% IR. The IRs and PRs in the 6 months after the biopsy showed that pregnancy was not related to the local injury. Twenty-two RIF patients (25.9%) were NR, and in 15 of them a second ERA validated

A decade of ERA clinical application

The hypothesis was that **implantation failure of endometrial origin** is **not a pathology or an endometrial dysfunction** (conditions that stigmatize a patient), but rather a **failure to synchronize the developing embryo with a patient's individual WOI**.

The study group included:

- **85 patients** with **RIF** (4.8 ± 2.0 previous failed cycles) at least **four** total **morphologically high-grade embryos or blastocysts** transferred **no other explanation for the implantation failures**.
- The **control** group was **25** patients.

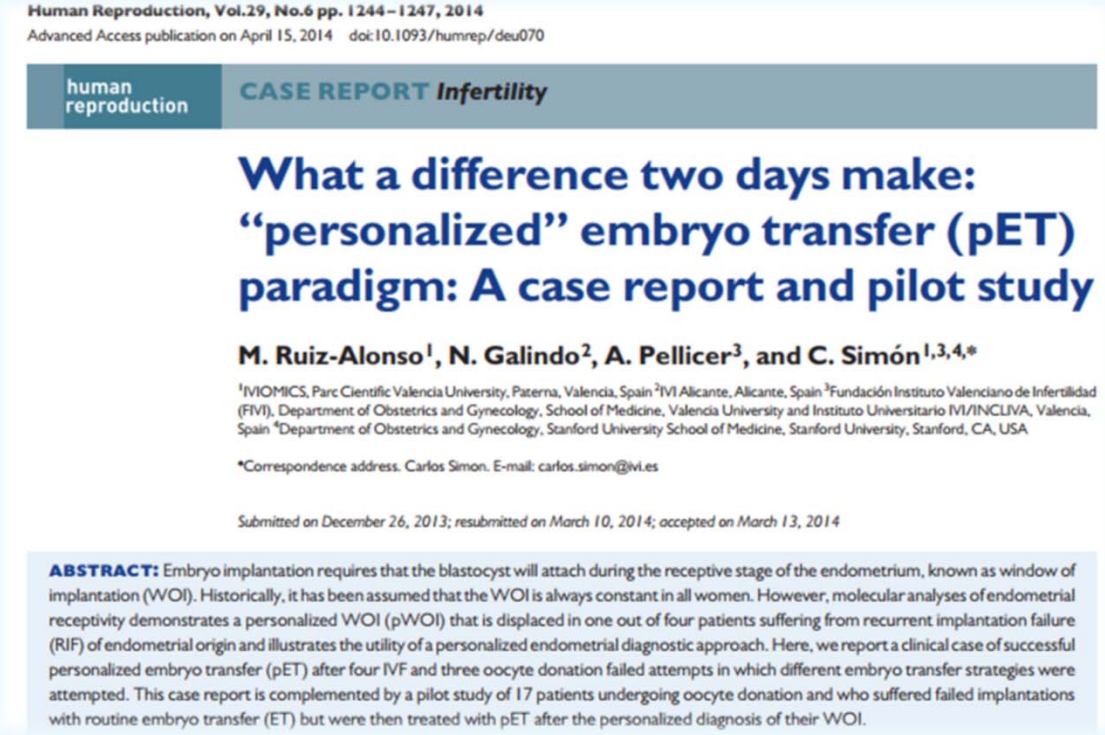
They detected that **25.9% of patients with RIF** showed a **displaced WOI** (advanced or delayed), while only **12%** of **control** patients had such displacement.

A decade of ERA clinical application

- They concluded that **one in four patients** with **RIF** have a **displaced/asynchronous WOI**.
- Their computational algorithm classified these patients as **non-receptive endometrium** either **pre-** (84%) or **post-receptive** (16%), which was further verified by a second ERA test.
- We **translated these genomic results to the clinic** by **transferring embryo(s)** according to the **WOI of the individual patient**, providing a '**personalized embryo transfer**' (**pET**) resulting in a **50.0% pregnancy rate** (PR) and **38.5% implantation rate** (IR), similar to that of controls.
- These results suggested that **normal pregnancy** and **implantation rates** may be achieved in patients with **RIF** of endometrial origin if **synchrony between the embryo and receptive endometrium is accomplished**.

A decade of ERA clinical application

- This initial study was further validated by the report of a clinical case of successful pET after seven previous failed IVF attempts (four with autologous oocytes and three with donor oocytes).



Human Reproduction, Vol.29, No.6 pp. 1244–1247, 2014
Advanced Access publication on April 15, 2014 doi:10.1093/humrep/deu070

human reproduction

CASE REPORT *Infertility*

What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study

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Submitted on December 26, 2013; resubmitted on March 10, 2014; accepted on March 13, 2014

ABSTRACT: Embryo implantation requires that the blastocyst will attach during the receptive stage of the endometrium, known as window of implantation (WOI). Historically, it has been assumed that the WOI is always constant in all women. However, molecular analyses of endometrial receptivity demonstrates a personalized WOI (pWOI) that is displaced in one out of four patients suffering from recurrent implantation failure (RIF) of endometrial origin and illustrates the utility of a personalized endometrial diagnostic approach. Here, we report a clinical case of successful personalized embryo transfer (pET) after four IVF and three oocyte donation failed attempts in which different embryo transfer strategies were attempted. This case report is complemented by a pilot study of 17 patients undergoing oocyte donation and who suffered failed implantations with routine embryo transfer (ET) but were then treated with pET after the personalized diagnosis of their WOI.

A decade of ERA clinical application

- The case report was soon complemented by a **pilot study** of:

➢**17 patients** undergoing **oocyte donation**

➢who experienced from **1 to 6 failed implantations** (2.9 ± 2.1) with **routine embryo transfer** (ET),

But

- were subsequently **treated with pET** after diagnosis of their WOI.

- Results **after pET** showed that these patients (with up to six previous failures) reached a **60% clinical PR**, while a **19% PR was achieved after routine ET** in a non-receptive endometrium diagnosed by ERA.

After these initial reports, **independent groups** started to publish their own data **using ERA** to **guide pET** in their clinical practice.

➢ In **2015**, a **retrospective study** in an **Indian population** (Mahajan, 2015) analyzed data from **three different groups**:

- patients with RIF
- patients with one previous failed cycle
- patients with atrophic (thin) endometrium (<6 mm)

Endometrial receptivity array: Clinical application

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ABSTRACT

Human implantation is a complex process requiring synchrony between a healthy embryo and a functionally competent or receptive endometrium. Diagnosis of endometrial receptivity (ER) has posed a challenge and so far most available tests have been subjective and lack accuracy and a predictive value. Microarray technology has allowed identification of the transcriptomic signature of the window of receptivity window of implantation (WOI). This technology has led to the development of a molecular diagnostic tool, the ER array (ERA) for diagnosis of ER. Use of this test in patients with recurrent implantation failure (RIF) has shown that the WOI is displaced in a quarter of these patients and use of a personalized embryo transfer (pET) on the day designated by ERA improves reproductive performance. Our results in the Indian population revealed an endometrial factor in 27.5% RIF patients, which was significantly greater than the non-RIF group 15% ($P = 0.04$). After pET, the overall ongoing pregnancy rate was 42.4% and implantation rate was 33%, which was at par with our *in-vitro* fertilization results over 1-year. We also performed ERA in patients with persistently thin endometrium, and it was reassuring to find that the endometrium in 75% of these patients was receptive despite being 6 mm or less. A pregnancy rate of 66.7% was achieved in this group. Though larger studies are required to validate these results ERA has become a useful tool in our diagnostic armamentarium for ER.

KEY WORDS: Endometrial receptivity, ERA, *in-vitro* fertilization, recurrent implantation failure, thin endometrium

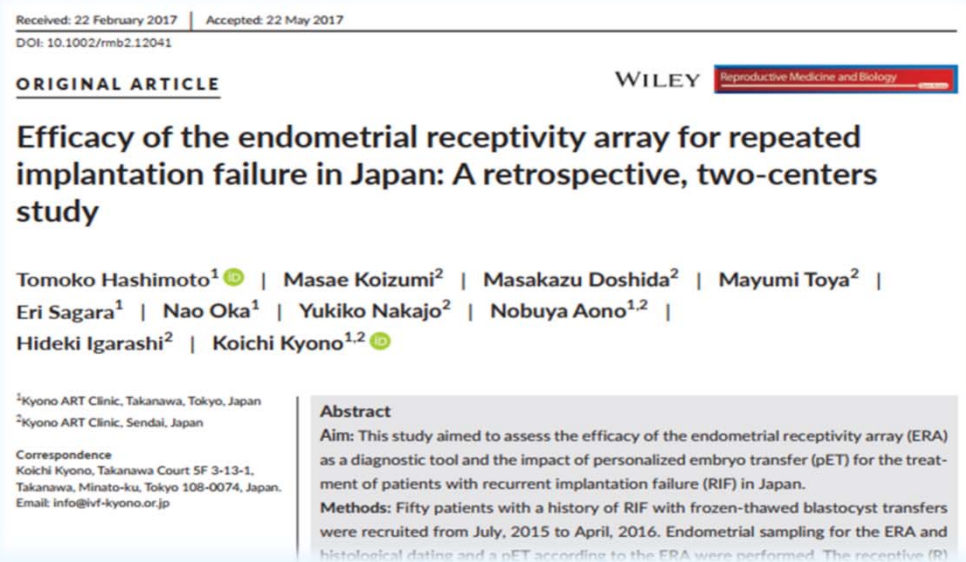
A decade of ERA clinical application

- Their results revealed that **27.5% of patients with RIF** had a **displaced WOI**, while only **15%** of patients with **one previous failure** had a **displacement** (similar to data published in 2013).
- **After pET**, the overall ongoing **PR** in the **RIF group** was **42.4%** and **IR** was **33%**, which was similar to that in the group of patients with one failure.
- This finding again suggested that results in patients with RIF can be normalized after pET.
- Interestingly, the ERA test revealed **displaced WOIs** in **25%** of those with **atrophic endometrium**, but **after pET** their **PR** was **66.7%** despite having an endometrial thickness <6 mm. Similar cases have been reported for unresponsive 4-mm endometrium (Cruz and Bellver, 2014).

A decade of ERA clinical application

➢ In **2017**, a retrospective analysis of **50 patients** with **RIF** assessed **the impact of pET guided by ERA** in a **Japanese** population (Hashimoto et al., 2017).

- Approximately **24%** of patients in the **RIF** group had **a displaced WOI**,
- but after pET they reached a **50% PR**, similar to that reported in previous studies.



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ORIGINAL ARTICLE

WILEY Reproductive Medicine and Biology

Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: A retrospective, two-centers study

Tomoko Hashimoto[1] | Masae Koizumi[2] | Masakazu Doshida[2] | Mayumi Toya[2] | Eri Sagara[1] | Nao Oka[1] | Yukiko Nakajo[2] | Nobuya Aono[1,2] | Hideki Igarashi[2] | Koichi Kyono[1,2]

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Abstract

Aim: This study aimed to assess the efficacy of the endometrial receptivity array (ERA) as a diagnostic tool and the impact of personalized embryo transfer (pET) for the treatment of patients with recurrent implantation failure (RIF) in Japan.

Methods: Fifty patients with a history of RIF with frozen-thawed blastocyst transfers were recruited from July, 2015 to April, 2016. Endometrial sampling for the ERA and

A decade of ERA clinical application

➢In **2019**, Hromadova et al. (2019) reported similar findings in the **Czech Republic**.

- Retrospective data from **85 patients** (**74 RIF** cases and 11 controls) revealed that **36.5%** of **RIF** patients showed a **displaced WOI** and **69.2%** became pregnant after performing pET guided by ERA.

➢Ota et al. (**2019**) published a case report of a **Japanese** patient who achieved pregnancy with pET guided by ERA **after 11 previous failed attempts**.

➢Simrandeep and Padmaja (**2019**) reported **three severe cases of RIF** in **Indian** patients; two of the patients had a previous ERA performed at a different centre, and the recommendation for pET for a displaced WOI was not followed, resulting another failure. Once pET was implemented, successful clinical pregnancies were achieved in both patients

While these studies indicate the outcomes for patients who received pET based on their WOI, what is the **clinical outcome** in patients in whom transfers occur **outside of their WOI** according to ERA?

- Such data were collected in a study (Ruiz-Alonso et al., 2014) comparing the clinical outcome of pET in **205 receptive** (R) patients versus embryo transfers performed in **52 non-receptive** (NR) patients according to the ERA test.

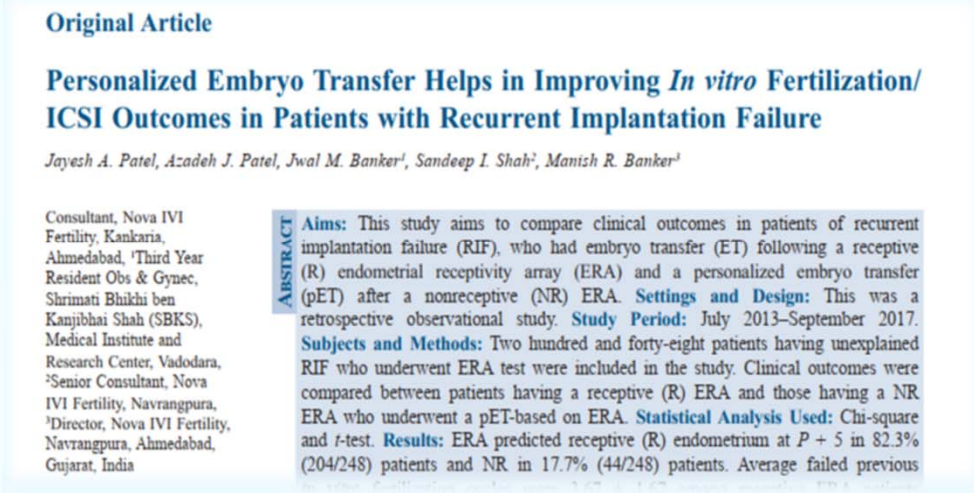
The clinical outcome

Groups	Pregnancy Rate (PR)	Implantation Rate (IR)	Ongoing Pregnancy Rate (OPR)
Receptive (R)	60%	45%	74%
Non-receptive (NR)	23%	13%	0%

A decade of ERA clinical application

- However, other retrospective publications have **not found statistical clinical differences** in **pET** versus **ET** in patients with **RIF** (Patel et al., 2019).

