Informational biometrics in human gametes and embryos.

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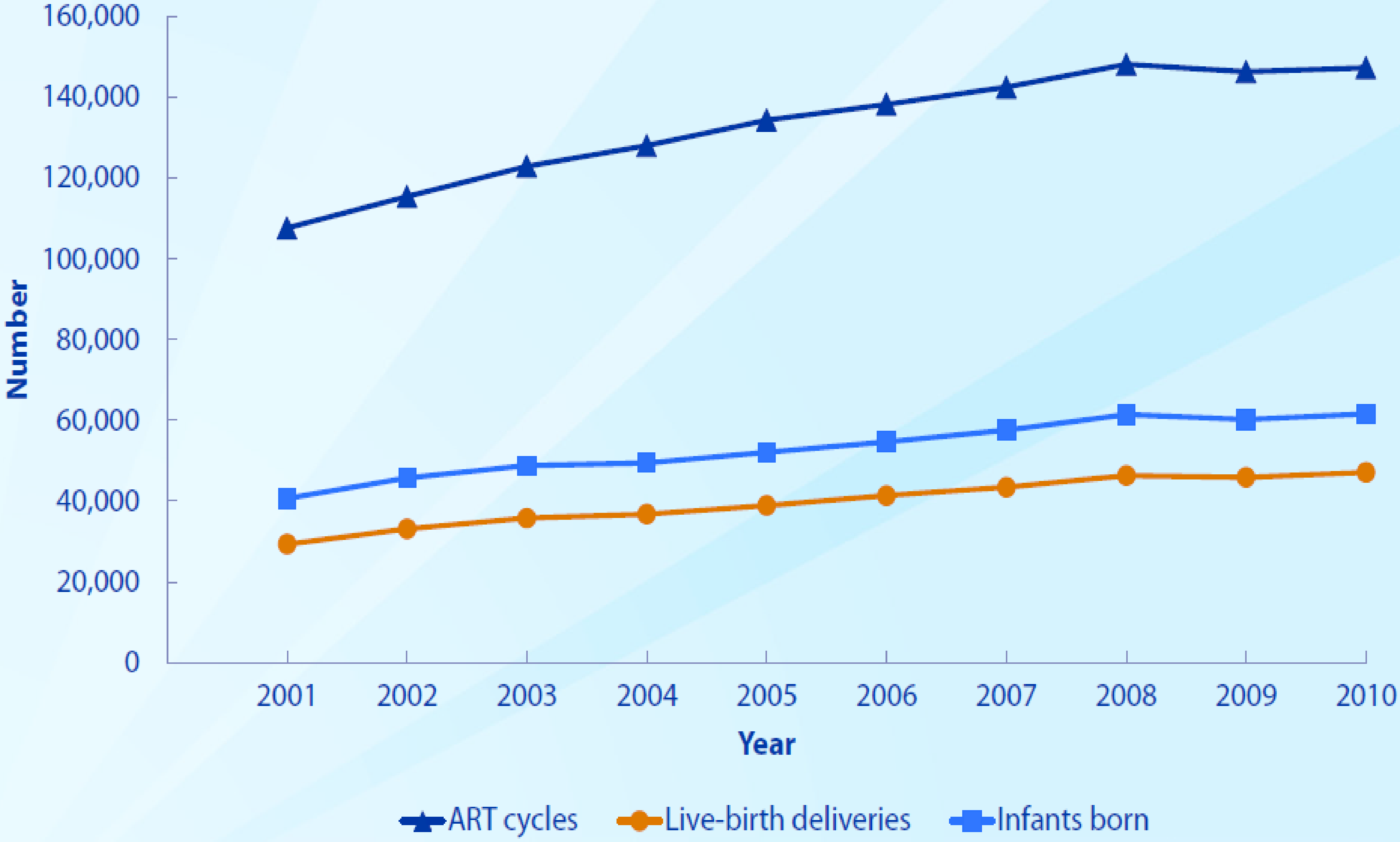
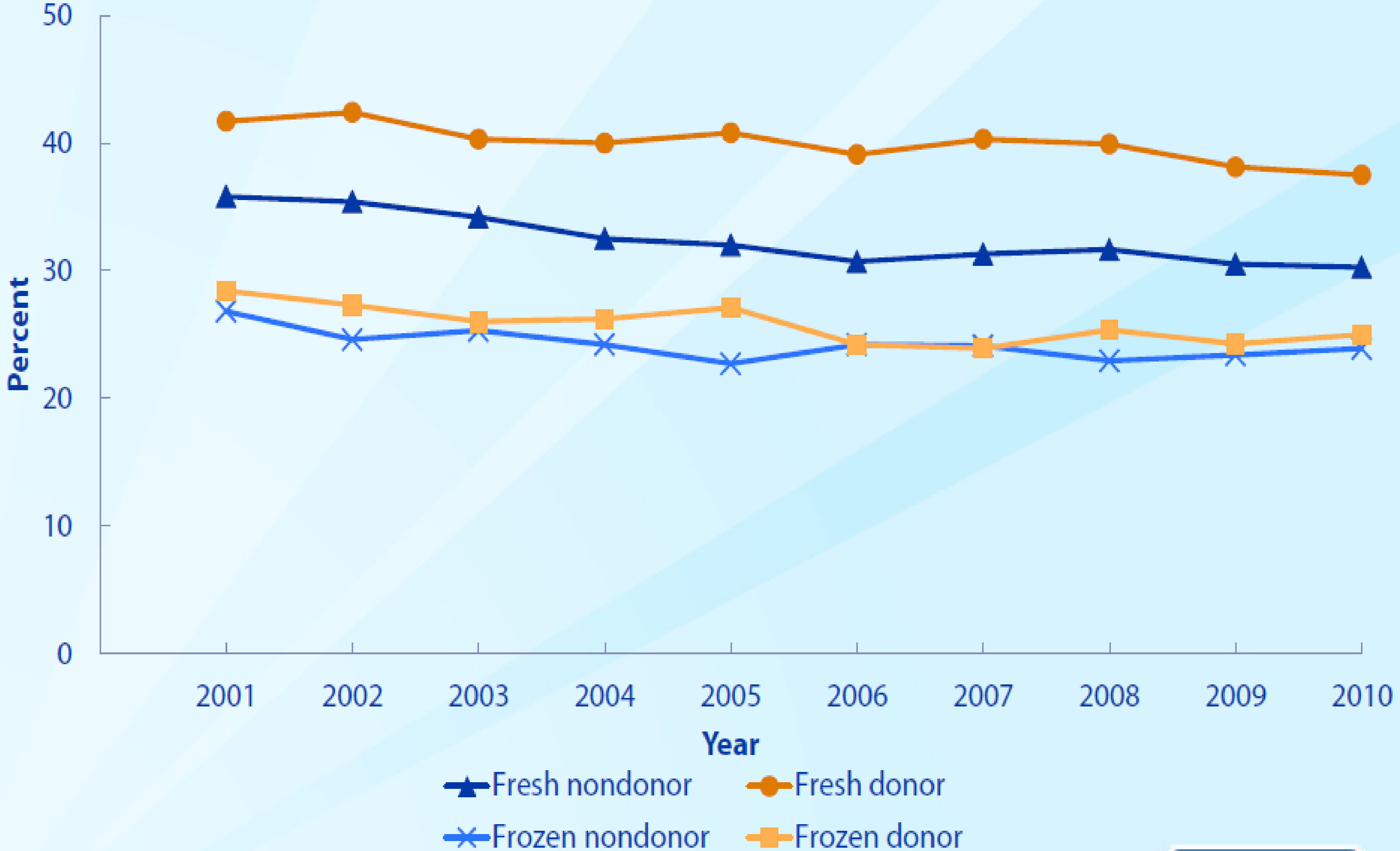
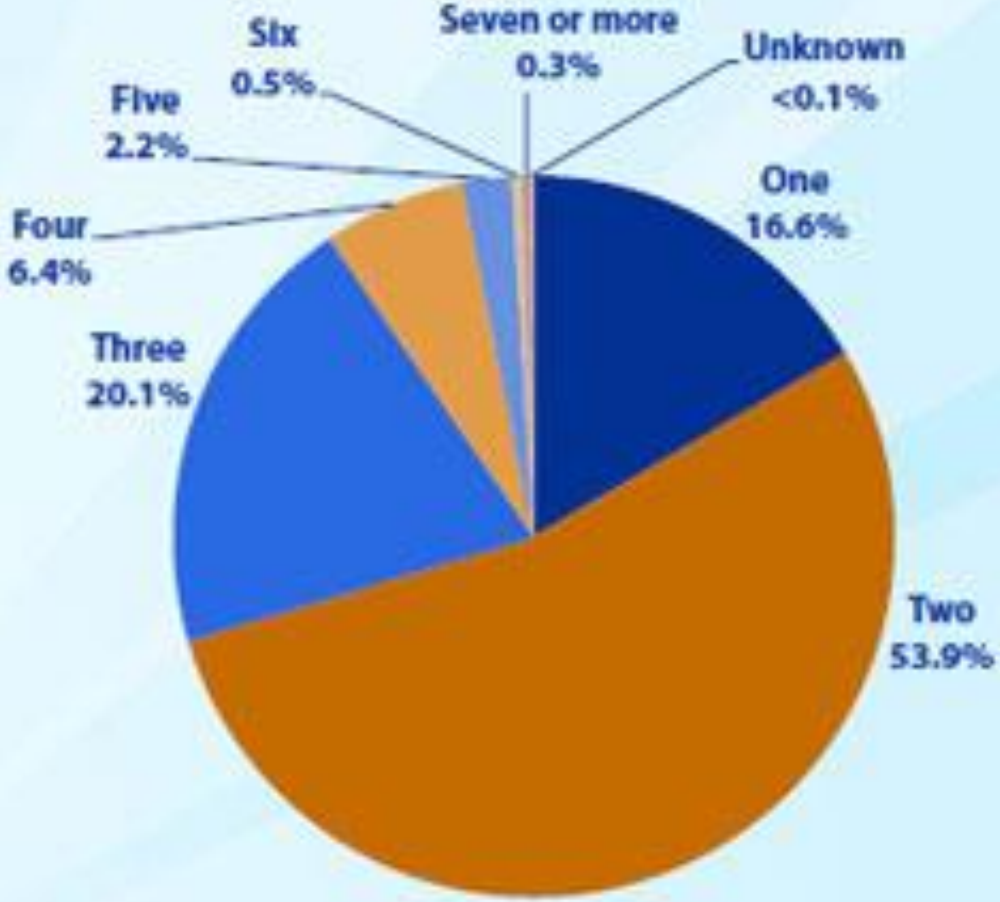
Numbers of ART Cycles Performed, Live-Birth Deliveries, and Infants Born Using ART, 2001–2010



