Non-Invasive PGD to Improve the Result of ART

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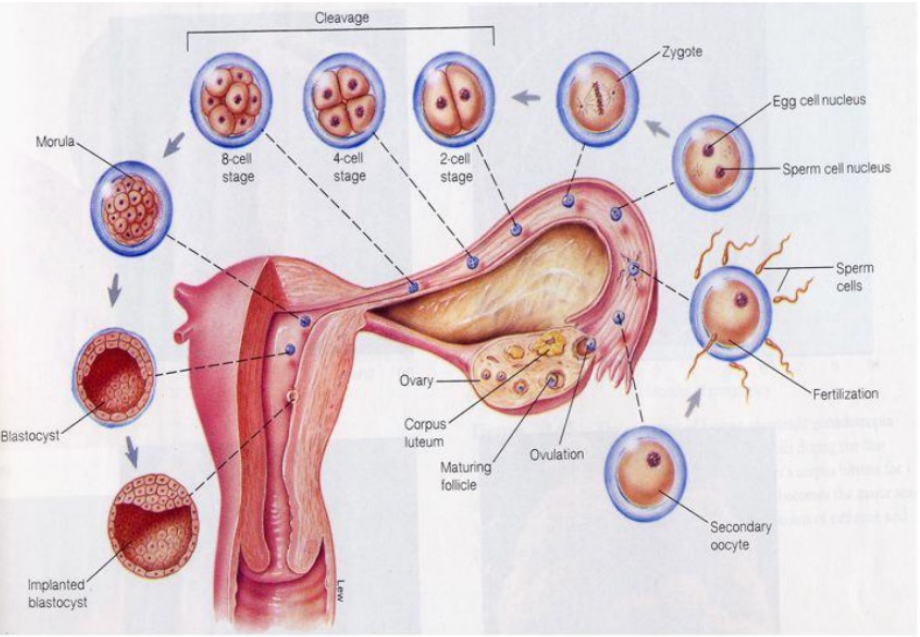
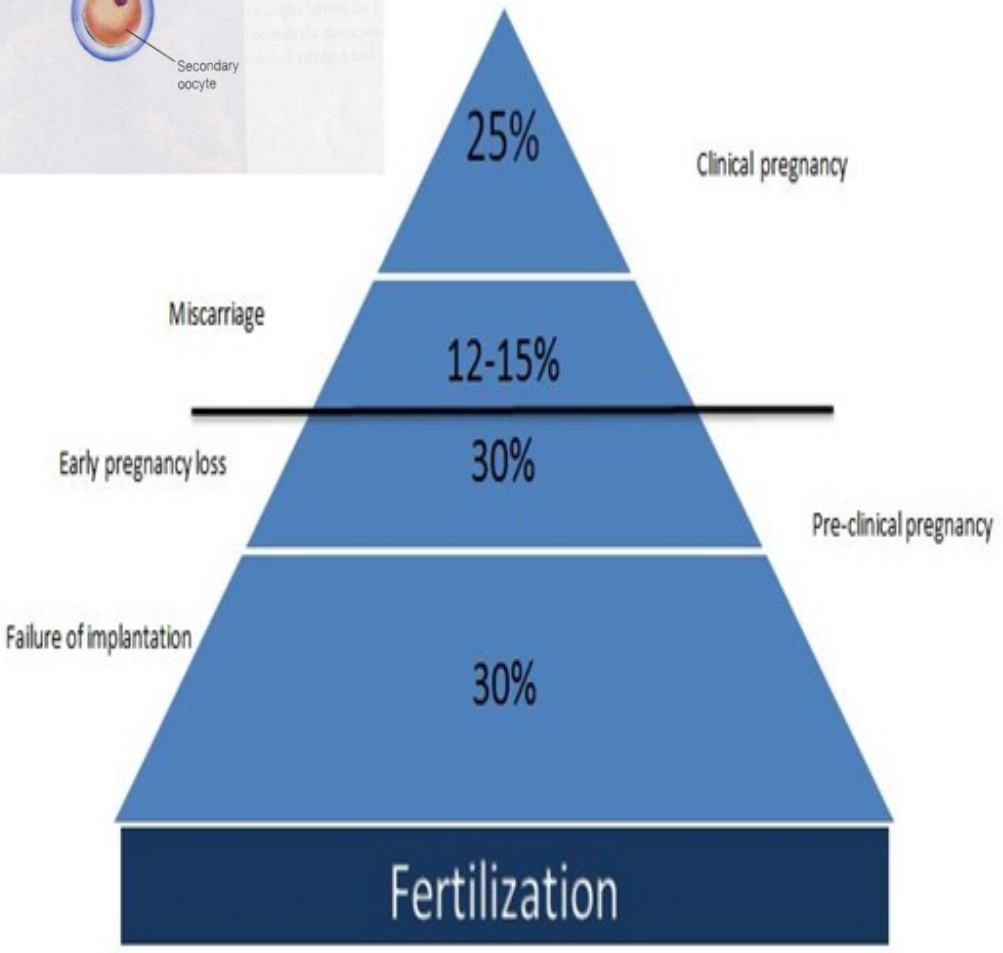
INFERTILITY

Childless couples (C.L.C)
I.RPL
II. Infertility

- **Q: What is the problem?**


Cleavage
Zygote
Egg cell nucleus
Sperm cell nucleus
Morula
8-cell stage
4-cell stage
2-cell stage
Sperm cells
Fertilization
Ovary
Corpus luteum
Maturing follicle
Ovulation
Blastocyst
Secondary oocyte
Implanted blastocyst


25%
Clinical pregnancy
Miscarriage
12-15%
Early pregnancy loss
30%
Pre-clinical pregnancy
Failure of implantation
30%
Fertilization

Introduction

- Miscarriages are very common, affecting about **15% of all known pregnancies**.
- Much higher incidence if those PRs that are lost before the 1st missed period included.
- Recurrent pregnancy loss (RPL) is devastating to couples who desire children.

Pregnancy losses can be classified as:

1-**Occult**(preclinical or chemical) pregnancy loss prior to missed menses.(40% of implantation embryos)

2-**Early** pregnancy loss before 12 wk.(13%)

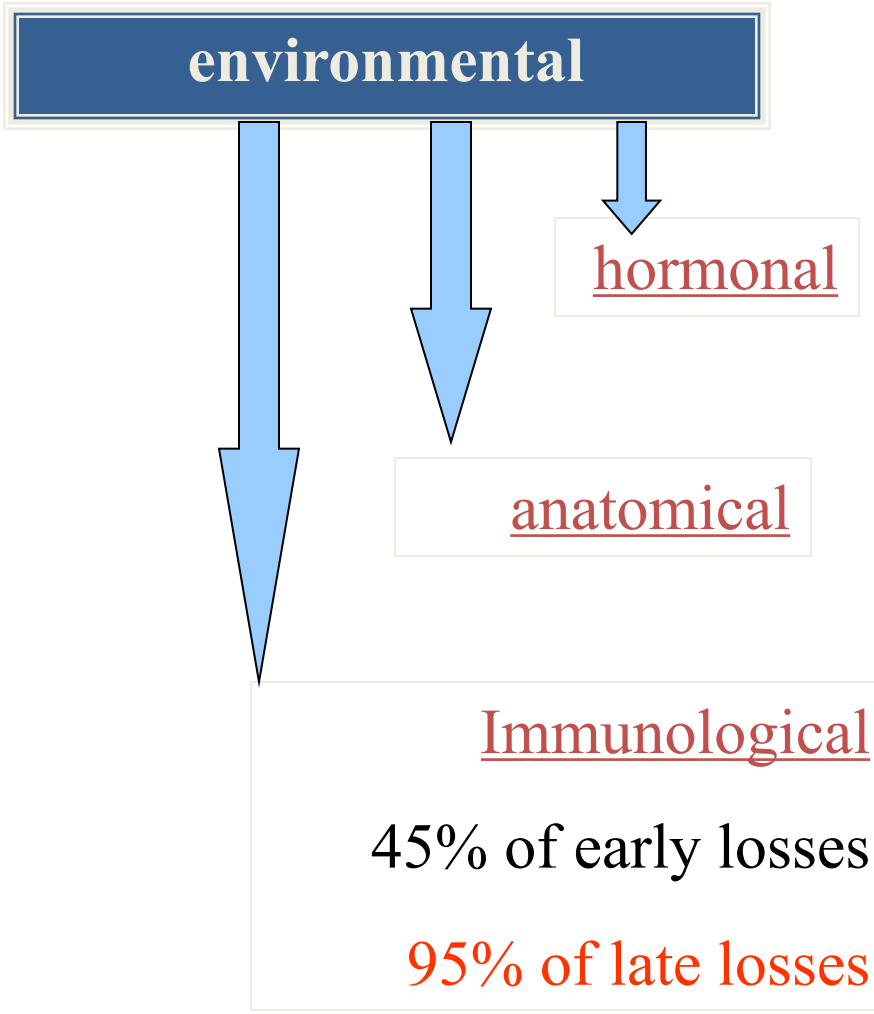
3-**Late** pregnancy loss after 12 wk. (1%)

