BLASTOCYST CULTURE

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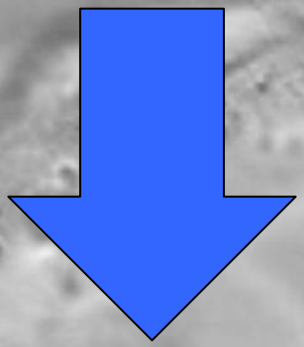
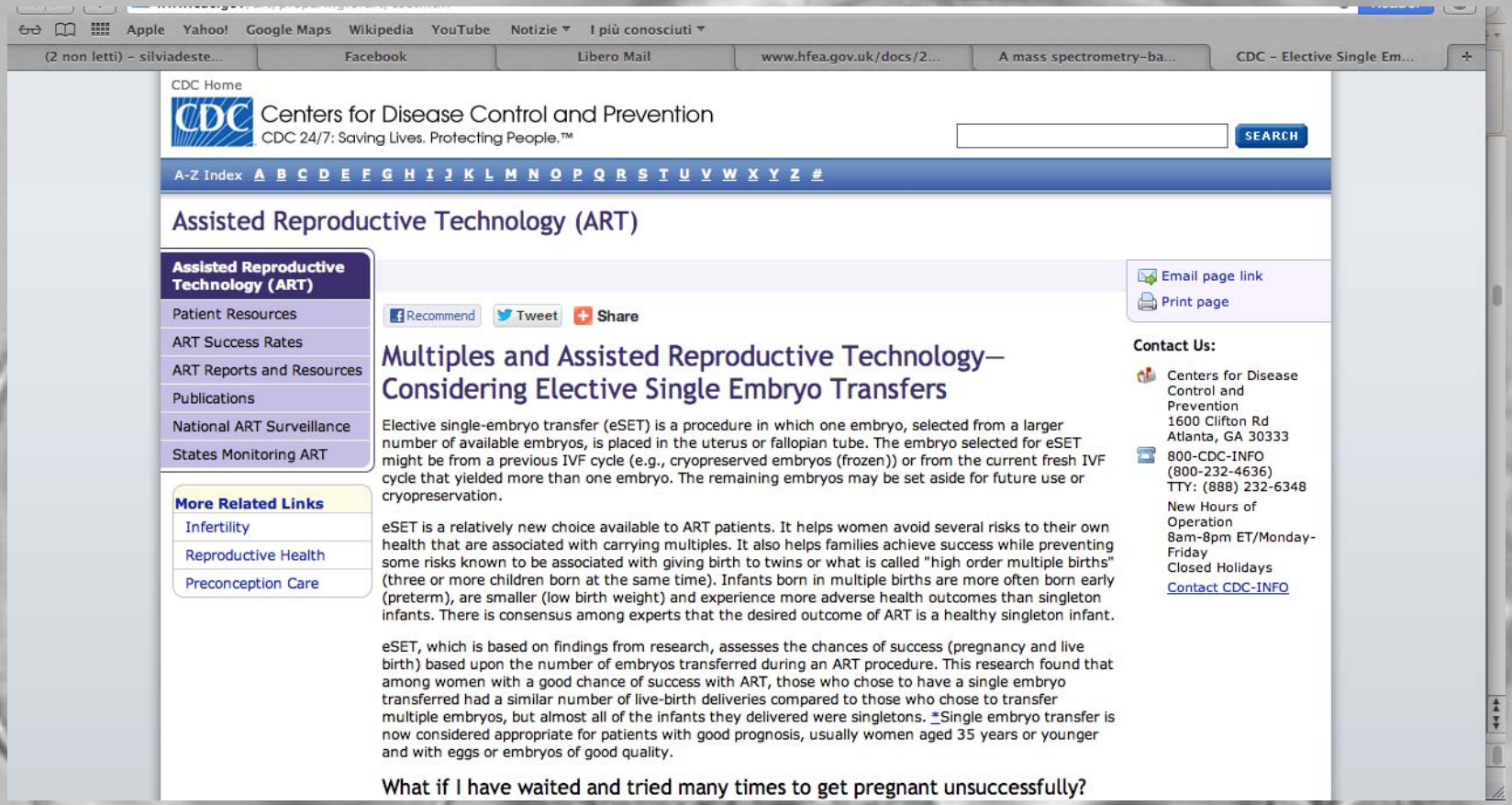
MULTIPLE PREGNANCY



GUIDELINES
eSET POLICY

Trasferire un embrione top quality e criopreservare i restanti

MULTIPLE PREGNANCY



MULTIPLE PREGNANCY

Elective single-embryo transfer

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

American Society for Reproductive Medicine, Birmingham, Alabama

As in vitro fertilization implantation rates have improved, the practice of transfering multiple embryos must be evaluated. The purpose of this document is to reassess the literature on elective single-embryo transfer, to provide guidance for patient selection, and to discuss barriers to utilization. (Fertil Steril® 2012;97:835–42. ©2012 by American Society for Reproductive Medicine.)

In 2000, more than two-thirds of all IVF transfer procedures in the United States were of three or more embryos. Practice guidelines from SART and the American Society for Reproductive Medicine (ASRM) recommending maximum numbers of embryos to transfer were first published in 1998 and have been periodically revised and adjusted downward as implantation rates im- proved, most recently in 2009. With the release of these guidelines, the frequency of transfers of three or more embryos has declined steadily. In the 10-year period from 1999 to 2008, the proportion of transfers with three or more embryos declined from 70% to 39%, with transfers of four or more embryos declining by more than one-half from 36% to 14%. Before 2002, only 1% of transfers were eSET

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

MULTIPLE PREGNANCY

HFEA data shows that about one in four IVF pregnancies resulting in live birth babies were multiple pregnancies. In other words, two out of five (or 40%) live born babies from IVF were from multiple pregnancies. These figures contrast with the statistics for spontaneously conceived pregnancies in which an incidence of one in 80 (approximately 1%) pregnancies being multiple pregnancies and one out of 40 (approximately 2%) live born babies coming from multiple pregnancies.

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

MULTIPLE PREGNANCY

The risks of multiple births

A **multiple birth (twins and triplets)** is the single biggest health risk associated with fertility treatment. Multiple births carry risks to both the health of the mother and the babies:

- Mothers have a higher risk of miscarriage and other complications in pregnancy
- The babies are more likely to be premature and to have a low birth weight
- The number of deaths within the first month of life increases from 3 deaths per 1,000 live births for singletons, to 19 deaths per 1,000 live births for multiple babies[1]
- The risk of cerebral palsy increases from 1.7 cases per 1,000 live births for singletons to 6.2 cases per 1,000 live births for twins[2][3]

The birth of a healthy singleton child, born at full term, is the safest outcome of fertility treatment for both mother and child.

[1] Office for National Statistics (2009) Mortality Statistics: Childhood, Infant and Perinatal 2007.
[2] Surman, G, *et al*. (2009) Four Counties Database of Cerebral Palsy, Vision Loss, and Hearing Loss in Children: Annual Report University of Oxford/NPEU

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

eSET POLICY

Not all patients are eligible for eSET and every patient needs to be treated as an individual. **However, for good prognosis patients, eSET can maximise the chance of a healthy singleton baby born at term[4] and improve the health outcomes for mother and child[5]**. Careful patient selection, and taking into account fresh and subsequent frozen embryo transfers, can maintain overall live birth rates whilst minimising multiple births[3].

In January 2009 the HFEA introduced a policy to promote eSET and minimise the risk of multiple births from IVF treatment. All clinics must have their own strategy around eSET, which sets out how they will lower their multiple birth rate to within a maximum rate set by the HFEA. The HFEA lowers the maximum multiple birth rate each year, after careful evaluation, towards an ultimate aim of a multiple birth rate of not more than 10% each year.

Year	Target
January – December 2008	No target, acting as a benchmark
January 2009 – March 2010	No more than 24% multiple births
April 2010 – March 2011	No more than 20% multiple births
April 2011 – March 2012	No more than 15% multiple births

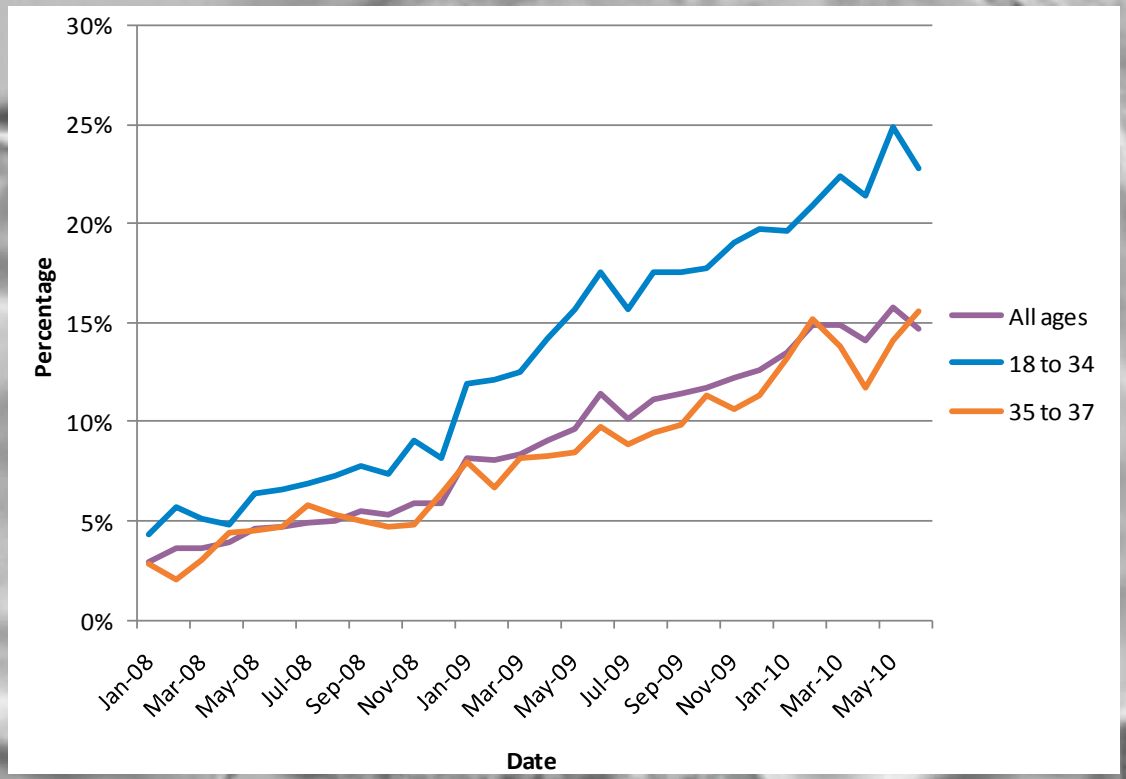
HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

eSET POLICY


One at a time
Better outcomes from fertility treatment
search
Search
Home
Patients
Professionals
Research and evidence
Latest developments
Who supports this?
The single biggest risk of fertility treatment is multiple pregnancy.
One at a time is a professionally-led site aimed at reducing the risks of multiple pregnancies from fertility treatment.
Read the new guidelines for eSET
The professional guidelines for eSET provide essential information to help clinics introduce eSET policies.
The guidelines cover every aspect of practice: patient education and selection, embryo selection and freezing, and costs and funding issues.
>>Read the guidelines
Patients
Professionals
What should I know?
What should I know?
Latest developments