Percentages of ART Cycles That Resulted in Multiple-Infant Live Births, by Type of ART Cycle, 2001–2010

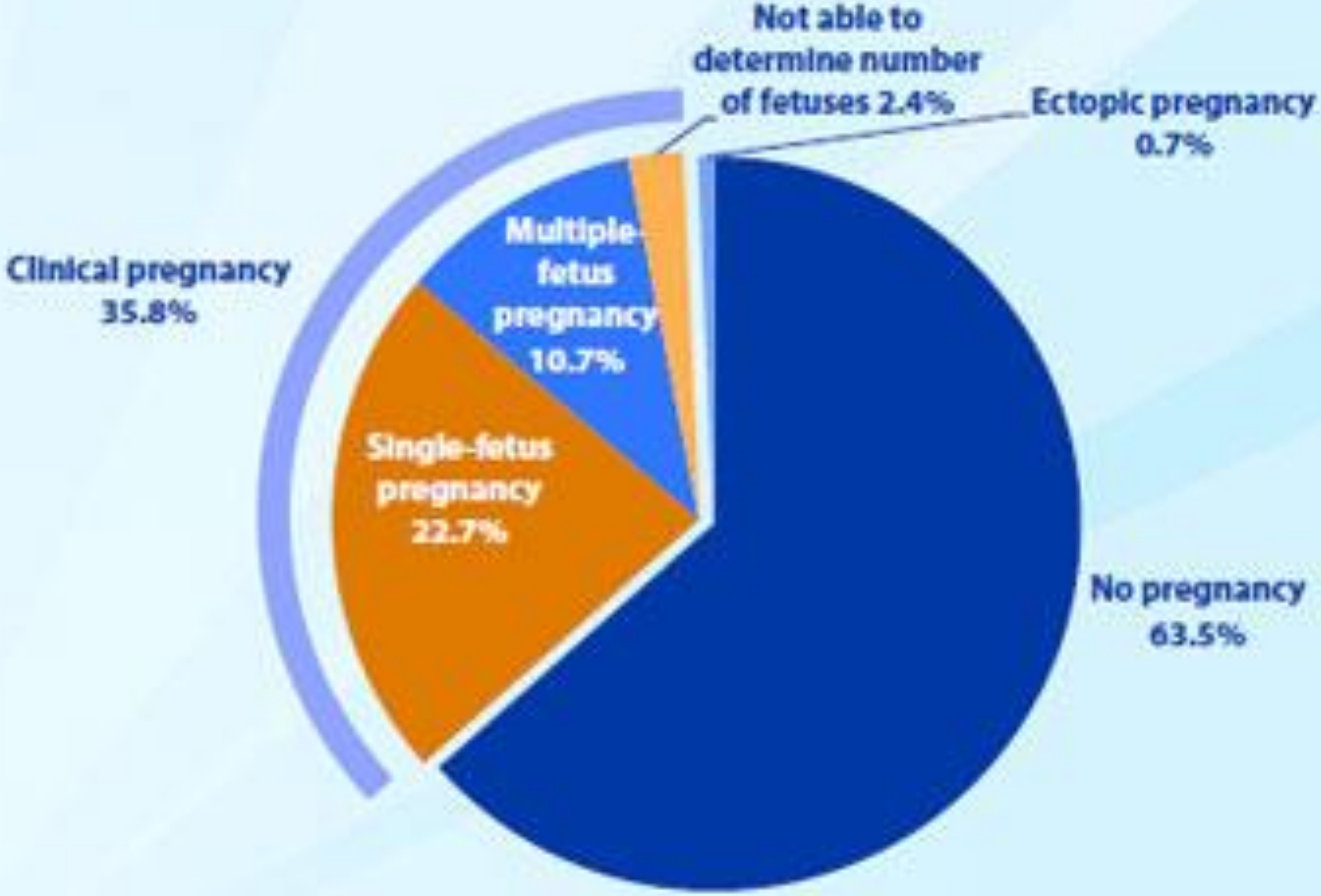


Numbers of Embryos Transferred During ART Cycles Using Fresh Nondonor Eggs or Embryos,* 2011

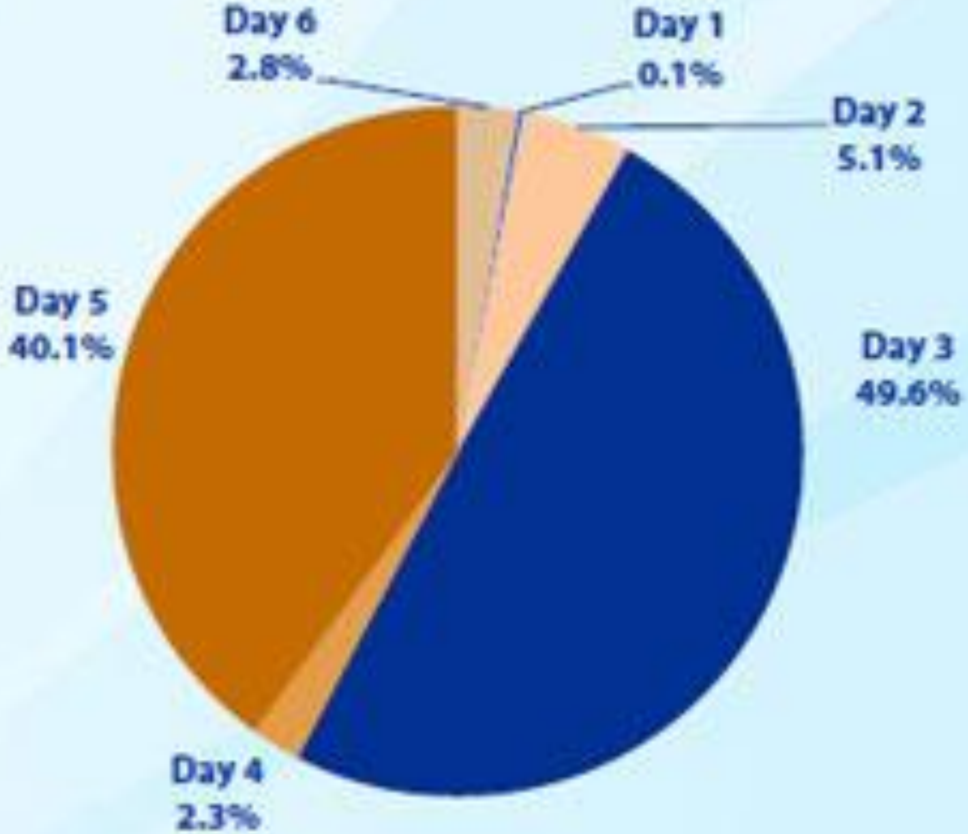


* Total does not equal 100% due to rounding.

Outcomes of ART Cycles Using Fresh Nondonor Eggs or Embryos, 2011



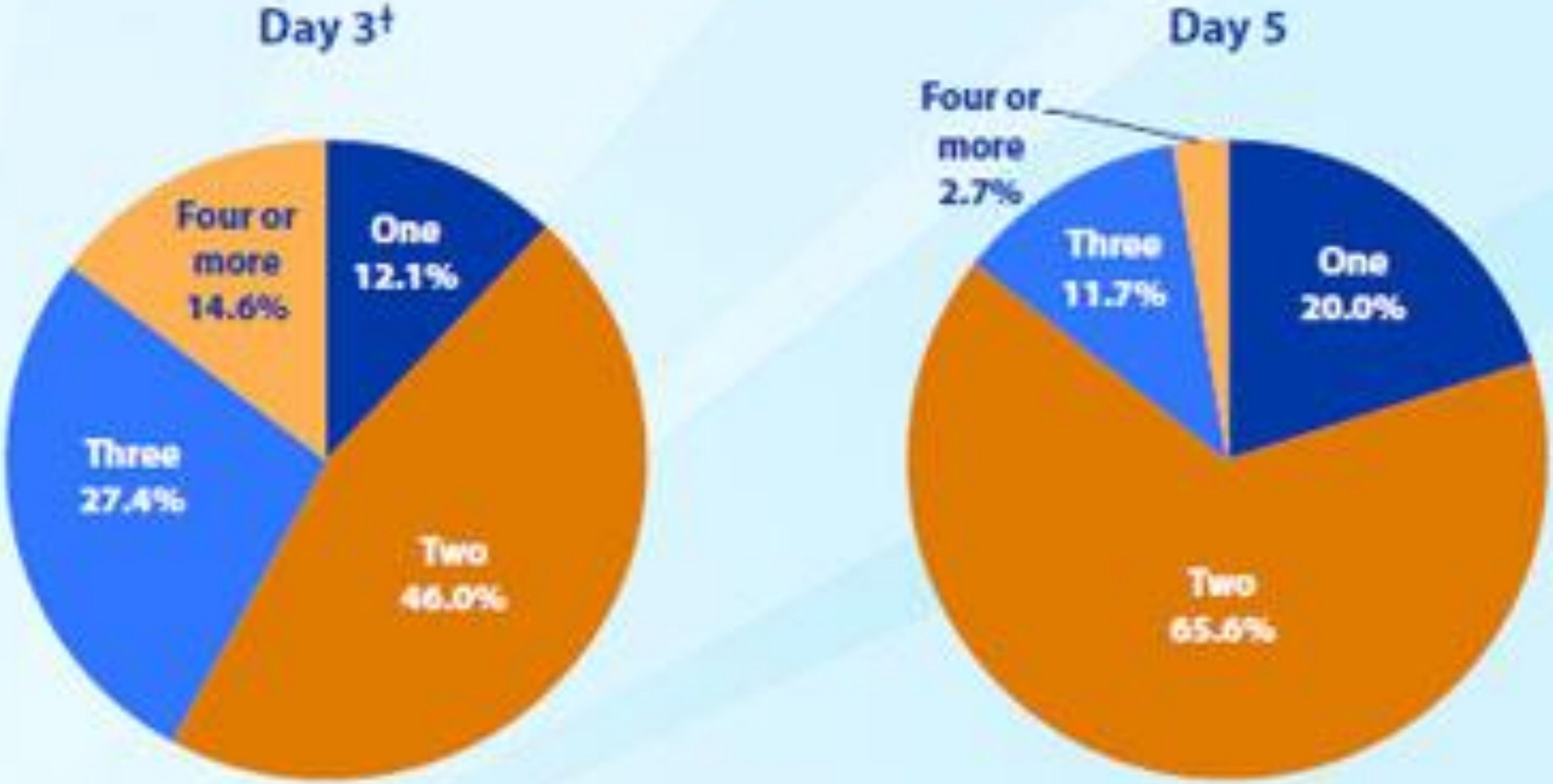
Day of Embryo Transfer* Among ART Cycles Using Fresh Nondonor Eggs or Embryos,† 2011



* Number of days following egg retrieval.