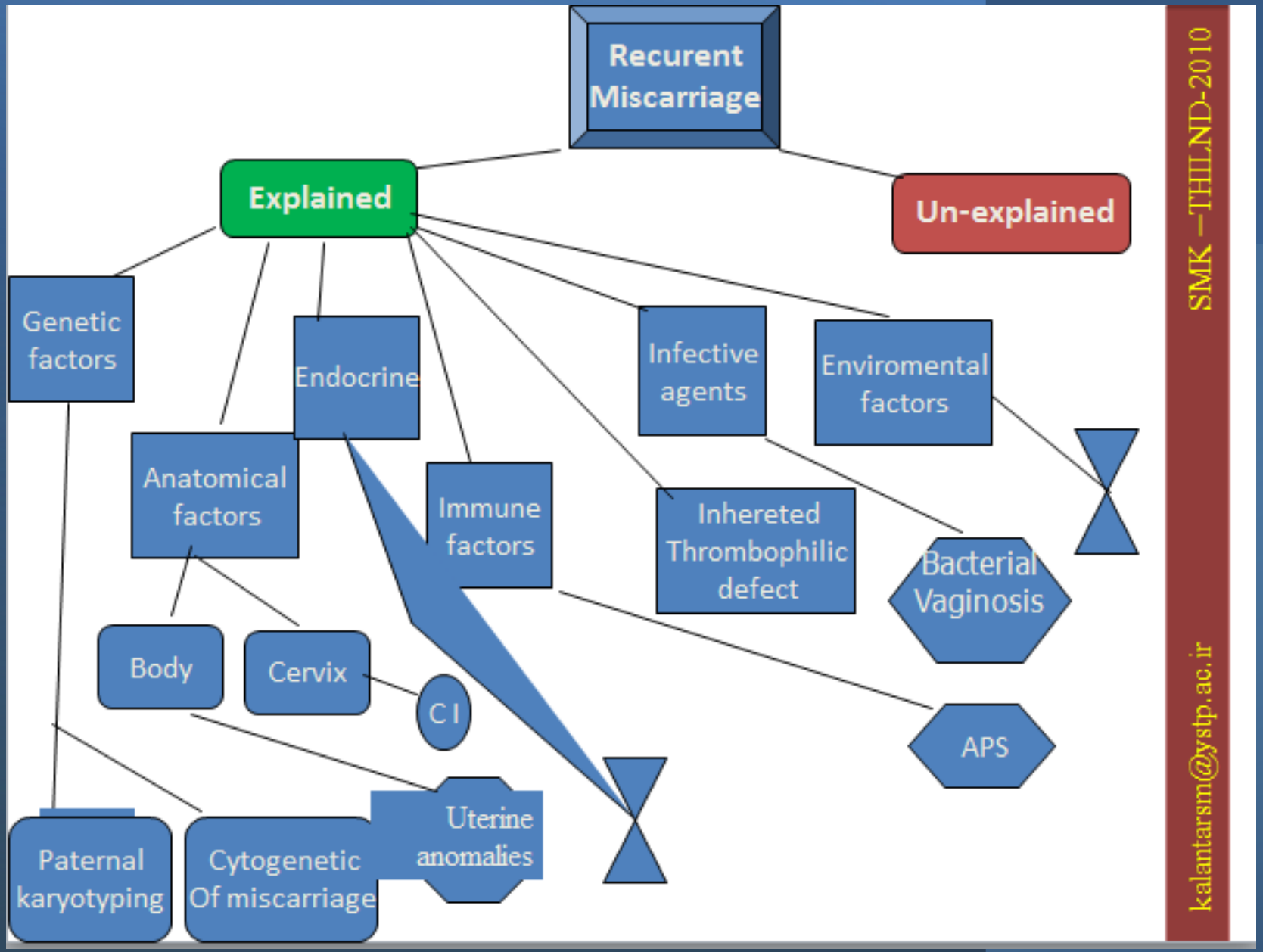
Causes of pregnancy loss

Chromosomal

55% of occult and early losses

5% of recurrent losses.




Recurent Miscarriage
Explained
Un-explained
Genetic factors
Endocrine
Infective agents
Enviromental factors
Anatomical factors
Immune factors
Inhereted Thrombophilic defect
Bacterial Vaginosis
Body
Cervix
CI
APS
Paternal karyotyping
Cytogenetic Of miscarriage
Uterine anomalies
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Chromosomal abnormalities

The genetic diseases are divided into two categories:

Chromosomal abnormalities

are caused by cells that

have extra or missing chromosomes (aneuploid)or parts of chromosomes (Deletions).

Gene abnormalities

occur when the genetic instructions stored in the DNA are altered so that the protein product coded for by the gene is less functional or nonfunctional

Chromosomal abnormalities POC

- Occurs in 50-60% of eggs retrieved for IVF.
- in 50% of all early preg. Loss .
- in 5% of late losses
- in 0.5% of live births.

Aneuploidy

Aneuploid fetus risk in women >35y age- AMA → 1/80

Inherent risk of fetal loss after amniocentesis → 1/200

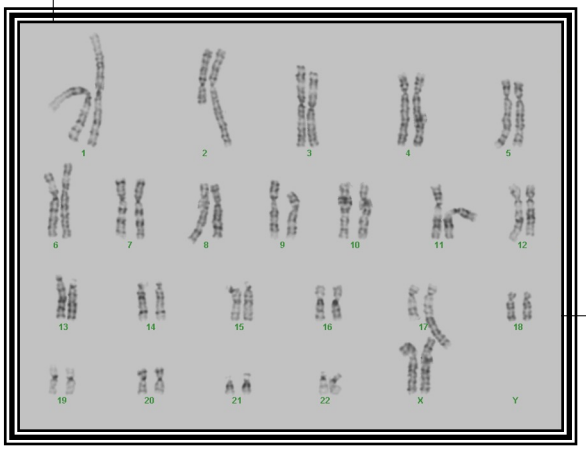
SO,The standard of care is to offer genetic amniocentesis for all pregnant women older than 35 years.

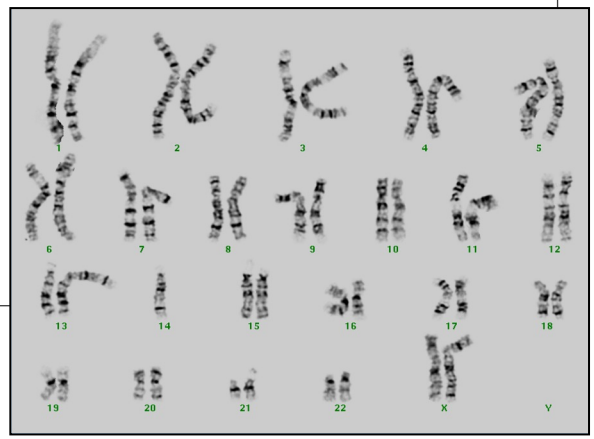
Translocations

A translocation is the transfer of genetic material from one chromosome to another.

Reciprocal translocations

Robertsonian translocations





Polyploidy

Polyploidy is a condition in which there is more than 2 sets of chromosomes. Triploids (3N), tetraploids (4N), pentaploids (5N) etc.

Polyploids have defects in nearly all organs.

Most die as embryos or fetuses. Occasionally an infant survives for a few days.

Nondisjunction

Nondisjunction occurs when chromosomes fail to "disjoin" during meiosis or mitosis.