eSET POLICY

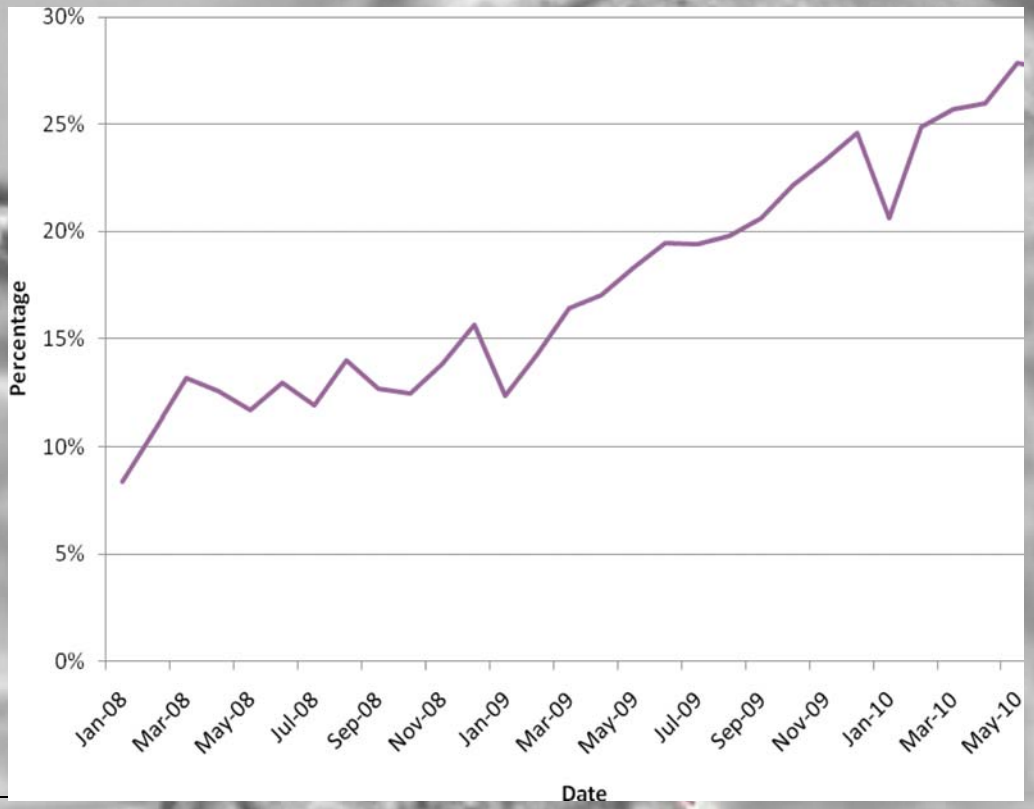
Figure 1: Percentage of embryo transfers which were eSET, January 2008 to June 2010



HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

MOVE TO BLASTOCYST TRANSFER

Figure 5: Blastocyst stage embryo transfers as a percentage of all embryo transfers, January 2008 to June 2009.



MOVE TO BLASTOCYST TRANSFER

Blastocysts are embryos grown in the laboratory incubator for five to six days after fertilisation.

Blastocyst transfer is a relatively new procedure in the UK; previously almost all embryos were transferred two to three days after fertilisation, when they are known as cleavage stage embryos.

Research has shown that transferring blastocyst stage embryos may increase the chance of having a live birth, particularly for patients with a higher likelihood of getting pregnant anyway. This may be because only high quality embryos will be successfully cultured by the embryologist to the blastocyst stage. It may also be easier at this stage for the embryologist to select the best quality embryo[10].

[10] Blake D, Farquhar C, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database of Systematic Reviews* 2007, Issue 4. Art. No.: CD002118. DOI: 10.1002/14651858.CD002118.pub3.

COLTURA A BLASTOCISTI

Le blastocisti non sono una novità!!!

Il primo lavoro sulle blastocisti risale al 1995

(Edwards 1995)

COLTURA A BLASTOCISTI

Perché si è comunque continuato ad eseguire transfer in D3?

1. poche conoscenze riguardo metabolismo blastocisti

2. gli embrioni umani sono in grado di sopravvivere in utero anche fuori della finestra di impianto *(Marston 1977)*

3. basso tasso di sviluppo allo stadio di blastocisti *(Blake D 2010)*

COLTURA A BLASTOCISTI

Con l'avanzare delle conoscenze sul metabolismo degli embrioni, si iniziò ad usare:

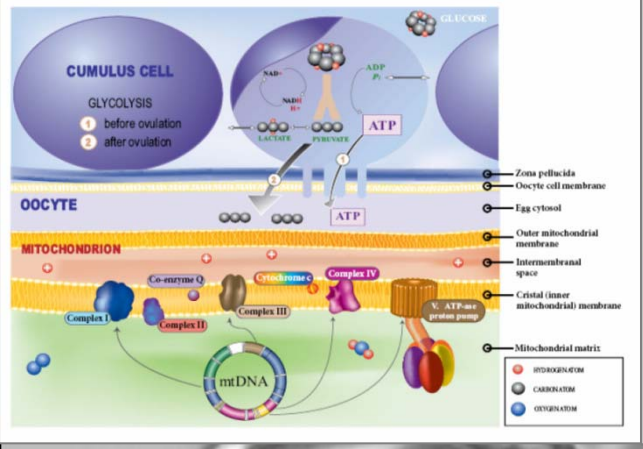
-co-colture *(Menezo 1990,Van Blerkom 1993, Yeung 1992)*

-terreni di coltura avanzati *(Scholtes 1996)*

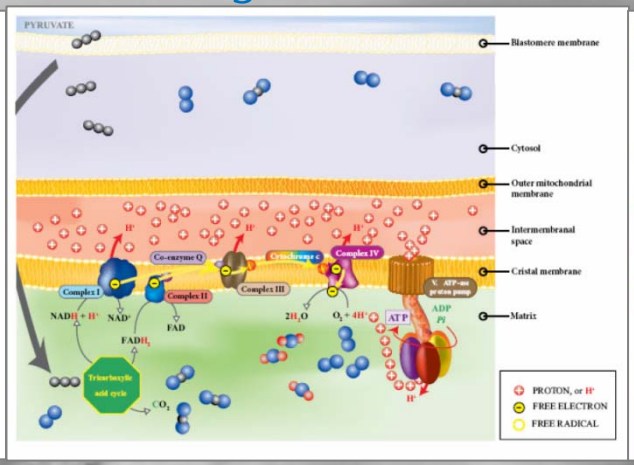
- terreni sequenziali G1/G2 con Glucosio invece del Piruvato e AA fondamentali *(Gadner 1998)*

COLTURA A BLASTOCISTI

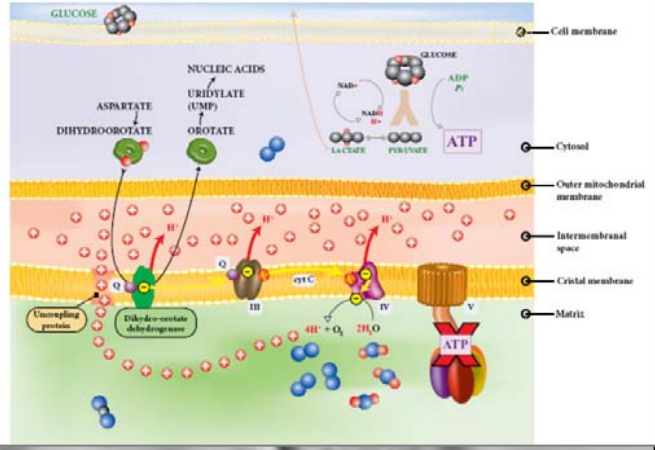
Fertilization Medium/IVF

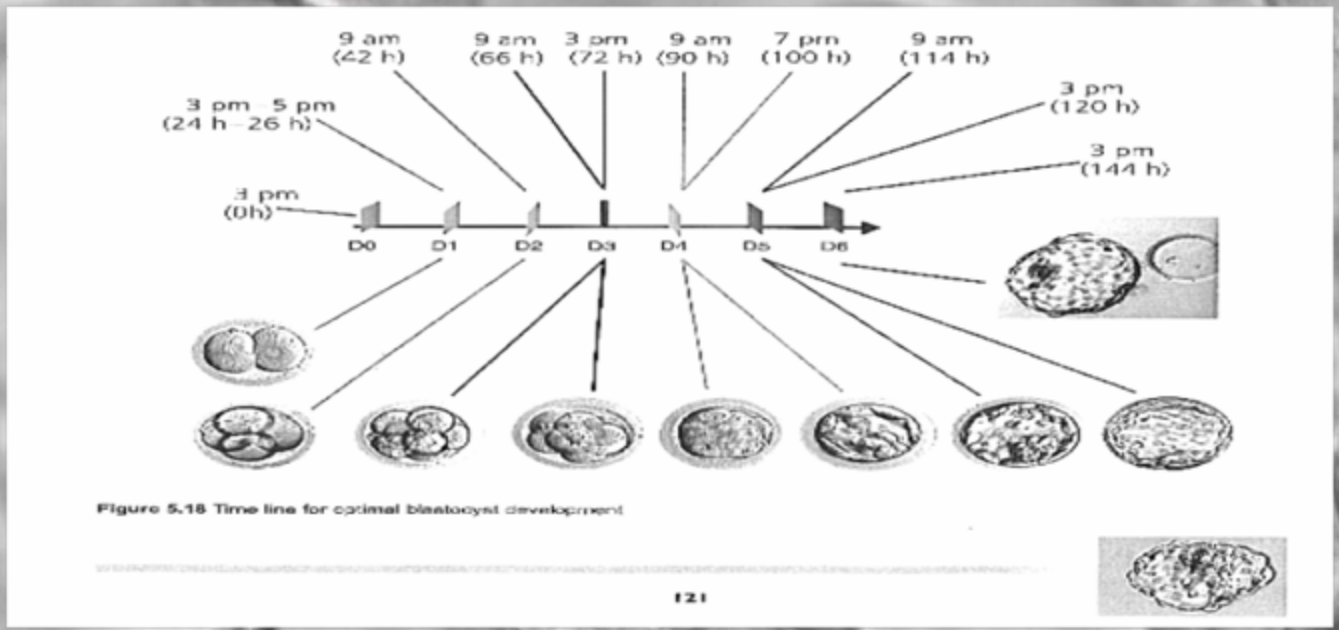


Cleavage Medium/G1



Blastocyst Medium/G2





COLTURA A BLASTOCISTI

Avere dei terreni di supporto allo sviluppo delle BL è condizione ***NECESSARIA MA NON SUFFICIENTE***

E’ necessario anche avere una organizzazione adeguata di TUTTO il sistema:

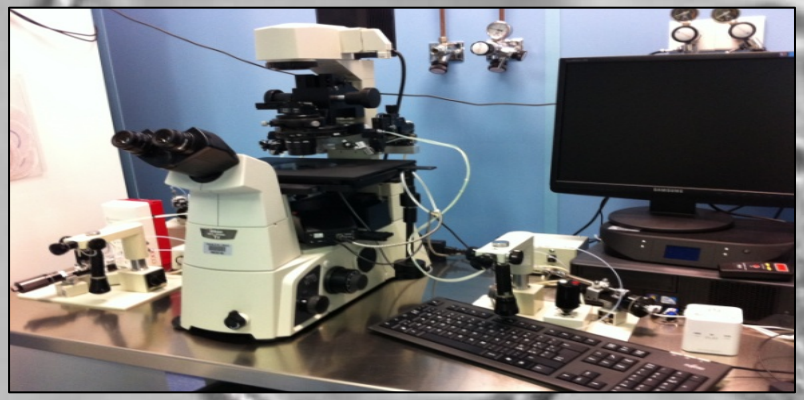
Strumentazione di laboratorio

Controllo parametri

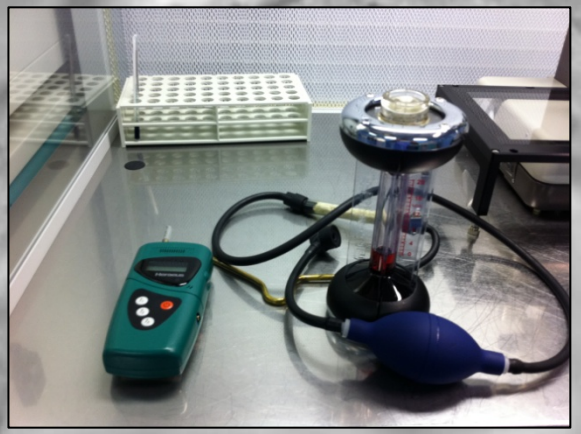
Mantenimento condizioni

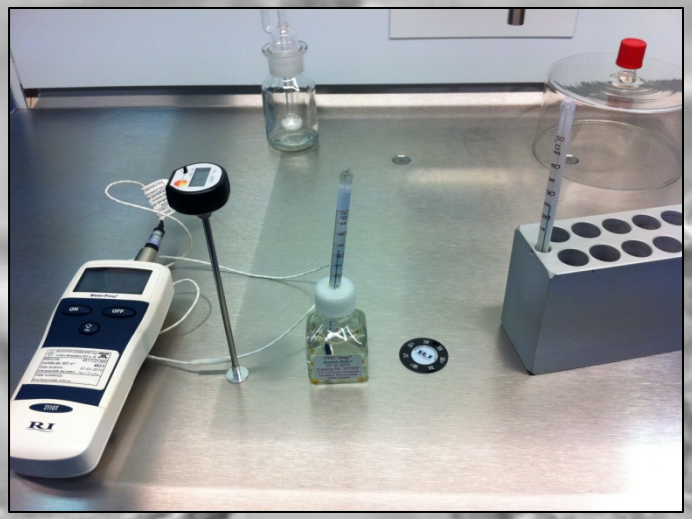
Studio della singola paziente da parte di medico e biologo