† Cycles using GIFT or ZIFT are excluded. Missing or implausible values for day of embryo transfer (i.e., 0 or >6) are not included.

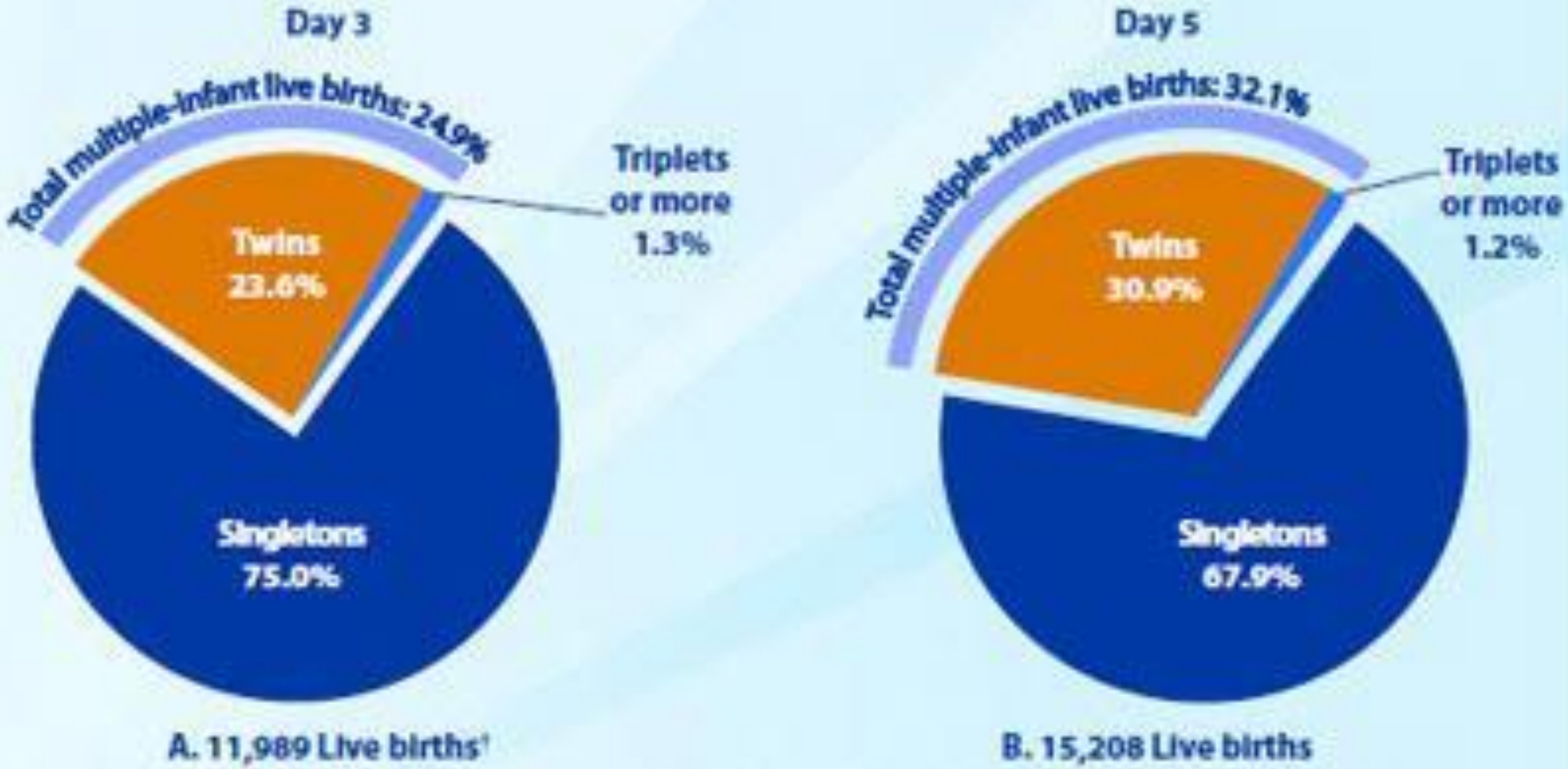
Numbers of Embryos Transferred Among ART Cycles Using Fresh Nondonor Eggs or Embryos for Day 3 and Day 5 Embryo Transfers,* 2011



* Cycles using GIFT or ZIFT are excluded. Embryo transfers performed on days 1, 2, 4, and 6 are not included because each of these accounted for a small proportion of procedures.

‡ Total does not equal 100% due to rounding.

Distribution of Multiple-Infant Live Births Among ART Cycles Using Fresh Nondonor Eggs or Embryos for Day 3 and Day 5 Embryo Transfers,* 2011



* Cycles using GIFT or ZIFT are excluded. Embryo transfers performed on days 1, 2, 4, and 6 are not included because each of these accounted for a small proportion of procedures.

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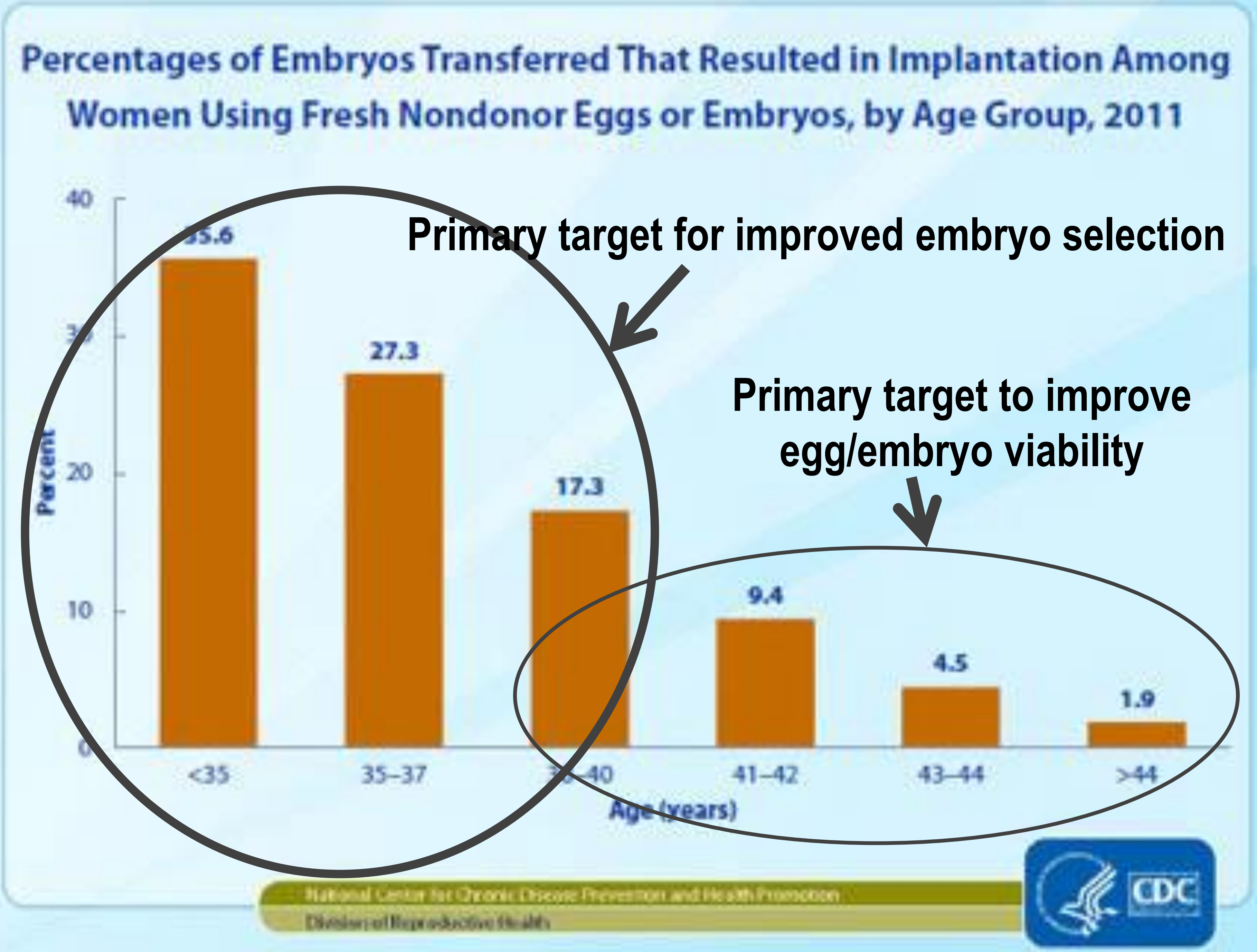


Percentages of Embryos Transferred That Resulted in Implantation Among Women Using Fresh Nondonor Eggs or Embryos, by Age Group, 2011
Primary target for improved embryo selection
Primary target to improve egg/embryo viability
40
20
10
0
Percent
27.3
17.3
9.4
4.5
1.9
<35
35–37
41–42
43–44
>44
Age (years)
National Center for Chronic Disease Prevention and Health Promotion
Division of Reproductive Health
CDC

Techniques and equipment

- Oocyte / sperm preparation
- Insemination (ICSI)
- Embryo culture
- Embryo assessment / diagnostic
- Embryo transfer

The goal of assessing embryos is to identify those that are viable and can contribute to a healthy (singleton) pregnancy after fresh (or frozen) transfer

Contribution of ART to all Deliveries

Proportion of all singletons	3.2%
Proportion of all twins	38.1%
Proportion of all triplets	79.6%

IVF pregnancy rates: 10%-50% - 1/3 multiple

Ombelet et al. 2005.