- Tan et al. observed that when embryos were chromosomally analyzed, a higher IR and OPR was observed in **pET** versus **ET** (66.7 vs. 44.4% and 58.3 vs. 33.3%, respectively), but these differences were **not statistically significant** due to the small sample size (Tan et al., 2018).



Original Article

Personalized Embryo Transfer Helps in Improving *In vitro* Fertilization/ ICSI Outcomes in Patients with Recurrent Implantation Failure

Jayesh A. Patel, Azadeh J. Patel, Jwal M. Banker[1], Sandeep I. Shah[2], Manish R. Banker[3]

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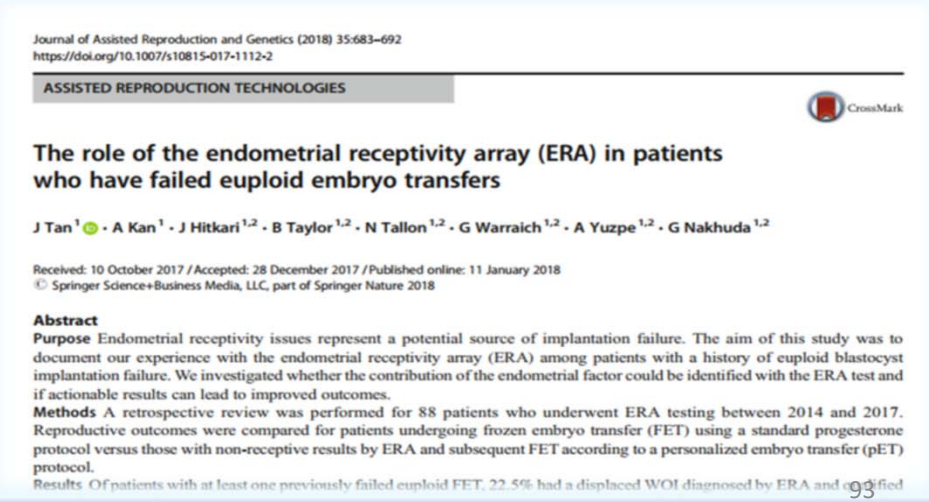
ABSTRACT

Aims: This study aims to compare clinical outcomes in patients of recurrent implantation failure (RIF), who had embryo transfer (ET) following a receptive (R) endometrial receptivity array (ERA) and a personalized embryo transfer (pET) after a nonreceptive (NR) ERA. **Settings and Design:** This was a retrospective observational study. **Study Period:** July 2013–September 2017. **Subjects and Methods:** Two hundred and forty-eight patients having unexplained RIF who underwent ERA test were included in the study. Clinical outcomes were compared between patients having a receptive (R) ERA and those having a NR ERA who underwent a pET-based on ERA. **Statistical Analysis Used:** Chi-square and *t*-test. **Results:** ERA predicted receptive (R) endometrium at $P + 5$ in 82.3% (204/248) patients and NR in 17.7% (44/248) patients. Average failed previous



Journal of Assisted Reproduction and Genetics (2018) 35:683–692
https://doi.org/10.1007/s10815-017-1112-2

ASSISTED REPRODUCTION TECHNOLOGIES

The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers

J Tan[1] · A Kan[1] · J Hitkari[1,2] · B Taylor[1,2] · N Tallon[1,2] · G Warraich[1,2] · A Yuzpe[1,2] · G Nakhuda[1,2]

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Abstract

Purpose Endometrial receptivity issues represent a potential source of implantation failure. The aim of this study was to document our experience with the endometrial receptivity array (ERA) among patients with a history of euploid blastocyst implantation failure. We investigated whether the contribution of the endometrial factor could be identified with the ERA test and if actionable results can lead to improved outcomes.

Methods A retrospective review was performed for 88 patients who underwent ERA testing between 2014 and 2017. Reproductive outcomes were compared for patients undergoing frozen embryo transfer (FET) using a standard progesterone protocol versus those with non-receptive results by ERA and subsequent FET according to a personalized embryo transfer (pET) protocol.

Results Of patients with at least one previously failed euploid FET, 22.5% had a displaced WOI diagnosed by ERA and rectified

A decade of ERA clinical application

- Some authors undertook a different approach to evaluate the clinical efficiency of ERA, using retrospective cohort studies **comparing** patients with an **indication of ERA** treated by **pET** to those **without an ERA indication**, and yielding **similar clinical results** between these groups.
(Bassil et al., 2018; Neves et al., 2019; Cozzolino et al., 2020)

Journal of Assisted Reproduction and Genetics (2018) 35:1301–1305
https://doi.org/10.1007/s10815-018-1190-9

ASSISTED REPRODUCTION TECHNOLOGIES

CrossMark

Does the endometrial receptivity array really provide personalized embryo transfer?

Rawad Bassil[1] · Robert Casper[1,2] · Nivin Samara[1] · Tzu-Bou Hsieh[1] · Eran Barzilay[3] · Raoul Orvieto[3,4] · Jigal Haas[1,3]

Received: 31 January 2018 / Accepted: 13 April 2018 / Published online: 8 May 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract
Purpose The aim of the present study was to determine the percentage of inf… receptive endometrium according to the endometrial receptivity array (ERA) te… transfer day according to the proposed shift in the window of implantation impr… tested patients.
Methods A single-center retrospective cohort study, including 53 consecutive… embryo transfers) admitted to our IVF unit for a mock cycle prior to their froze… mock cycle included an endometrial biopsy for both the ERA test and histologica… The next cycle frozen embryo transfer (FET) in the study group was adjusted a… consisted of patients who underwent FET cycles at our clinic during the same pe… and ERA testing.
Results During the study period, 503 patients (control group) underwent FET cy…

Journal of Assisted Reproduction and Genetics (2020) 37:2989–2997
https://doi.org/10.1007/s10815-020-01948-7

ASSISTED REPRODUCTION TECHNOLOGIES

Check for updates

Evaluation of the endometrial receptivity assay and the preimplantation genetic test for aneuploidy in overcoming recurrent implantation failure

Mauro Cozzolino[1,2,3] · Patricia Diaz-Gimeno[1] · Antonio Pellicer[1,4,5] · Nicolas Garrido[1]

…blished online: 24 September 2020
…ature 2020

…endometrial receptivity array (ERA) and the preimplantation genetic test for …derate recurrent implantation failure (RIF).
…s conducted in patients who failed to achieve implantation following transfer of … single embryo transfers; patients were classified as moderate or severe RIF, …ared based on the testing they received: PGT-A, ERA, or PGT-A+ERA versus a …ate and ongoing pregnancy rates per embryo transfer were considered primary …

https://doi.org/10.1007/s10815-019-01535-5

ASSISTED REPRODUCTION TECHNOLOGIES

Check for updates

What is the clinical impact of the endometrial receptivity array in PGT-A and oocyte donation cycles?

Ana Raquel Neves[1] · Marta Devesa[2] · Francisca Martínez[2] · Sandra Garcia-Martinez[2] · Ignacio Rodriguez[2] · Nikolaos P. Polyzos[2,3,4] · Buenaventura Coroleu[2]

Received: 28 May 2019 / Accepted: 16 July 2019 / Published online: 27 July 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract
Purpose To evaluate the influence of the endometrial receptivity array (ERA) test on the implantation rate (IR) and pregnancy rate (PR) in patients with previous failed euploid embryo transfers (Euploid-ET) or oocyte donation embryo transfers (Donor-ET).
Methods Single-center retrospective study of patients with ≥ 1 previous failed Euploi-ET ($n = 24$) or ≥ 2 failed Donor-ET ($n = 32$) who underwent an ERA test and a post-ERA Euploid-ET/Donor-ET between 2012 and 2018. Controls were patients with ≥ 1 previously failed Euploid-ET ($n = 119$) or ≥ 2 failed Donor-ET ($n = 158$) who underwent Euploid-ET/Donor-ET during the same …

A decade of ERA clinical application

We should bear in mind that, until 2020, patients with indication for ERA were

the most difficult cases with several previous failures, as **no explanation** was found for their RIF of endometrial origin even **after a thorough infertility workup**.

Therefore,

the fact that **pET** in this **RIF population** was **able to obtain similar clinical results** to those in '**control patients**' is confirmatory of previous results, due to

improved outcomes for **the most difficult patients**.

Recently, the **effectiveness** of **personalized embryo transfer** guided by **ERA** compared to **frozen ET** (FET) or **fresh embryo transfer** (ET) were explored (Simón *et al.*, 2020).

- This **prospective** open label randomised clinical trial (**RCT**)
- included **458** patients younger than 37 years undergoing IVF with blastocyst transfer
- **at their first appointment**
- across **16 reproductive centers** from **Europe**, **America** and **Asia**, and involved 30 co-authors together with the support of the ERA RCT Consortium.

402 RBMO VOLUME 41 ISSUE 3 2020

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ELSEVIER

ARTICLE

A 5-year multicentre randomized controlled trial comparing personalized, frozen and fresh blastocyst transfer in IVF

BIOGRAPHY

Professor Carlos Simón is Board Certified and Professor of Obstetrics and Gynaecology at the University of Valencia, Spain, and Senior Lecturer, Beth Israel Deaconess Medical Center Harvard University, Boston, USA. His main interest is the human embryonic implantation process, including the embryo, maternal endometrium, and the cross-communication between them.

Carlos Simón[1,2,4,*,†], Carlos Gómez[3,†], Sergio Cabanillas[4], Iavor Vladimirov[5], Gemma Castillón[6], Juan Giles[4], Kubra Boynukalin[7], Necati Findikli[7], Mustafa Bahceci[7], Israel Ortega[8], Carmina Vidal[4],

A decade of ERA clinical application

- **Intention-to-treat analysis** revealed comparable clinical outcomes across transfer types;
- however, there was a significantly **higher cumulative pregnancy rate** (CPR) in the **pET** group (**93.6%**) than in **FET** (**79.7%**) ($P = 0.0005$) and **ET** (**80.7%**) groups ($P = 0.0013$).
- By **per-protocol analysis**, **pET** resulted in a **56.2%** **live-birth** (LB) rate after first embryo transfer compared to **42.4%** for **FET** ($P = 0.09$) and **45.7%** for **ET** (**45.7%,** $P = 0.17$).
- After 12 months, **pET** resulted in **significantly higher cumulative LB rate** (71.2%) compared to **FET** (55.4%, $P = 0.04$) and **ET** (48.9%, $P = 0.003$).
- **pET** also yielded **significantly higher PR** at the first embryo transfer (**72.5%**) compared to **FET** (54.3%, $P = 0.01$) and **ET** (58.5%, $P = 0.05$).
- **Similar outcomes** were observed for first-transfer **IRs**, which were 57.3% for pET versus 43.2% ($P = 0.03$) and 38.6% ($P = 0.004$) for FET and ET, respectively.
- All groups exhibited **similar** **obstetrical**, **delivery type** and **neonatal outcomes**.

A decade of ERA clinical application

While the RCT experienced an **unexpectedly high patient drop-out** (observed, 50%; expected, 30%), the **per-protocol analysis comparing pET to FET and ET arms** revealed significantly better **cumulative LB rates**, **PR** and **IR**.

These findings support that **using the ERA test at the first appointment** to **guide pET** may have clinical benefit.

Intention-to-treat analysis	Per-protocol analysis
Shows **no beneficial effect** of the **ERA test** except for a **statistically significant cumulative pregnancy rate (CPR)** compared with FET and fresh embryo transfer	Demonstrates a **significant improvement** in **pregnancy rates** at the **first** and **cumulative rates** up to 12 months, and **implantation rates** at the first attempt

The **potential of the ERA test** to **diagnose** the **endometrial factor** in the **work-up of the infertile couple**.

These findings **need to be confirmed** in a **larger randomized clinical trial**.

What percentage of women might need the ERA test?

- **RIF of endometrial origin** is recognized as a **concern** by all clinicians who transfer **euploid embryos** that ultimately **fail to achieve pregnancy**.
- The **ERA** test was **initially** created to **solve** the problem of our **most difficult patients**, namely **RIF of endometrial origin** that is estimated to be present in **10% of all IVF cycles.**
- The **RCT** exploring, at the **first appointment**, the **cost-effectiveness** of this approach compared to FET or fresh ET has been published.
- **Per protocol analysis** demonstrated that **pET increases** the **IR** at the first embryo transfer by 14.1% (pp) versus FET (P = 0.03) and by 18.7% versus fresh ET (P = 0.004). **LB rates**, while not statistically significant, were increased by 13.8% versus FET and 10.5% versus fresh ET (Simón et al., 2020).
- Thus, it is **up to readers to consider if this approach is reasonable to use** in all patients.

