UNIVERSITÀ DEGLI STUDI DI TERAMO

Second-Cycle Degree Course in "REPRODUCTIVE BIOTECHNOLOGIES"

«ICSI Procedure and advanced techniques in medically-assisted procreation»

Teramo, 28-30 Marzo 2022

A.Y. 2021 - 2022

Ilaria Listorti
Head of Villa MafaldaART lab
ilistorti@unite.it

MAIN TOPICS

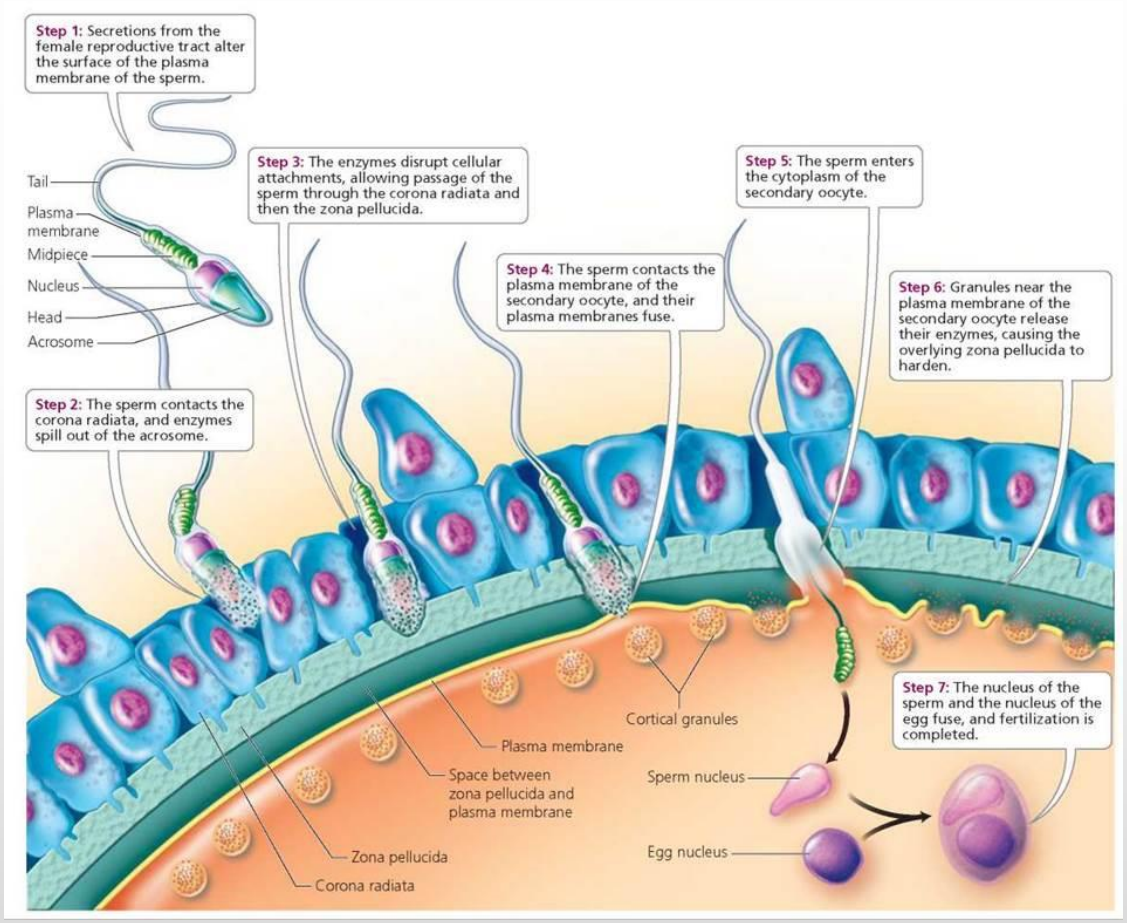
- **ASSISTED REPRODUCTIVE TECHNIQUES**

•The oocyte retrieval (*Pick-Up*);
•*In-vitro* insemination;
•Intracytoplasmatic Sperm Injection (*ICSI*);
•Assisted zona hatching;
•Blastocyst biopsy;
•Pre-implantation genetic diagnosis and embryo screening;
•Vitrification of oocytes and embryos;

THEROICAL LESSON

ASSISTED ZONA HATCHING

ZONA PELLUCIDA: acellular matrix composed of sulfated glycoproteins (ZP1, ZP2, ZP3)



BINDING OF REACTED SPZ DURING FERTILIZATION

PREVENTION OF POLYSPERMIC FERTILIZATION

PROTECTION OF THE EMBRYO AND INTEGRITY MAINTENANCE

PREVENTION OF ECTOPIC PREGNANCY

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ZP SIZE AND SHAPE

MII oocyte normal in shape

MII oocyte with a thick and dense ZP

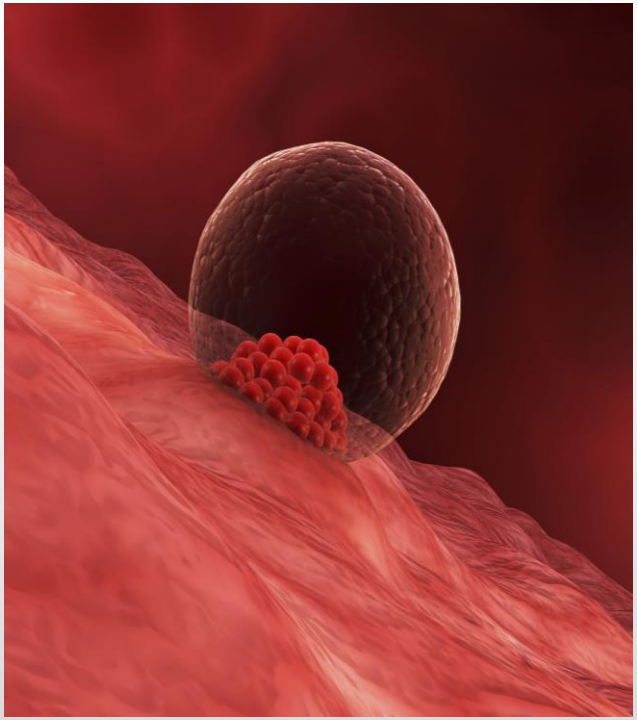
Oocyte with an abnormally shaped ZP and with what appears to be a duplication of the ZP. The oocyte has a regular shape.

ASSISTED ZONA HATCHING

In-vivo

EMBRYO HATCHING: a spontaneous rupture of the ZP that allow the embryo to interact with the endometrial layer of the uterus. *In-vivo*, hatching occurs at blastocyst stage and is due to:

- CHEMICAL DIGESTION OF ZP (principal hypotesis): lysins from the embryo and/or the uterus are involved;
- MECHANICAL LYSIS OF ZP (secondary hypotesis): due to contraction and expansion cycles.



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In-vitro

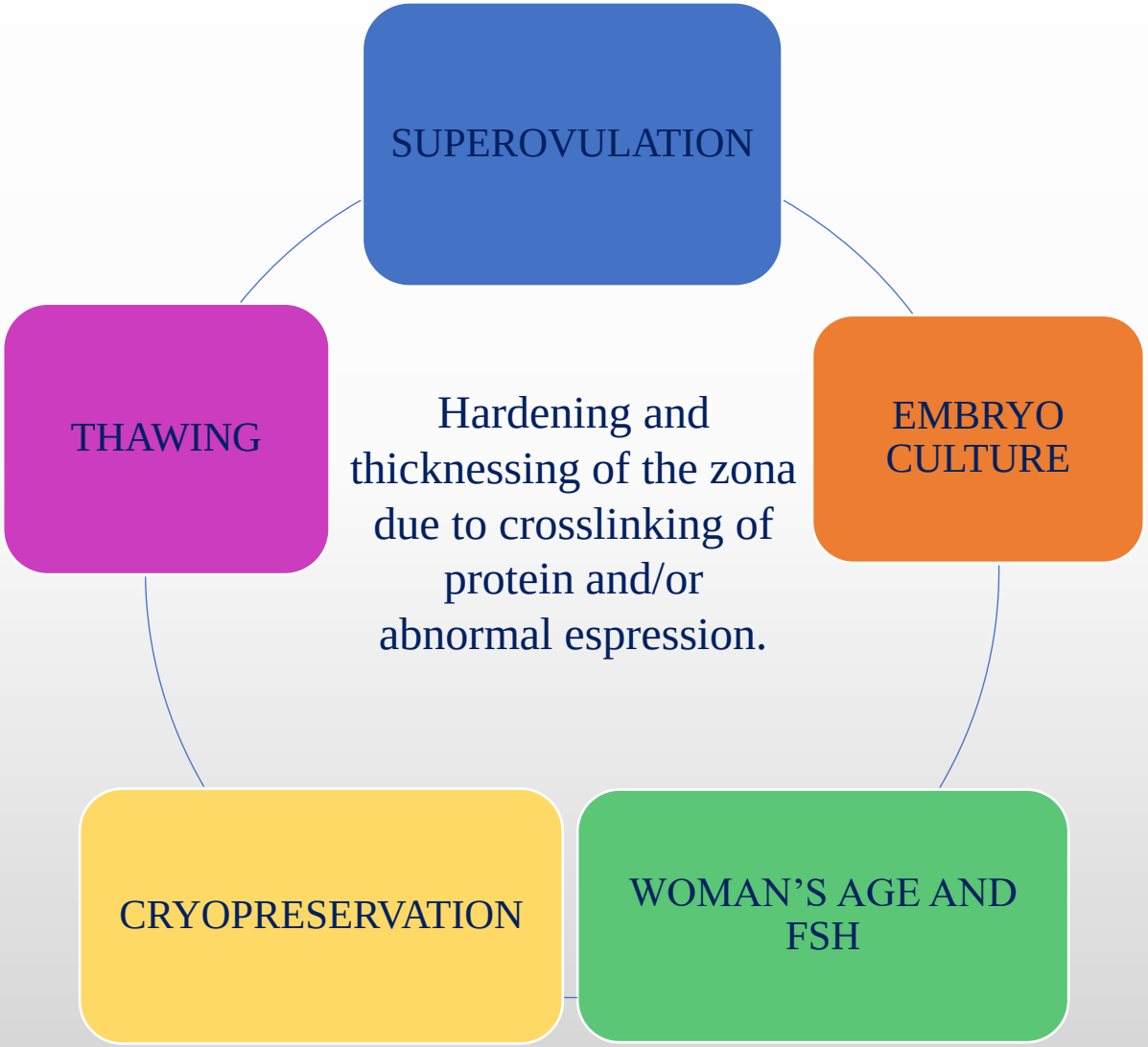
IN-VITRO GENERATED EMBRYOS DEVELOP MORE SLOWLY THAN THE *IN-VIVO* ONES;

MANIFEST A RELATIVE HIGH DEGREE OF GENETIC ABNORMALITIES;

UNDERGO CELL FRAGMENTATION;

HATCH AND IMPLANT AT A LOWER RATE THAN NATURAL.