Infant Complications from Multiple Pregnancy

	Singleton	Twin	Triplet
Ave. Week @ Birth	39 wks	36 wks	32 wks
% Very Premature	1.7%	14%	41%
Ave. Birth Weight	3357 gms	2390 gms	1735 gms
% Severe Handicap	1.9%	3.4%	5.7%
% Infant Mortality	1.1%	6.6%	19.7%
Expense	$15K	$30K	$152K

Oleszczuk et al. 2003; Callahan et al. New Engl J Med 1994;331:244

Contemporary Goals of IVF

- Reduce number of embryos transferred
- Maintain high pregnancy rates per cycle

Need for Optimal Embryo Selection

Which Embryo to Choose?

Best morphology

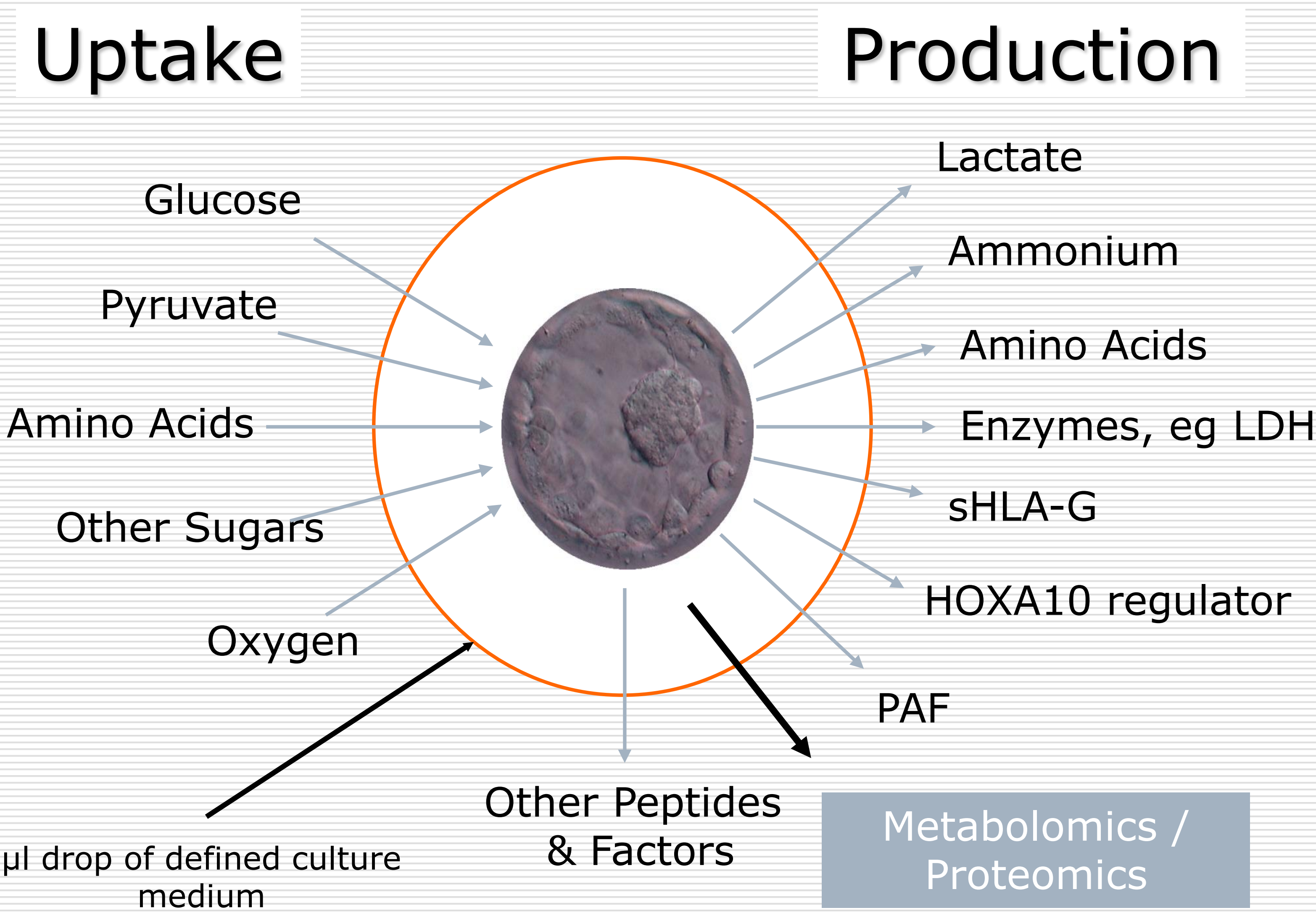
Non Viable by PGS/aCGH/qPCR

Best by “omics”/time lapse

Non-invasive Embryo Assessment Approaches
Possible Targets to Use for Testing

Morphology	□ Birefringence (SpindleView) □ EmbryoScope/Monitoring System
Metabolic Activity	□ Pyruvate/Glucose uptake □ Amino acids* □ Oxygen consumption (Respirometry)
Constituents	□ Genome □ Transcriptome (cumulus cells) □ Proteome* □ Metabolome*
Secreted Factors	□ PAF □ HLAg □ "Secretome"*

Nagy; RBMo, 2008: Symposium: innovative techniques in human embryo viability assessment.



Modified from: Gardner and Leese (1993) Assessment of embryo metabolism and viability. In: Handbook of In Vitro Fertilization. Eds Trounson & Gardner CRC Press. pp195-211.

Single or specific molecule targeting

Target Molecule	Method of analysis	Embryonic stage tested	Clinical practicality	Outcome	References
Pyruvate	Ultramicrofluorescence	Day 0-5	High technicality, less practical.	Contrasting results	(Hardy, Hooper et al. 1989; Gott, Hardy et al. 1990; Conaghan, Handyside et al. 1993; Gardner, Lane et al. 2001; Jones, Trounson et al. 2001)
Glucose	Ultramicrofluorescence	Oocytes, Day 0-5 embryos	High technicality, less practical.	Contrasting results	(Hardy, Hooper et al. 1989; Gott, Hardy et al. 1990; Gardner, Lane et al. 2001; Jones, Trounson et al. 2001; Gardner et al, 2011)

Nel-Themaat L, Nagy ZP; Placenta; 2011: A review of the promises and pitfalls of oocyte and embryo metabolomics.

Single or specific molecule targeting

Target Molecule	Method of analysis	Embryonic stage tested	Clinical practicality	Outcome	References
Oxygen	Microspectrophotometry Respirometry	Oocytes, blastocysts Oocytes	High technicality, impractical. Expensive equipment.	Acquired oxygen consumption rates Respiration rates correlated to maturation and viability of oocytes.	(Magnusson, Hillensjo et al. 1986) (Scott, Berntsen et al. 2008)
HLA-G	Enzyme-linked immunoabsorbent assay	Follicular fluid, Day 0-5	High technicality, impractical	Contrasting findings.	(Fuzzi, Rizzo et al. 2002; Warner, Lampton et al. 2008; Tabiasco, Perrier d'Hauterive et al. 2009)
Leptin	Enzyme-linked immunoabsorbent assay	Day 5 embryos	High technicality, impractical	Positive correlation between leptin secretion and blastocyst development	(Gonzalez, Caballero-Campo et al. 2000)

Groups of molecules targeted

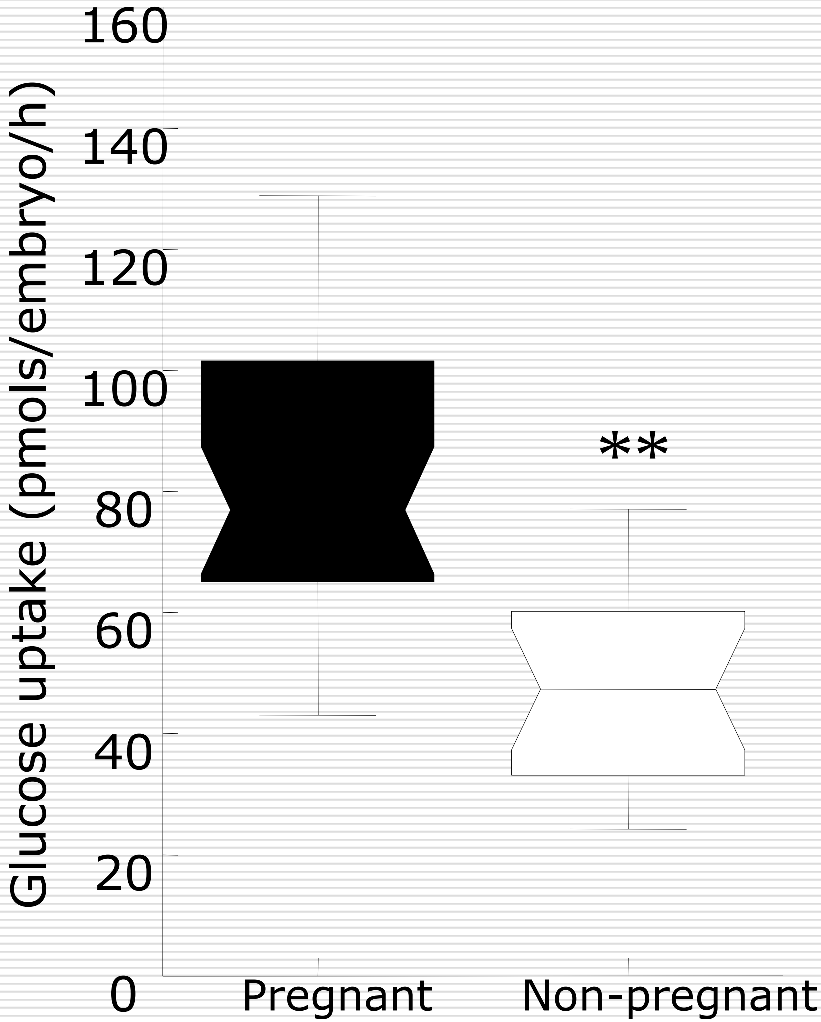
Target Molecule	Method of analysis	Embryonic stage tested	Clinical practicality	Outcome	Selected references
Protein comple-ment	Surface-enhanced laser desorption ionization time-of-flight mass spectrometry Protein microarray	Day 5 embryos Day 5 embryos	High technicality, impractical. Expensive equipment. High technicality, impractical. Expensive equipment.	Protein profiles are related to blastocyst morphology. Implantation potential corresponds to specific protein secretion levels.	(Katz-Jaffe, Gardner et al. 2006) (Dominguez, Gadea et al. 2008)