Multigene panel tests

Multigene panel tests

- **Multigene panel tests** might **reveal the mechanisms** and lead us to a better understanding.
- **Interventions** can be offered on the basis of the **multigene panel test** results and **clinical evidence.**
- Since **genes** at **high risk** and **genes of lesser magnitude** are all tested, **multigene panel tests** can **reduce** the **likelihood of false-negative assessment** and are **cost-effective** and **time-efficient** in **oncology**.
- As an **ideal goal of reproductive medicine**, identifying **pathogenic genetic variants** for patients with **specific infertility phenotypes**, such as **ovarian dysgenesis** or **azoospermia**, can be realized with **focused gene panels** (such as those offered by **Centogene** and **Evolve Gene**).

CENTOGENE
THE RARE DISEASE COMPANY

Infertility panel
GENETIC TESTING

Diagnostic strategy

WHOM TO TEST?	TESTING STRATEGY	ACTION
Men Men/Women Women	**Infertility Panel** *ANOS1, AR, ARL13B, ARL6, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BMP15, C8ORF37, CATSPER1, CC2D2A, CCDC28B, CEP164, CEP290, CFTR, CHD7, DUSP6, ENPP1, FEZF1, FGF17, FGF8, FGFR1, FLRT3, FMR1, FSHB, FSHR, GNRH1, GNRHR, HESX1, HEXA, HFE, HS6ST1, IFT172, IFT27, IL17RD, INPP5E, KIF7, KISS1, KISS1R, LEP, LEPR, LHB, LHCGR, LHX3, LHX4, LZTFL1, MKKS, MKS1, MYO7A, NPHP1, NPHP3, NR0B1, NR0B2, NR5A1, NSMF, OFD1, PCSK1, PHF6, PNPLA6, POLR3B, POMC, POU1F1, PPARG, PROK2, PROKR2, PROM1, PROP1, PRPH2, RDH5, RHO, RLBP1, RPGRIP1L, SDCCAG8, SEMA3A, SOX10, SOX2, SOX3, SPRY4, SRY, TAC3, TACR3, TMEM67, TRIM32, TTC21B, TTC8, TUBB8, WDPCP, WDR11, ZP1* Complementary assays: Repeat expansion analysis for *AR*, *FMR1* and *MLPA* for aneuploidy and *AZF* region.	Genetic counseling Adapt treatment

Turnaround time: **25 days**

CENTOGENE

Infertility Panel

➢ **Recommended for:**

- Patients trying to conceive for one year or longer
- with known fertility problems
- who have experienced more than one miscarriage
- with irregular or absent menstruation
- with low sperm count, form, or movement
- with absence of development of secondary sexual features

➢ **Panel includes:**

The most important genes related to infertility in males and females

➢ **Common syndromes & disorders covered:**

- Female infertility
- Male infertility

- CNV analysis included (For 34 genes)
- Repeat expansion analysis: *AR, FMR1*
- MLPA: Aneuploidy, AZF region
- mtDNA analysis included

No. of genes: 276
TAT: 25 days
Coverage: ≥99.0% ≥20x

Fertilome

Fertilome is a new option that tests for **genetic factors** that may be leading to troubles with conception or carrying a pregnancy full-term.

While **it can't diagnose exact conditions**, it can **point to risks** that patients may have for disorders that make pregnancy more difficult.

➢ The Fertilome test scans for **49 variants** across **32 genes**, indicating risks for conditions like:

- Diminished or decreased ovarian reserve (DOR)
- Early menopause
- Endometriosis
- Hyperandrogenism
- Idiopathic/unexplained infertility
- Polycystic ovary syndrome (PCOS)
- Primary ovarian insufficiency (POI)
- Recurrent implantation failure (RIF)
- Recurrent pregnancy loss (RPL)

✓ **The genes included in the Fertilome test function in just 10 biological processes:**
immune response regulation, hormone regulation, ovarian follicle development, blood circulation, cell proliferation/differentiation, DNA replication and repair, glucose homeostasis, tissue remodeling, steroidogenesis, and oxidative stress regulation.

Fertilome fertility test could help these patients:

Many patients could benefit from these advances in genetic fertility tests for women, including patients who:

- Are **older than 35** and debating when to start their family
- Are considering **fertility preservation procedures**
- Have a **family history of reproductive conditions**, like PCOS or endometriosis

- Even with genetic fertility tests for women, it's likely that **genetic defects** will be responsible for **only a small percentage of infertility cases**.

- However, if the Fertilome test suggests an increased risk for certain conditions, **a patient can be proactive rather than reactive** in their life choices.

- For example, they may consider oocyte or embryo cryopreservation or move forward with pregnancy attempts at an earlier time.

Genetic risk assessment for causes of infertility with multigene panel testing

Focused gene panels
(such as those offered by Centogene)

Identify pathogenic genetic variants
for patients with
specific infertility phenotypes, such as ovarian dysgenesis or azoospermia,
&
Screen for rare mutations
which **directly cause overt infertility**, such as impaired oocyte maturation and fertilization defects.

Genetic assessment for infertility risk
(rather than diagnosis of Mendelian fertility disorders)

Predominantly focused on
Screening for the *FMR1* premutation
to identify a risk of **primary ovarian insufficiency**.

More recently

Multigene panel testing for risk assessment
has been introduced into the field of reproductive medicine.
The first **commercially available product** in the USA:
Fertilome (Celmatix Inc., New York, NY)

By **limiting the genetic analysis to only selected variants**, the Fertilome panel **avoids the dilemma of VUS detection** that encumbers many multigene test panels.

Personalized medicine in female infertility

Diagnosis of POI

The **diagnosis** Premature Ovarian Insufficiency is based on the presence of

Menstrual disturbance

Oligo/Amenorrhea for **at least 4 months**
In women **< 40 years old**

Biochemical confirmation

An elevated **FSH level > 25 IU/l** on two occasions **> 4 weeks apart**

The second part of the diagnostic work-up is to **establish a cause** for POI.

Establishing causation may have implications for the **management options** for symptoms associated with POI, and/or associated conditions.

Etiology of POI

- Unknown mechanisms (65%)
- Structural and numerical abnormalities of the X chromosome (13%)
- Iatrogenic (12%)
- Gene mutations (6%)
- Autoimmunity (4%)
- Systemic or Syndromic disorders (2%)

Causes of POI

(Genetic, Iatrogenic, Autoimmune, Metabolic, Infectious, or Environmental)

- Premature ovarian insufficiency is usually designated as **spontaneous** or **idiopathic** POI because

its etiology is mostly underdetermined

The identified causes of POI, potentially involved in those mechanisms, have been grouped into **two categories**:

Genetic

Genetic causes entail **various genetic abnormalities**,

Non-genetic

non-genetic causes include **autoimmune** and **metabolic disorders**, **infections**, **environmental factors**, and **iatrogenic procedures**

Genetic etiology

Genetic causes account for approximately **20% to 25%** of patients with **POI**.

Heritability

It is possible to estimate **what proportion of the etiology can be ascribed to genetic factors** as opposed to **environmental factors**

an indication of **the relative importance of genetic factors in its causation**, so that **the greater the value for th heritability the greater the role of genetic factors**.

The reported **hereditability** estimate of **0.52** for **age** at **natural menopause** suggests that **genetic effects** explain at least **half** of the **interindividual variation** in **age** at natural menopause.

Genetic etiology

Early menopause in a **mother**, **sister**, **aunt**, or **grandmother** was associated

6-fold increased odds of **early menopause**

8-fold increased risk of **premature menopause**

Twin studies

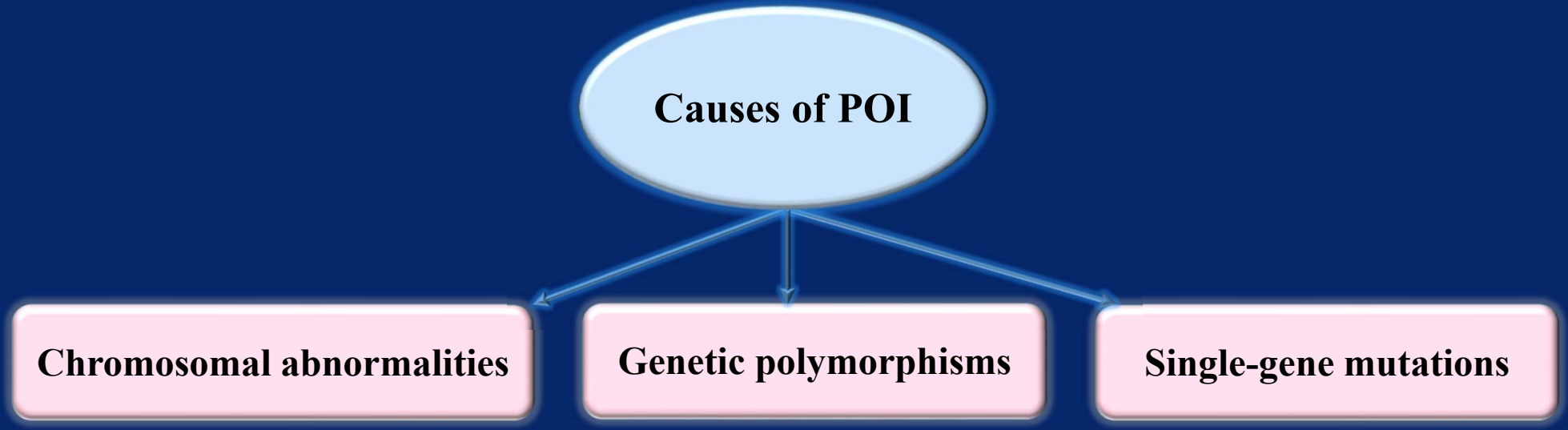
Twins have a **significantly higher prevalence** of **POI** than the **general population**, with a **3-fold greater prevalence**.

Menopause before age 40 in one of the twin → **7-fold increased** risk of Menopause before age 40 in her identical sister

Up to 90% of nonsyndromic POI cases are estimated to be **idiopathic**, with **about 30% having an affected first-degree relative**, supporting **a potential underlying genetic etiological basis**.

Genetic Aspect of POI

Genetic causes of POI
are **highly heterogeneous** and may involve **interactions** of **various genetic defects**.



Chromosomal Abnormality

Chromosomal abnormalities are a well-established cause of POI, and their frequency is approximately 10–13%.

Numerical defects

- **X monosomy**
(45,X; Turner syndrome)
- **Mosaic forms**
(45,X/46,XX and 45,X/47,XXX)
- **Trisomy X**
(47,XXX)

Structural defects

- **X-deletions/duplications**
- **X-autosomal translocations**
- **Small or large rearrangements**

A region of the **long arm** of the **X chromosome** that seems **critical for the POI** phenotype extends from **Xq13-Xq21 (POI2)** to **Xq23-Xq27 (POI1)**.

Genetic mutation

FMR1 Premutation

Another **congenital abnormality** resulting in POI is the **presence of CGG repeats** (in the range of approximately **55–199 repeats**) in the fragile-X mental retardation (***FMR1***) gene.

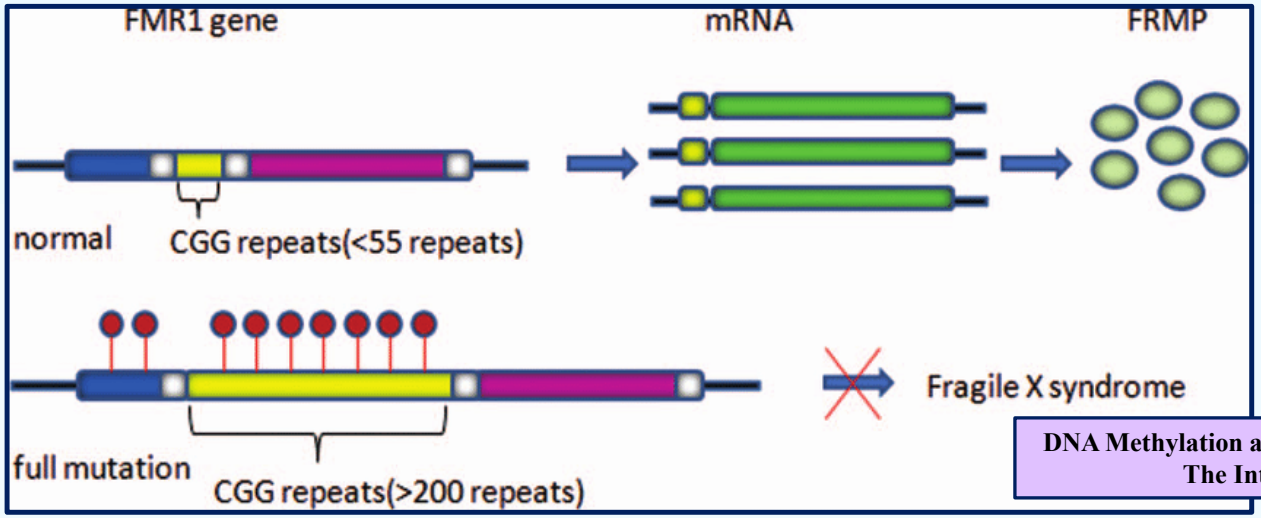
Fragile X syndrome is caused by the **deficiency** or **absence** of fragile X mental retardation protein (**FMRP**), a widely expressed **RNA-binding protein** that also **regulates translation**.

The FMRP is expressed in **neurons** and **granulosa cells**.

Theoretically, the deficiency or absence of FMRP can occur through any type of deletion or inactivation mutation, but in more than 99% of cases there is an **expansion of a segment of CGG repeats in the 5' untranslated region of the *FMR1* gene** that leads to DNA **hypermethylation** and **inhibition of transcription**.