ASSISTED ZONA HATCHING

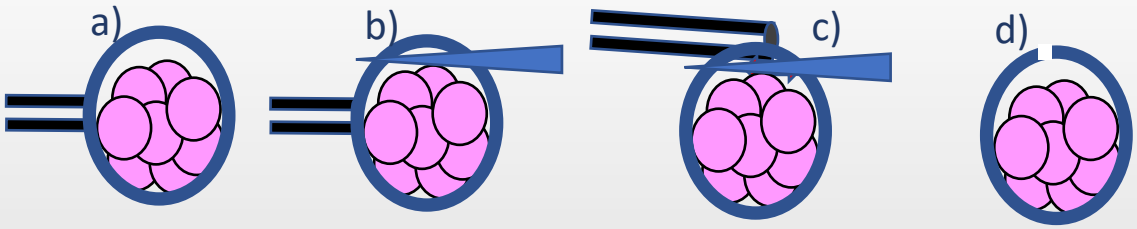


ASSISTED ZONA HATCHING

In order to help the embryo to implant:

1) MECHANICAL PARTIAL "ZONA DISSECTION" a) the embryo is held with a holding pipette and b) the ZP is tangentially pierced with a needle (from 1 to 11 o'clock position). c) the embryo is released from the holding pipette and the part of the ZP between the two points is rubbed against the holding d) until a slit is made in the ZP.

(Cohen et al., 1990)



Small hole
Skilled embryologyst
Labour intensive
Expensive

We use this technique for day 3 or day 4 embryos to avoid osmolarity imbalance caused by larger hole

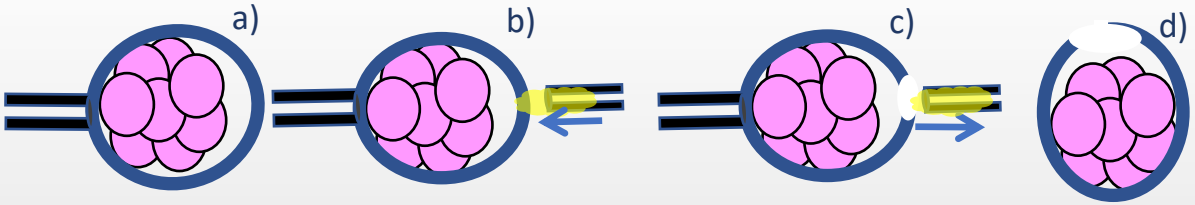
https://www.youtube.com/watch?v=GQVA0xJhre8

ASSISTED ZONA HATCHING

In order to help the embryo to implant:

2) CHEMICAL "ZONA DRILLING" a) the embryo is held with a holding pipette and b) an acid solution (Tyrode's pH 2.2 – 2.6) is gently delivered over a small area of the ZP c) as soon as a hole in the ZP is created c) a suction is applied to avoid damage arisen from toxic solution.

(Cohen et al., 1992)



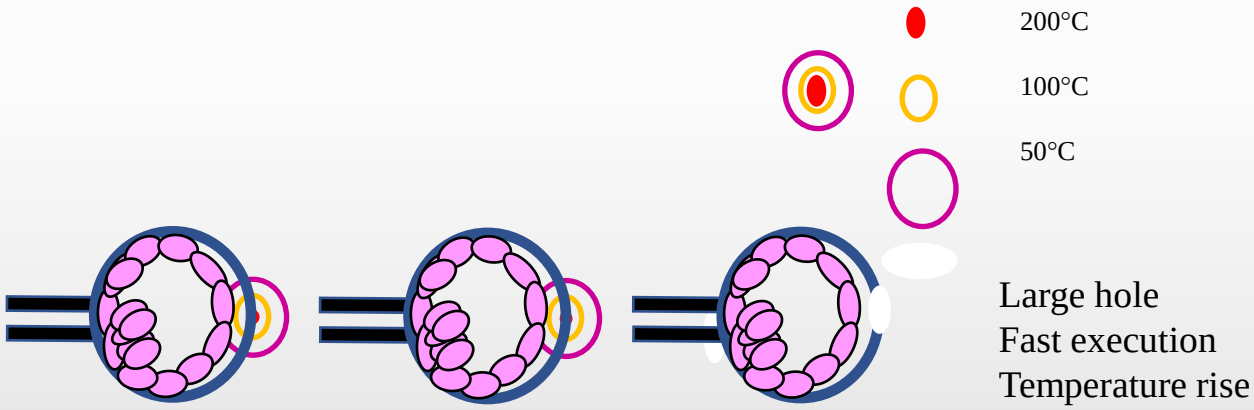
Large hole
Skilled embryologyst
Labour intensive
Expensive
Toxic effect

We never use this technique due to low pH solution toxicity to the embryos

ASSISTED ZONA HATCHING

In order to help the embryo to implant:

3) "LASER ASSISTED" HATCHING a) the embryo is held with a holding pipette and b) four 200 – 450 μs impulses are applied until a hole in the ZP is made. *(Obruca et al., 1994)*



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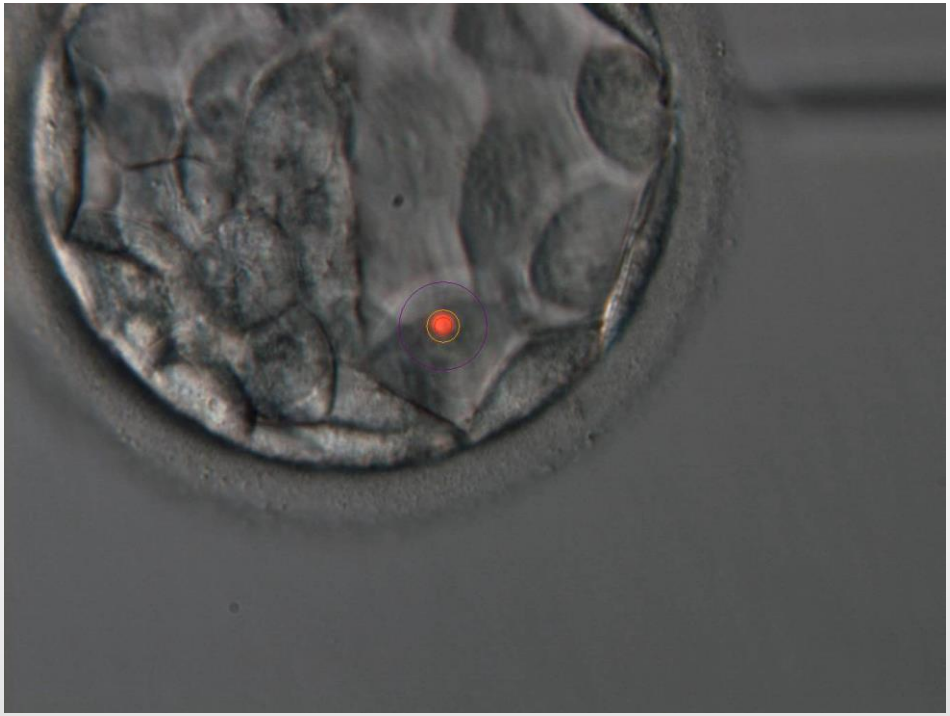
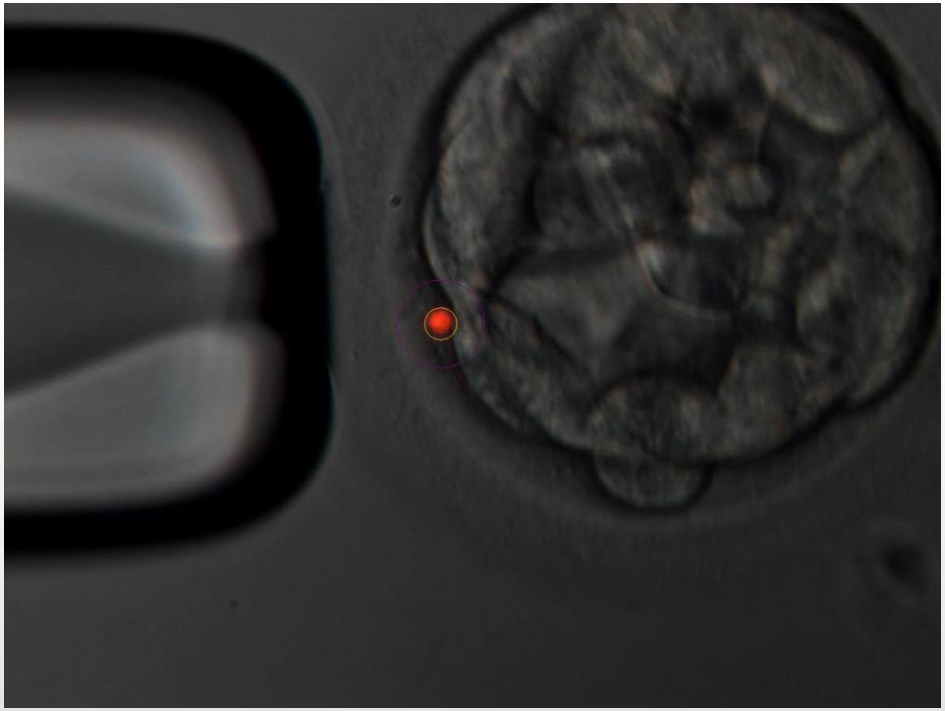
LASER ASSISTED HATCHING