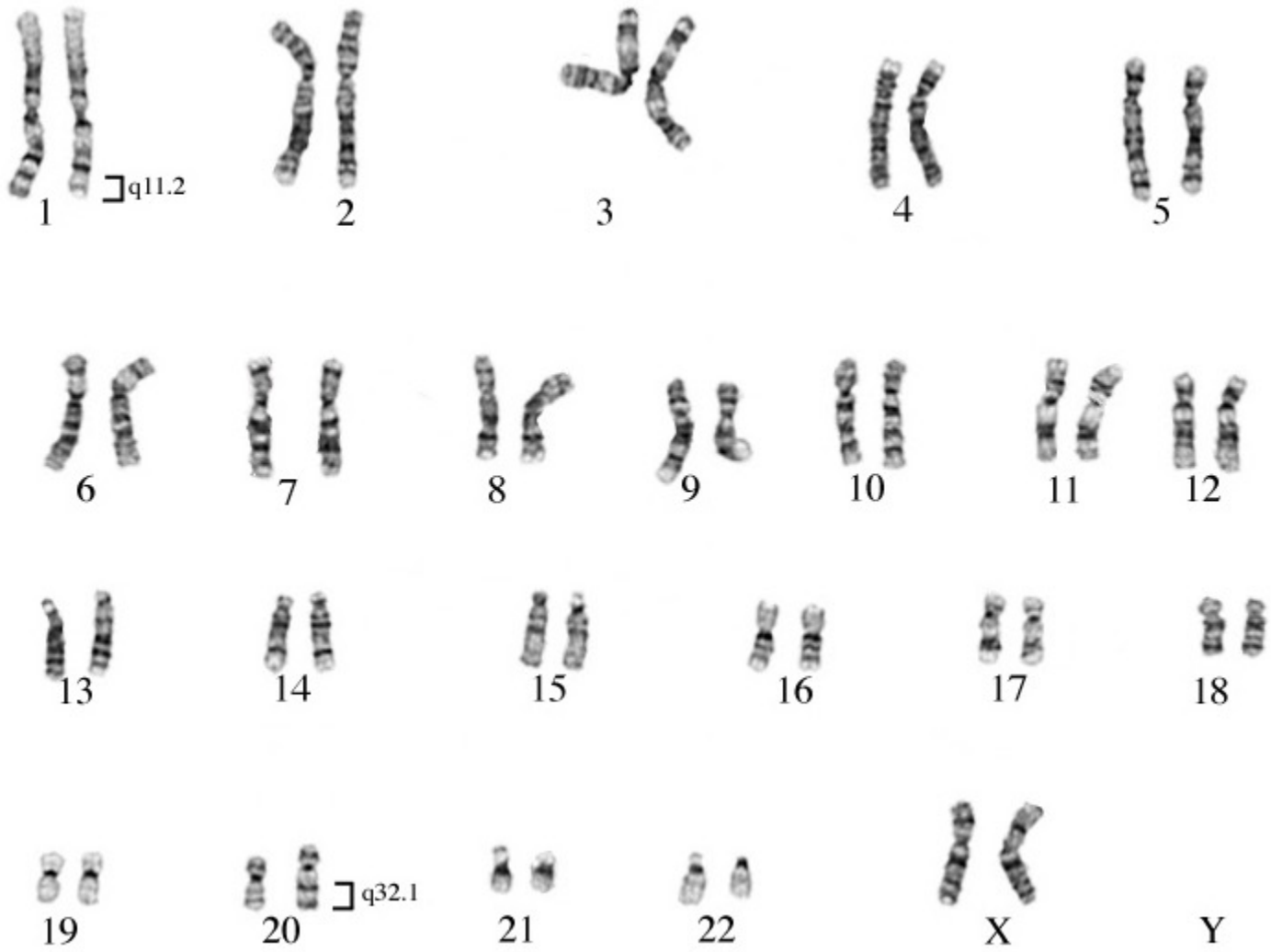
Frequency of Cytogenetic finding

TYPE	FREQUENCY	PERCENT
46,xx/45.xo	3	3.5
47,xxx	1	1.1
46,xx,t (14,13)	1	1.1
46,xx,del 13p	1	1.1
46,xy,del 13p	1	1.1
46,xx,del . 1q (end ter.)	1	1.1
46,xx/46.xx,del 3p	1	1.1
46,xx,add 13p	1	1.1
46,xy,dup. 4 (q31.1,q32)	1	1.1
47,xx +mar	1	1.1
46,xy/47,xy +mar	1	1.1
normal	73	84.9
total	86	100

46,XX,inv 9 46,XY,inv 9

Q: Different population?
No chromosomal for gene disorders (possible)

ZWK99027 KEY



46, XX, t(120)(q32.1q11.2)

Genetic counseling after a miscarriage with CA

- risk for fetal aneuploidy in couples with recurrent miscarriages is 1.6%, similar to the aneuploidy risk in a woman older than 40 years.
- In a woman with a prior trisomic livebirth, an approximately 1% increased risk exists for subsequent trisomic birth.

Solution

- Dependent of cause
- PND
- PGD
- Aspirin, Heparin
- others

- Nearly 72.4 million people or 15% of couples experience fertility problems
- For couples and clinicians, a diagnosis of infertility signals the start of investigations and possible treatment.
- Infertility, defined as failure to conceive a clinically detectable pregnancy after >12 months of unprotected intercourse, is a common condition, reported by 1 in 6 couples

- As infertility is a **heterogeneous condition**, caused by various underlying pathologies,
- it is possible that some of the mechanisms leading to infertility also play a role in the etiology of this outcome
- In recent years, several advancements have been made in assisted reproduction treatment and now more than **80% of couples** experiencing infertility issues can **conceive a child**

Childless couples
The cause of Infertility

- Male infertility
- Female infertility
- Male & Female Infertility
- idiopathic Infertility

Male infertility

- Reproductive tract
  - Varicocel , BAVD
- Oligospermia,
- sever oligo,
- Azoospermia
- OAT

Genetic:

Chromosomal (numeric, structural)

Gene abnormalities

mtDNA

Female Infertility

- Before FR:
  - Reproductive tract
  - Hormonal abnormality
  - Ovarian problem
- Post FR
  - Cleavage stage
- Implantation
  - Post implantation

Genetic:

Chromosomal (numeric, structural)
Gene abnormalities
mtDNA

Solution

- IUI
- IVF
- ICSI
- ZIFT, GIFT
- IVM
- Donation (Sperm, Egg, Embryo)
- Surrogate

Ejaculation

PESA

TESE

Ovarian stimulation

Puncture

ET

Introduction

- **Pre-implantation genetic diagnosis (PGD) was first applied in 1988 using a polymerase chain reaction (PCR) protocol to amplify a sequence on the Y chromosome for embryo sexing for patients carrying X-linked disease.**

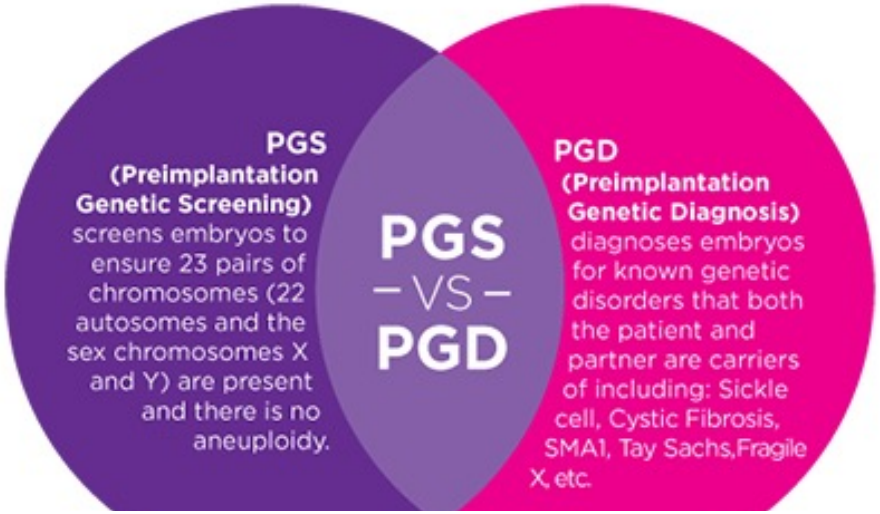
Q: Problem?
A: Success rate

- Based on **CDC's 2019 Fertility Clinic Success Rates** Report, there were **330,773*** ART cycles performed at **448 reporting clinics** in the United **States during 2019**, resulting in **77,998 live births** (deliveries of one or more living infants).
- 77998/330773= **23.6%**