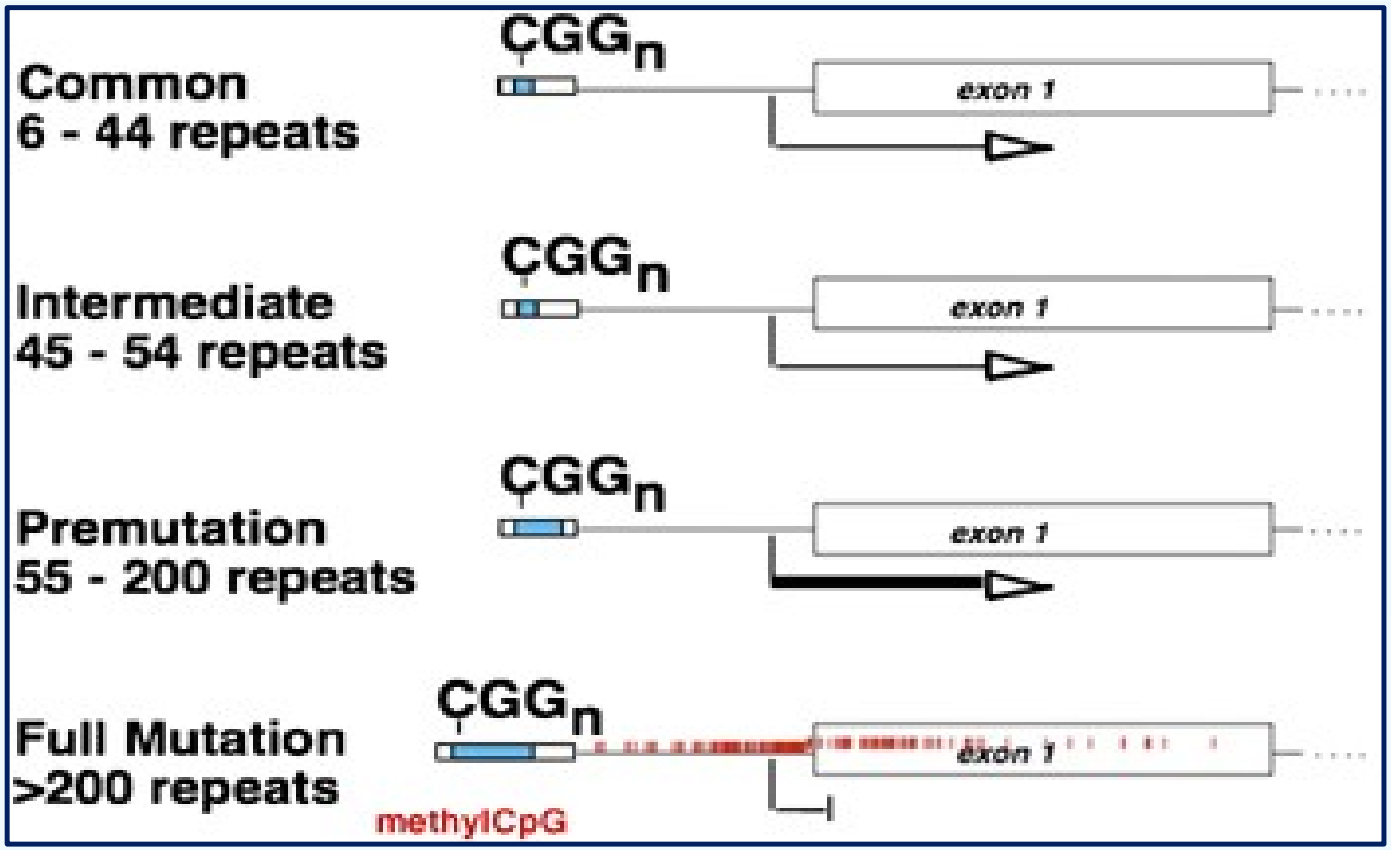
Fragile X syndrome

- **The most common single gene cause** of **intellectual disability** and **autism.**
- Clinical features include **mental retardation**, **characteristic facial features** with **large ears** and **prominent jaw**, **connective tissue findings** (joint hypermobility), **large testes** after puberty and **behavioral abnormalities**.
- Fragile X syndrome occurs in **men** when **CGG repeats** number is **>200**.
- Around **70% of women** with **more than 200 CGG** repeats show intellectual disability.
- The **incidence** of fragile X syndrome is approximately **1:4000 in men** and **1:8000 in women.**
- Interestingly, **ovarian function remains normal** in women with **full mutation range** repeat alleles.



DNA Methylation as a Biomarker for Neuropsychiatric Diseases, Ai et al., **The International Journal of Neuroscience, 2011**

The *FMR1* gene has **4 types of alleles**: **normal**, **intermediate**, **premutation**, and **full mutation**



Fragile X syndrome, Garber et al., **European Journal of Human Genetics, 2008**

Allele types of *FMR1* gene

Normal alleles

- Range from about **5** to about **44** **repeats**
- The **most common** repeat length is **29** or **30** CGG repeats
- Normal alleles have **no meiotic** or **mitotic instability**
  (in stable, normal alleles, the CGG region is interrupted by an AGG triplet after every 9 or 10 CGG repeats; the AGG triplets are thought to anchor the region during replication and prevent strand slippage)

Intermediate alleles

- Range from about **45** to about **54** **repeats**
- Can be **considered normal**
  given that such alleles are not associated with fragile X syndrome and have not been observed to expand to a full mutation in 1 generation.
- However, a grey-zone allele of **52 repeats** was reported to **expand to a premutation** allele of **56 repeats** in 1 generation, which subsequently expanded to a full mutation allele in the next generation.

No evidence to support an association between high normal and intermediate range *FMR1* alleles with a risk of POI

Allele types of *FMR1* gene

Premutation alleles

- Range from about **55** to about **200** **CGG repeats**
- These alleles are **long repeat tracks** that are **unstably transmitted** from patient to child.
- **Expansions** from the premutation size range to the full mutation typically occur during **maternal transmission**. In fact, with extremely rare exceptions, the patient of **origin of the expansion** to the full mutation is **woman**.
- Most individuals with the premutation do **not** **show fragile X syndrome-related features**;

however, some with high repeat sizes (>100 repeats) have been identified with learning difficulties, emotional problems, or even intellectual disability.

- **POI** occurs in **20% of women** with alleles in the **premutation** **range of *FMR1*** gene (usually **more than 80** **CGG repeats**).
- It did **not** **find** a **correlation** between the ***FMR1*** **CGG intermediate repeat length** and the **severity of idiopathic POI**.

Single genes causing non-syndromic POI

- **Hundreds of genes** have been involved in **POI etiology** by their participation in **key biological processes in the ovary**, such as:

  meiosis and **DNA damage repair**, **homologous recombination**, **follicular development**, **granulosa cell differentiation** and **proliferation**, and **ovulation**.

- The other traditional strategy to identify candidate genes in POI is to **study genes whose product is known and plays a role in human folliculogenesis** or shows an **organ-specific effect** based on murine knockout models (candidate genes). Many genes have been interrogated for these reasons.

Genes on the X chromosome

- *Bone morphogenetic protein 15 (BMP15) (Xp11.2)*
- *Progesterone receptor membrane component 1 (PGRMC1) (Xq22-q24)*
- *Androgen receptor (AR) (Xq12)*
- *Forkhead box O4 (FOXO4) (Xq13.1)*
- *Premature ovarian failure, 1B (POF1B) (Xq21.2)*
- *Dachshund family transcription factor 2 (DACH2) (Xq21.3)*
- *Fragile X mental retardation 1 (FMR1) (Xq27.3)*

Genes on autosomes

- *Growth differentiation factor 9 (GDF9) (5q31.1)*
- *Folliculogenesis specific bHLH transcription factor (FIGLA) (2p13.3)*
- *Newborn ovary homeobox gene (NOBOX) (7q35)*
- *Nuclear receptor subfamily 5, group A, member 1 (NR5A1); Steroidogenic factor-1(SF-1) (9q33)*
- *FSH receptor (FSHR) (2p21-p16)*
- *TGF, beta receptor III (TGFBR3) (1p33-p32)*
- *G protein-coupled receptor 3 (GPR3) (1p36.1-p35)*
- *Wingless-type MMTV integration site family, member 4 (WNT4) (1p36.23-p35.1)*
- *Inhibins: inhibin, alpha (INHA) (2q35); inhibin, beta A (INHBA) (7p15-p13)*
- *POU class 5 homeobox 1 (POU5F1) (6p21.31)*
- *MutS homolog 4 (MSH4) (1p31) and MSH5 (6p21.3)*
- *Forkhead box O3 (FOXO3) (6q21)*
- *Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) (6q23.3)*
- *Spermatogenesis and oogenesis specific basic helix-loop-helix transcription factor 1 (SOHLH1) (9q34.3) and SOHLH2 (13q13.3)*
- *Phosphatase and tensin homolog (PTEN) (10q23.3)*
- *Nanos homolog 1, 2, 3 (Drosophila) (NANOS1, 10q26.11; NANOS2, 19q13.32; NANOS3, 19p13.13)*
- *Cyclin-dependent kinase inhibitor 1B (CDKN1B) (12p13.1-p12)*
- *Anti-Mullerian hormone receptor, type II (AMHR2) (12q13)*
- *KIT ligand (KITLG) (12q22)*
- *Forkhead box O1 (FOXO1) (13q14.1)*
- *Spalt-like transcription factor 4 (SALL4) (20q13.2)*
- *Meiotic protein covalently bound to DSB (SPO11) (20q13.31)*
- *DNA meiotic recombinase 1 (DMC1) (22q13.1)*

Causative genes in POI

Gene	Mutation rate (%)	Functional category	Regulatory mechanism	Reference
LHX8	N.A.	Transcription factor	Germ-cell-specific critical regulator of early oogenesis	Rossetti et al., 2017
SOHLH1*	N.A.	Transcription factor	Early folliculogenesis	Zhao et al., 2015
FOXO3A	2.2	Transcription factor	Regulating primordial follicle growth activation	Watkins et al., 2006; Gallardo et al., 2008
NOBOX(7q35)	1.0–8.0	Transcription factor	Follicle development	Rajkovic et al., 2004
FMR1(Xq27)	0.5–6.7	highly polymorphic CGG repeat in the 5′ untranslated region (UTR) of the exon 1	Transcriptional regulation	Oostra and Willemsen, 2009
PGRMC1(Xq22-q24)	0.5–1.5	Heme-binding protein	Regulation of apoptosis	Venturella et al., 2019
POLR3H	1.5	RNA polymerase III subunit H	Regulation of cell cycle, cell growth, and differentiation	Franca et al., 2019
GDF9(5q31.1)	0.5–4.7	Growth factor	Growth and differentiation of granulosa cell proliferation	Patiño et al., 2017b
BMP15(Xp11.2)	1.0–10.5	Growth factor	Growth and differentiation of granulosa cells (GCs)	di Pasquale et al., 2004
BMPR2	N.A.	BMP receptor	Signal transduction between oocytes and somatic cells	Patiño et al., 2017a
AMH(19p13.3)	2.0	Anti-Müllerian hormone	Control of the formation of primary follicles by inhibiting excessive follicular recruitment by FSH	Alvaro Mercadal et al., 2015
AMHR2(12q13)	1.0–2.4	AMH receptor	AMH signal transduction	Yoon et al., 2013
FOXL2(3q23)	1.0–2.9	Transcription factor	Differentiation and growth of granulosa cells	Boulily et al., 2016
WT1(11p13)	0.5	Transcription factor	Granulosa cell differentiation and oocyte–granulosa cell interaction	Gao et al., 2014
NR5A1(9q33)	0.3–2.3	Transcription factor	Steroidogenesis in ovaries	Jiao et al., 2017
FSHR (2p21-p16)	0.1–42.3	Receptor	Follicular development and ovarian steroidogenesis	Welt, 2008
KHDRBS1	N.A.	Signal transduction activator	Alter mRNA expression level and alternative splicing	Wang et al., 2017
FIGLA (2p13.3)	0.5–2.0	bHLH transcription factor	Regulation of multiple oocyte-specific genes, including genes involved in folliculogenesis and those that encode the zona pellucida	Zhao et al., 2008
INHA variants	0–11	Growth factor	Maturation of ovarian follicles by FSH inhibition	Dixit et al., 2004
ESR1	N.A.	Estrogen receptor	Regulation of follicle growth and maturation and oocyte release	de Mattos et al., 2014
LHR	N.A.	Lutropin-choriogonadotropic hormone receptor	Regulation of ovarian follicle maturation, steroidogenesis, and ovulation	Simpson, 2008

Premature Ovarian Insufficiency: Past, Present, and Future, *Chon et al.* , **Frontiers in Cell and Developmental Biology**, **2021**

Genetic Mutations

- **Alterations** in the newborn ovary homeobox (***NOBOX***) and the factor in germline alpha (***FIGLA***), both **oocyte-specific transcription factors**, are involved in POI.
- ***NOBOX*** gene mutations cause **oocyte loss after birth**, while ***FIGLA*** mutations **impair the regulation of zona pellucida genes**, thus causing **postnatal loss in primordial follicles**.
- **Other mutations** associated with POI are those occurring in forkhead box L2 (***FOXL2***), ***WT1*** (Wilms tumor 1), ***NR5A1*** (nuclear receptor subfamily 5 group A member1), the **transcription factors** affecting **folliculogenesis**, are associated with POI.
- **Abnormalities** in bone morphogenetic protein 15 (***BMP15***), growth differentiation factor 9 (***GDF-9***), **transforming growth factor-β superfamily**, is also critical for POI.
- **Mutations** of the **FSH receptor** cause **amenorrhea** in the POI. It is also associated with FSH resistance hence raise serum FSH.