Groups of molecules targeted

Target Molecule	Method of analysis	Embryonic stage tested	Clinical practicality	Outcome	Selected references
Metabo-lomic comple-ment	Non-optical spectroscopy (Proton nuclear magnetic resonance) Vibrational spectroscopy (Near infrared; Raman)	Day 3 embryos Oocytes, Day 3-5 embryos	High technicality, impractical. Expensive equipment. Simple, rapid procedure, inexpensive, high practicality for clinical setting.	Metabolomic profile correlates with reproductive potential of embryos. Oocyte viability score correlates to developmental potential. Embryo viability score predicts pregnancy independent of morphology.	(Seli, et al. 2008) (Nagy et al. 2009) (Agrawal et al. 2006; Seli, et al.; 2006; Seli et al. 2007; Scott et al, 2008; Vergouw et al. 2008, 2012) Hardarson et al., 2012)

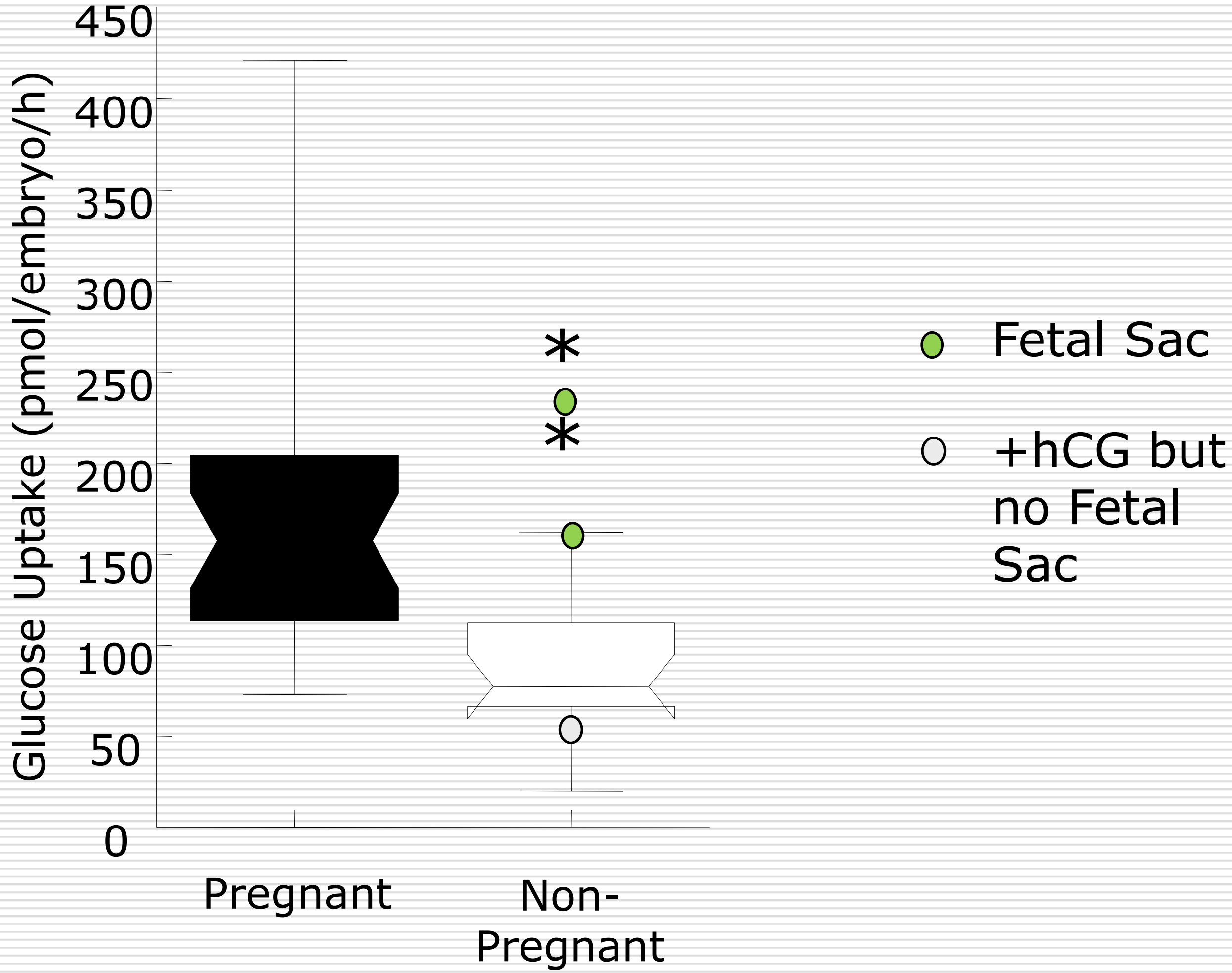
Glucose

Glucose Uptake on day 4 of development and pregnancy outcome



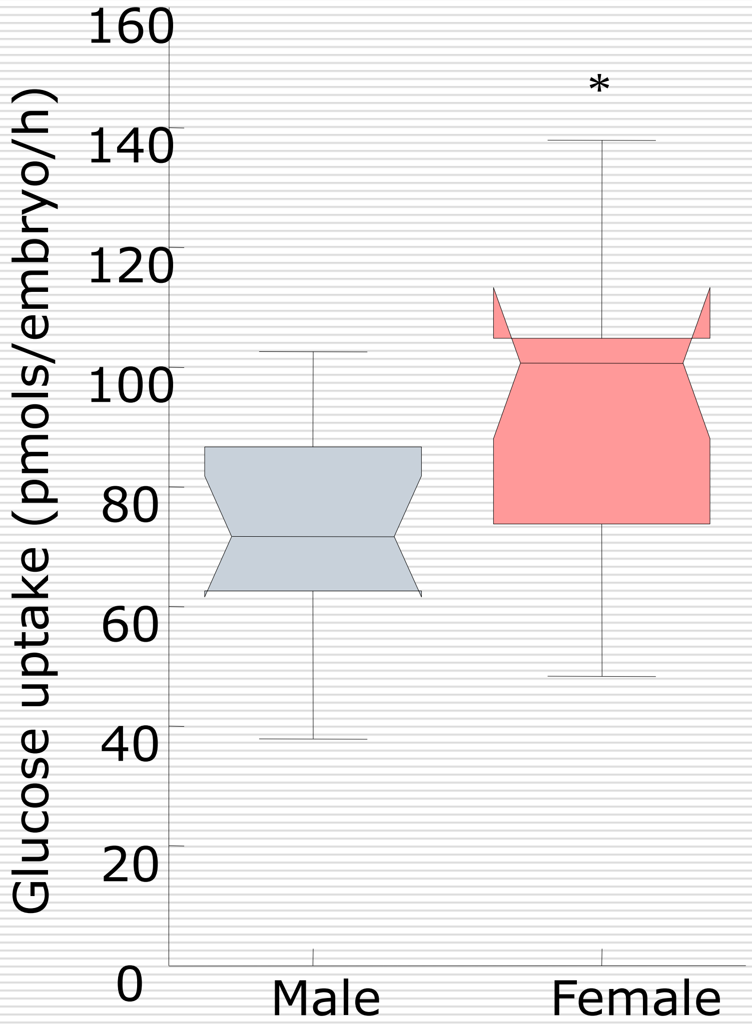
Gardner et al. (2011) Human Reproduction 26: 1981-1986