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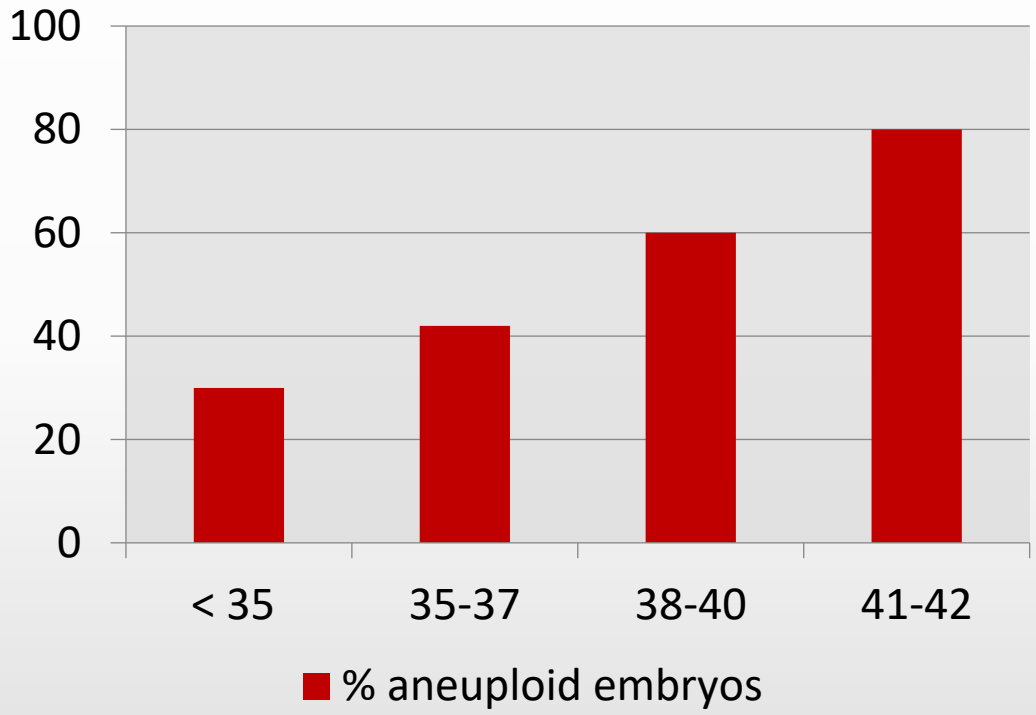
THEROICAL LESSON

BLASTOCYST BIOPSY

MOST LOSS OF IMPLANTATION IS CAUSED BY CHROMOSOME ABNORMALITIES



2014. Data from Reprogenetics, our partner in PGS/PGD >19000 blastocysts analyzed.

BLASTOCYST BIOPSY

- The mean percentage of aneuploid blastocysts is 50%
- Aneuploidy increase with maternal age
- Maternal age is inversely proportional to implantation

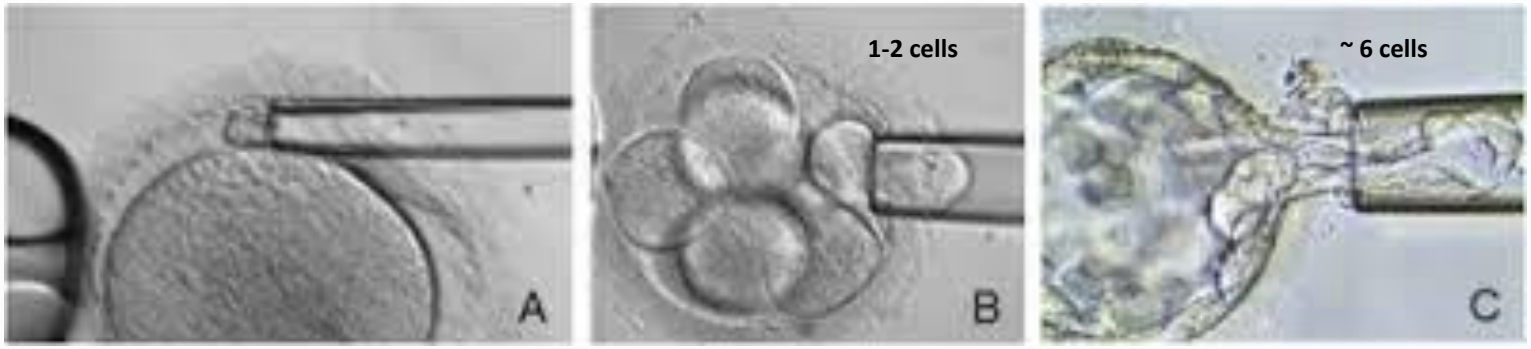
- The error rate of CCS diagnosis is low (<2%)

CCS: comprehensive chromosome analysis such as aCGH, qPCR, NGS

- Blastocyst biopsy is non detrimental to the embryo

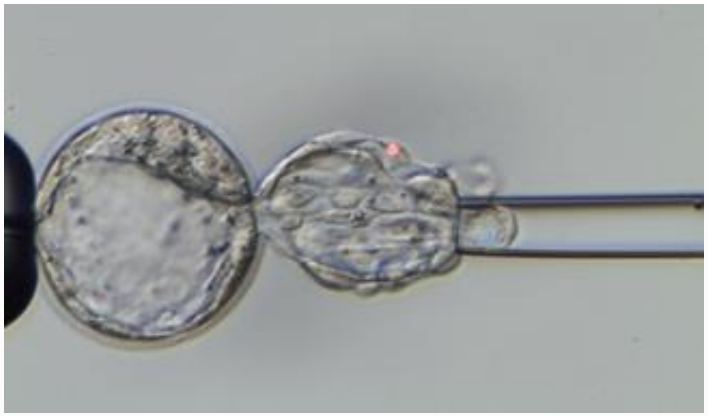
Genetic diagnosis in blastocyst embryo improve implantation rates and eliminate maternal age effect on implantation (miscarriage; birth defects)

WHEN TO BIOPSY



EFFECT OF DAY 3 AND BLASTOCYST BIOPSY					
	cleavage stage		***blastocyst stage***		
Implantation rate	biopsy	not	biopsy	not	
	31%	**53%**	**54%**	41%	

BLASTOCYST BIOPSY



DISADVANTAGES:

- Not all embryos reach the blast stage and not all the same day
- 4,5% monozygotic twins after hatching

ADVANTAGES:

- More DNA: less NO results
- Less mosaicisms = low error rate
- Reduced impact of embryo biopsy
- Less embryos to process (only blasts!)
- Uterine environment optimized after thaw
- The trophoectoderm is representative of ICM (97%)

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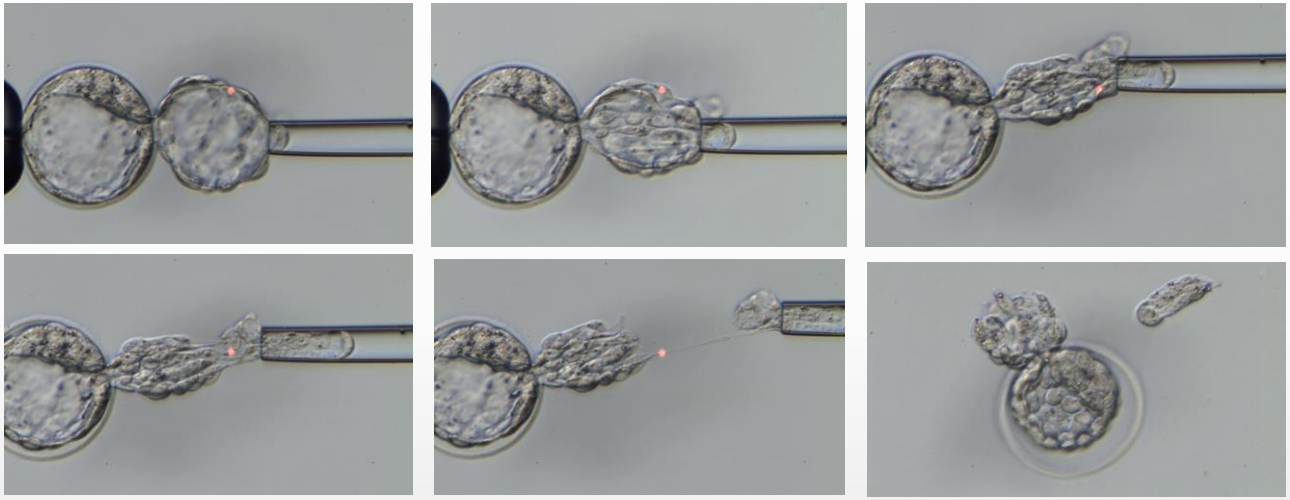
BLASTOCYST BIOPSY