- Although the national average was about 29%, the percentage of ART cycles that resulted in live births varied somewhat depending on the couple's diagnosis.
- However, the use of these diagnostic categories may vary from clinic to clinic, and the definitions are imprecise

- Presently, assessment of embryo quality based on morphologic
- criteria is the predominant non-invasive technique for selecting
- viable embryos and this provides valuable information for the prediction
- of IVF/ICSI-ET outcomes

Preimplantation Genetic Diagnosis (PGD) or Screening(PGS)



- ***Solution:***

Contents lists available at ScienceDirect

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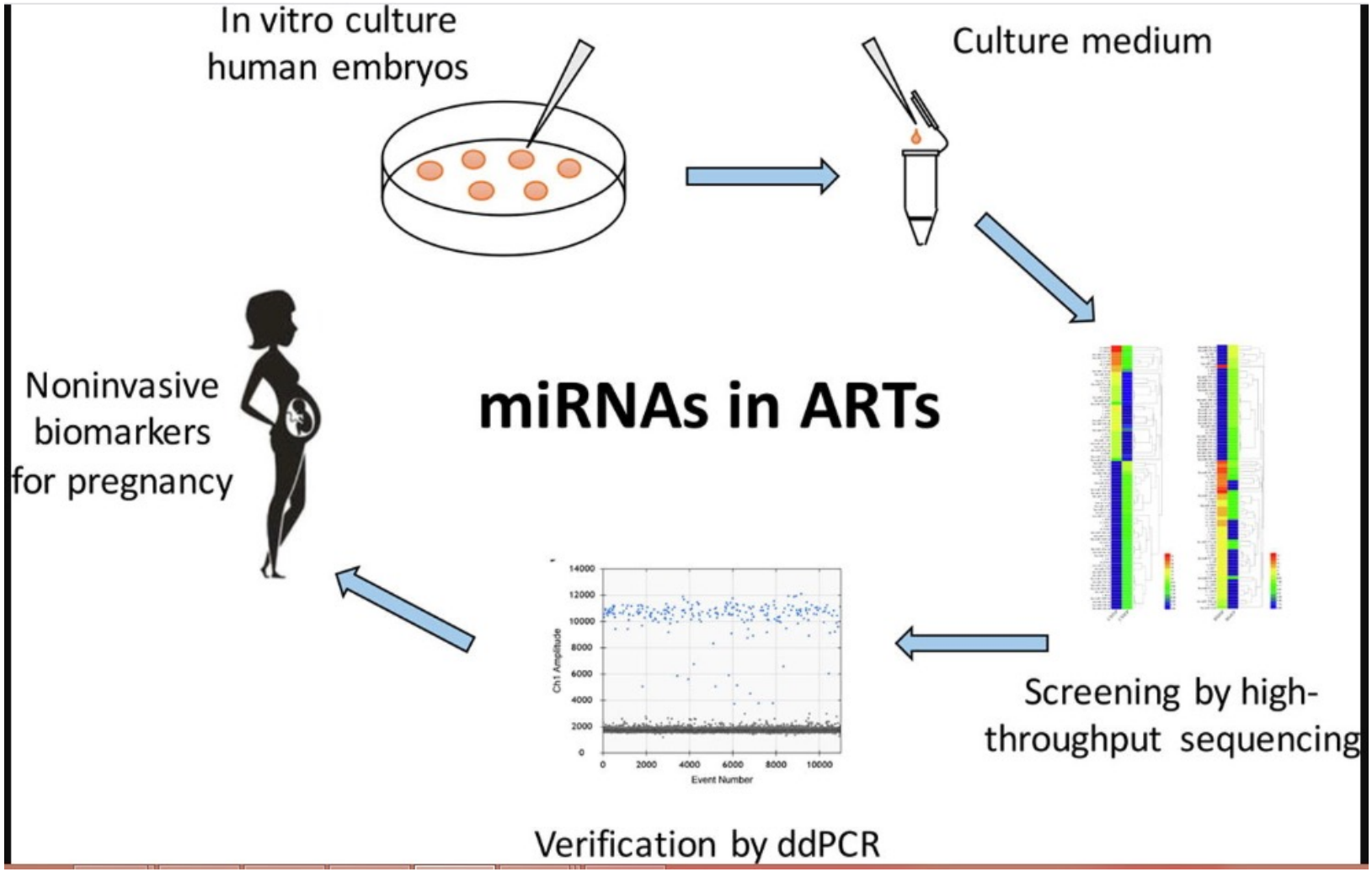
MicroRNAs secreted by human embryos could be potential biomarkers for clinical outcomes of assisted reproductive technology



- **High-throughput proteomics and metabolomics technologies are valuable tools for noninvasive embryo analysis. The biggest advantages of such technology are that it can differentiate between the embryos that appear morphologically identical and has the potential to identify the ploidy status noninvasively prior to transfer in a fresh cycle or before verification for a later frozen embryo transfer.**

International Journal of *Molecular Sciences*

MDPI

Review

Current Advancements in Noninvasive Profiling of the Embryo Culture Media Secretome

Raminta Zmuidinaite [1], Fady I. Sharara [2] and Ray K. Iles [1,3,*]

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JBRA Assisted Reproduction 2019;**23**(4):352-360
doi: 10.5935/1518-0557.20190039

Original article

Expressed proteins and activated pathways in conditioned embryo culture media from IVF patients are diverse according to infertility factors

Tatiana CS Bonetti[1], Debora CM Haddad[2], Thais S Domingues[1,3], Jose Roberto Alegretti[3], Eduardo LA Motta[1,3], Kent Seeley[4], Ismael DCG Silva[1]

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[3]Huntington - Medicina Reprodutiva. Brasil
[4]Proteomics and Mass Spectrometry Facility, Center for Drug Discovery and Innovation (CDDI), University of South Florida (USF). USA

Conclusions: Proteomic embryonic secretome will advance our knowledge of early embryogenesis and additionally could lead to improved selection of embryos for transfer warrants further investigation.

Journal of
Clinical Medicine

MDPI

Article

Non-Invasive Human Embryo Metabolic Assessment as a Developmental Criterion

Marjan Motiei [1,*], Katerina Vaculikova [2], Andrea Cela [3], Katerina Tvrdonova [4,5], Reza Khalili [6], David Rumpik [7], Tatana Rumpikova [7], Zdenek Glatz [3] and Tomas Saha [2]

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3 Department of Biochemistry, Faculty of Science, Masaryk University, 62500 Brno, Czech Republic;

- **Conclusion:** The different significant ratios of metabolomic depletion/appearance between the embryos confirm their potential for the improvement of the prospective selection of the developed single embryos, and also suggest the fact that pyruvate and alanyl-glutamine are the most critical ATP suppliers on the fifth day of blastocyst development.

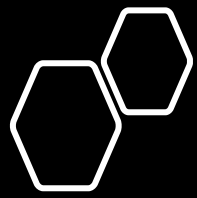
SCIENTIFIC REPORTS
nature research

OPEN

Minimally Invasive Cell-Free Human Embryo Aneuploidy Testing (miPGT-A) Utilizing Combined Spent Embryo Culture Medium and Blastocoel Fluid –Towards Development of a Clinical Assay

Valeriy Kuznyetsov[1,5]✉, Svetlana Madjunkova[1,5]✉, Rina Abramov[1], Ran Antes[1], Zenon Ibarrientos[1], Gelareh Motamedi[1], Afsaneh Zaman[1], Iryna Kuznyetsova[1] &

- Regarding the origin of embryonic cfeDNA, the average amount of miPGT-A WGA-DNA we obtained from blastocysts with different morphological grades, as well as the size miPGT-A WGA-DNA fragmentssuggest that it is unlikely that apoptosis and necrosis are only mechanisms of DNA release from the inner cell mass (ICM) and TE (trophectoderm) into BF (blastocoel fluid) and SEM (spent embryo culture medium) .



Xie *et al. Journal of Translational Medicine* (2022) 20:396
https://doi.org/10.1186/s12967-022-03596-0

Journal of Translational Medicine

RESEARCH Open Access

Non-invasive preimplantation genetic testing for conventional IVF blastocysts

Pingyuan Xie[1,3,5†], Shuoping Zhang[4†], Yifang Gu[2,3,4], Bo Jiang[5], Liang Hu[2,3,4,5], Yue-qiu Tan[2,3,4,5], Yaxin Yao[6], Yi Tang[2,3,4], Anqi Wan[6], Sufen Cai[3,4], Yangyun Zou[6], Guangxiu Lu[2,3,4], Cheng Wan[6], Fei Gong[2,3,4,5], Sijia Lu[6*] and Ge Lin[2,3,4,5*]

- **Conclusions:** Our research results preliminarily confirm that the niPGT approach using spent culture medium (SCM) from conventional IVF has comparable performance with ICSI and might broadening the application scope of niPGT.