Glucose Uptake on day 5 of development and pregnancy outcome



Gardner et al. (2011) Human Reproduction 26: 1981-1986

Glucose Uptake on day 4 of development and relation to embryo sex

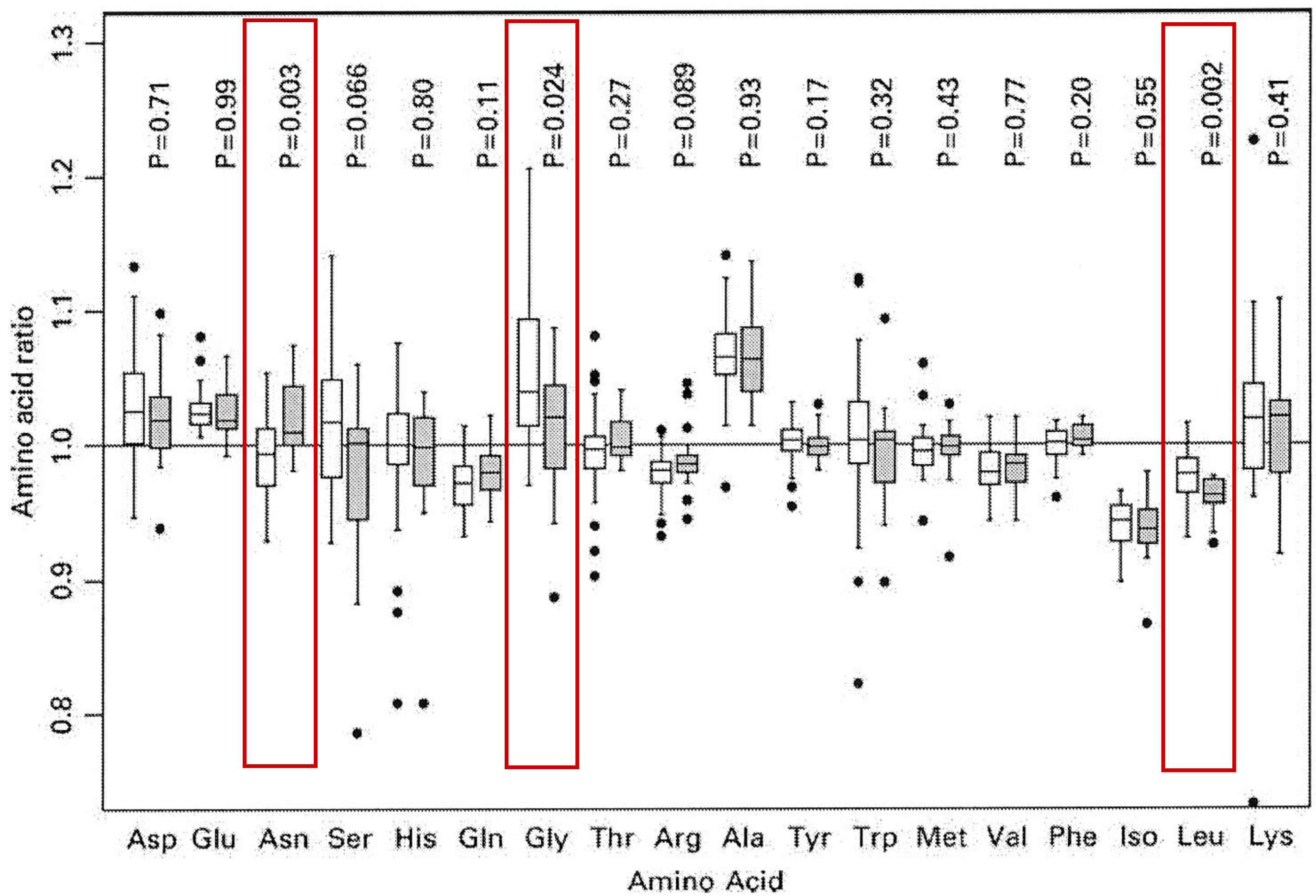


27% increase by females

Gardner et al. (2011) Human Reproduction 26: 1981-1986

Amino Acids

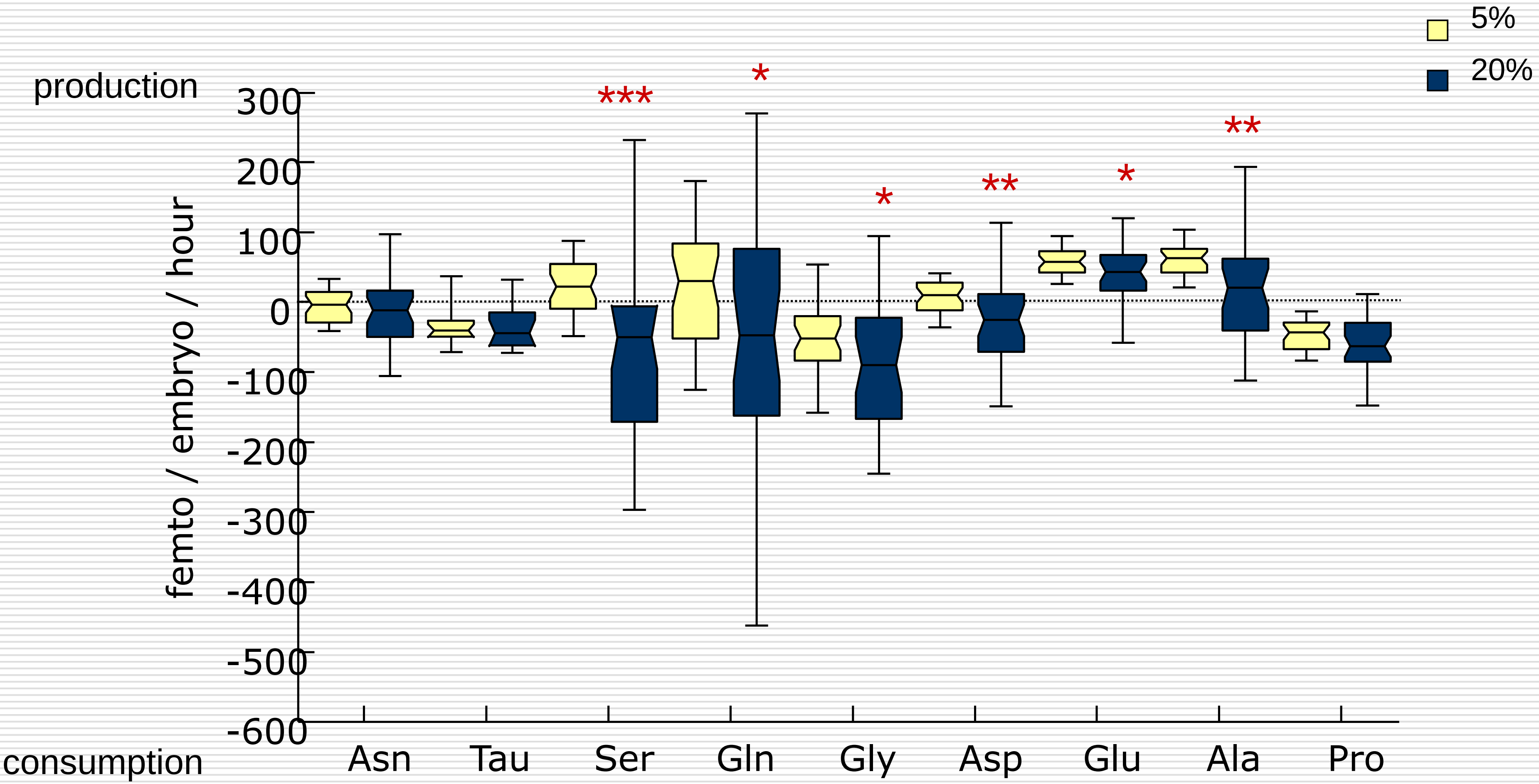
The turnover of Asn, Gly and Leu was correlated with clinical pregnancy rates



All studies on amino acid utilisation have been performed at 20% oxygen

Brison et al. (2004) Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover Hum Reprod 19:2319-2324

Box-plots: cleavage stage individual amino acid utilisation

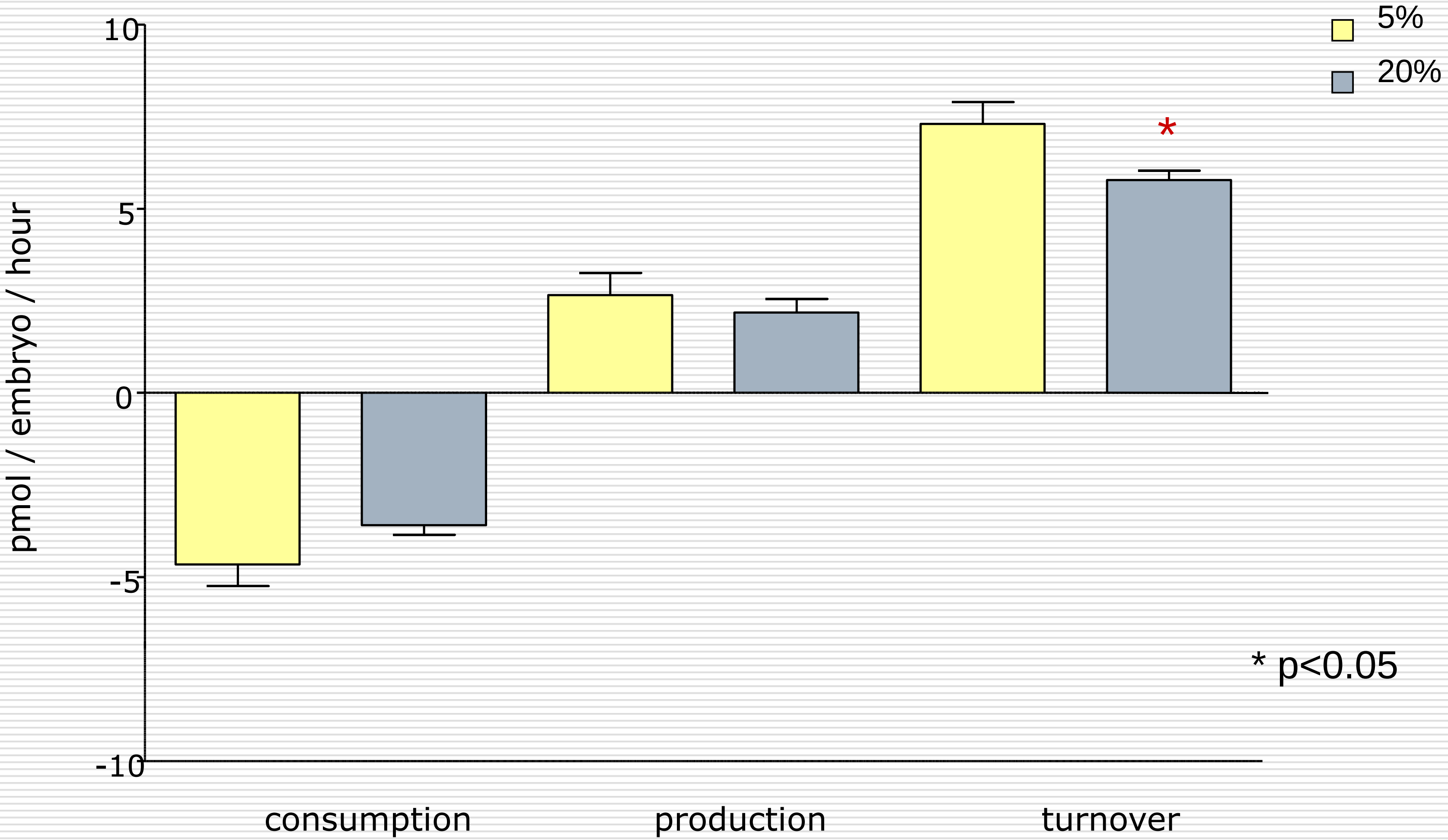


n = 330 (33 replicates, 10 embryos per sample) per treatment

* p<0.05, ** p<0.01 , *** p<0.001

Decreased amino acid turnover from post-compaction embryos cultured atmospheric O_2