Genetic Mutations

- The steroidogenic factor 1 gene (***SF-1***, ***NR5A1***) is important for **gonadal differentiation** and **controls steroidogenesis** by **regulating** steroidogenic acute regulatory protein (***StAR***), cytochrome P450, family 19, subfamily A, polypeptide 1 (***CYP19A1***), lutropin-choriogonadotropic hormone receptor (***LHR***), and inhibin alpha subunit (***INHA***) genes, which function in the **hypothalamic–pituitary–steroidogenesis axis**.
- Recent discoveries have shown a link between **POI development** and **spermatogenesis and oogenesis-specific basic helix-loop-helix (SOHLH) 1 and 2 sequence-specific DNA-binding factors**. These mutations cause **infertility** and **loss** of **follicles**.

Pleiotropic single gene disorders in POI

Candidate genes responsible for Mendelian disorders that manifest POI

Gene	Location	Mendelian syndrome	Somatic features	Reference
FMR1	Xq27.3	Fragile X syndrome	Attention deficits, hyperactivity, social deficits, anxiety disorder, deficits in cognitive flexibility.	Reiss and Hall (2007) and Spath *et al.* (2010)
FOXL2	3q23	Blepharophimosis-ptosis-epicanthus BPE type I syndrome, BPES I	BPES type I is a complex eyelid malformation associated with POI. The major features of the eyelid malformation involve (i) narrowed horizontal aperture of the eyelids (blepharophimosis), (ii) drooping of the upper eyelid (ptosis), (iii) the presence of a fold of skin arising from the lower eyelid that runs inward and upward (epicanthus inversus), and (iv) lateral displacement of the inner canthi (telecanthus).	Zlotogora *et al.* (1983) and Oley and Baraitser (1988)
GALT	9p13	Galactosemia	Cataracts, speech defects, poor growth, poor intellectual function, neurologic deficits (predominantly extrapyramidal findings with ataxia).	Schadewaldt *et al.* (2004)
AIRE	21q22.3	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome, APECED	Candidiasis, Addison's disease, hypoparathyroidism, type 1 diabetes, alopecia, vitiligo, ectodermal dystrophy, celiac disease and other intestinal dysfunctions, chronic atrophic gastritis, chronic active hepatitis, autoimmune thyroid disorders, pernicious anemia.	Fierabracci *et al.* (2012)
EIF2B	EIF2B2 -14q24.3; EIF2B4-2p23.3; EIF2B5-3q27.1	Central nervous system leukodystrophy and ovarian failure, ovarioleukodystrophy	Neurological disorder characterized by involvement of the white matter of the central nervous system. When Leukodystrophies associated with premature ovarian failure referred to as ovarioleukodystrophy.	Mathis *et al.* (2008)
POLG	15q25	Progressive external ophthalmoplegia, PEO	Manifestations range from involvement limited to the eyelids and extraocular muscles.	Graziewicz *et al.* (2007)
NOG	17q22	Proximal symphalangism, SYM1	Ankylosis of the proximalinterphalangeal joints.	Kosaki *et al.* (2004)
PMM2	16p13	PMM2-CDG CDG-1 (a previously known as congenital disorder of glycosylation type 1a)	Cerebellar dysfunction (ataxia, dysarthria, dysmetria), non-progressive cognitive impairment, stroke-like episodes, peripheral neuropathy with or without muscle wasting, absent puberty in females, small testes in males, retinitis pigmentosa, progressive scoliosis with truncal shortening, joint contractures, and premature aging	Sparks and Krasnewich (2005)
HSD17B4 *HARS2* *CLPP* *LARS2* *C10orf2*	5q21 5q31.3 19p13.3 3p21.3 10q24	Perrault syndrome, PS	Sensorineural deafness in both males and females, and neurological manifestations in some patients.	Jenkinson *et al.* (2013), Morino *et al.* (2014), Pierce *et al.* (2011), Pierce *et al.* (2013) and Pierce *et al.* (2010)
BLM	15q26.1	Bloom syndrome	Chromosomal breakage leading to early onset of aging, short stature and elevated rates of most cancers.	Ellis and German (1996)
ATM	11q22-q23	Ataxia telangiectasia, A-T	Progressive cerebellar degeneration, telangiectasias, immunodeficiency, recurrent infections, insulin-resistant diabetes, premature aging, radiosensitivity, and high risk for epithelial cancers in surviving adults.	Gatti *et al.* (1991) and Su and Swift (2000)
WRN	8p12	Werner syndrome	Premature aging of the skin, vasculature, and bone and elevated rates of certain cancers, particularly sarcomas.	Epstein *et al.* (1966)
RECQL4	8q24.3	Rothmund–Thomson syndrome, RTS	Cutaneous rash, sparse hair, small stature, skeletal and dental abnormalities, cataracts, premature aging, and an increased risk for cancer, especially malignancies originating from bone and skin tissue.	Wang *et al.* (2001)

Genetics of primary ovarian insufficiency: new developments and opportunities,
Qin et al.,
Human Reproduction Update,
Vol.21, No.6 pp. 787–808, **2015**

Mitochondrial genes causing POI

- Perturbations of **mitochondrial genes** or **nuclear genes affecting mitochondria** are **good candidates for POI** because the **mature oocyte** has the **greatest number** of **mitochondria** of any human cell.
- Mature oocytes readily **accumulate mitochondria** during **oogenesis**.
- **Mitochondrial biogenesis** playing an essential role in **oocyte maturation**, **fertilization** and **embryo development**.
- **Dysregulation of mitochondrial dynamics** contributes to **excess oxidative stress** and **initiation of apoptosis**, thus **accelerating follicle depletion**.

Mitochondrial genes causing POI

- A **marked quantitative decrease** of mitochondrial DNA (**mt DNA**) in **oocytes** and **peripheral blood cells** has been well documented in women with **ovarian insufficiency**.
- Thus, **any gene affecting mitochondria** involving muscular and neurological disturbance is a candidate, because these systems are so dependent on mitochondrial integrity.
- Genes governing mitochondrial functions may be located in the **nucleus**, or in **mitochondria** itself (mt DNA).
- To date, those of relevance to POI have been nuclear genes.

  - Progressive external ophthalmoplegia (PEO): polymerase (DNA directed), gamma (***POLG***) (15q25)
  - Perrault syndrome: ***HARS2***, 5q31.3; ***LARS2***, 3p21.3; ***CLPP***, 19p13.3; ***C10orf2***, 10q24

Genome-wide studies in POI

- Contemporary genetic strategies applied to locate susceptible loci or genes causing POI have extended beyond suspected candidate gene interrogations to genome-wide approaches.

➢ Approaches include:

- Linkage analysis in families with multiple affected members
- aCGH for CNV
- Genome-wide association studies (GWAS)
- Genome-wide sequencing of exomes (WES)
- Whole genome sequencing (WGS)

Genome-Wide Association Study in POI

GWAS involves **genome-wide approaches** to **search for susceptible loci or genes** that cause the disease.

In GWAS, one examines **many common genetic variants** in different individuals to see if **any variant is associated with a trait**.

In GWAS, one examines **many common genetic variants** in different individuals to see if **any variant is associated with a trait in similar ethnicities**.

These data will allow **further elucidation of the genetic mechanism** underlying POI.

Genome-Wide Association Study in POI

➢ known POI candidates:

- ***SOHLH1***, ***FSHR***, DNA damage repair-, homologous recombination-, and meiosis-related [stromal antigen 3 (***STAG3***)], synaptonemal complex central element protein 1 (***SYCE1***), scaffolding protein involved in DNA repair (***SPIDR***), PSMC3 interacting protein (***PSMC3IP***), ATP-dependent DNA helicase homolog (***HFM1***), MutS protein homolog 4 and 5 (***MSH4*** and ***MSH5***), minichromosome maintenance component 8 and 9 (***MCM8*** and ***MCM9***), fusion protein (***CSB-PGBD3***), and nucleoporin 107 kDa (***NUP107***); and mRNA transcription- and translation-related [eukaryotic translation initiation factor 4E nuclear import factor 1 (***eIF4ENIF1***), and KH Domain-Containing, RNA-Binding, Signal Transduction-Associated Protein 1 (***KHDRBS***).

Genome-wide association studies for POI

	Kang et al. (2008)	Knauff et al. (2009)	Qin et al. (2012b)	Pyun et al. (2012)	Oldenburg et al. (2008)	Caburet et al. (2012)
Ethnicity	Korean	Caucasian (Dutch)	Chinese	Korean	Dutch	Middle-Eastern
Discovery set						
No. of cases	24	99	391	24	10	5
No. of controls	24	181	895	24	5	4
Associations in discovery set	*PTHB1* at 7p14 showed strongest association. Ht1 GAAAG: POI-susceptible haplotype; Ht2 TGTGC: POI-resistant haplotype.	rs246246 mapped to *ADAMTS19* intron	8q22.3 (10^{-6})	22 SNPs in *LAMC1* associated with POI	Susceptible locus: 5q14.1-q15	Susceptible loci: 7p21.1-15.3, 7q21.3-22.2
Replication set						
No. of cases	101	60	400	98	–	–
No. of controls	87	90	800	218	–	–
Results of replication set	*PTHB1* associated with POI; Ht1 confers susceptibility to POI.	Association not confirmed.		Frequencies of 9 SNPs and 1 haplotype were higher in POI than in control.	–	Sequencing three candidate genes *DLX5*, *DLX6* and *DSS1* did not reveal causal mutations

PTHB1: Bardet–Biedl syndrome 9 (BBS9); ADAMTS19: ADAM metallopeptidase with thrombospondin type 1 motif, 19; LAMC1: laminin, gamma 1; DLX5, 6: distal-less homeobox 5 and 6; DSS1: split hand/foot malformation (ectrodactyly) type 1.

Genetics of primary ovarian insufficiency: new developments and opportunities,Qin et al., **Human Reproduction Update**, Vol.21, No.6 pp. 787–808, **2015**

Common POI-related genes in humans and mice

Genes	Full name	Function	References
ATM	Ataxia telangiectasia mutated	A member of the phosphatidylinositol-3 kinase-like protein kinase (PIKK) family	Liu H. et al., 2020
BMP15	Bone morphogenetic protein 15	Growth factor beta	di Pasquale et al., 2004
BMPR1A/1B	Bone morphogenetic protein receptor, type IB	Growth factor beta	Renault et al., 2020
CLPP	CIPP caseinolytic peptidase, ATP-dependent, proteolytic subunit homolog (Escherichia coli)	Cytochrome P450 family 19 subfamily A member 1	Jenkinson et al., 2013
CSB-PGBD3*	CSB-PGBD3 fusion protein	DNA damage repair	Qin et al., 2015a
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	Cytochrome P450 family 19 subfamily A member	Kim et al., 2011
eIF4ENIF1*	Eukaryotic translation initiation factor 4E nuclear import factor 1	Regulates translation and stability of mRNAs in processing bodies	Zhao et al., 2019
ERCC6	DNA excision repair protein ERCC-6	Essential factor involved in transcription-coupled nucleotide excision repair	Qin et al., 2015b
FANCA	Fanconi anemia, complementation group A	DNA repair protein	Pyun et al., 2014
FOXL2	Forkhead box L2	Transcription factor	Park et al., 2014
FSHR	Follicle stimulating hormone receptor	Receptor	Aittomäki et al., 1995; Huang et al., 2019
HFM1*	HFM1, ATP-dependent DNA helicase homolog (Saccharomyces cerevisiae)	Receptor	Zhe et al., 2019
MCM8/9*	Minichromosome maintenance component 8	DNA-repair gene	Wood-Trageser et al., 2014; AlAsiri et al., 2015; Desai et al., 2017
MSH4/5*	MutS protein homolog 4/5	Homologous recombination (HR) repair for DNA double strand breaks	Carlosama et al., 2017; Guo et al., 2017; Wang et al., 2020
NANOS3	Nanos homolog 3 (Drosophila)	Signaling molecule	Wu et al., 2013
NBN	Nibrin	DAN-repair gene	Chrzanowska et al., 2010; Tucker et al., 2018
NR5A1	Nuclear receptor subfamily 5, group A, member 1	Receptor	Lourenço et al., 2009
NUP107*	Nucleoporin 107 kDa	Receptor	Weinberg-Shukron et al., 2015
PGRMC1	Progesterone receptor membrane component 1	Cell cycle gene meiotic recombination	Peluso, 2013
PRIM1	DNA primase small subunit	DNA replication	Stolk et al., 2012
PSMC3IP*	PSMC3 interacting protein	Cell cycle genes	Zangen et al., 2011
SALL4	Spalt-like transcription factor 4	Oogenesis	Wang et al., 2019
SGO2	Shugoshin 2	Transcription factors	Faridi et al., 2017
SOHLH1	Spermatogenesis and oogenesis specific basic helix-loop-helix 1	Cell cycle genes	Zhao et al., 2015
SPIDR*	Scaffolding protein involved in DNA repair	Homologous recombination repair during meiosis	Smirin-Yosef et al., 2017
STAG3*	Stromal antigen 3	DNA-damage	Heddar et al., 2019
StAR	Steroidogenic acute regulatory protein	Acute regulation of steroid hormone synthesis	Jehaimi et al., 2010
SYCE1*	Synaptonemal complex central element protein 1	Growth factor beta	de Vries et al., 2014
WRN	Werner syndrome protein; Werner	Caseinolytic mitochondrial matrix peptidase	Du et al., 2004

Premature Ovarian Insufficiency: Past, Present, and Future, *Chon et al.* , **Frontiers in Cell and Developmental Biology**, **2021**

Regulatory genes and networks

- Seeking POF genes has to date largely focused on **coding variants**, presuming plausible protein disruption.
- However, only **1.5%** of the genome is **protein-coding**.
- Indeed, many **POF-associated variants** in whole genome studies map within, or in linkage disequilibrium to, **intronic** or **intergenic regions**; thus, **these regions likely contain causative regulatory genes or networks**. In one POI GWAS, for example, '**gene desert**' **8q22.3** was the **region of most significance by association**.
- Non-coding variants must be more robustly interrogated.