The “PULLING” method: ideal for hatching blastocyst



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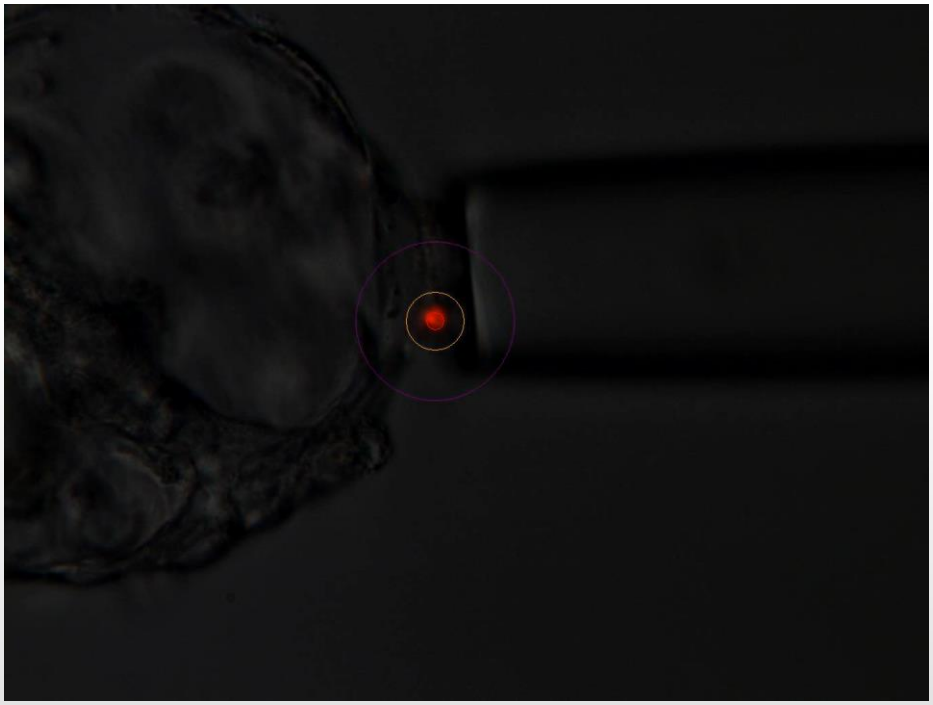
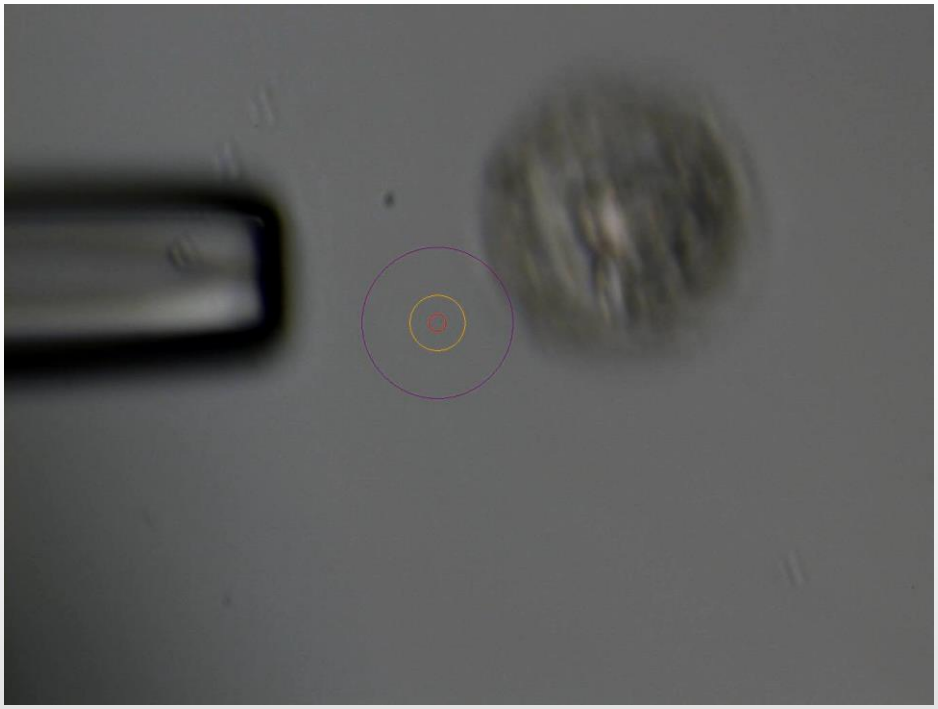
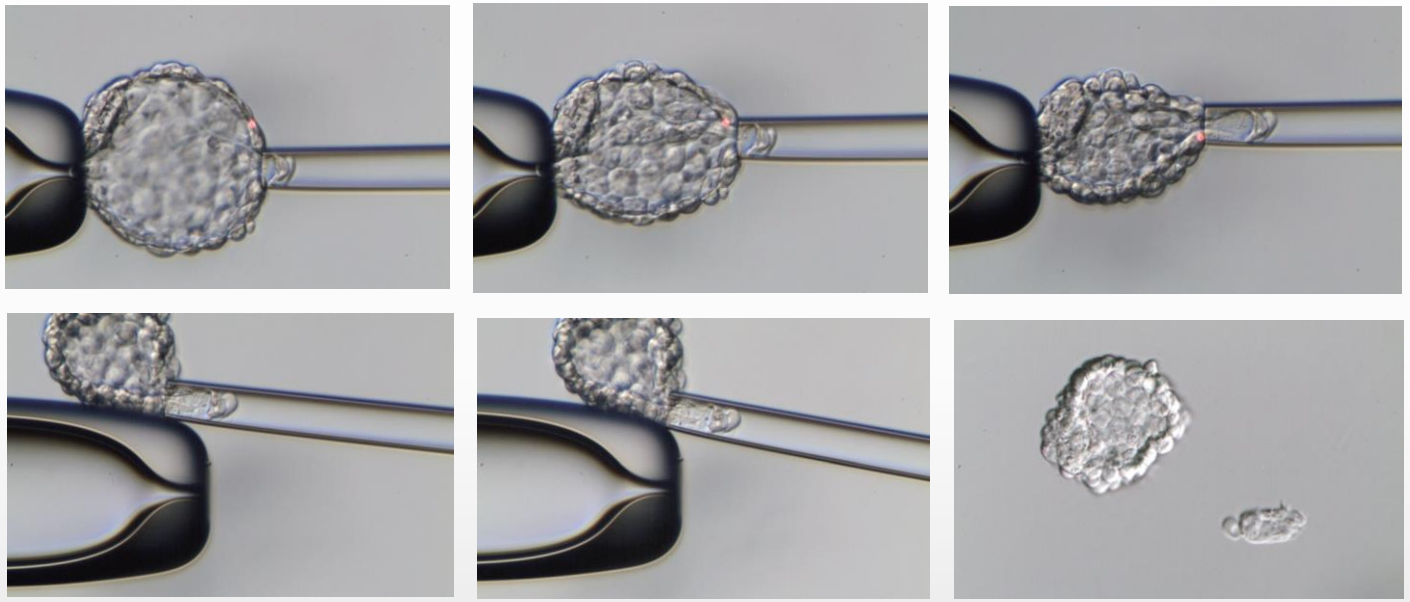
BLASTOCYST BIOPSY





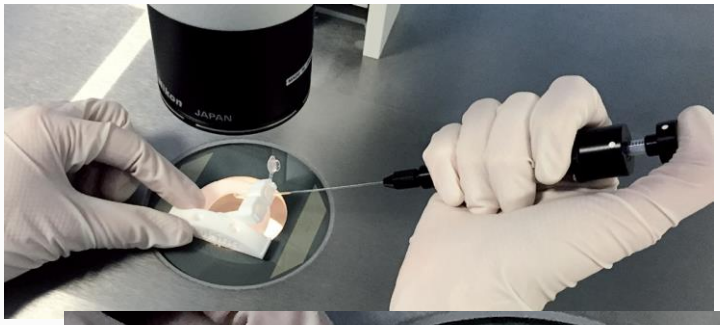
BLASTOCYST BIOPSY

The “FLICKING” method: ideal for fully hatched blastocyst



https://www.youtube.com/watch?v=g8CFQRLJxks

BLASTOCYST BIOPSY



TUBING

https://www.youtube.com/watch?v=zhBxyEVEkIs

050- 2.34

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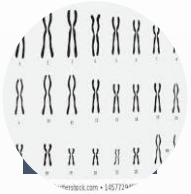
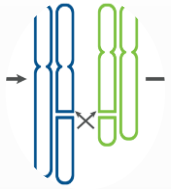
THEROICAL LESSON

PREIMPLANTATION GENETIC TESTING



PGT-A



PGT-SR

PGT- M

PGT-A

Preimplantation Genetic Testing for Aneuploidy

Simultaneous testing of the entire set of chromosomes of an embryo on the day 5 of development by ***aCGH*** *(array Comparative Genomic Hybridization)* on microchips or by ***NGS*** *method (Next generation sequencing)* before the embryo is transferred into the uterus.

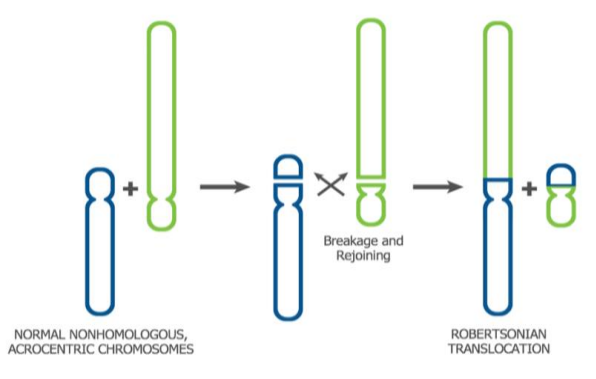
Indications:
•Repeated failed IVF cycles;
•Pregnancy occurs, but then repeatedly interrupted or stopped (Repeated miscarriages);
•Age after 40 years (Advanced maternal age).
In such cases, embryos with an altered chromosome set often develop, which are not able to be implanted into the uterus or stop in their development in the early stages of pregnancy.

PGT-A is carried out:
to screen the entire set of chromosomes for abnormalities;
to improve IVF results;
without personalized test preparation.

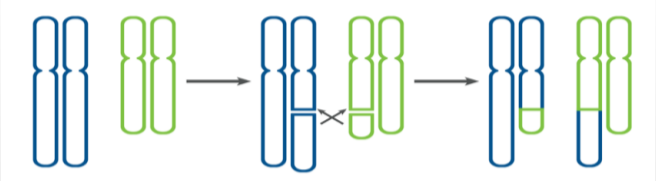
PGT- SR

Preimplantation Genetic Testing for chromosomal Structural Rearrangements

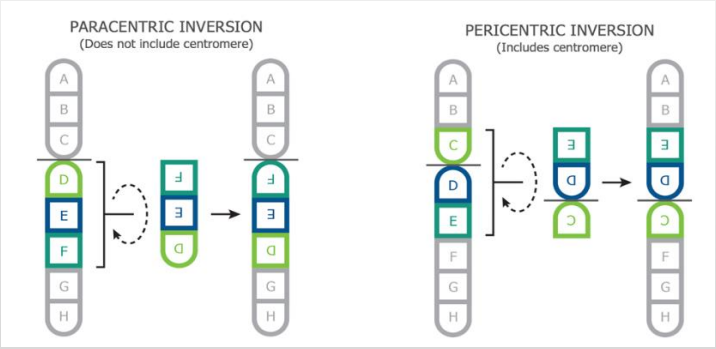
Robertsonian translocations



Reciprocal translocations



Inversion



Chromosomal rearrangements are changes from the normal size or arrangement of chromosomes.