COLTURA A BLASTOCISTI
STRUMENTAZIONE di LABORATORIO

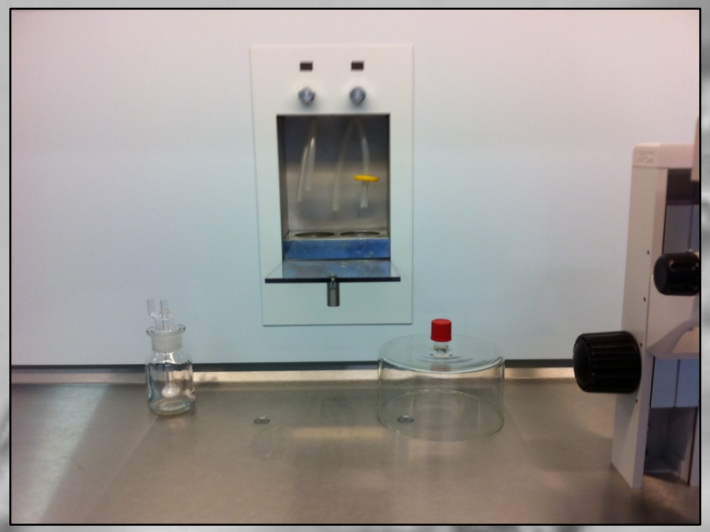


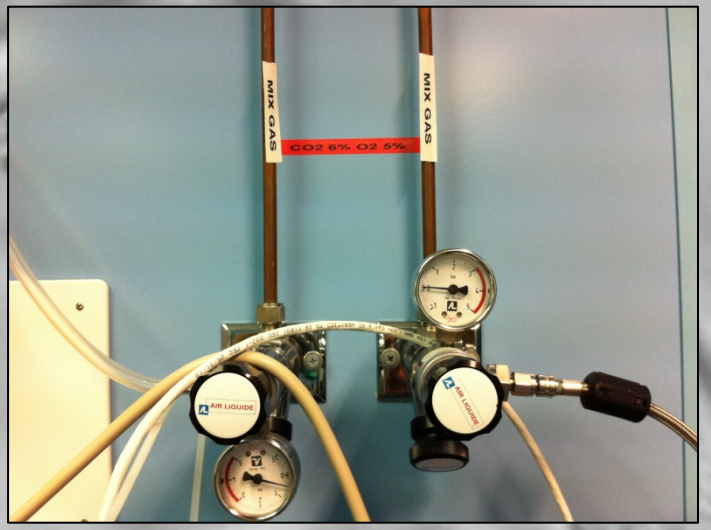
COLTURA A BLASTOCISTI
CONTROLLO PARAMETRI

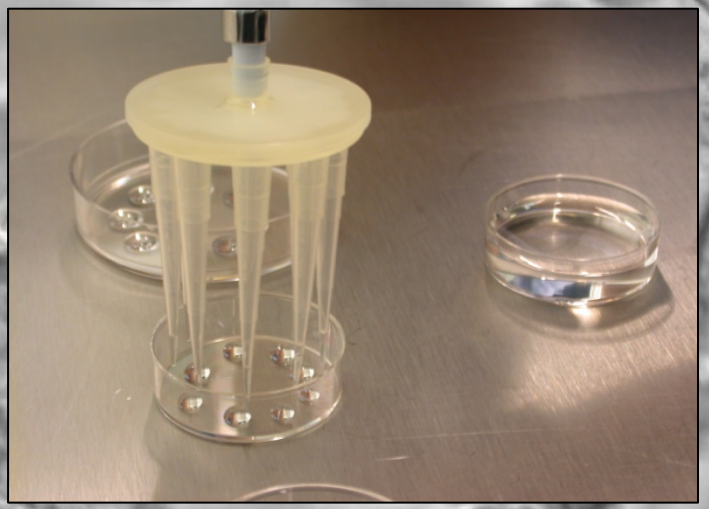




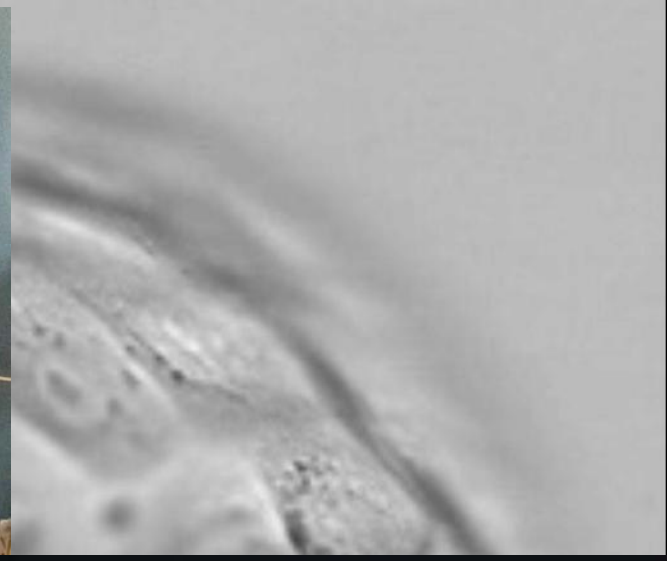
COLTURA A BLASTOCISTI
MANTENIMENTO CONDIZIONI

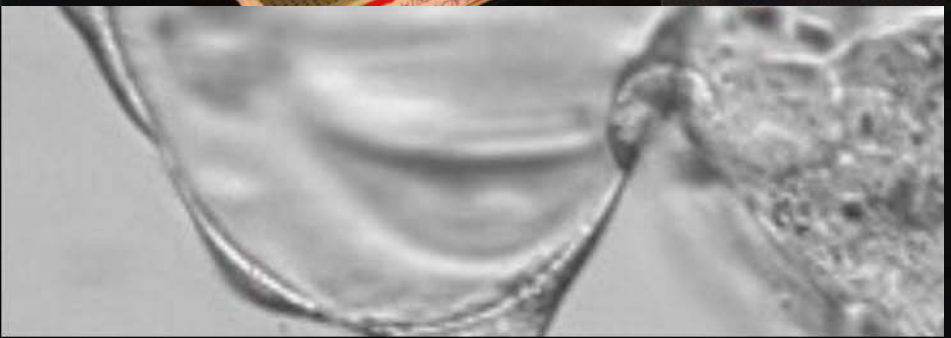






IL LABORATORIO

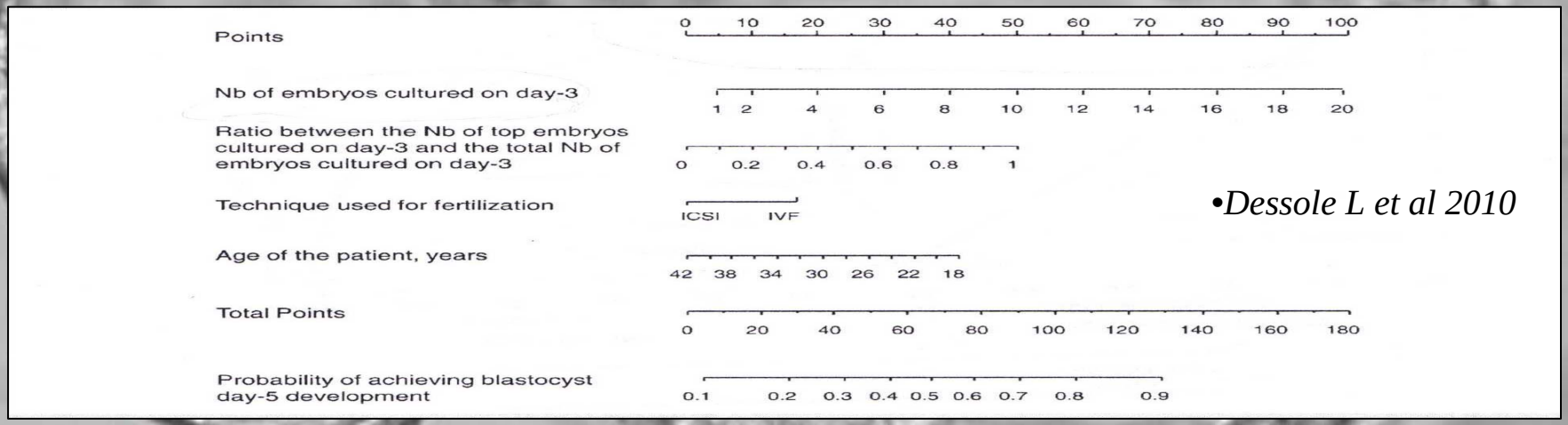




IL LABORATORIO

EVIDENCE BASED MEDICINE

- *Rachel Cutting, BFS and ACE 2008* (Linee guida Inglesi)
- *Papanikolaou EG 2005* (+ di 4 top Quality D3)
- *Dessole L et al 2010*
- *NICE 2013*