Non-coding RNA in POI

- The role of non-coding RNAs (ncRNAs) in biology has become an area of intense focus, since they have already been extensively studied for the determination of altered protein function in various diseases.

- RNAs that do **not encode conventional proteins** are collectively referred to as **ncRNAs**, which function as **epigenetic regulators**.

- MicroRNAs (**miRNAs**) are endogenously present in mammalian ovaries and **their expression patterns** are **altered** throughout **ovarian development** and **folliculogenesis**, implying their functional roles in the ovarian cycle.

List of miRNA-related POI studies

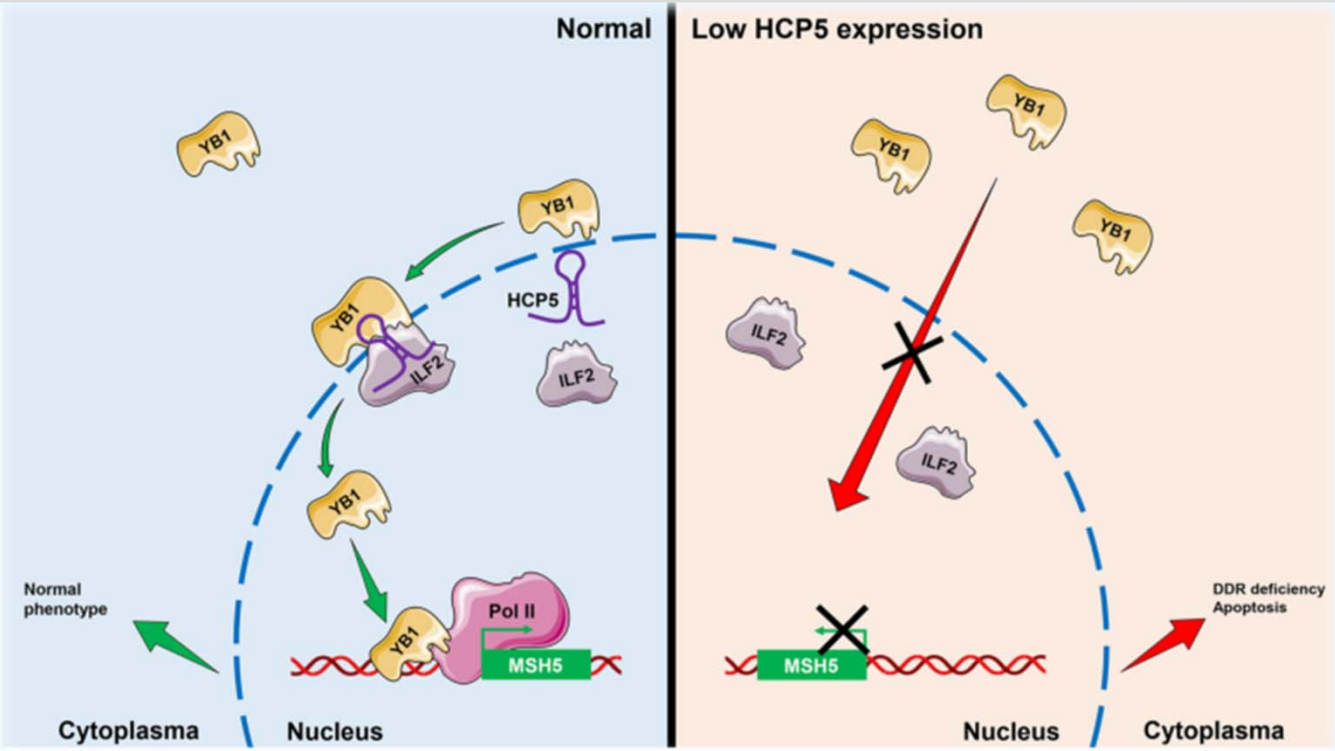
miRNAs	Association with POI	Reference
miR-146 miR-196a2	Putative gene-gene interaction between miR-146 and miR-196a2 may be involved in POF development of Korean women.	Rah et al., 2013
	MiR-146aC > G and miR-196a2T > C change the mRNA expression patterns in granulosa cells.	Cho et al., 2017
miR-146	The expression of miR-146a in plasma and in ovarian granulosa cells of patients with POI was significantly upregulated.	Chen et al., 2015
miR-23a	Mir-23a may play important roles in regulating apoptosis via decreasing XIAP expression in human ovarian granulosa cells of POI patients.	Yang et al., 2012
miR-22-3p	The decreased expression of miR-22-3p in plasma of POI patients may reflect the diminished ovarian reserve and be a consequence of the pathologic process of POI.	Dang Y. et al., 2015
miR-379-5p	MiR-379-5p, PARP1, and XRCC6 were differentially expressed in granulosa cells of biochemical POI.	Dang et al., 2018
miR-21	Low expressions of miR-21 and Pelil were detected in autoimmune POI mice and patients.	Li et al., 2020
miR-127-5p	The upregulation of miR-127-5p was also detected in plasma of bPOI (biochemical POI) individuals.	Zhang et al., 2020

Premature Ovarian Insufficiency: Past, Present, and Future, *Chon et al.* , **Frontiers in Cell and Developmental Biology**, **2021**

Non-coding RNA in POI

➢ Recently, the mechanism of **lncRNA *HCP5*** was reported to be responsible for human POI.

HCP5 **regulated *MSH5* expression** and **granulosa cell function**
by **directly binding** with **YB1** and **modulating its subcellular localization.**

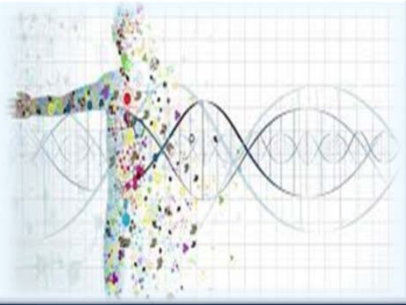


Long noncoding RNA HCP5 participates in premature ovarian insufficiency by transcriptionally regulating MSH5 and DNA damage repair via YB1,
Wang **et al.**, ***Nucleic Acids Research, 2020***, *Vol. 48, No. 8*

Non-coding RNA in POI

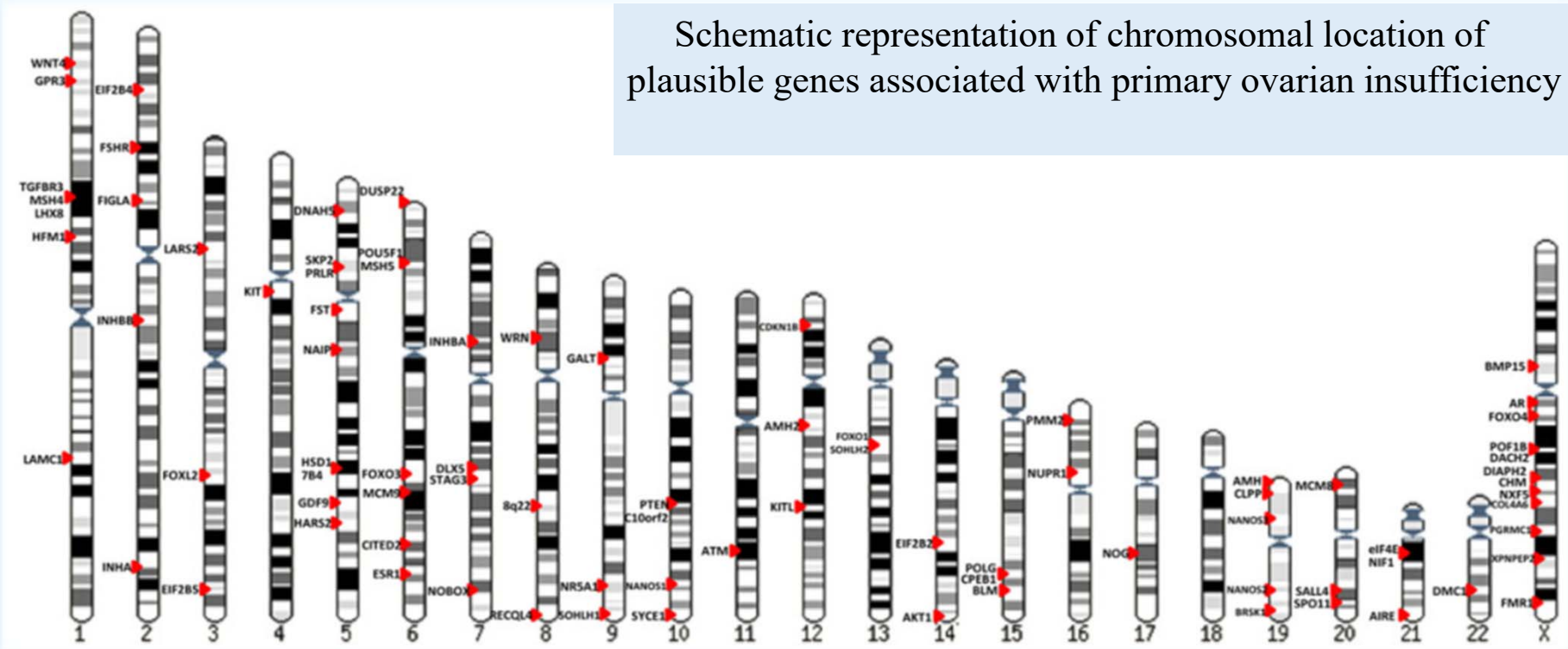
- This study also discovered a novel lncRNA *HCP5* that contributes to dysfunctional granulosa cells by **transcriptionally regulating *MSH5*** and **DNA damage repair via *YB1***, providing a **novel epigenetic mechanism** for POI pathogenesis.
- Multiple studies have reported that **downregulation of nc-RNAs** has also been found in **POI** women, which may lead to **new clinical markers to identify POI**, as well as potential therapeutics for POI.
- Nc-RNA has provided a novel foundation for the discovery of markers for specific diseases, and therapeutics have developed pipelines for treating POI.
- An improved understanding of ncRNA biology and the development of a delivery system will contribute to the identification and treatment of multiple diseases in patients.

Current status of genes causing POI



Current status of genes causing POI

❖ ***First***, **many genes have emerged as POI candidates.**



Schematic representation of chromosomal location of plausible genes associated with primary ovarian insufficiency

Genetics of primary ovarian insufficiency: new developments and opportunities, Qin et al., **Human Reproduction Update**, Vol.21, No.6 pp. 787–808, **2015**

Current status of genes causing POI

➢ But in **non-syndromic POI** only a **minority** have been proven **equivocally causative** by **functional validation.** These include:

***BMP15*, *PGRMC1*, and *FMR1* premutation on the X chromosome**

***GDF9*, *FIGLA*, *FSHR*,*NOBOX*, *NR5A1*, *NANOS3*, *STAG3*, *SYCE1*, *MCM8/9* and *HFM* 1 on autosomes.**

✓ **No perturbations** have been **found**

in a dozen other **plausible candidates** for which **murine knockouts** show **ovarian failure**,

but

this may simply reflect **small sample sizes** or interrogation **restricted to a single ethnic group**.

Current status of genes causing POI

❖ ***Second***, **notable differences in frequency exist among different populations.**

- This is predictable for any genetic condition, and in POI this has already been observed in: *FSHR*, *BMP15*, *NOBOX*, *FOXL2*, *TGFBR3*, *CDKN1B* and *FOXO3A*.
- **Future genetic studies** should involve **different ethnic groups** and **larger sample sizes**.
- Clinically, **caution should apply** when **counseling** on the basis of **data derived from an ethnic group different** from that of the counseled individual.

Current status of genes causing POI

❖ ***Third***, **causative POI genes are increasingly being shown not only to be restricted to expression in ovaries but also expressed ubiquitously.**

- True, ***POF5*** (*NOBOX*) and ***POF6*** (*FIGLA*) are restricted to the ovaries.
- However, ***PTEN*** is a **major regulator** of the **PI3K pathway** involved in: **systemic cell proliferation**, **survival**, **migration** and **metabolism**.
- ***PTEN*** also plays a **vital role** in the **activation of primordial follicles**.
- The spectrum of candidate genes potentially causing POI is being enriched.

Current status of genes causing POI

❖ ***Fourth***, **many genes that currently appear isolated in function actually may be interrelated within yet to be defined pathways.**

- It is logical to stratify by gene function in ostensibly distinct systems: endocrine, folliculogenesis, cell cycle, meiosis, mitochondrial, as examples.

- More difficult are gene-gene or protein-protein interactions, acting in ways not yet evident.

- Until recently, exome studies had to be restricted to sequencing individual candidate genes. Continued advances in sequencing techniques and contemporary bioinformatives will facilitate finding additional genes responsible for POI in other portions of the genome.

Current status of genes causing POI

❖ ***Fifth*, elucidating the etiology and molecular basis of POI is of paramount importance not only in understanding ovarian physiology**

but also in

providing genetic counseling and fertility guidance.

- Once additional **variants are detected**, it will be increasingly possible to **predict the age of menopause**.

- Women having certain perturbations of POI can be **offered** the option of **oocyte cryopreservation**, with later thawing and use in assisted reproductive technology at the appropriate age.

Current status of genes causing POI

Conclusion

POI is a **highly heterogeneous disorder** associated with **mutations** in **more than 75 genes** that are **mainly related** to **meiosis** and **DNA repair**, each of which affects only a few women.

Some of the genes have **not yet** been **proven** to be **associated with POI** etiology, and **functional studies** or **additional reports** on affected women are warranted to confirm their associations with POI etiology.