Indications:

- Changes in the parental karyotype
- Child or pregnancy with a chromosome rearrangement

The majority of rearrangement cases:
Require no extra test preparation
Require no extra family member testing

PGT-M

Preimplantation genetic testing for monogenic/single gene diseases

The examination of monogenic hereditary diseases (which appeared because of changes in a single gene).

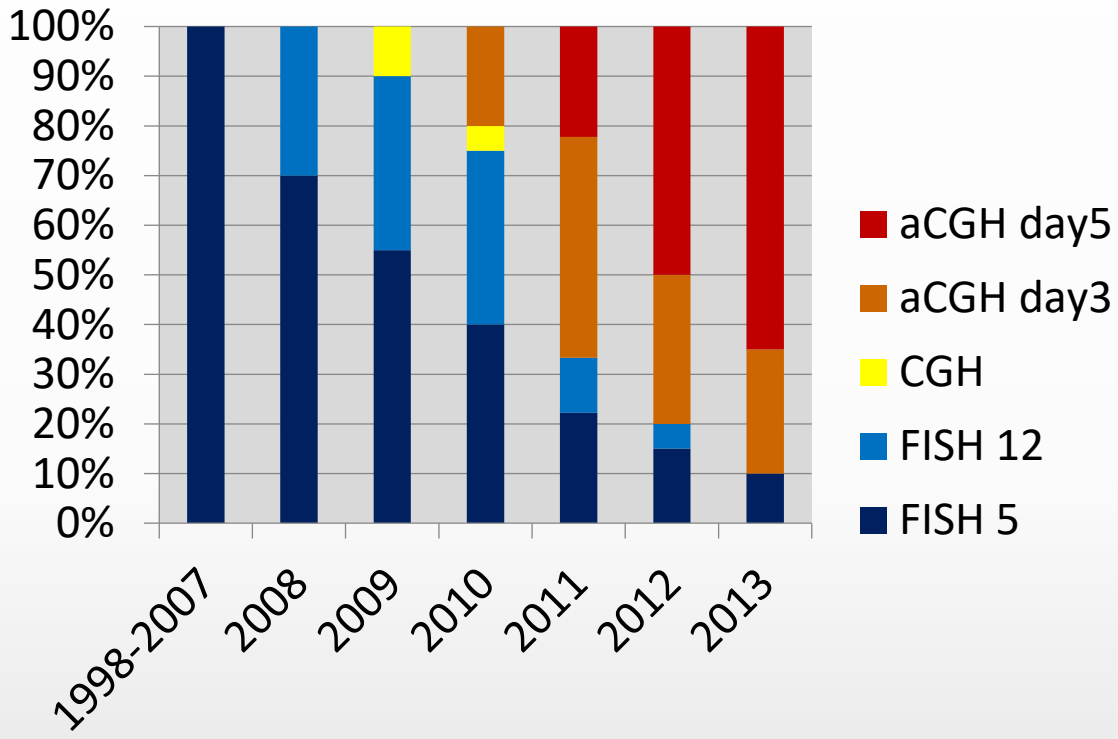
Indications:

- Confirmed monogenic hereditary diseases, which are transmitted from generation to generation;
- A monogenic disease has already been detected in one of family children

(PGT-M) is carried out:

- to reduce genetic disorder risk;
- to check for monogenic hereditary diseases;
- with personalized test design & preparation.

PGT EVOLUTION



2014. Data from Reprogenetics, our partner in PGS/PGD >19000 blastocysts analyzed.

NEXT GENERATION SEQUENCING

TABLE 1

A comparison of current preimplantation genetic screening platforms for comprehensive chromosomal screening.

Characteristics	qPCR	aCGH	SNP array	High resolution NGS
Total independent data signals[a] (reads per sample)	96	2,700	32,000	700,000
Resolution in million megabytes	20	6	6	3
Misdiagnosis of aneuploidies (4, 9, 12, 13, 15)	1%	2%	2%	0
Unbalanced translocations (16)	No	Yes	Yes	Yes
Partial aneuploidies	No	Yes	Yes	Yes
Polyploidy	No	No	Yes	Yes
Percent mosaicism detectable (17, 18, 19)	No	40%–60%	No	20%–80%

Note: aCGH = array comparative genomic hybridization; NGS = next generation sequencing; qPCR = quantitative polymerase chain reaction; SNP = single nucleotide polymorphism.
[a] Number of reads per run × number of samples per run × percent of reads lost = number of reads per sample.

Friedenthal. NGS increases ongoing PRs. Fertil Steril 2017.

Next generation sequencing is the newest platform for PGT, which performs high throughput and high resolution sequencing by synthesis.

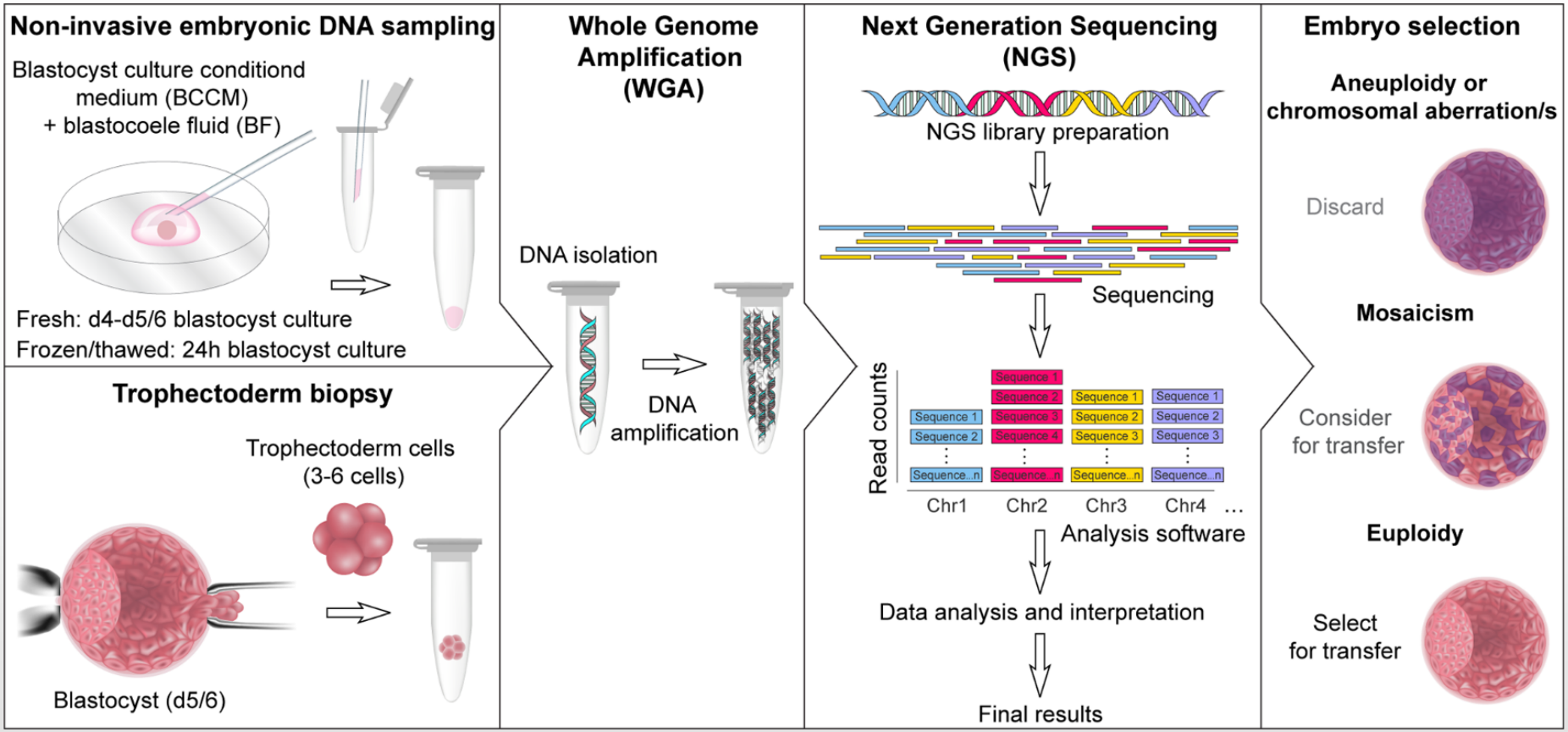
It can assess: aneuploidy of full chromosomes with low error rates, unbalanced translocations, segmental aneuploidies, some triploidies(20), and lower levels of mosaicism

THE MOSAIC EMBRYO

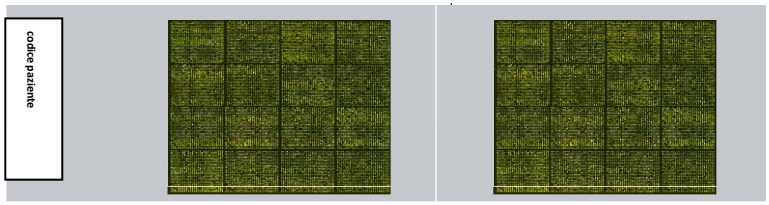
Mosaicism is defined as the presence of two or more populations of cells, each with different genotypes, within the same embryo and results from mitotic errors occurring after fertilization