•*Dessole L et al 2010*

EVIDENCE BASED MEDICINE

Fertility:
assessment and treatment for people with fertility problems

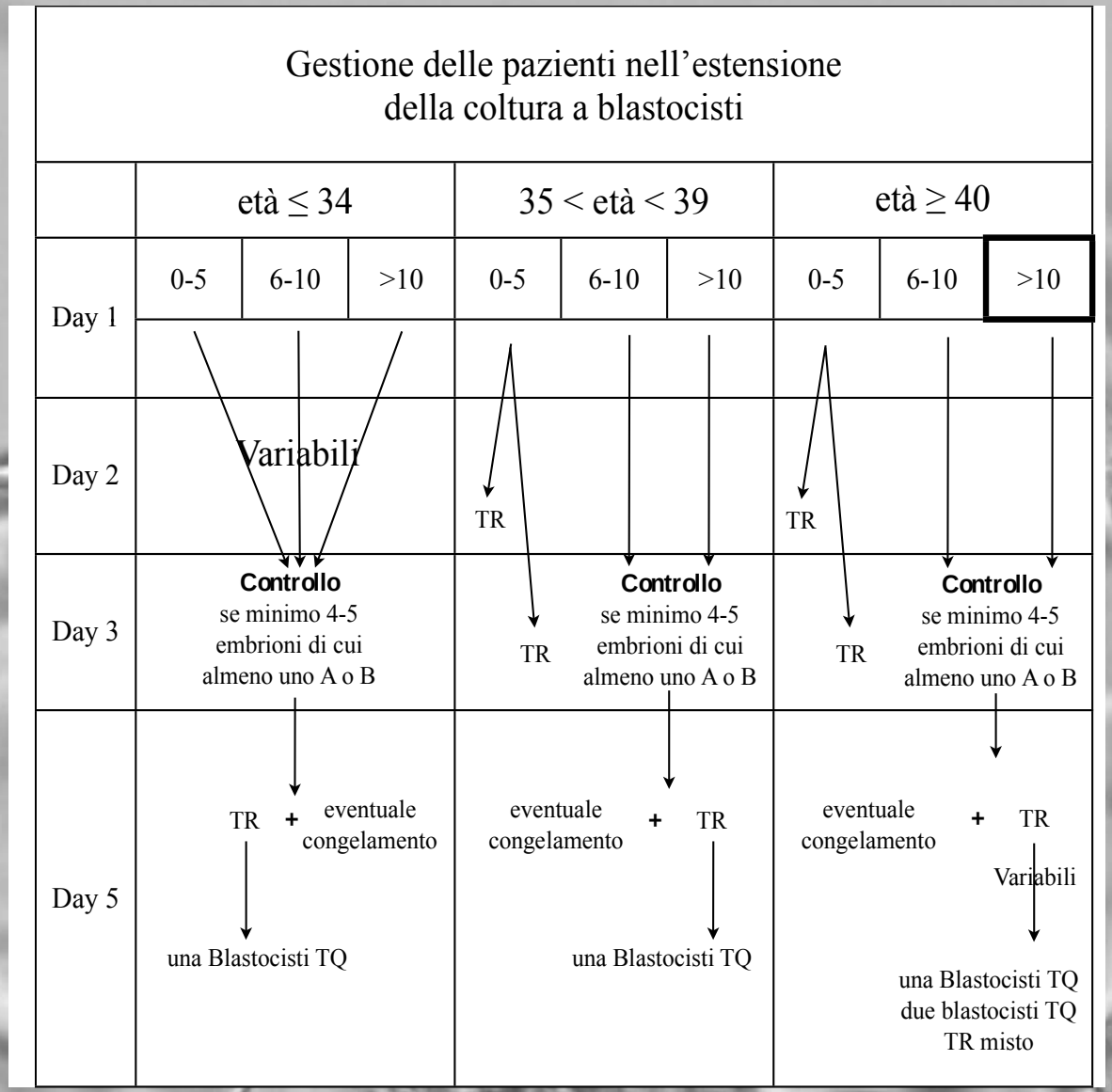
February 2013
NICE Clinical Guideline

Table 15.30 Results of consensus survey for embryo transfer strategies

Cycle	Women's age (years)	Number and grade of embryos available at cleavage stage	SET	DET
1st cycle: no previous IVF cycles	36 or under	Embryos (2 plus) available but none are top grade	~√	
		1 to 3	√	
		4 plus	√	
	37–39	Embryos (2 plus) available but none are top grade	=	
		1 to 3	√	
		4 plus	√	
	40–42	Embryos (2 plus) available but none are top grade		√
		1 to 3	=	
		4 plus	~√	
2nd cycle: 1 previous failed full cycle of IVF	36 or under	Embryos (2 plus) available but none are top grade	=	
		1 to 3	√	
		4 plus	√	
	37–39	Embryos (2 plus) available but none are top grade	=	
		1 to 3	=	
		4 plus	√	
	40 - 42	Embryos (2 plus) available but none are top grade		√
		1 to 3		~√
		4 plus	=	
3rd cycle: 2 previous failed full cycle of IVF	36 or under	Embryos (2 plus) available but none are top grade	=	
		1 to 3	=	
		4 plus	=	
	37–39	Embryos (2 plus) available but none are top grade		~√
		1 to 3	=	
		4 plus	=	
	40–42	Embryos (2 plus) available but none are top grade		√
		1 to 3		√
		4 plus		√

DET double embryo transfer, IVF in vitro fertilisation, SET single embryo transfer
√ consensus ≥70% agreement or disagreed with an embryo transfer strategy
~√ 'near consensus' 60–69% agreement
= no consensus 50–59% agreement

EVIDENCE BASED MEDICINE



ISS-SIERR

EVIDENCE BASED MEDICINE

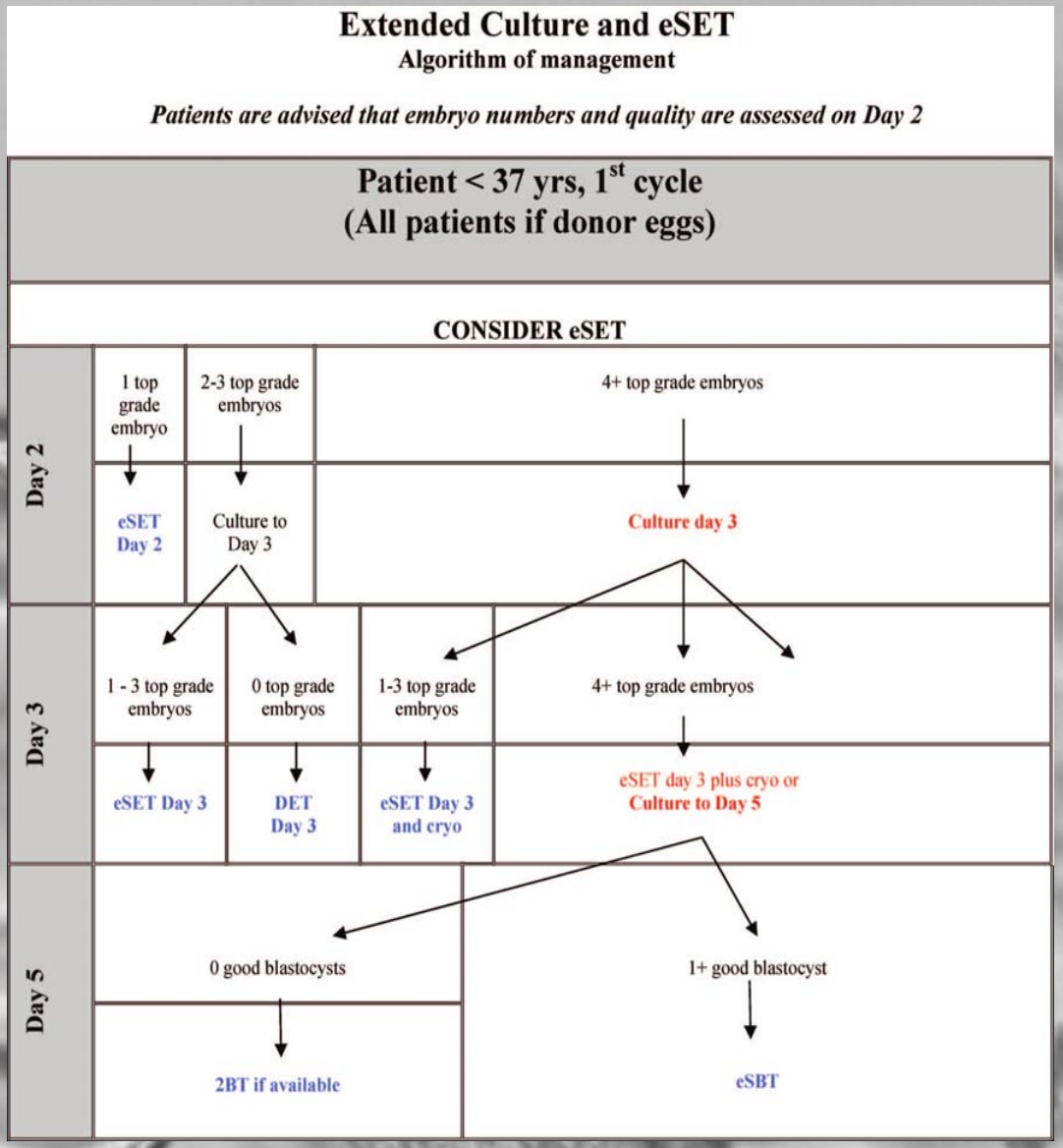


Figure 5. Algorithm for extended culture and eSET.

POTENZIALITA' BLASTOCISTI

Perché le blastocisti dovrebbero dare dei "*VANTAGGI*"?

1 ↑ risposta infiammatoria

2 è giusto aspettare il D5 sia per preparazione endometriale sia per eseguire il transfer nello stadio di impianto fisiologico

3 ↓ contrattilità uterina

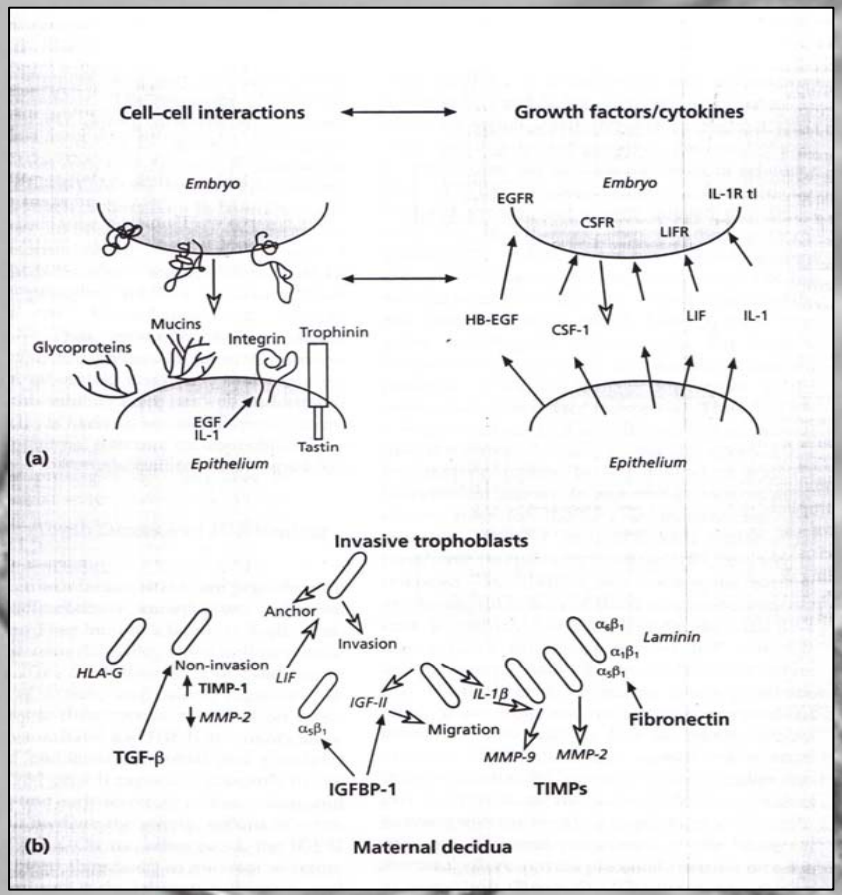
4 self selection e possibilità di eseguire eSET (elective single embryo transfer)

5 IR (Implantation Rate), PR (Pregnancy Rate)

6 analisi profilo metabolico e genetico sul blastocele

POTENZIALITA' BLASTOCISTI

1↑ risposta infiammatoria aumenta all'aumentare delle dimensioni dell'embrione *(LindaC.Giudice in Reproductive Medicine, Hertig 1968)*