Current Advancements in Noninvasive Profiling of the Embryo Culture Media Secretome

by Raminta Zmuidinaite [1], Fady I. Sharara [2] and Ray K. Iles [1,3,*]

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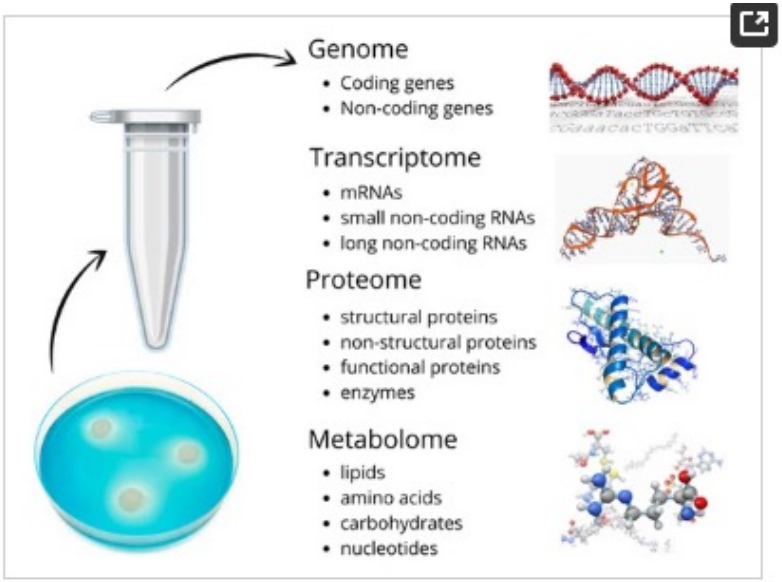


Figure 1. Schematic of materials found in spent blastocyst medium (SBM) that pose the diagnostic potential for embryo quality and ploidy status.

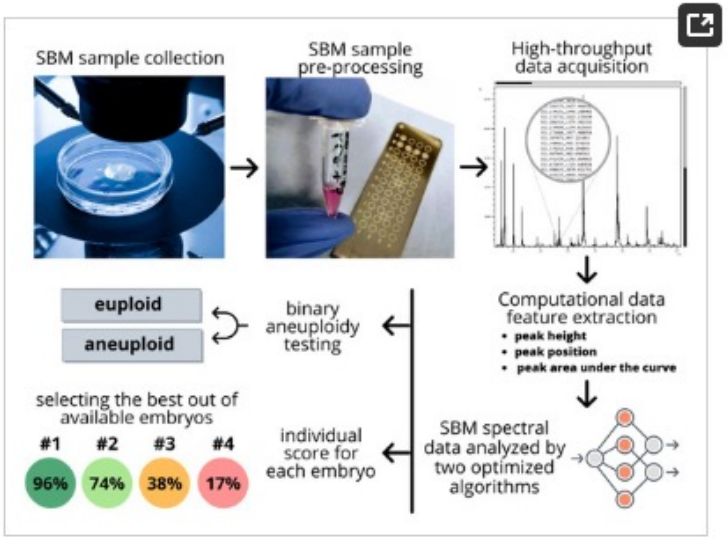
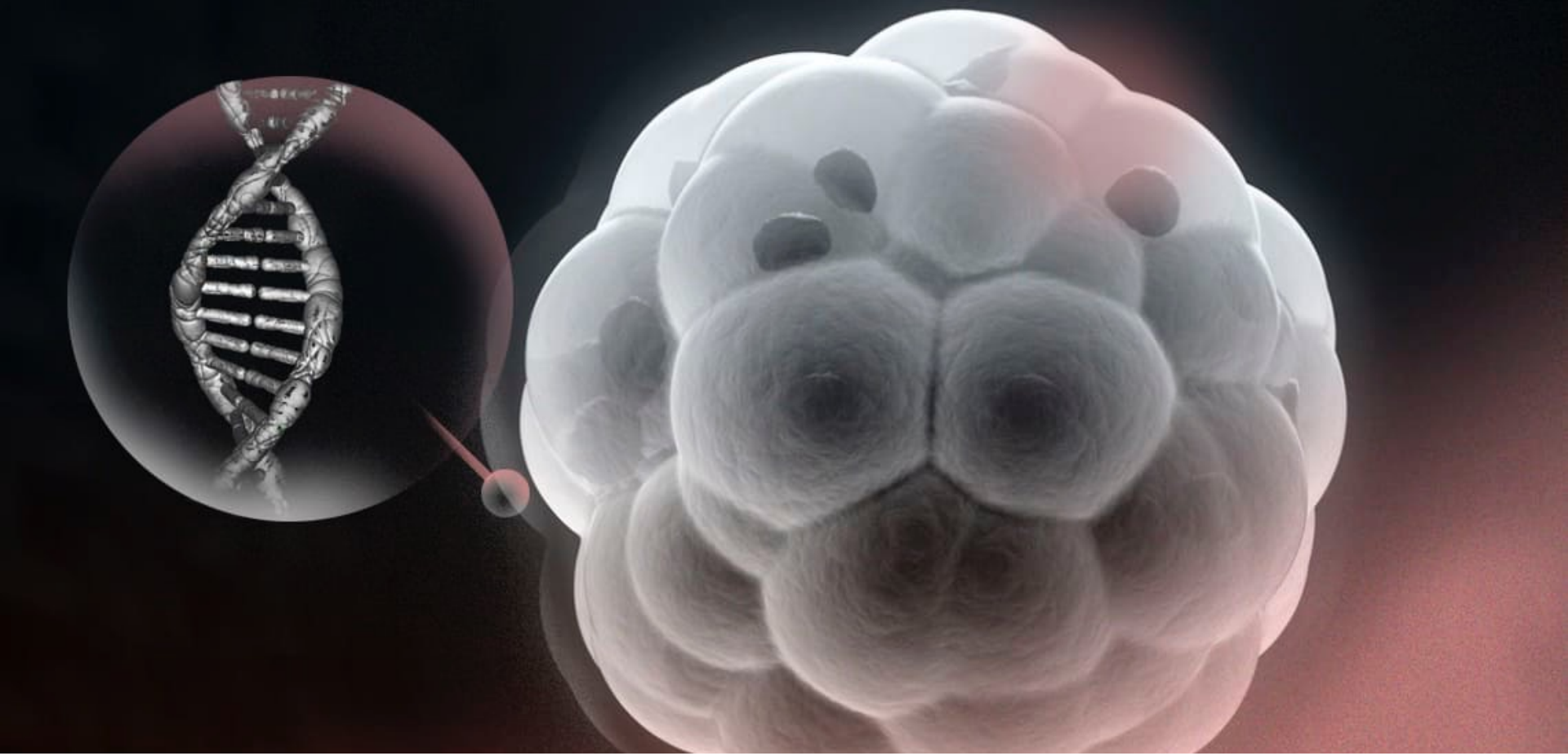


Figure 2. Pipeline of a high-throughput embryo analysis from a petri dish to a computational score outcome for the individual embryos. The pipeline goes from a non-invasive spent blastocyst medium (SBM) sample collection to the obtainment of final scoring that facilitated selection of the best available embryo for implantation.