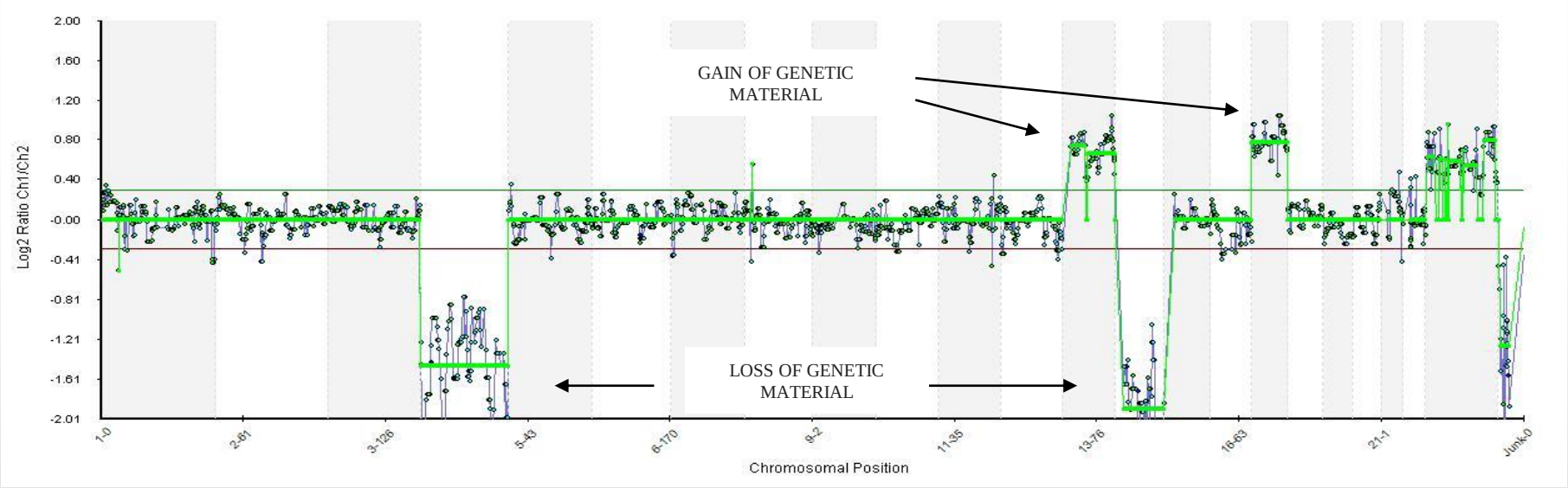
https://blog.vitrolife.com/togetherallthe way/pgt-a-and-mosaicism-the-good-the-bad-and-the-ugly

TIMING OF THE TECHNIQUE



DATA ANALYSIS AND INTERPRETATION





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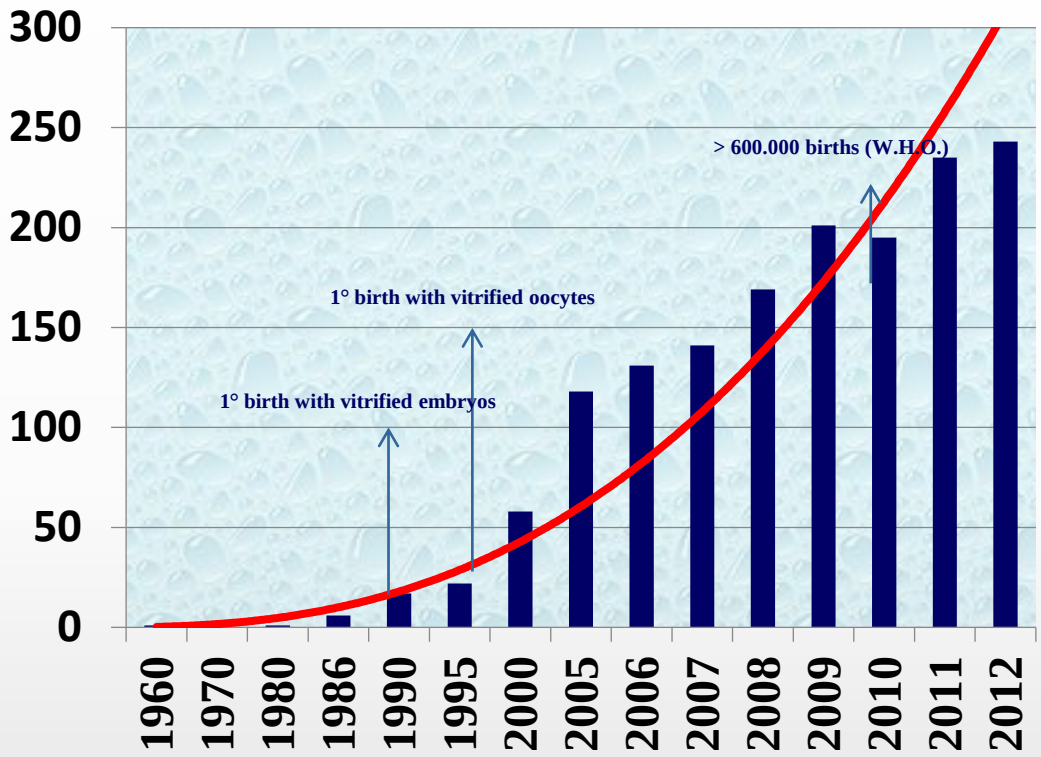
THEROICAL LESSON

VITRIFICATION



http://www.ncbi.nlm.nih.gov/pubmed/?term (Key Words)=oocyte+embryo+vitrification.

VITRIFICATION

“supercooling”

37 °C Aqueous solution at liquid stage

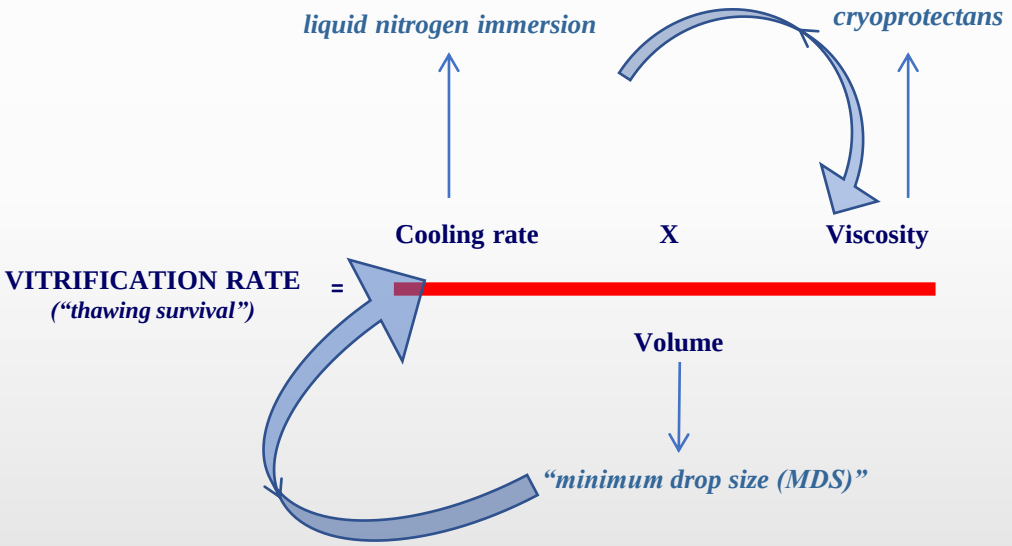
0 °C ~~Aqueous solution at crystalline~~ stage

-196 °C Aqueous solution at amorphous stage

“Glass”: liquid with very high viscosity

VITRIFICATION

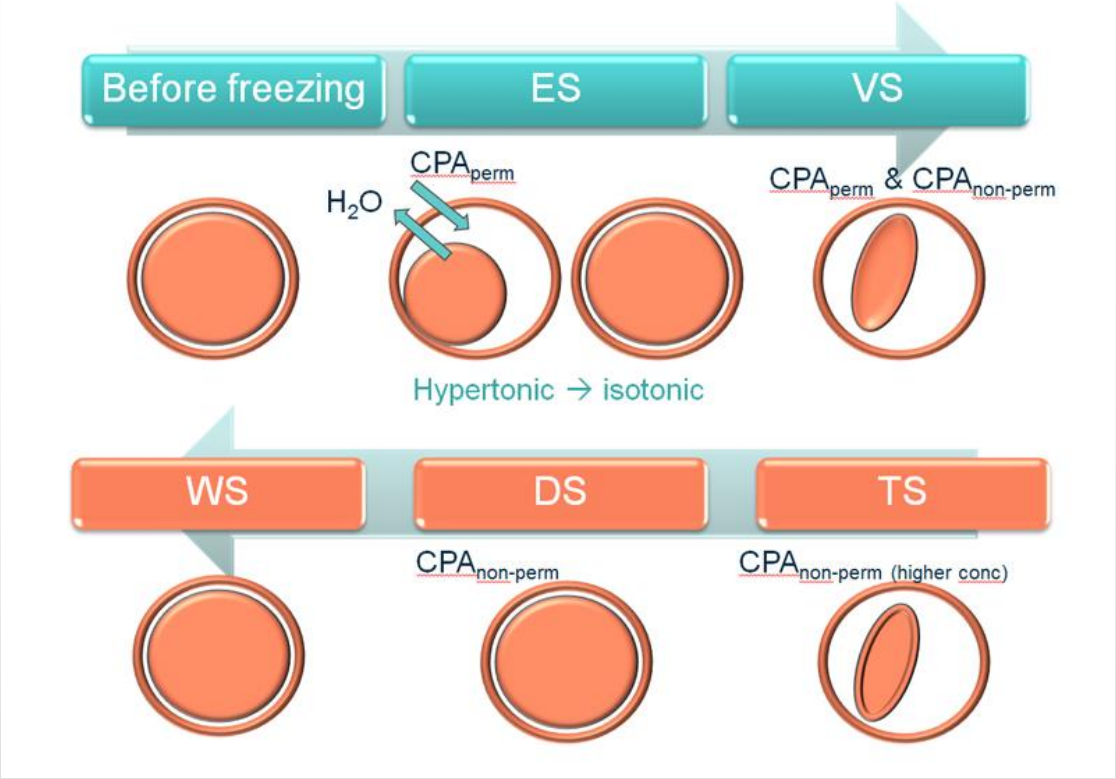
The successful of vitrification requires a balance of three major properties:
1) rapid cooling and warming;
2) CPA concentration (to increase viscosity and lower the freezing point of solutions);
3) limited media volume on cryodevice.



These three factors together prevent the intracellular crystallization of water!!