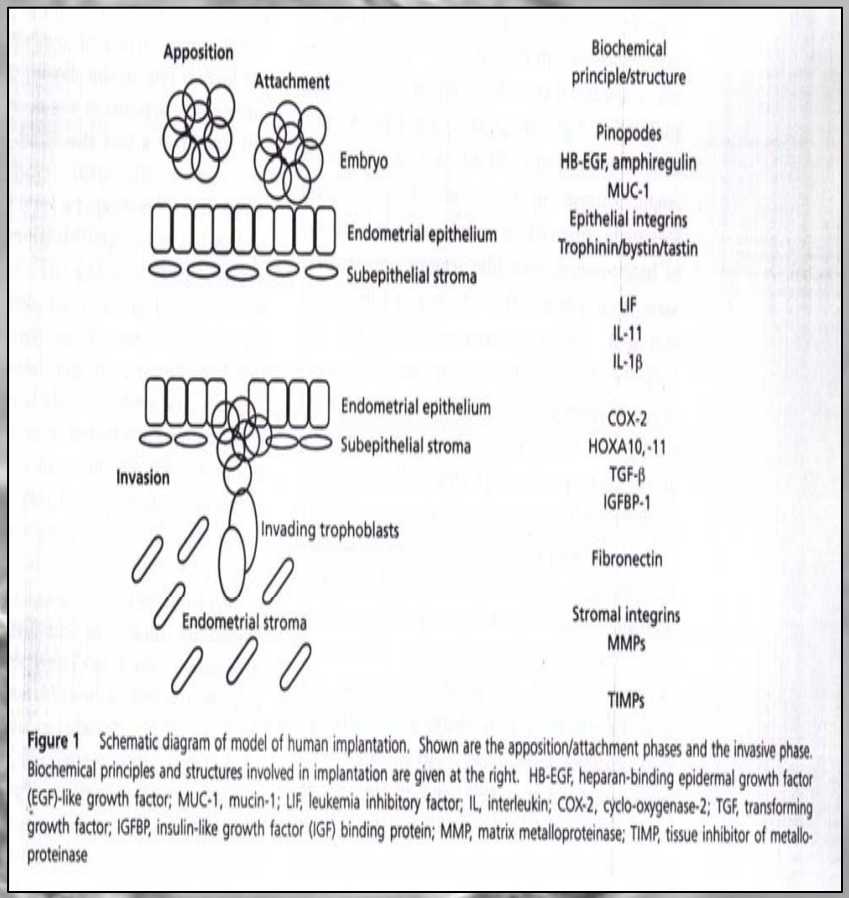
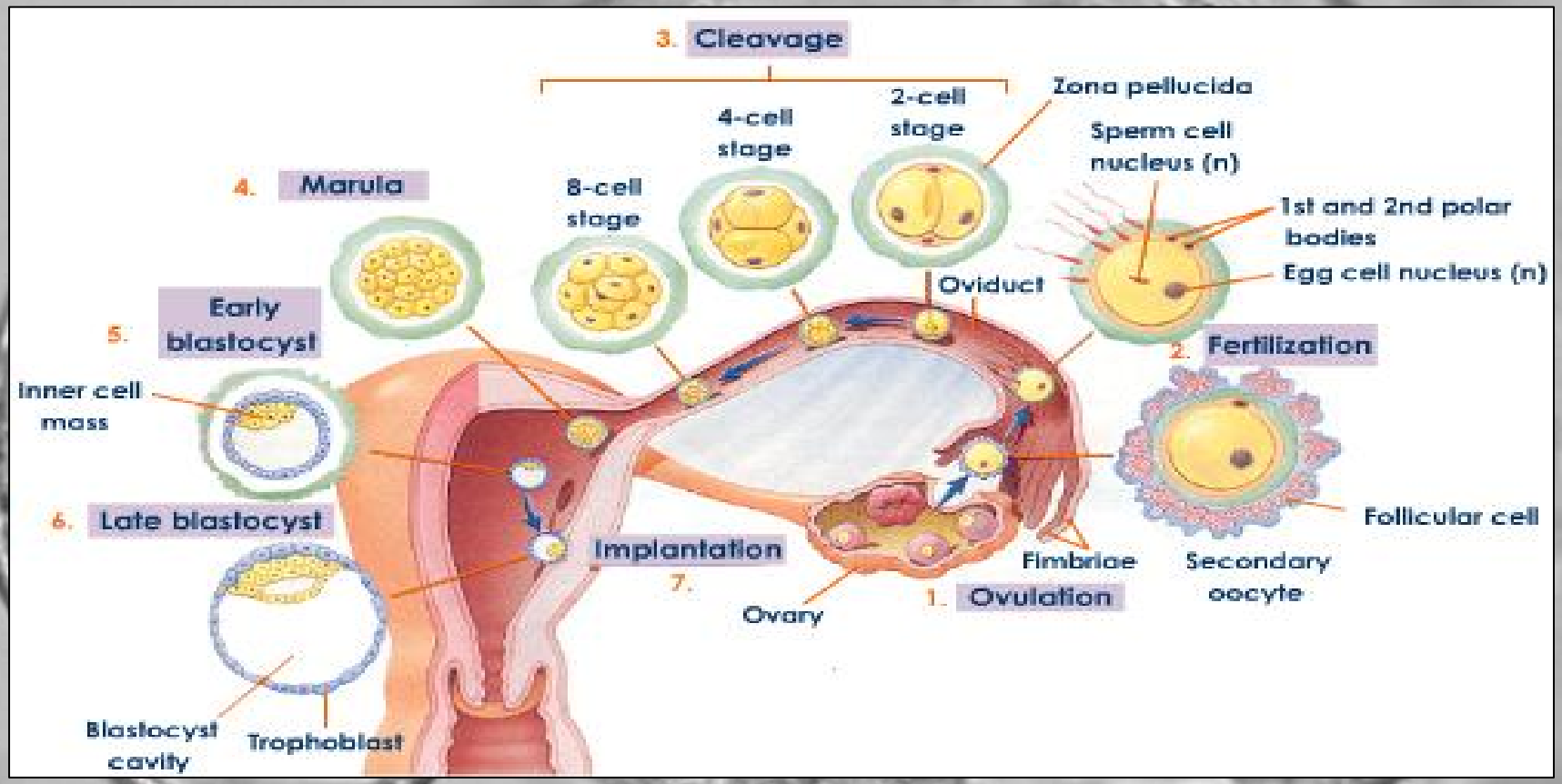


Figure 1 Schematic diagram of model of human implantation. Shown are the apposition/attachment phases and the invasive phase. Biochemical principles and structures involved in implantation are given at the right. HB-EGF, heparan-binding epidermal growth factor (EGF)-like growth factor; MUC-1, mucin-1; LIF, leukemia inhibitory factor; IL, interleukin; COX-2, cyclo-oxygenase-2; TGF, transforming growth factor; IGFBP, insulin-like growth factor (IGF) binding protein; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metallo-proteinase

POTENZIALITA' BLASTOCISTI

2 è giusto aspettare il D5 sia per preparazione endometriale sia per eseguire il transfer nello stadio di impianto fisiologico



POTENZIALITA' BLASTOCISTI

3 ↓ contrattilità uterina

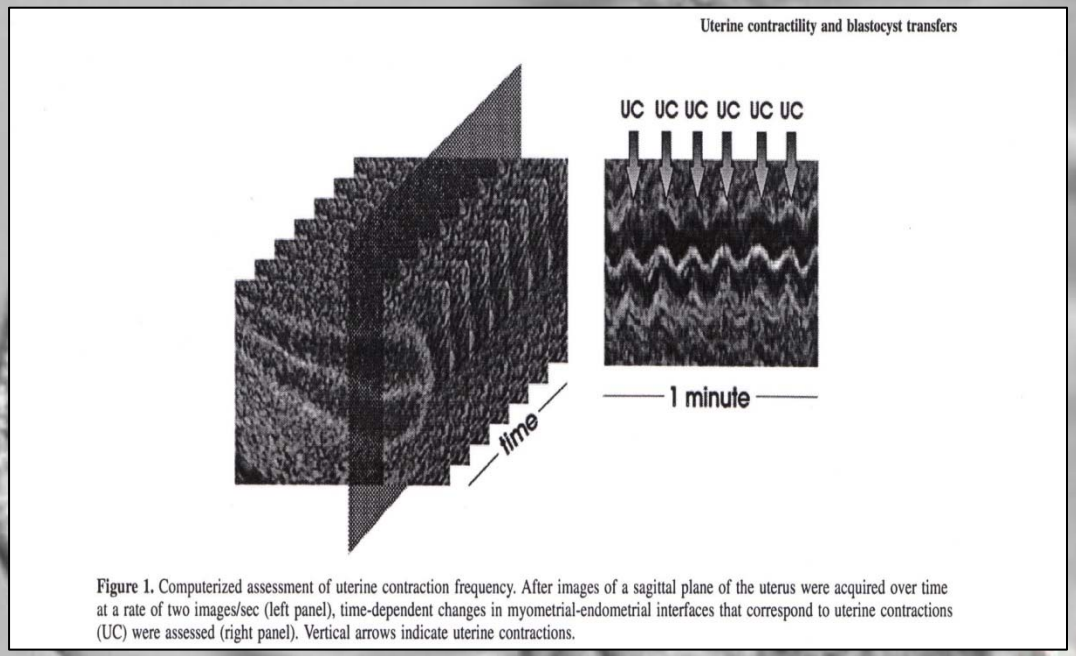


Figure 1. Computerized assessment of uterine contraction frequency. After images of a sagittal plane of the uterus were acquired over time at a rate of two images/sec (left panel), time-dependent changes in myometrial-endometrial interfaces that correspond to uterine contractions (UC) were assessed (right panel). Vertical arrows indicate uterine contractions.

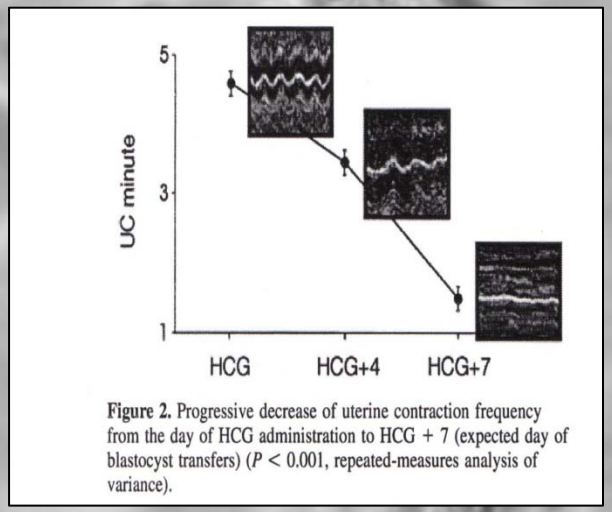


Figure 2. Progressive decrease of uterine contraction frequency from the day of HCG administration to HCG + 7 (expected day of blastocyst transfers) ($P < 0.001$, repeated-measures analysis of variance).