RUNNING THE PROJECT IN YAZD TO BE DISCUSSED:

Phase I

Evaluating cell-free DNA in spent embryo culture media in cleavage and blastocyst stage

STEP 4

Nanodrop

STEP 3

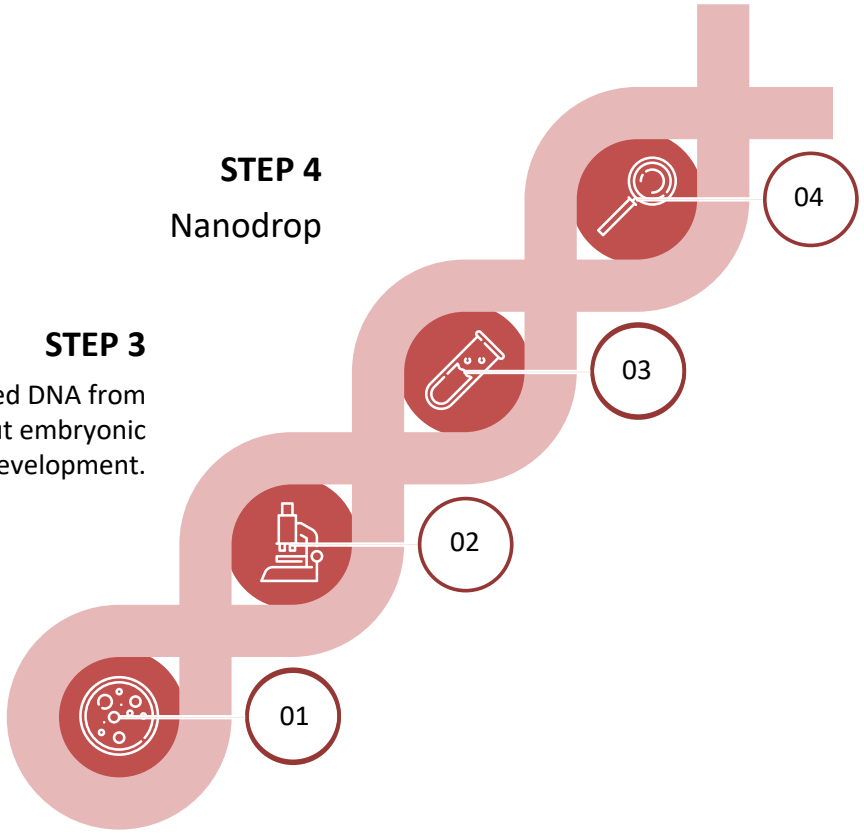
Contaminated with purified DNA from human blood / Without embryonic development.

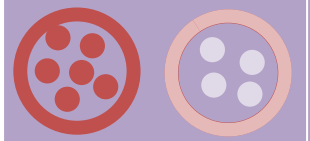
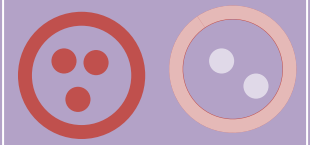
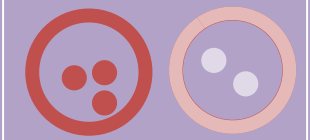
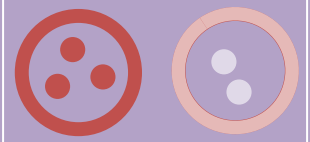
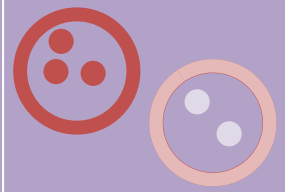
STEP 2

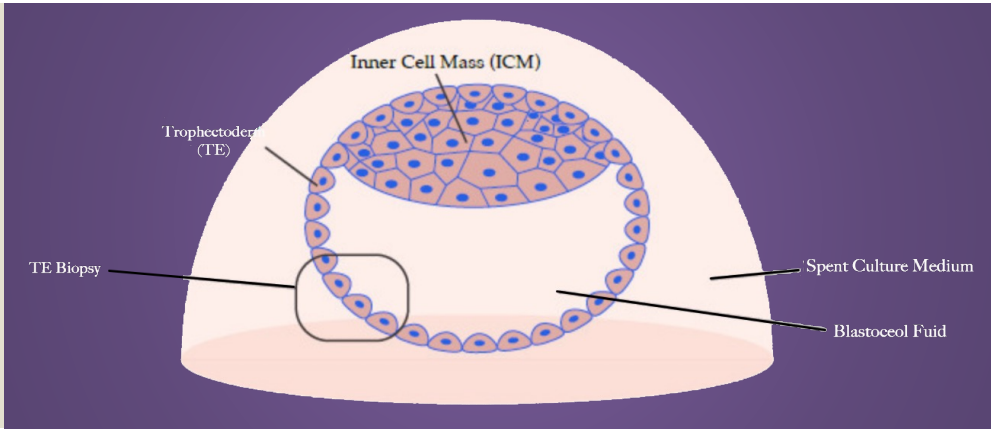
Spent embryo culture mediums In 5 groups. (Day 3 and Day 5-7)

STEP 1

in vitro fertilization, and each embryo was in one drop of culture medium



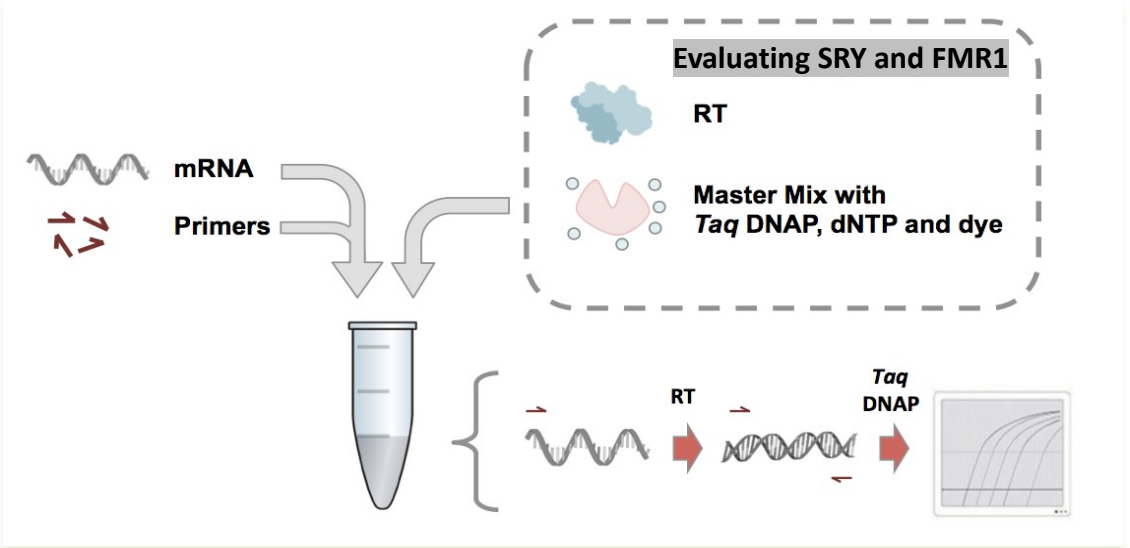
Group 1	Group 2	Group 3	Group 4	Group 5
 Day 3 Day 5-7				
Concentrated by heating	Genet bio kit	YTzol pure DNA kit (yekta Tajhiz)	Pure Viral Nucleic Acid extraction kit (Roche)	whole genome amplification without purification



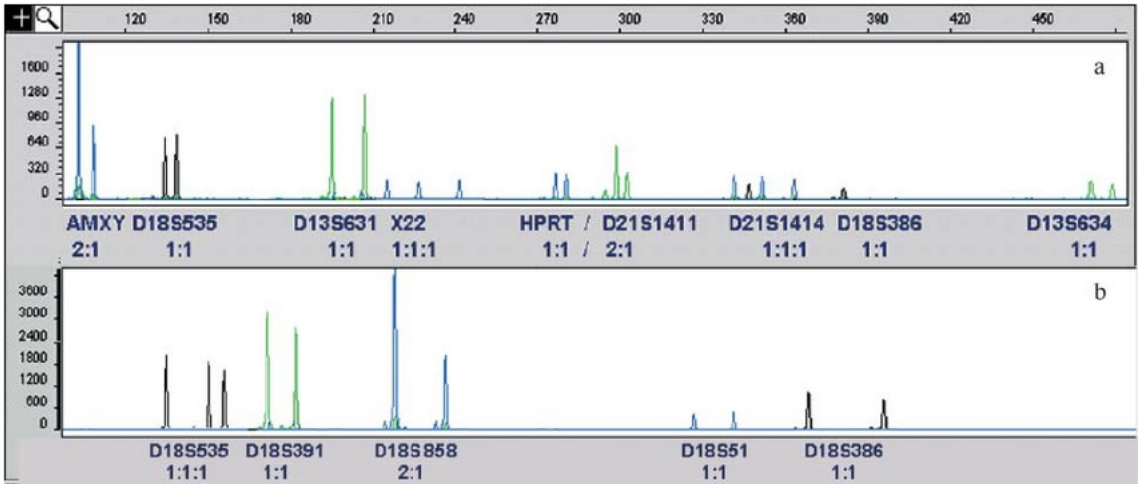
Result

Although cell-free DNA was confirmed in the samples using nanodrop (With a range of 160 to 225 ng per microliter), the cycle of threshold (CT) did not observe in the real-time PCR product of group 1. The Purified samples were amplified in groups 2,3 and 4 for SRY and FMR1 genes with real-time PCR and observed only acceptable CT in the fourth group.