VITRIFICATION AND THAW

During vitrification step, an embryo is exposed to Equilibration Solution (ES) containing CPA_{perm} from 6 to 10 min and undergoes osmotic change (hypertonic → isotonic state). When osmotic pressure reaches equilibrium, the embryo is placed in Vitrification Solution (VS) containing higher concentrations of CPA_{perm} and 0.5M sucrose ($CPA_{non\text{-}perm}$) for 30 seconds to dehydrate, loaded onto a cryodevice with a very small volume of vitrification medium, and plunged into liquid nitrogen within 80 seconds.



In the warming steps, the embryo is rapidly warmed in Thawing Solution (TS) for 1 minute at 37°C and undergo further dehydration. Stepwise reduction of sucrose concentrations from 1M in TS to 0.5M in Dilution Solution (DS) induces rehydration of the cells while providing an osmotic buffer. In Wash Solution, the embryo is pre-equilibrated before transferring to culture medium

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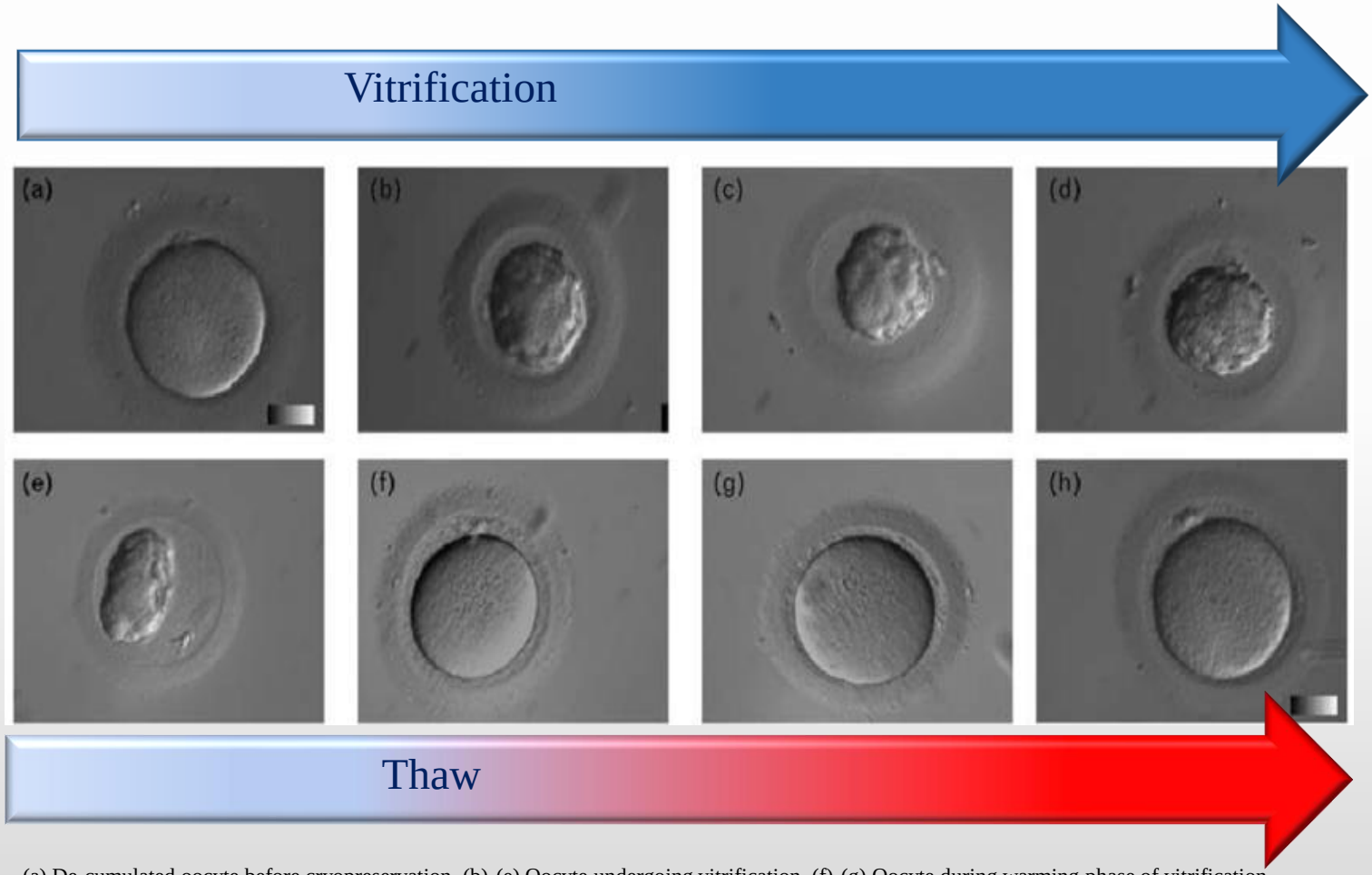
OOCYTE VOLUME CHANGES DURING VITRIFICATION AND THAW.



(a) De-cumulated oocyte before cryopreservation. (b)-(e) Oocyte undergoing vitrification. (f)-(g) Oocyte during warming phase of vitrification protocol. (h) Oocyte after cryopreservation. Images courtesy of Herrero et al., 2011.

VITRIFICATION & THAWING MEDIA COMPOSITION

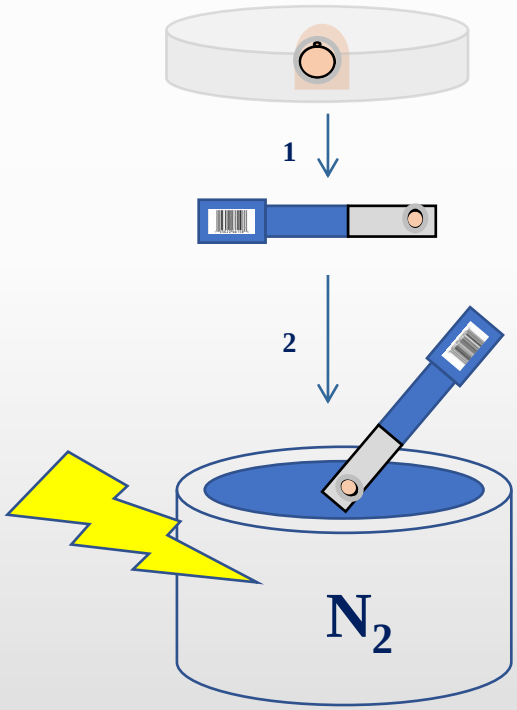
	Solution	DMSO (v/v)	EG (v/v)	Sucrose
Vitrification Kit	Equilibration solution (ES)	7.5 %	7.5 %	0
	Vitrification Solution (VS)	15 %	15 %	0.5M
Thawing Kit	Thawing Solution (TS)	0 %	0 %	1.0M
	Dilution Solution (DS)	0 %	0 %	0.5M
	Washing Solution (WS)	0 %	0 %	0

CPA_{perm} $CPA_{non\text{-}perm}$

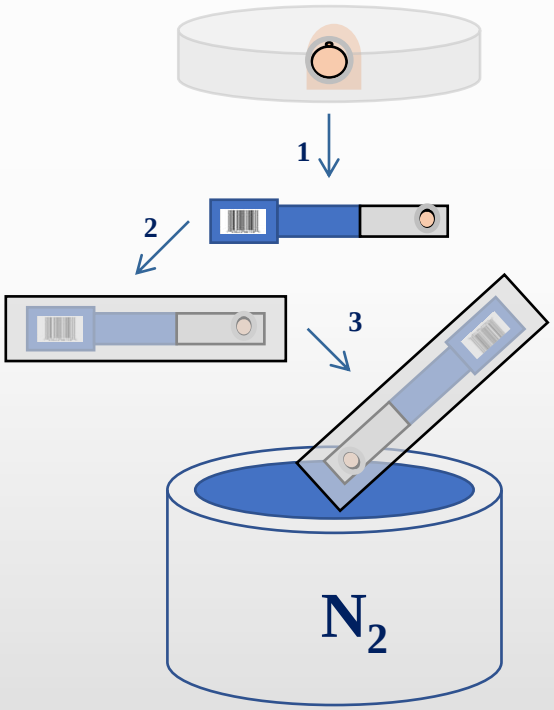
Irvine Scientific Vit Kit® – Freeze and –Thaw are supplemented with 20% DSS and 35 µg/mL gentamicin.

VITRIFICATION DEVICES

A) OPEN SYSTEM

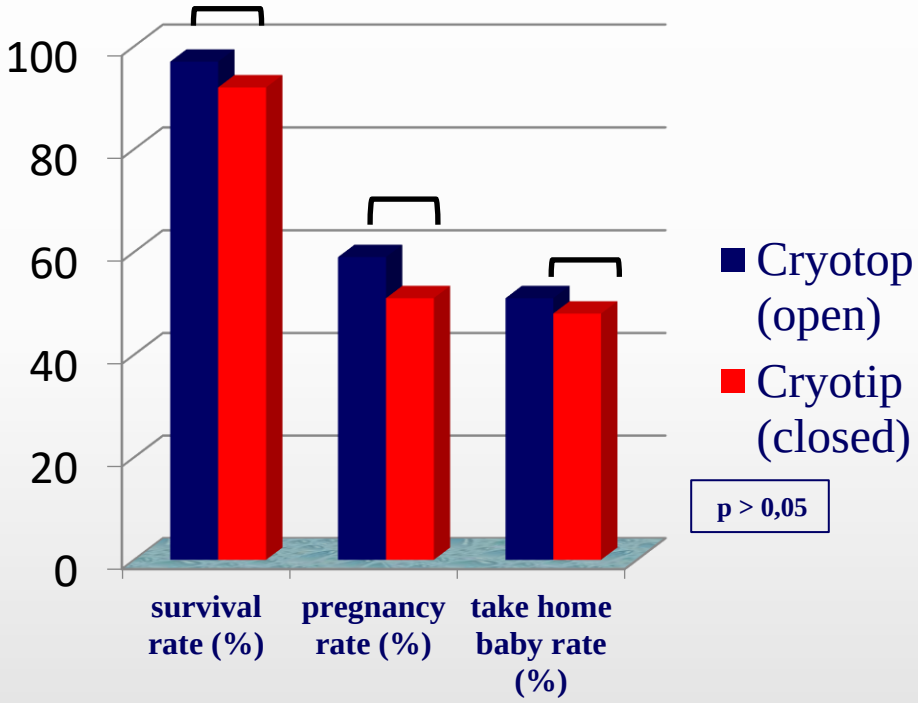


B) CLOSED SYSTEM



VITRIFICATION

OPEN SYSTEM VS CLOSED SYSTEM



Kuwayama et al.."Comparison of open and closed methods for vitrification of human embryos and the elimination of Potential contamination". 2005. *RBM Online,* Vol.11, N.5 pp. 608–614.