Franchin R. Hum Rep. 2001

POTENZIALITA' BLASTOCISTI

4 Self selection e possibilità di eseguire eSET

Grading criteria for human blastocysts

Cavitating morula < 50% cavity

Blastocyst ≥ 50% cavity

Degree of expansion and hatching status:

(1) Early blastocyst; the blastocoel filling more than half the volume of the conceptus, but no expansion in overall size as compared to earlier stages

(2) Blastocyst; the blastocoel filling more than half of the volume of the conceptus, with slight expansion in overall size and notable thinning of the zona pellucida

(3) Full blastocyst; a blastocoel more than 50% of the conceptus volume and overall size fully enlarged with a very thin zona pellucida

(4) Hatching blastocyst; non-preimplantation genetic diagnosis. The trophectoderm has started to herniate through the zona

(5) Fully hatched blastocyst; non-preimplantation genetic diagnosis. Free blastocyst fully removed from zona pellucida

(6) Hatching or hatched blastocyst; preimplantation genetic diagnosis

Inner cell mass (ICM) grading:

(A) Tightly packed, compacted cells

(B) Larger, loose cells

(C) No ICM distinguishable

(D) Cells of ICM appear degenerative

Trophectoderm grading:

(A) Many healthy cells forming a cohesive epithelium

(B) Few, but healthy cells, large in size

(C) Poor, very large, or unevenly distributed cells; may appear as few cells squeezed to the side

(D) Cells of the trophectoderm appear degenerative

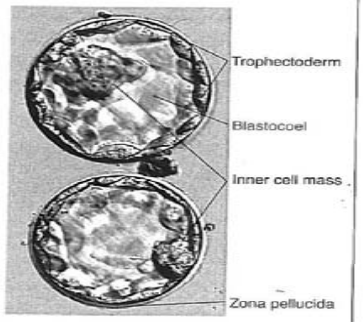


Figure 5.11 Blastocyst grading schemes used by the Cornell program. (a) Grading system detailed in text and photograph; (b) grading system detailed by individual photographs (*see next page*)

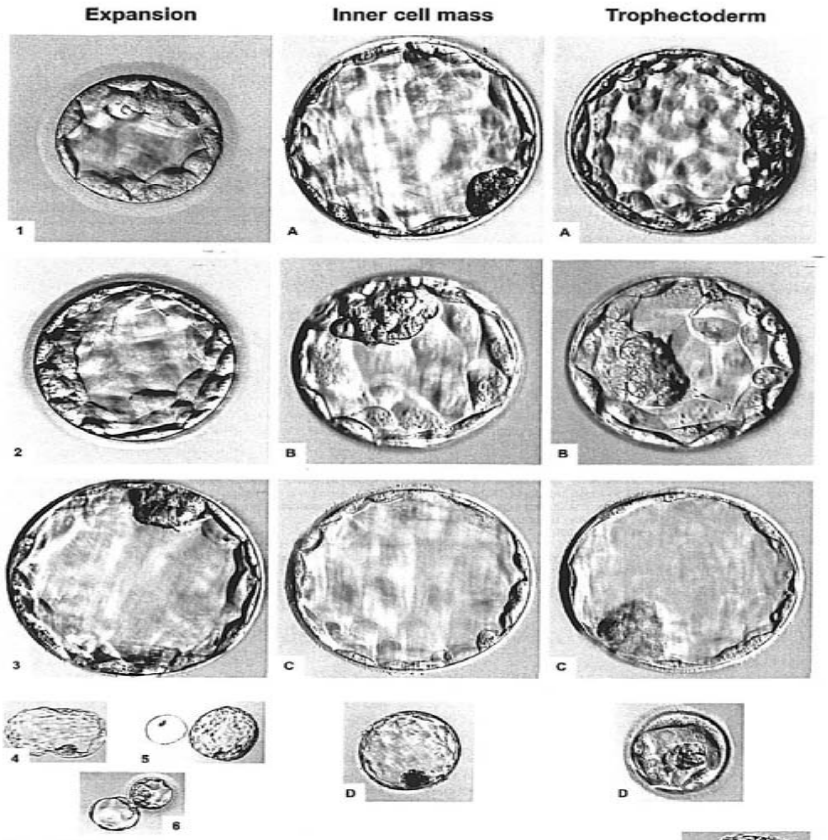
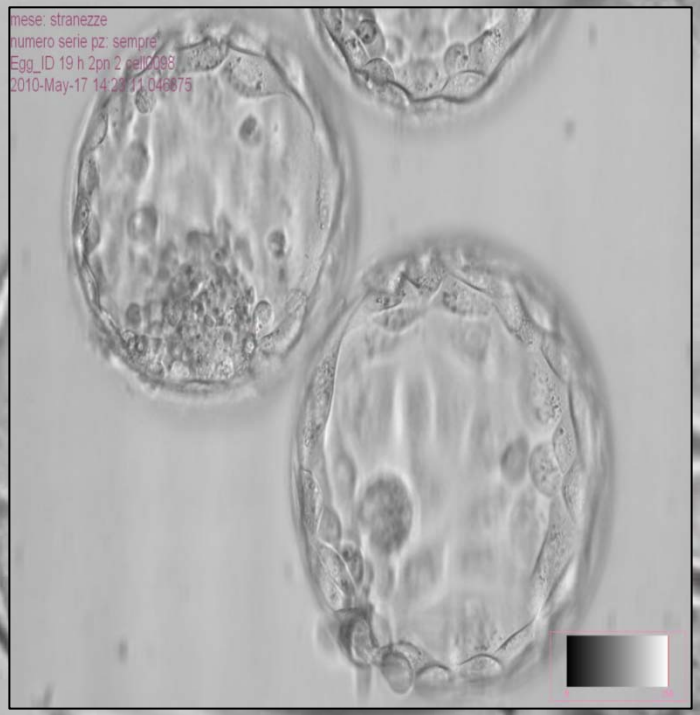
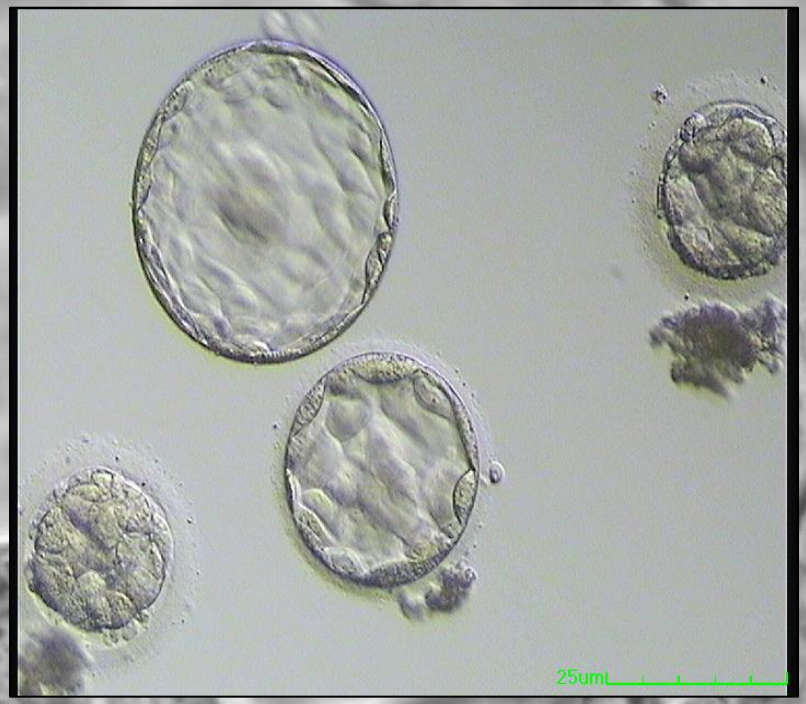