Real-time RCR

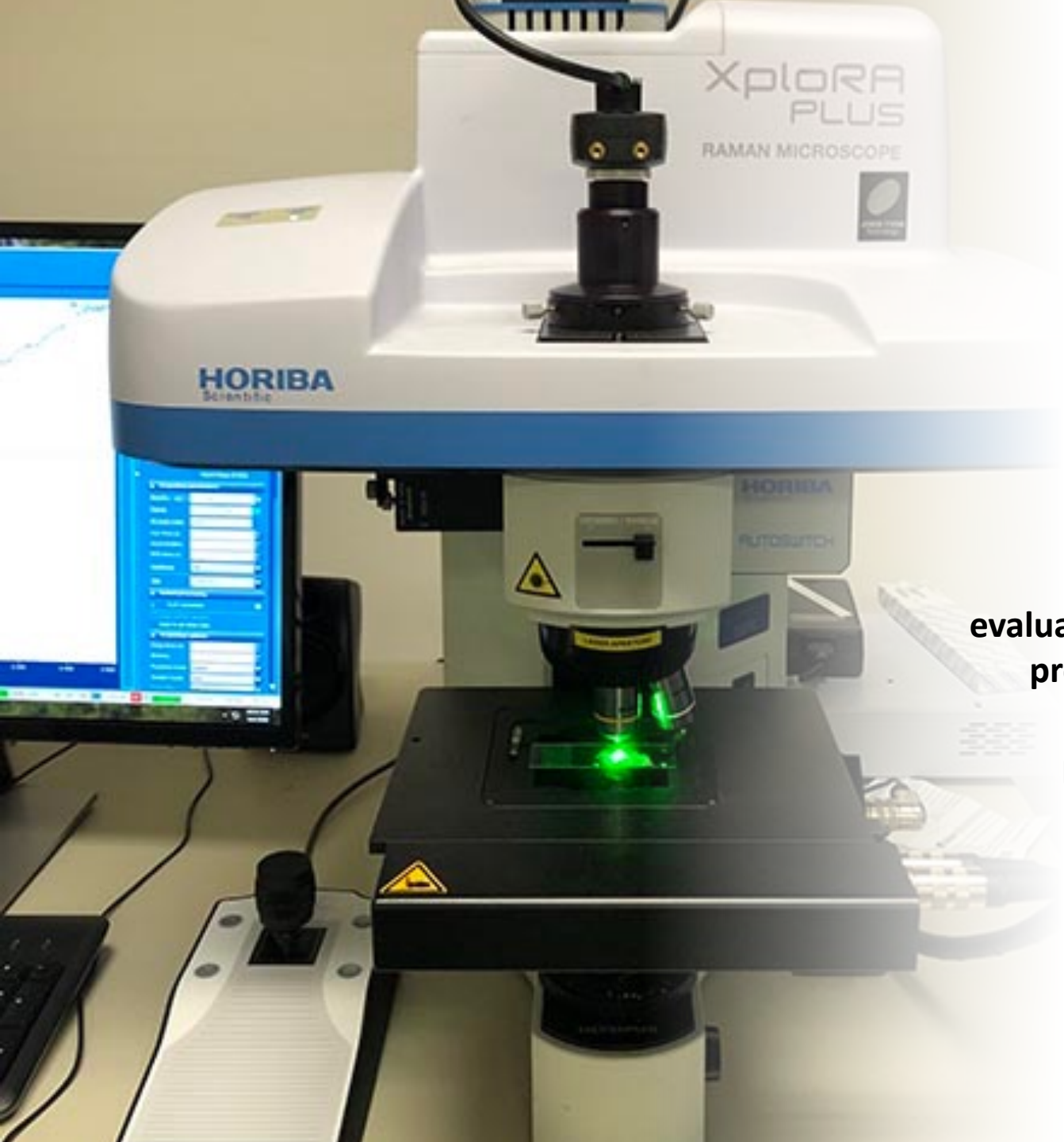


qF-RCR



Cell-Free DNA

- Spent culture medium is a potential alternative source of embryo DNA and a promising procedure for the genetic testing of all developing embryos.
- detection/amplification and concordance rates need to be improved before implementing Spent culture medium-PGT in routine clinical practice.
- The high protein and solutes in the culture medium and the low amount and quality of DNA are Restrictive.
- it is necessary to purify the genomic DNA and amplify it with precise kits.
- Our research is underway to improve DNA collection, amplification, and testing to isolate genomic DNA.

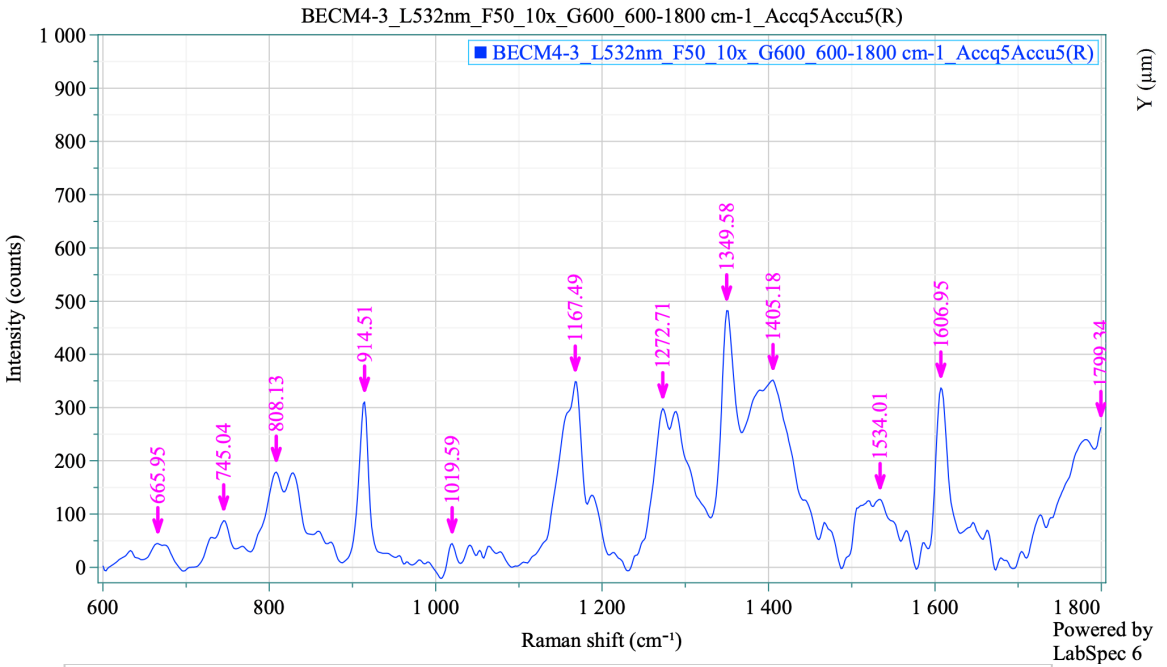


Phase II

evaluate the quality of embryos produced during in vitro fertilization by

Raman spectroscopy

Conclusion: This study suggests that chromosomal abnormalities in embryos lead to changes in metabolic footprints in embryo growth medium. This feature could be detected by Raman spectroscopy and, using machine learning, was analyzed by Raman- based footprint profiling of spent culture media.