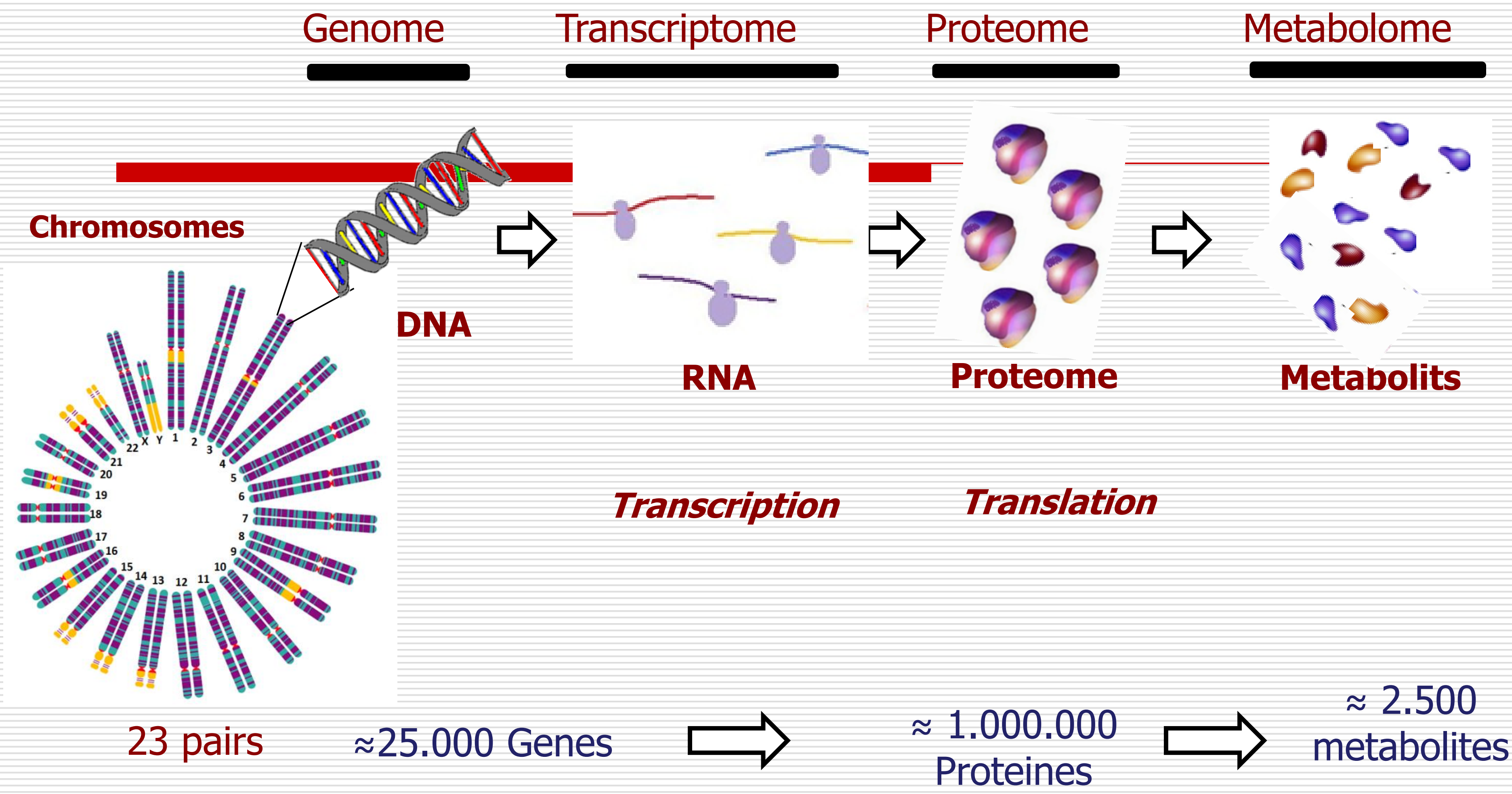
Oxygen has a different impact pre and post compaction



mean ± SEM, n = 75 (25 replicates, 3 embryos per sample) per treatment

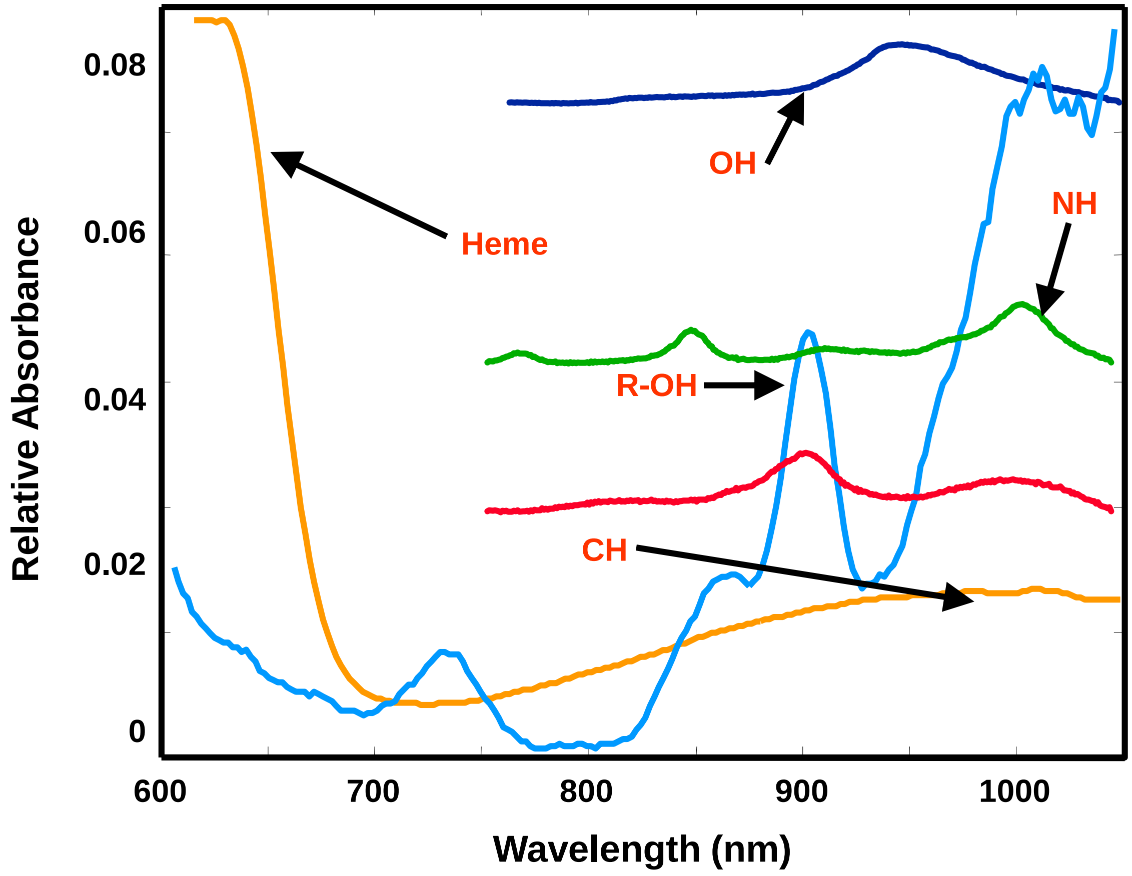
‘OMICS’ Technologies

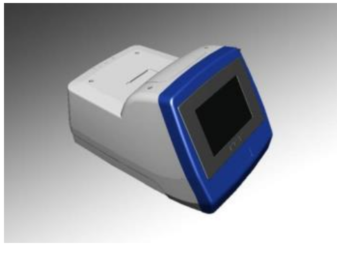
'OMICS' Technologies



Metabolomics
Biomarker Spectral Signatures (by NIR)

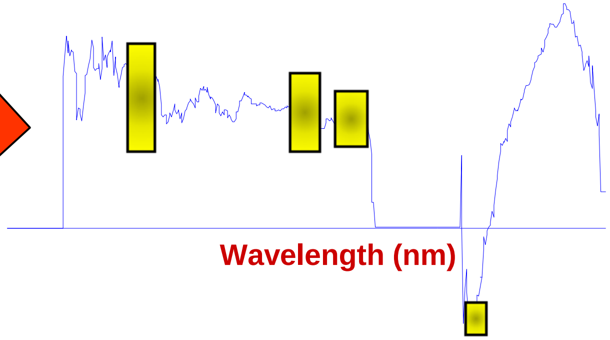
What is measured?





Algorithm Development and Blind Assessment

Step 1: Develop Predictive Algorithms on Day 2, 3 or 5 Embryo Culture Media with known Fetal Cardiac Activity outcome

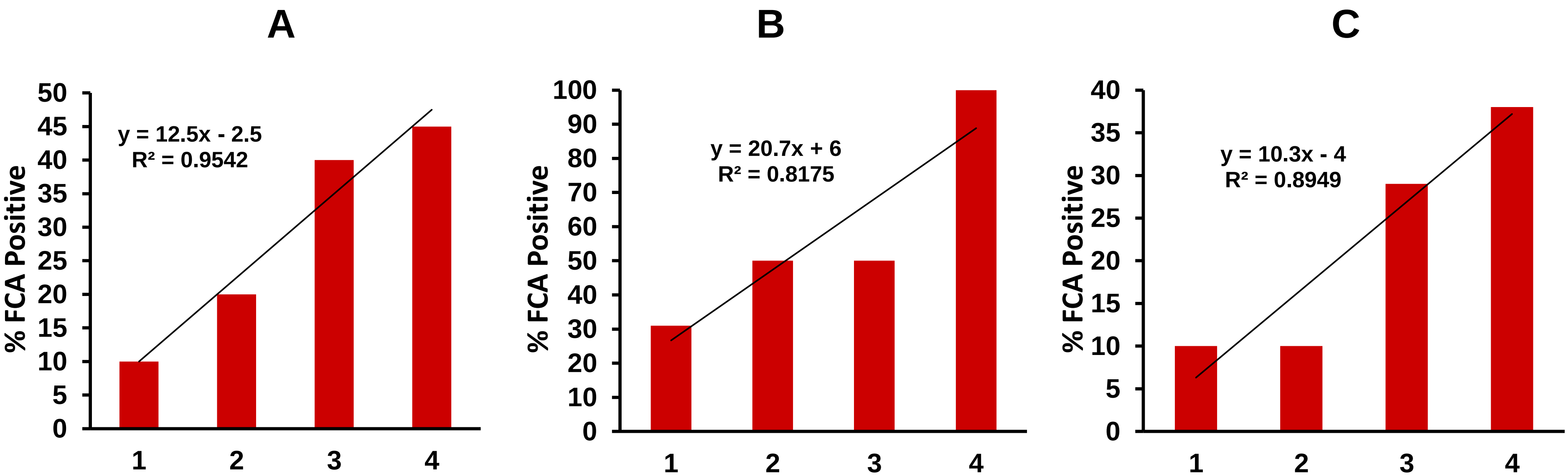


Step 2: Develop Predictive Formula
Viability Score =
α (Wα) + β(Wβ) + γ(Wγ) + δ(Wδ)