Figure 5.11 Continued

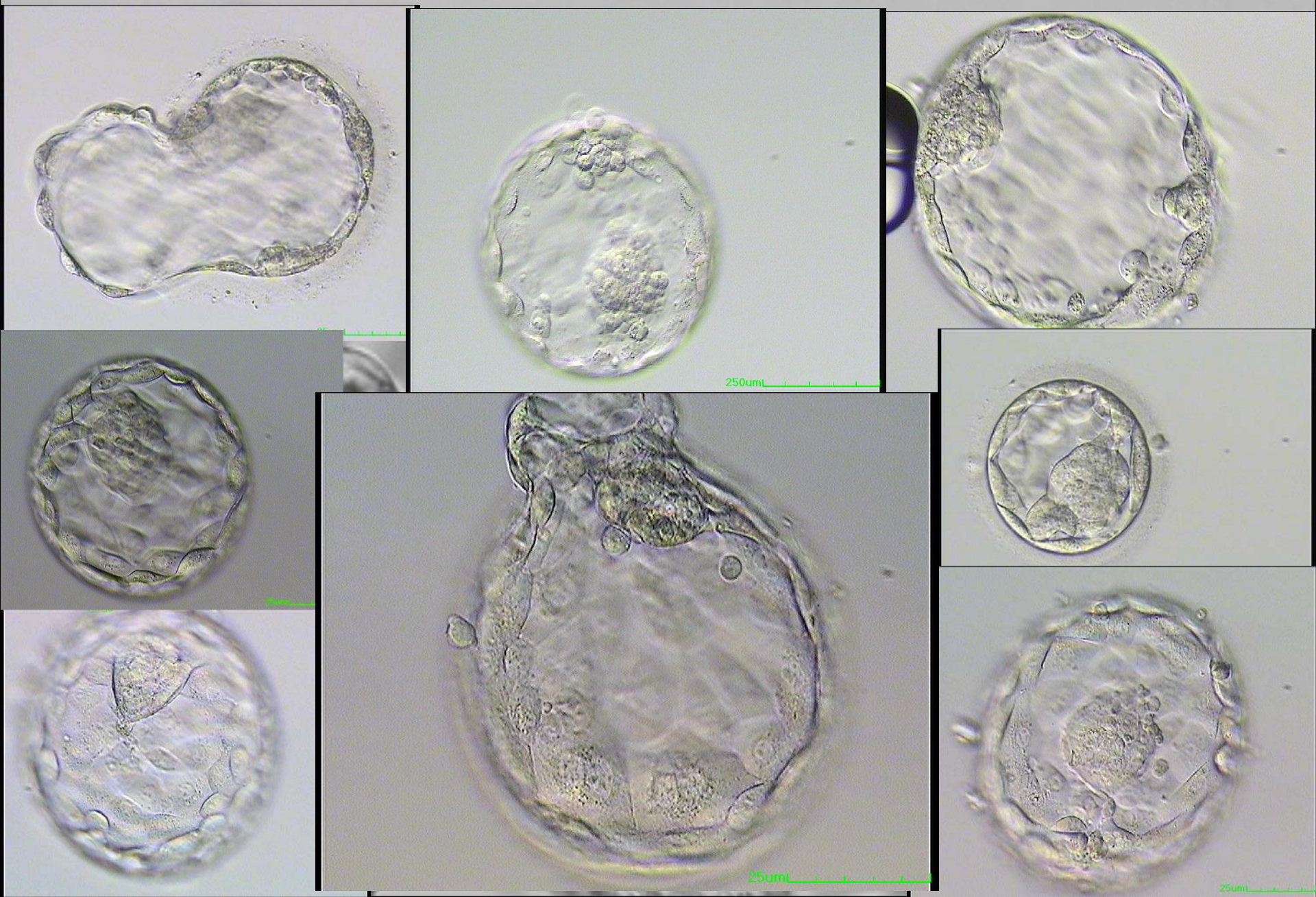
POTENZIALITA’ BLASTOCISTI

4 Self selection e possibilità di eseguire eSET

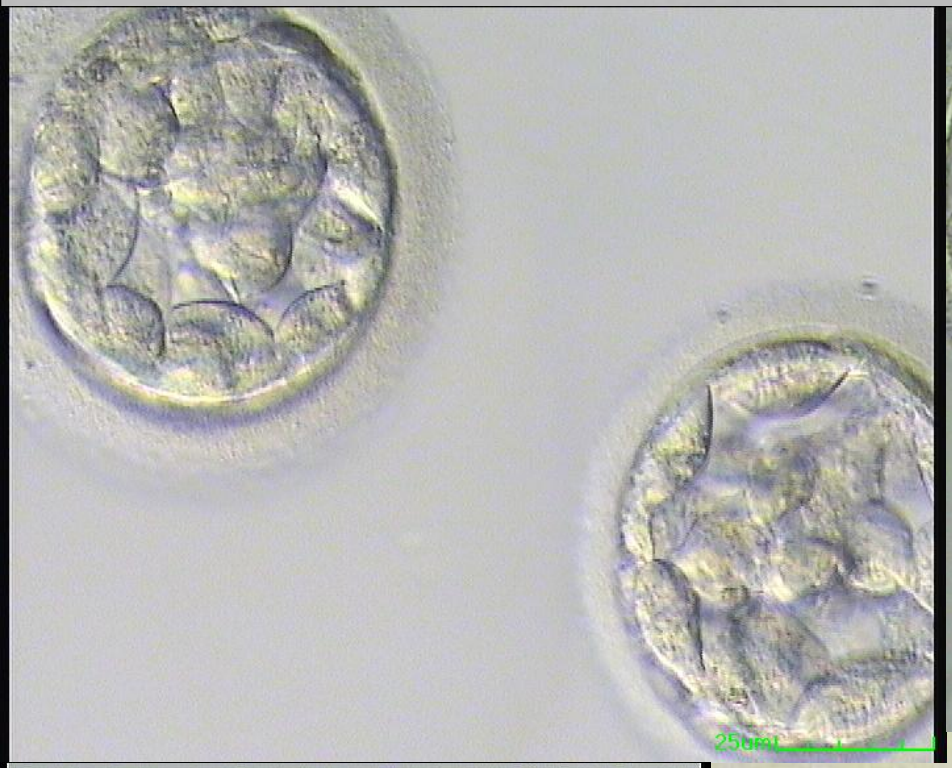


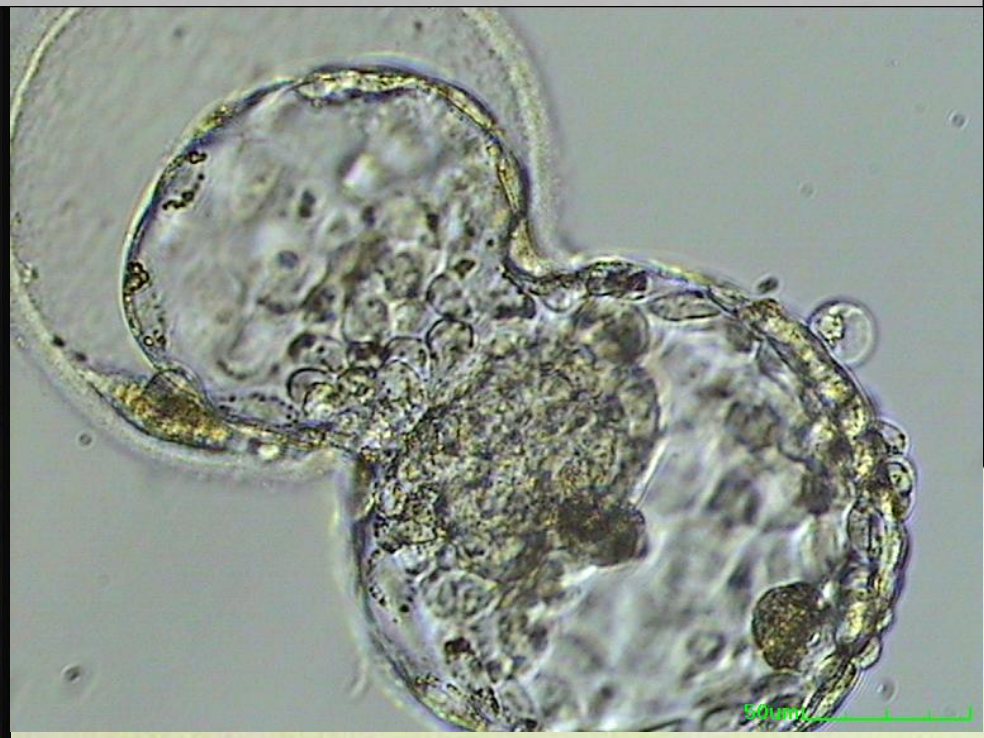


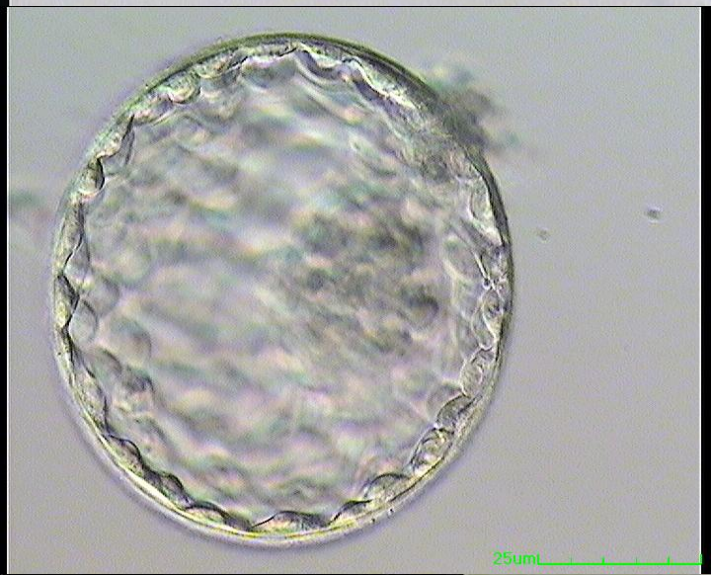
POTENZIALITA’ BLASTOCISTI

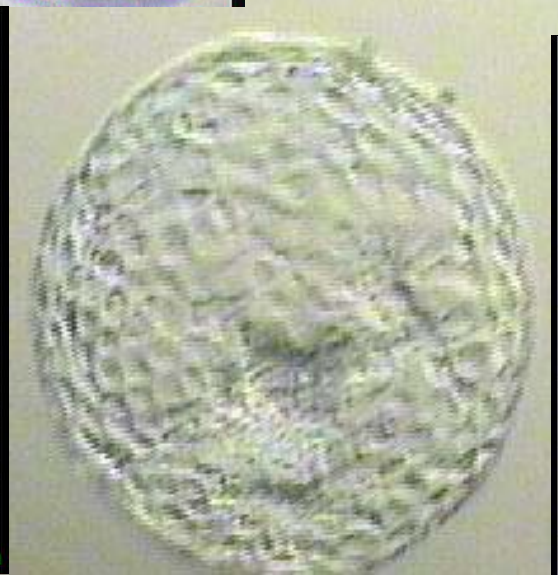


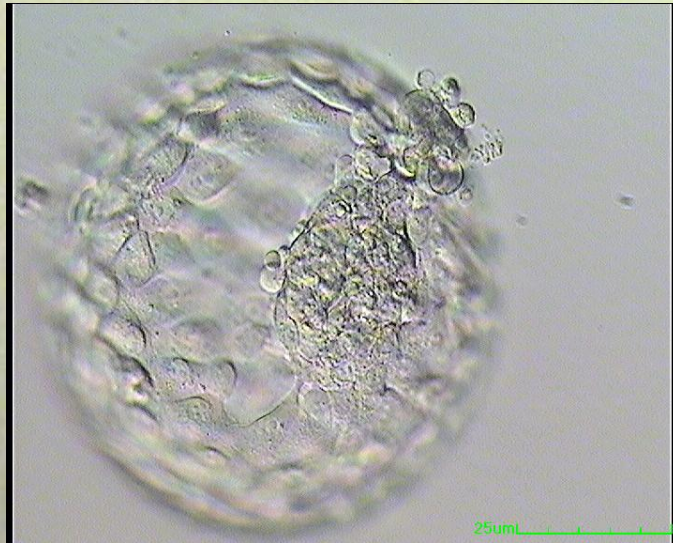
POTENZIALITA’ BLASTOCISTI











POTENZIALITA' BLASTOCISTI

5 ↑ IR e PR

Table 3. Blastocyst and implantation rate (in day 5/6 transfers)

Study	Blastocyst rate	Implantation D2/3	Implantation D5/6	Other
Bungum 2003	55.2%	50/114 43.9%	44/120 36.7%	2/61 patients had only 1 blastocyst
Coskun 2000	28%	50/235 21.3%	52/218 23.9%	77% patients had at least 1 blastocyst
Devreker 2000	Not stated	1/34 2.9%	8/19 42.1%	
Emiliani 2003	48%	57/197 28.9%	50/168 29.8%	
Frattarelli 2003	Not stated	18/69 26.1%	23/53 43.4%	
Gardner 1998	46.5%	64/174 36.8%	53/95 55.8%	85% patients had at least 2 blastocysts
Hreinsson 2004	33%	29/139 20.9%	24/114 21.1%	2 morula replace (one implanted). 60% preg rate when top quality blasts transferred
Karaki 2002	33%	37/291 12.7%	37/142 26.1%	9/80 cancelled due to lack of blastocysts (unselected)
Kolibianakis 2004	50.7%	96/234 41.0%	94/226 41.6%	
Levitas 2004	43%	4/56 7.1%	10/24 4.2%	Day 5-7 26% cancelled due to lack of blastocysts (poor prog)
Levron 2002	34.2%	53/137 38.7%	20/99 20.2%	6.5% cancelled due to lack of blastocysts (good prog)
Livingstone 2002	Not stated			
Motta 1998	Not stated	51/262 19.5%	36/120 30.0%	6/58 cycles cancelled D5 no blastocysts
Papanikolaou 2005	Not stated	35/170 20.6%	59/158 37.3%	4/158 women had only 1 blast transferred due to lack of availability and 1 had it on request.
Papanikolaou 2006	Not stated	38/156 24%	58/149 38.9%	Number of patients with no embryos avail D3: 8 and D5: 11
Rienzi 2002	44.8%	34/96 35.4%	38/100 38.0%	Good prognosis
Schillaci 2002	60.3%	23/168 13.7%	26/110 23.6%	Unselected population nil cancellations D5
Van der Auwera	44.7%	31/106 29.2%	41/90 45.6%	27% cancellation D5 (unselected population)

Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology Blake D 2010 ***Cochrane***

POTENZIALITA' BLASTOCISTI

6 analisi profilo metabolico e genetico sul blastocele

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Reprod Biomed Online. 2013 Mar 13. pii: S1472-6483(13)00118-1. doi: 10.1016/j.rbmo.2013.02.012. [Epub ahead of print]

Genomic DNA in human blastocoele fluid.

Palini S, Galluzzi L, De Stefani S, Bianchi M, Wells D, Magnani M, Bulletti C.

IVF Unit, 'Cervesi' Hospital Cattolic

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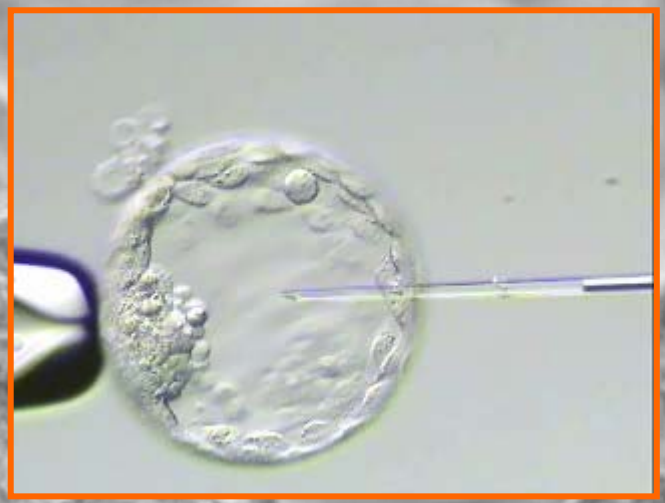
Mol Biosyst. 2012 Apr;8(4):953-8. doi: 10.1039/c1mb05358b. Epub 2011 Oct 21.

A mass spectrometry-based targeted metabolomics strategy of human blastocoele fluid: a promising tool in fertility research.

D'Alessandro A, Federica G, Palini S, Bulletti C, Zolla L.

Department of Environmental Sciences, University of Tuscia, Largo dell'Università snc, 01100 Viterbo, Italy.



eSET ?

Fertil Steril. 2011 Feb;95(2):520-4. doi: 10.1016/j.fertnstert.2010.04.003. Epub 2010 May 26.

The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender.

Alfarawati S, Fragouli E, Colls P, Stevens J, Gutiérrez-Mateo C, Schoolcraft WB, Katz-Jaffe MG, Wells D.

Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, United Kingdom.

Abstract

OBJECTIVE: To assess correlation between blastocyst morphology and chromosomal status.

DESIGN: Observational research study.

SETTING: An IVF clinic and a specialist preimplanation genetic diagnosis (PGD) laboratory.

PATIENT(S): Ninety-three couples undergoing IVF treatment in combination with chromosome screening of embryos.