Although the genetic etiology of POI has been studied by several groups, and although NGS techniques have increased the numbers of known genes identified to play roles in POI etiology and have allowed the discovery of new players in POI etiology, **most cases remain without a clear genetic diagnosis**.

In the next few years, new genetic etiologies will be identified for POI phenotypes, considering the strong genetic background of this disorder and the **widespread** use of **low-cost**, **high-throughput parallel sequencing techniques**.

Personalized medicine in male infertility

What is known about male infertility?

- According to the American Society for Reproductive Medicine (**ASRM**), **male factor infertility** is responsible for **30% of cases of infertility** in the couple.
- Male infertility can be considered as a **multifactorial disorder**.
- In some cases caused by **known and specific causes** such as:
  - Chromosomal abnormalities
  - Infections
  - Gene mutations
  - Varicocele
  - Hormonal disruption
  - Reproductive tract obstructions
- These causes can be **temporary** or **permanent**, and can also be divided between men able to produce **low numbers** and/or **physiologically incompetent spermatozoa**, or those **unable to complete spermatogenesis**.
- From this *variety of possibilities*, it seems obvious that there is a **need to approach each case individually.**

Standard Male Infertility Workup

Currently, the **routine evaluation** of **male infertility** is mainly based on **semen analysis**.

By evaluating semen quality

- **sperm cell density**
- **motility**
- **viability**
- **morphology**

The World Health Organization has established standard ranges of normal, based on population studies:

- Sperm concentration: ≥15 million sperm per milliliter of semen
- Sperm volume: greater than 39 million
- Semen volume: ≥ 1.5 mL
- Morphology: greater than 4% normal form
- Motility: ≥ 40%

Standard Male Infertility Workup

✓However, **semen analysis** does **not** provide **predictive information** on the **fertile potential** in males, nor for **fertilization** or **the assisted reproduction treatment success**.

- A **normal** **result of semen analysis** does **not guarantee fertility** and none of the semen parameters indicate a proper sperm physiologic function.

- In fact, **30%** **of** **normozoospermic** men are **unable** to achieve **pregnancy**.

✓ This limitation as a predictive test does not imply that

basic semen analysis results are **not** a **cost-effective** way to **estimate fertility** potential, decide which are the most convenient therapeutic approaches and assisted reproduction techniques to be used, and also detect cases where additional tests may be required to better discern the causes of infertility and/or avoid reproductive risks to the offspring.

Facing to male infertility, a more detailed physical and clinical examination should be performed.

➢ The **absence of spermatozoa** or the **presence of abnormal sperm** in the ejaculate may reflect **chromosomic disorders**.

The clinically relevant test to investigate the genetic origin of a disrupted spermatogenesis

karyotype

- Used to complement male infertility evaluation where the sperm counts are low.
- Permits the chromosome structure to be examined.
- The karyotype anomalies are related to chromosomic deletions or translocations, that, ultimately, affect sperm production, showing reduction in sperm concentration.

Y-linked microdeletions assays

- Used to inspect the chromosome integrity.
- These microdeletions affect azoospermia factor genes and it is associated with severe oligospermic and azoospermic men.
- It shows the opportunity to find sperm in a testicular biopsy, and also the possibility to transmit this condition to the progeny. Genetic counseling is needed in these cases.

CURRENT GENETIC TESTING

GENETIC TESTS
FOR
NON-OBSTRUCTIVE
AZOOSPERMIA/SEVERE
OLIGOSPERMIA

➢ **Karyotype**
- Klinefelter syndrome (47,XXY or 47,XXY/46,XY)
- Chromosomal Translocation
- 46,XX Male

➢ **Y chromosome microdeletion testing**

GENETIC TESTS
FOR
OBSTRUCTIVE
AZOOSPERMIA

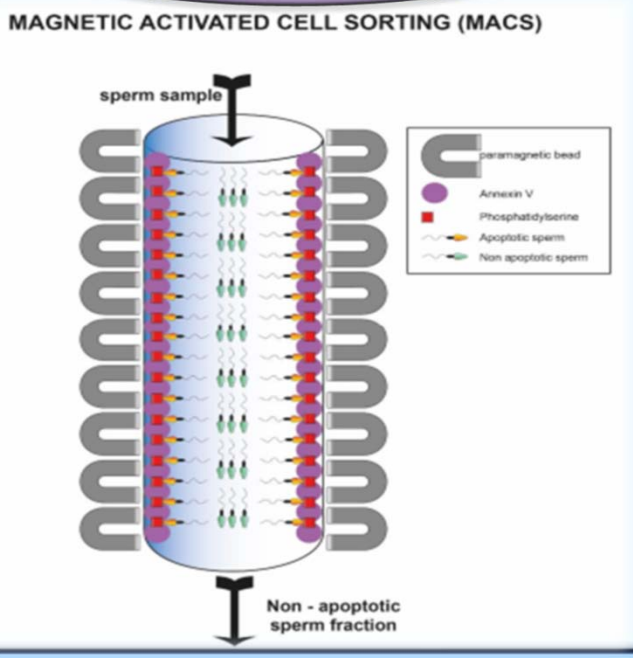
➢ ***CFTR* mutations**
- Congenital bilateral absence of the vas deferens (CBAVD)
- Mutations (>1300) in **CFTR** gene
  (Cystic Fibrosis Transmembrane Conductance Regulator)
- 27 exons

Sperm Cells Selection

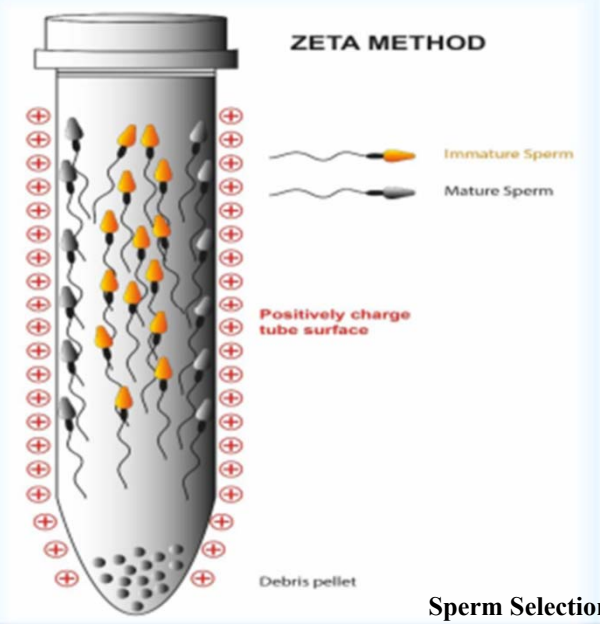
Some selection techniques assess the **male gamete morphology.**

These techniques select or deselect sperm based on their **molecular characteristics**, such as:

Apoptosis markers



Sperm surface charge



Ability to bind to hyaluronic acid

Physiologic Intracytoplasmic Sperm Injection (PICSI)

Sperm Selection for ICSI: Do We Have a Winner?, Baldini et al., Cells, **2021**

Sperm Cells Selection

- Nevertheless, **despite the theoretical benefit** of these **selection methods**, the latest reviews noted that **clinical outcomes** (implantation, pregnancy, and live birth rates) **cannot be enhanced** by means of **current sperm selection techniques**, in other cases, **clinical information** is still **lacking**.

- New **diagnostic techniques** are necessary to ascertain the **cause of infertility** and recognize both **semen and sperm quality**, and design appropriate strategies for **fertility treatment** or **sperm selection**, optimizing clinical outcomes.

This clinical evaluation of male infertility is to some extent superficial and limited, and does not examine the concomitant sperm physiology related to fertility.

- The **molecular factors** related to **fertilization failures**, **poor embryonic quality**, or **poor clinical outcomes** cannot be completely explained with **conventional semen analyses** so far.
- Besides, there are **other sperm intrinsic characteristics** impossible to be assessed only by means of **spermiogram** requiring other specific tests.
- The **molecular factors** related to **fertilization failures**, **poor embryonic quality**, or **poor clinical outcomes** cannot be completely explained with **conventional semen analyses** so far.
- Besides, there are **other sperm intrinsic characteristics** impossible to be assessed only by means of **spermiogram** requiring other specific tests.
- This fact clearly denotes **the need to develop new male infertility tests**.

New approaches

To be able to **personalize medical treatments**,
the **physiologic function** of the involved cells is mandatory.

Knowing the **exact causes of disease** may lead to defining the **exact way of treating** it.

In recent years, the development of **high-output technologies** permitted
a **detailed examination** of **infertility-related causes**, moving forward and advancing this path.

The **omics sciences** study **molecules** and **their interactions**, and the **processes** that occur
from DNA to biological function.

This technology provides **large-scale information** about **genes**, **proteins**, and **metabolites**,
at a relatively **low cost** and **effort**.

Genomics

Genomics studies the **set of genes** of an **individual.**

More than a thousand genes have been correlated with **human male fertility**, so far.

Genomic and **GWAS** studies have concluded definitely that **male infertility** is frequently a **heterogeneous disorder**, which makes its **diagnosis** and **management** extremely **complicated**.

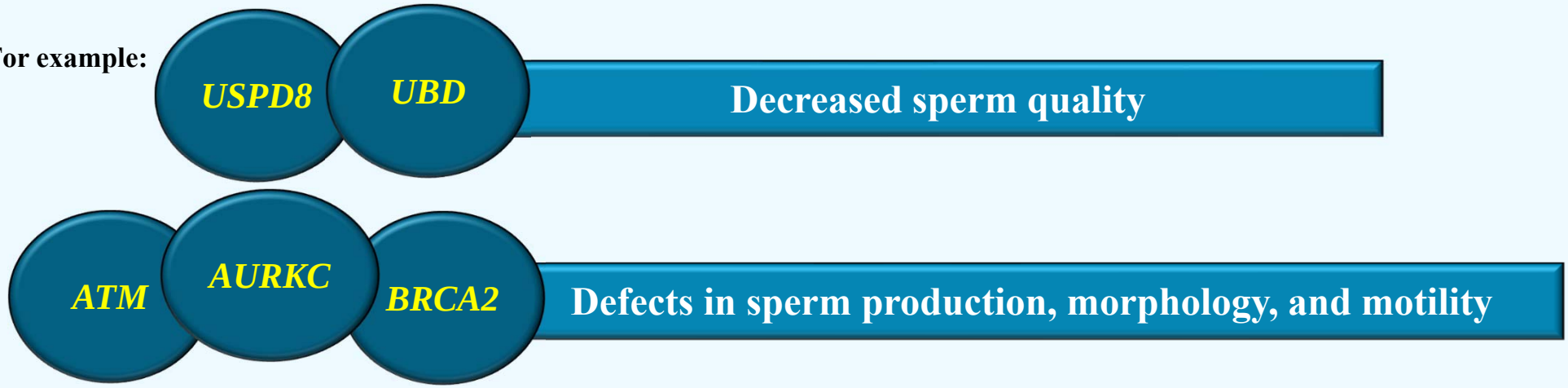
Last-generation technology such as **aCGH** and **NGS** allowed for the **analysis** of **a large set of genes**, to identify in **infertile** individuals **which genes are mutated** and **which are not**.

These **genes** and **their genetic variants** are likely to be **diagnostic biomarkers** of **male infertility**.

Genomics

A review of the items published to date shows **a large number of genes involved**,
but
none definitively causing infertility by themselves.

For example:



Some polymorphisms in the **hormone-sensitive lipase**
modify the sperm lipid's metabolism and conferred a greater risk for infertility in carrier individuals.

Genomics

The difficulty of genomic studies

The **huge number of genes** that are analyzed

which of these might be used as biomarkers, to define therapies related to each specific alteration.

- An **understanding of the number of genes involved** and **their interaction with others** to **increase the risk of infertility** should be one of the subsequent **objectives** of reproductive male genetics.
- Pinpointing these risk genes or their variants could be used to create a **multigene panel testing** for male infertility.
- With this analysis, it would be possible to **screen a hundred or more risk alleles simultaneously**.
- **Multigene panel testing** will be able to use as a **personalized medicine tool** in male infertility diagnosis.
- **Further studies** into the **clinical benefit** and cost-effectiveness of these genetic tests are needed.

Transcriptomics

The term transcriptomics refers to the **science that evaluates the total content of RNAs**, which reflects **gene expression profiles within cells or tissues**.

It is well-known that **sperm RNAs** play a **pivotal role** in **fertilization** and **early embryonic development**, hence the importance of their evaluation.

Examination of the **mRNA profiles** of **sperm** samples can be used as a **diagnostic tool** in fertility.

Profound discrepancies in **mRNA sperm expression profiles** between **fertile** and **infertile** men (with normal semen parameters) were found.

The sperm RNA expression profile

Could be a **tool** to..

Assess seminal quality

Predict the reproductive success

This technology could..

Complement basic semen analysis

Evaluate the **individual molecular pattern** associated with **patient's fertile potential**

Despite the previous studies revealed that
the **individual's transcriptomic profile** may be a **potential diagnostic method**,
further investigation, and clinical validation are required.