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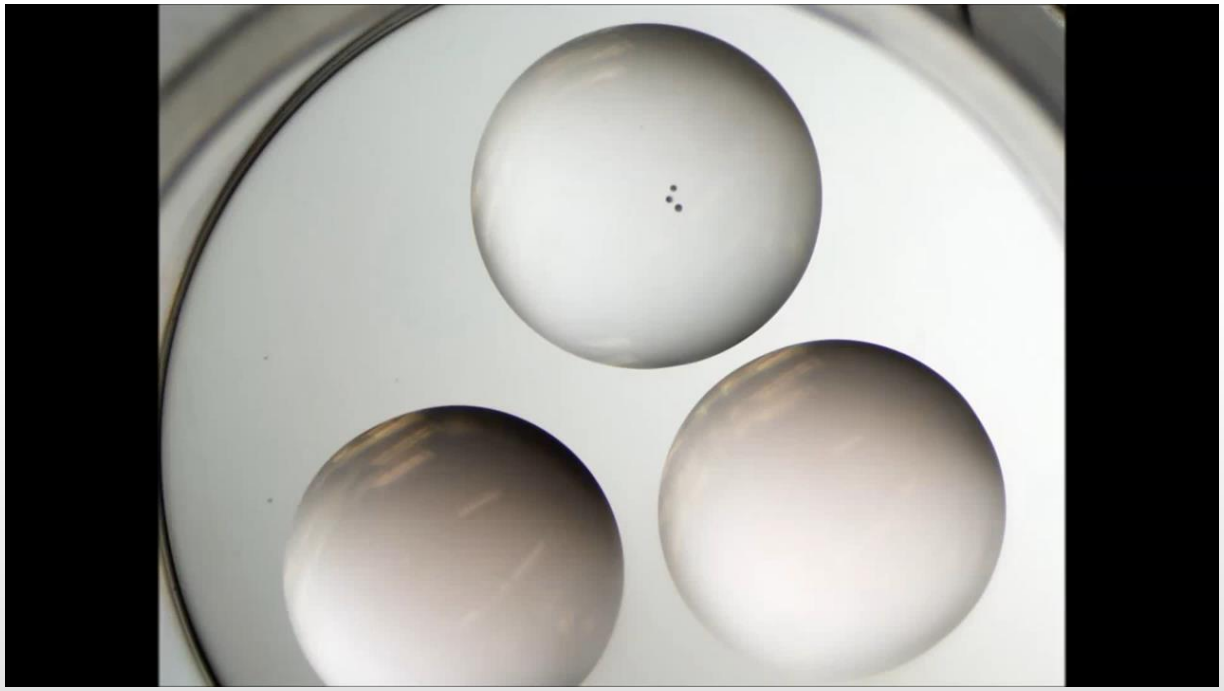
VITRIFICATION PROTOCOL



VITRIFICATION & THAWING PROTOCOL

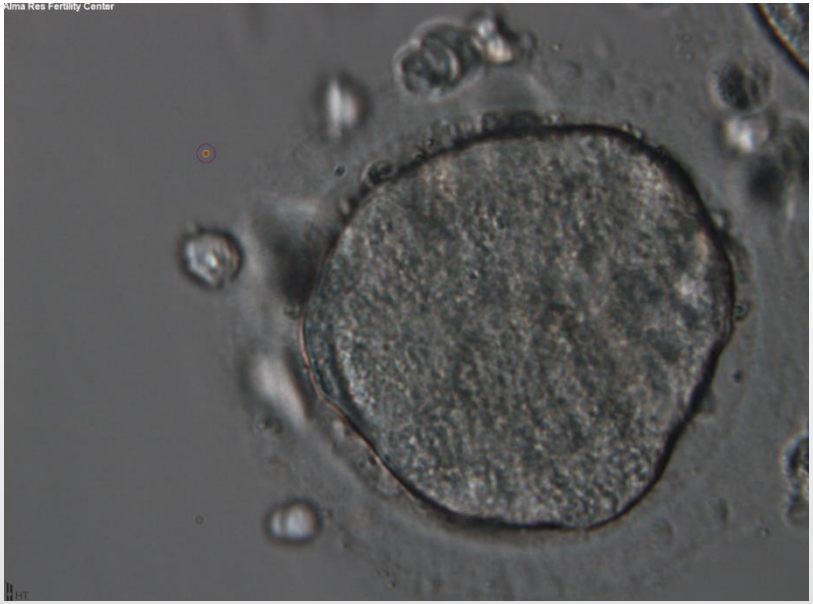
Kitazato Cryotop® Oocyte Vitrification+Thawing - Open System

https://www.youtube.com/watch?v=TUPfZhP64IA

Kitazato Cryotop® Embryo Vitrification+Thawing - Open System

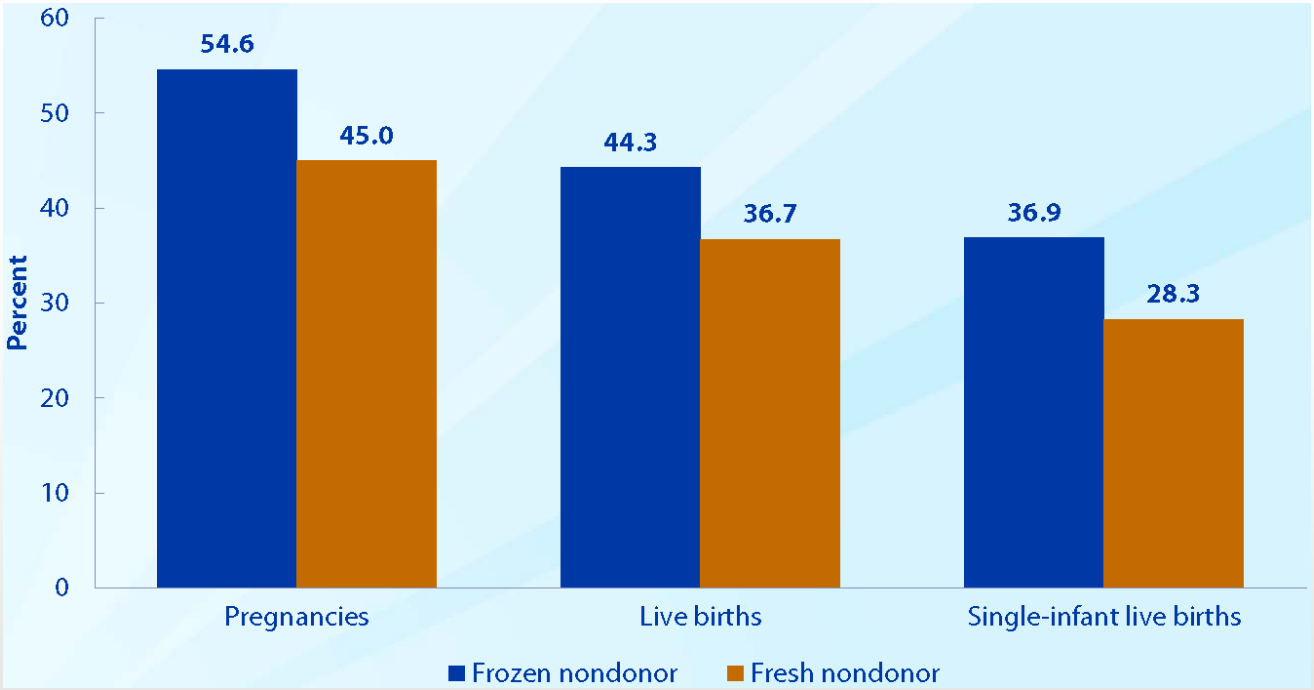
https://www.youtube.com/watch?v=g0m3xK-Zvaw

VITRIFICATION: When?



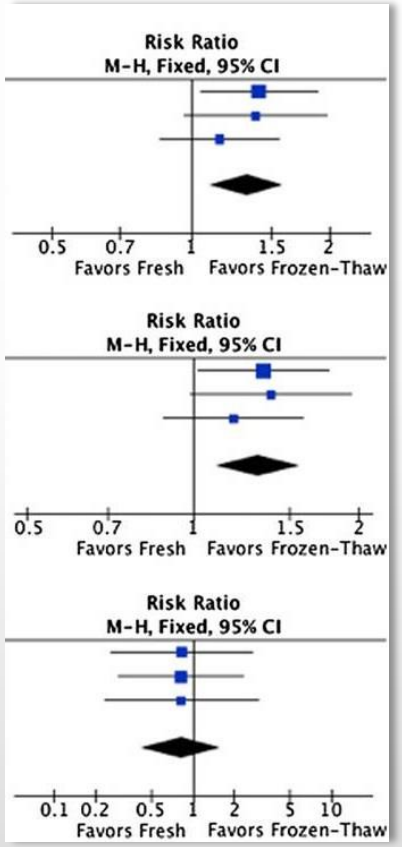
UNIVERSITÀ DEGLI STUDI DI TERAMO

FROZEN VS FRESH EMBRYO TRANSFER



VITRIFICATION

FROZEN EMBRYO TRANSFER (FET)



Ovarian hyperstimulation negativelly affect the endometrial receptivity during ART treatments.

High levels of E_2 e P_4 induce biochemical and morphologic alterations to the uterus. *(Simon et al., 1999)*

Oocytes or embryos from the same cohort give better results in recipient patients than in donor patients
(Simon et al., 1999; Shapiro et al., 2009)

Vitrified embryos give high percentage regardless embryo morphology rate.
(D'Angelo et al., 2010; Griesinger et al., 2011)

Roque et al.." Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis". 2013. *Fertility & Sterility*, Vol.99, N.1 pp. 156-162.