INTERVENTION(S): Five hundred blastocysts underwent trophectoderm biopsy and comprehensive chromosome screening using comparative genomic hybridization (CGH). The morphology of the embryos was evaluated using standard methods.

MAIN OUTCOME MEASURE(S): Association of aneuploidy and morphologic score.

RESULT(S): A total of 56.7% of blastocysts were aneuploid. One-half of the grade 5/6 blastocysts were euploid, compared with only 37.5% of embryos graded 1/2, suggesting an effect of aneuploidy on blastocyst development. Aneuploidy also had a negative effect on inner cell mass and trophectoderm grades. Morphologically poor blastocysts had a higher incidence of monosomy and abnormalities affecting several chromosomes. The gender ratio was significantly skewed in relation to morphology. A total of 72% of blastocysts attaining the highest morphologic scores (5AA and 6AA) were found to be male, compared with only 40% of grade 3 embryos.

CONCLUSION(S): Morphology and aneuploidy are linked at the blastocyst stage. However, the association is weak, and consequently, morphologic analysis cannot be relied on to ensure transfer of chromosomally normal embryos. A significant proportion of aneuploid embryos are capable of achieving the highest morphologic scores, and some euploid embryos are of poor morphology. Gender was associated with blastocyst grading, male embryos developing at a significantly faster rate than females.

NECESSITA' SISTEMA CRIOCONSERVAZIONE OTTIMALE

Quali indicatori di processo considerare?

% Beta+ eSET a fresco

% Beta+ da embrioni congelati

% Beta+ cumulativa

% aborti da fresco

% aborti da scongelato

% gemellari

% bambini in braccio/on going

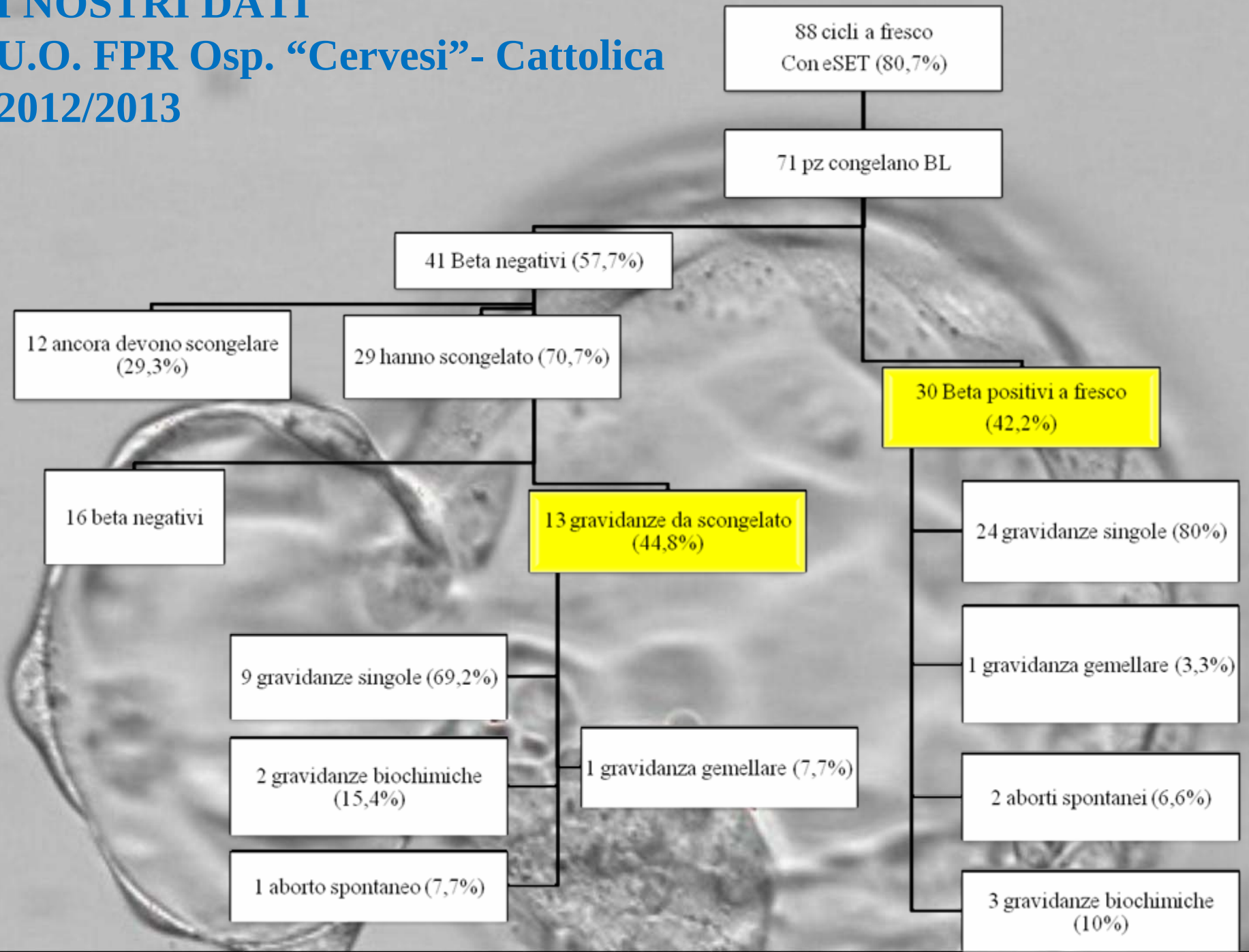
I NOSTRI DATI
U.O. FPR Osp. “Cervesi”- Cattolica
2012/2013

eSET BL D5 Cicli a fresco		
N° pazienti pickup TR D5	88	
N° ovociti recuperati	1320	
N° ovociti maturi	1143	86.6 %
Ovociti inseminati ICSI	843	86.5 %
Ovociti inseminati FIVET	132	13.5 %
2 PN	750	77.0 %
N° Ovociti Crioconservati	168	15.0 %
N° Embrioni ottenuti D3	537	55.0 %
N°BL ottenuti D5	355	66.1 %
N° pz crio D5/N°BL	31/113	42.3 %
N° pz crio D6/N°BL	51/154	57.7 %
N° gravidanze	36	40.1 %
Gravidanze Singole	35	
Gravidanze gemellari	1	0.8%
BCF	37	
N° Aborti	7	19.4 %
IR		42.0 %

I NOSTRI DATI
U.O. FPR Osp. “Cervesi”- Cattolica
2012/2013

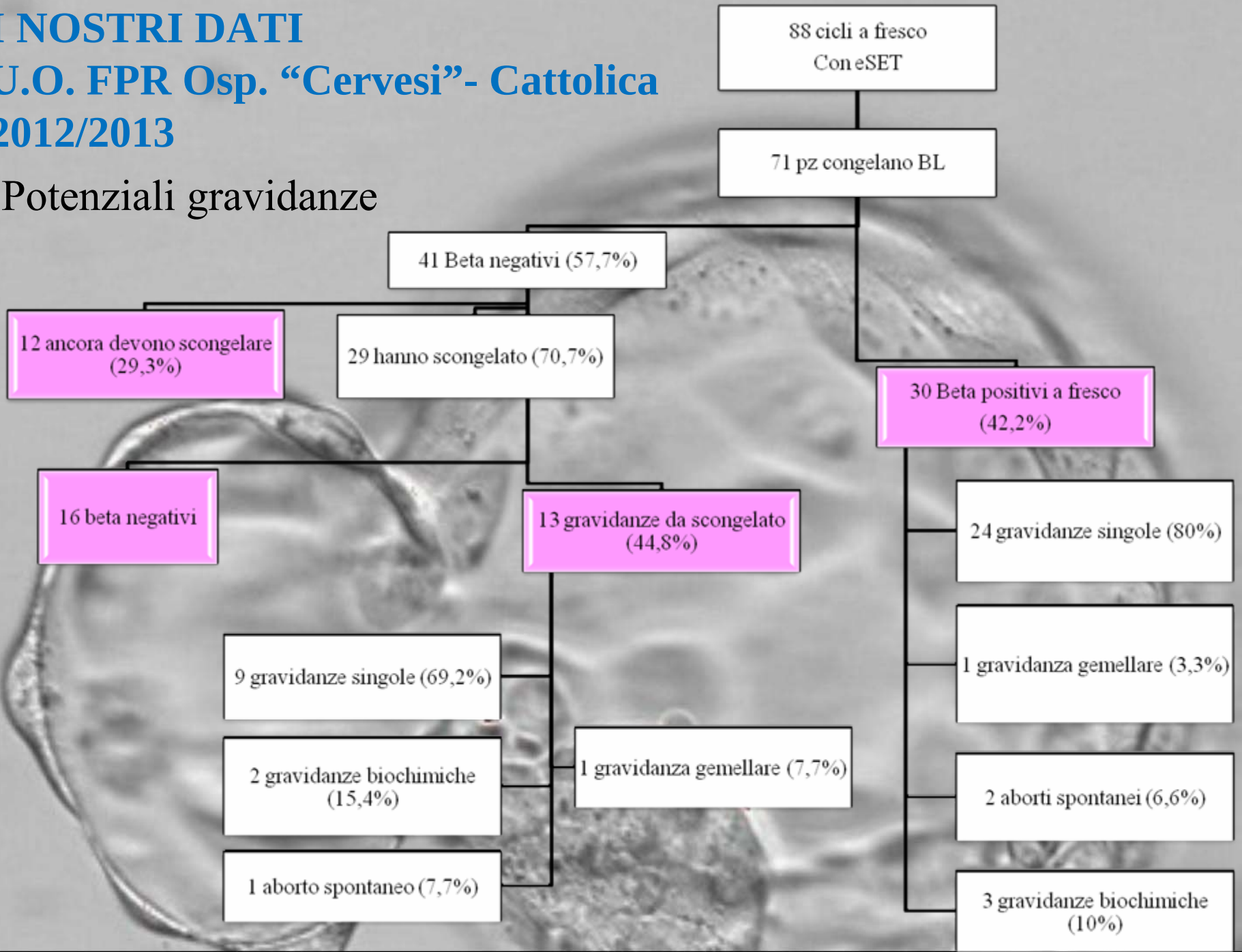
eSET warming BL D5/D6 Cicli di Scongelamento		
N° pazienti	118	
N°BL scongelati	164	
N°BL sopravvissuti	118	72.0 %
N°BL degenerati	46	28.0 %
N°TR D5	42	35.6 %
N°TR D6	76	64.4 %
N° gravidanze	30	25.4 %
Gravidanze Singole	29	
Gravidanze gemellari	1	0.8%
BCF	31	
N° Aborti	8	26.6%
IR		26.3%

I NOSTRI DATI
U.O. FPR Osp. “Cervesi”- Cattolica
2012/2013



I NOSTRI DATI
U.O. FPR Osp. “Cervesi”- Cattolica 2012/2013

Potenziali gravidanze



I NOSTRI DATI

Totale Gravidanze

43 (30 a fresco + 13 da scong) su 71 pazienti

GRAVIDANZE CUMULATIVE
60,6 %

37 BCF su 71 stimolazioni fatte 52%

con un Potenziale di12 pazienti ancora da scongelare e i non considerati altri embrioni da trasferire delle gia' gravide e di chi ancora non ha finito di scongelare ma non ancora gravide!!!

Quali indicatori di processo considerare?

% Beta+ eSET a fresco 42%

% Beta+ da embrioni congelati 44,8%

% Beta+ cumulativi 60,6%

% aborti da fresco 16,6%

% aborti da scongelato 23,1%

% gemellari 2,8%

% bambini in braccio/on going 52%

IN CONCLUSIONE…QUALI SONO I VANTAGGI REALI?

1. La letteratura e la nostra espertienza supportano la politica dell'eSET

2. Possibilita' di analizzare il profilo metabolico del contenuto del blastocele e PGD/PGS da Blastocele (studi registrati NCT01427413, NCT01780415)

3. Indicatore della qualità del sistema di lavoro