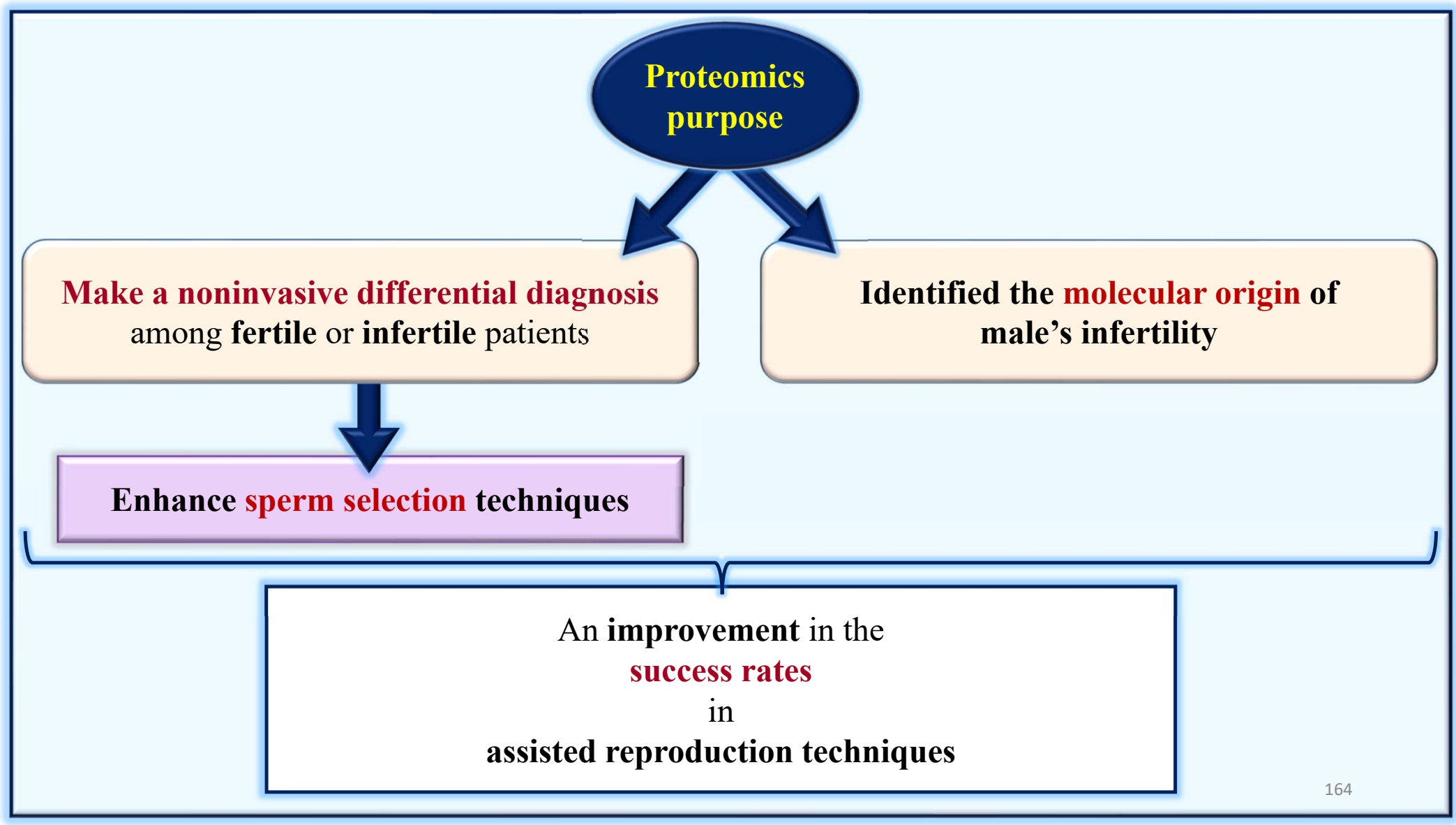
Proteomics

Proteomics evaluates **both** the **structure** and **function** of **cell and tissue's proteins**.

Giving rise to a **deeper insight** in all involved the **physiologic processes** and **molecules**.

It leads to the **discovery** of **numerous proteins** susceptible to be **biomarkers** or **therapeutic targets**.

Proteomics techniques allow **comparing protein profiles** in **2 different biological conditions**.


Proteomics purpose
Make a noninvasive differential diagnosis among fertile or infertile patients
Identified the molecular origin of male's infertility
Enhance sperm selection techniques
An improvement in the success rates in assisted reproduction techniques

The semen analysis by proteomic technology reveals proteins that may be engaged in the infertility condition.

- Because the **semen** contains the **sperm fraction** and **seminal plasma**, together with the fact that **proteins** may **belong** to **seminal plasma**, **sperm**, or **both**, the **analysis by proteomics** technology becomes **complex**.
- The **seminal plasma** is the result of the **secretion** of the **prostate**, **seminal vesicles**, and **bulbourethral glands**.
- It is a **protein-rich fluid** and creates an **ideal environment for spermatozoon survival**.
- Within **seminal plasma**, there are **only tissue-specific proteins** owing to the **blood–testis barrier**, thus generating **specific male biomarkers**.
- The **most abundant** seminal plasma proteins: **lactoferrin**, **semenogelin 1/2**, **transferrin**, **laminin**
- Seminal plasma proteins are used nowadays as **biomarkers** to **screen** the **men's health status: prostate-specific antigen**, **prostatic acid phosphatase**, and **semenogelin**.

Proteomics

- Several reviews expose which potential infertility biomarkers were found in the seminal plasma proteome.
- Eventually, there are **differently expressed proteins** in those **men with a pathologic condition**: abnormal seminal parameters (as oligospermia, asthenospermia, or teratospermia), azoospermia, varicocele, and idiopathic infertility.
- A number of reviews highlights some of the proteins likely to be biomarkers of male infertility, such as **prolactin-inducible protein**, **HAS**, **SPAG11B**, and **TEXT101**.
- Going into detail, the proteins **TEXT101** and **ECM1** are 2 **effective biomarkers** for the **noninvasive diagnosis** of **azoospermia type**.
- **Testicular biopsy** is the only method to **discern** between **obstructive azoospermia** (OA) and **NOA**, a highly invasive procedure.

TEXT101

Can **distinguish** an **OA** from an **NOA**
and
discriminate the **NOA subtypes**.

ECM1

Able to **differentiate**
an obstructive azoospermia from NOA
(or a **normal spermatogenesis**),
with **high sensitivity** and **specificity**.

Diagnoses a
hypospermatogenesis or **maturation arrest**,
in which it is possible to find
few foci of spermatogenesis,
from **Sertoli cell-only syndrome**, in which
there is **no sperm production**.

Clinical assays
of these 2 proteins
offer
a **noninvasive** and **differential diagnosis**,
establishing
the clinical action strategy.

What is its clinical value?

- **Able to assess vasectomy success**
- **Distinguish the NOA subtype**
- **Predict the outcome of sperm retrieval procedures**
- **Avoid testicular biopsy**.

Metabolomics

Metabolomics studies the **biochemical compounds** that **cell generates** and/or **uses** owing to its **metabolism**.

Metabolomic study **complements** the information provided by the analyses performed in **genomics** and **proteomics**, giving a **complete overview** of all the **involved molecules** and their **accurate cell functioning**.

Because metabolomic technology **analyzes** thousands of different types of **metabolites** (**carbohydrates**, **lipids**, **amino acids**, **nucleic acids**, **cofactors**, etc.), **multiple analytical platforms** to maximize the metabolome analysis are required.

The analysis of **seminal plasma** provides **several potential biomarkers** of **male infertility**, with the aim of being used both as **noninvasive diagnostic** and **predictive tool** of the assisted reproduction treatment success.

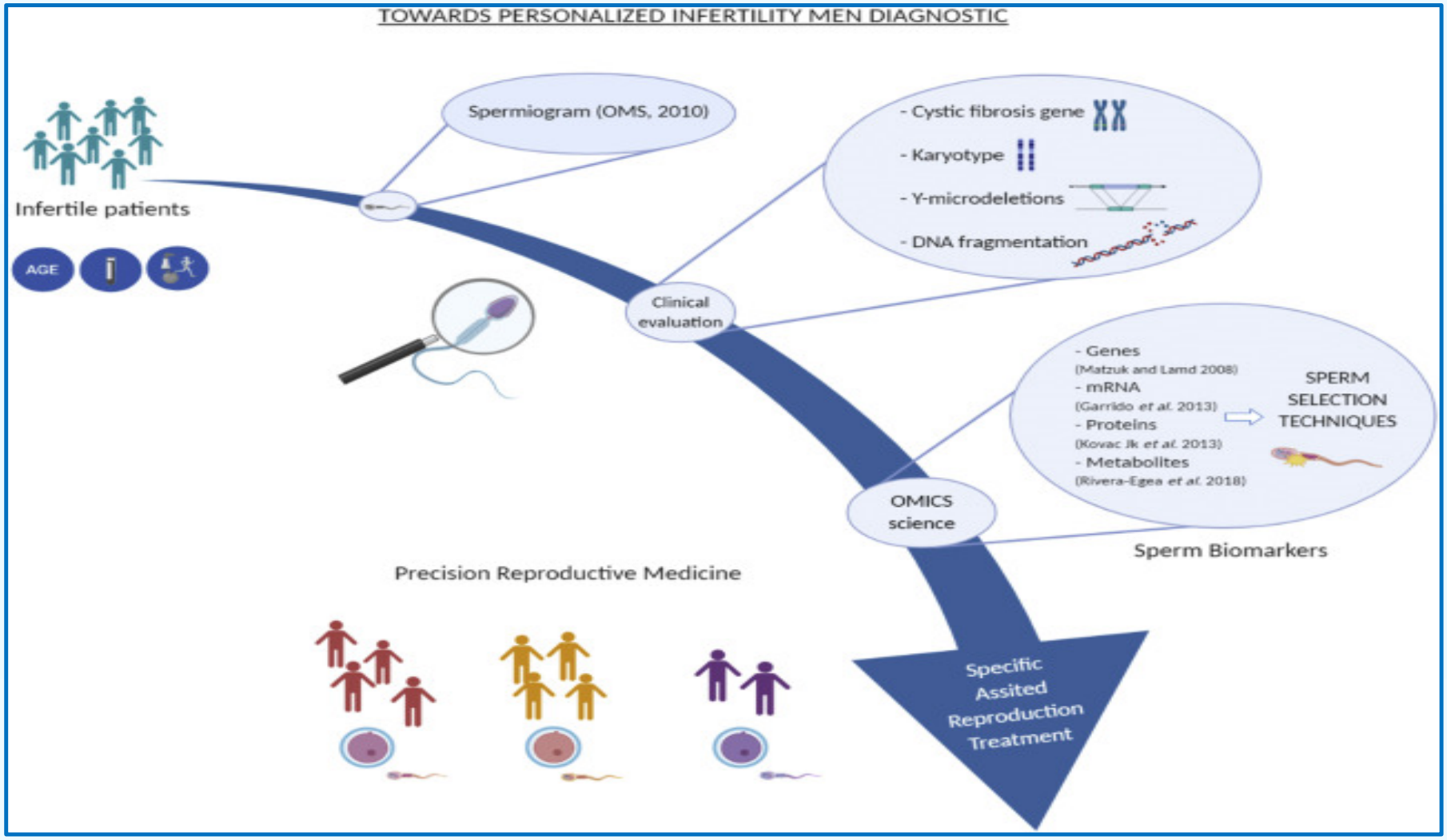
Future investigations will reveal whether these metabolic analyses can be included in the clinical routine.

New directions

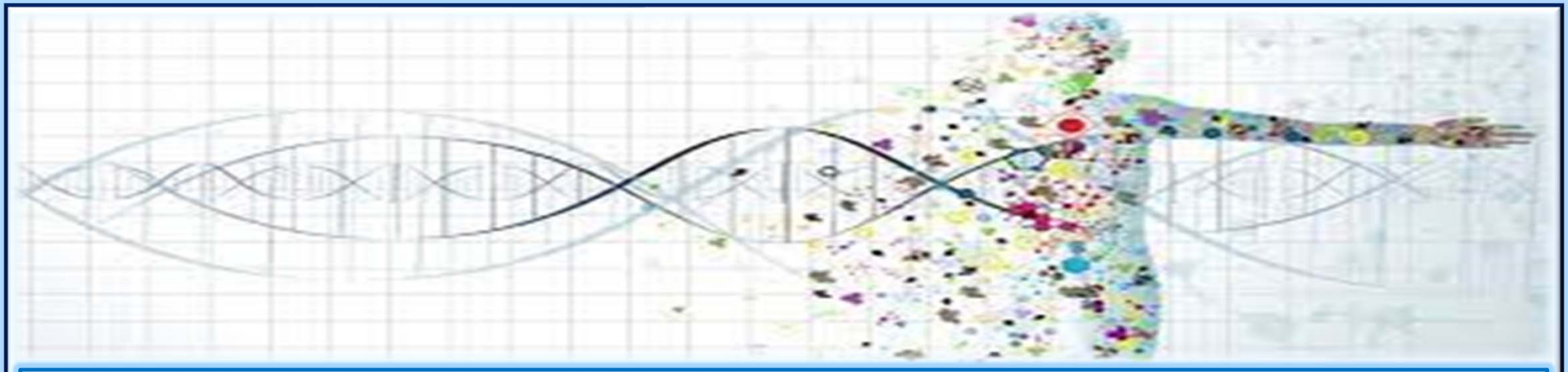
These **add-on treatments** are frequently being **criticized**, owing to the **lack of evidences supporting their use**.

It is necessary to conduct **randomized controlled trials** with the aim to demonstrate their **safety** and **efficacy** before introducing them into **clinical practice**.

In the near future,
there will be **multiparametric assays** able to measure **a set of sperm biomarkers** useful in the personalized diagnosis of male infertility,
and
a number of **specific**, **evidence-based**, **personalized** **sperm selection** or **therapeutic techniques** will improve the reproductive results of infertile males.



Personalized Medicine in Infertile Men, Garrido et al., **Urologic Clinics of North America, 2020**



Precision medicine
gathers the **most relevant data** involved in **human health**,
from the **genetic code** to **social behaviors**
to
specifically design medical solutions for **specific populations** or **cases**.
This new insight will allow **huge advances** in the **diagnosis** and **treatment** of reproductive diseases, which will be reflected in personalized health care for patients who comes to an assisted reproduction center.


Thank you for your attention