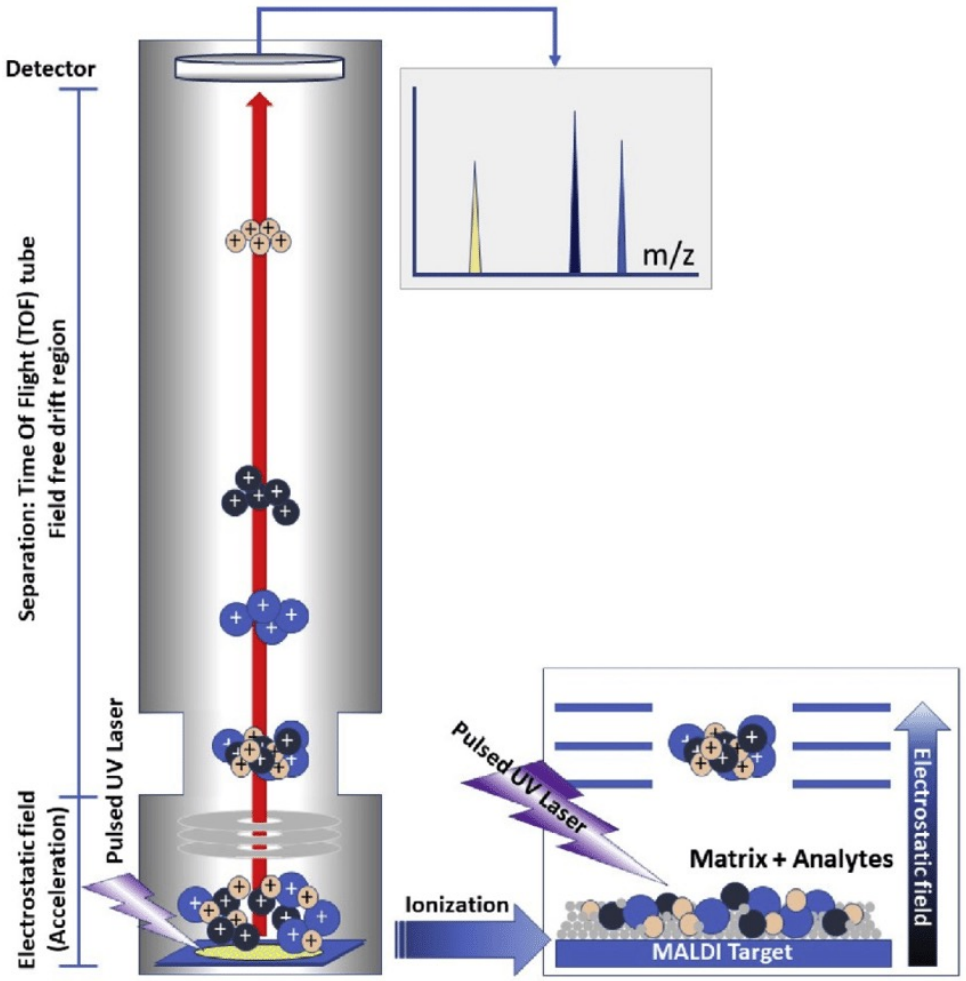
To establish the analysis protocol, 30 spent human embryo culture medium samples (blastocyst) with defined morphologic grades were collected and measured using Raman spectroscopy. Individual Raman spectra of the embryo culture medium was analyzed to find biological components. To validate the protocol via Principal Component Analysis, additional 250 Raman spectra from 30 embryo culture media were analyzed.

Phase III

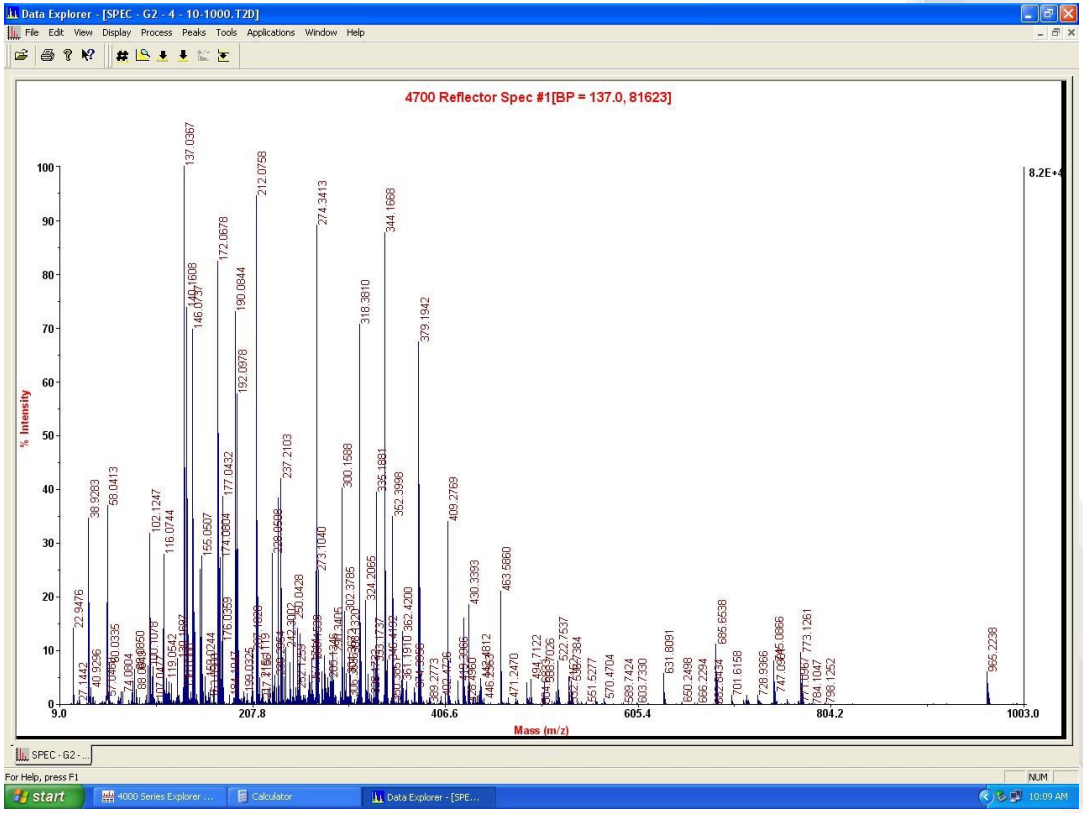
- **Evaluation of metabolomics profiles of human embryos**

The technique, metabolomics data, such as

- **MALDI-TOF-MS**
- **HP LC-MS/MS**

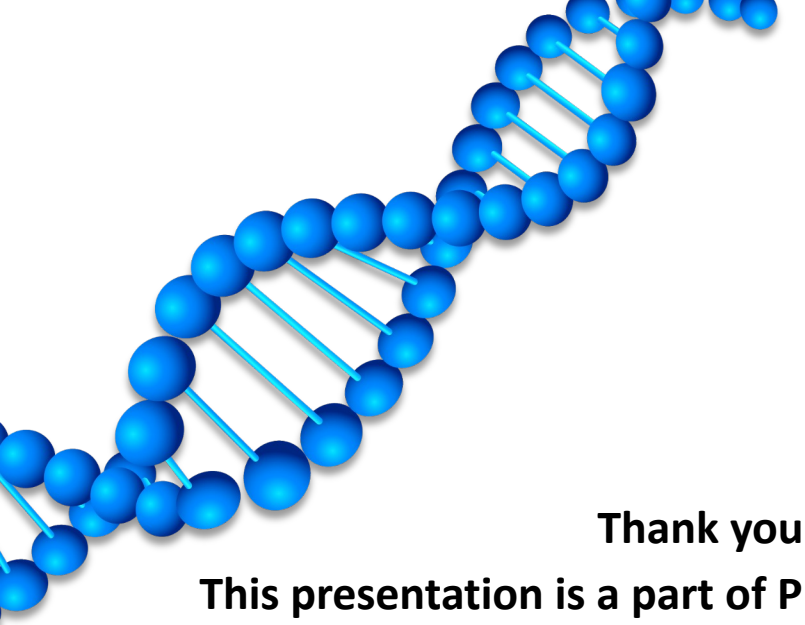


peaks were detected. More than 400 metabolites were identified of secreted metabolome from Day 3 and 5 embryo culture medium using Human Metabolome Database. However, only 9 identified metabolites overlapped between Day 3 and 5. Reactome analysis showed metabolites belonging to metabolism, signal transduction and transport of small molecules were enriched. However, the majority of metabolite's biological activity remains unknown.



9 identified metabolites were in overlap between Day 3 and 5

- L-Alanine
- Ascorbic acid
- Halocins
- Tryptophyl-Proline
- 2-Polyprenyl
- 3-methyl
- 5-hydroxy
- 6-methoxy
- 1,4-benzoquinone



Thank you

This presentation is a part of Ph.D. Thesis titled as:

Evaluation of QF-PCR, HPLC-MS/MS and Confocal Raman Microscopy in Medium-based noninvasive approach compared to Array CGH and FISH methods using human blastocyst biopsy for the diagnosis of chromosomal abnormality