Step 3: Blind Validation on unknown outcomes

MOLECULAR BIOMETRICS DAY 5 ALGORITHM
Blindly Validated on Day 5 Single Embryo Transfers (N = 133) from Clinics A, B and C

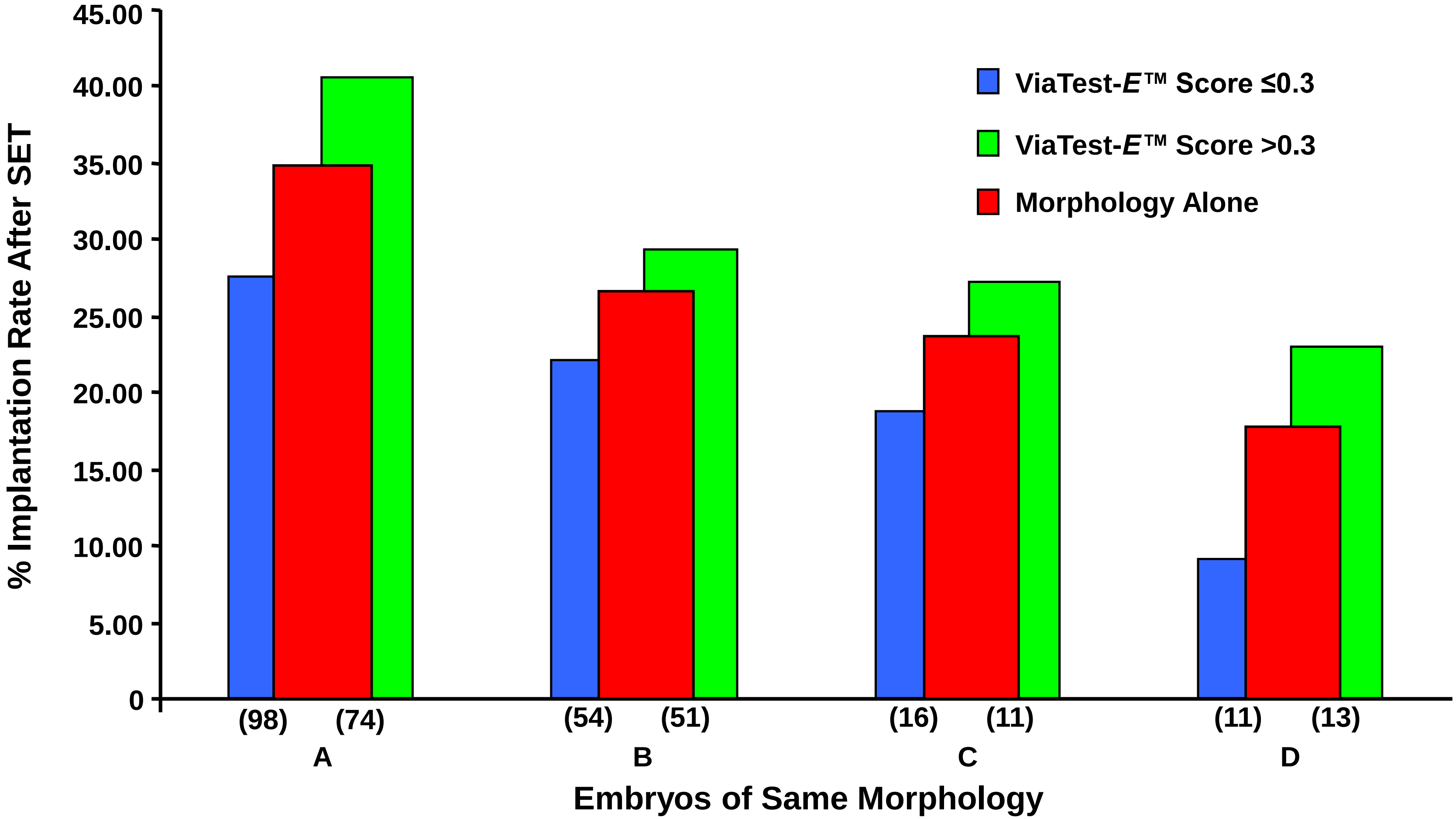
FCA +ve rates are plotted in relation to Quartiles of increasing Viability Scores for each Clinic



1 to 4 are Increasing viability scores

Data on file, courtesy of D. Sakkas
Molecular Biometrics Inc.

SET Implantation Rates of Day 3 Embryos Comparing the Same Morphology Grade and Metabolomic Score of ≤ or > 0.3



(number of SET in parentheses)

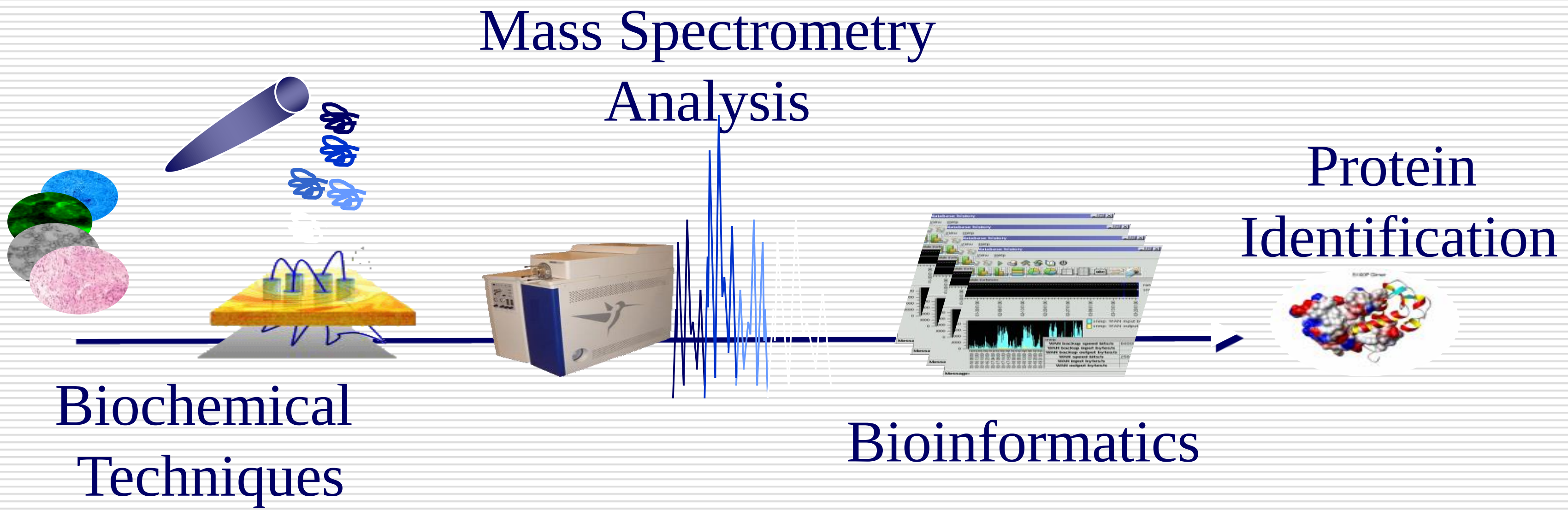
Metabolomics: Clinical Performance Summary

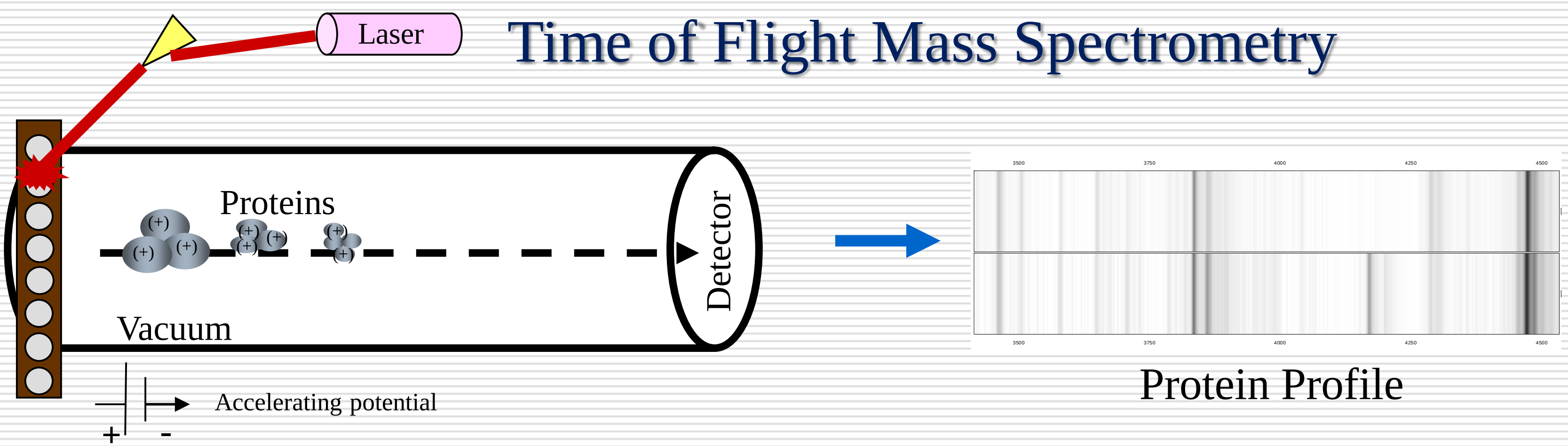
TYPE OF NIR INSTRUMENT	STUDY TYPE		MORPHOLOGY alone	MORPHOLOGY + VIAMETRICS (NIR)	BENEFIT
Prototype *Hardarson et al. (HR; 2012)*	SET	IR	Day 2: 22/83 (26.5%) Day 5: 36/80 (45.0%)	Day 2: 27/87[#] (31.0%) Day 5: 30/77[#] (39.0%)	YES NO
Prototype *Vergouw et al. (HR; 2012)*	SET	CPR	Day 3: 68/163 (41.7%)	Day 3: 61/146[#] (41.8%)	NO
Commercial *Economou et al. (ESHRE, 2011)*	DET	CPR	8/28 (29%)	16/28[#] (57%)	YES
Commercial *Sfontouris et al. (ESHRE, 2011)*	MET	CPR IR	41/86 (47.7%) 66/257 (25.7%)	21/39 (53.9%)[#] 35/102 (34.3%)*	YES

*$P<0.05$.
[#]Not Significant.

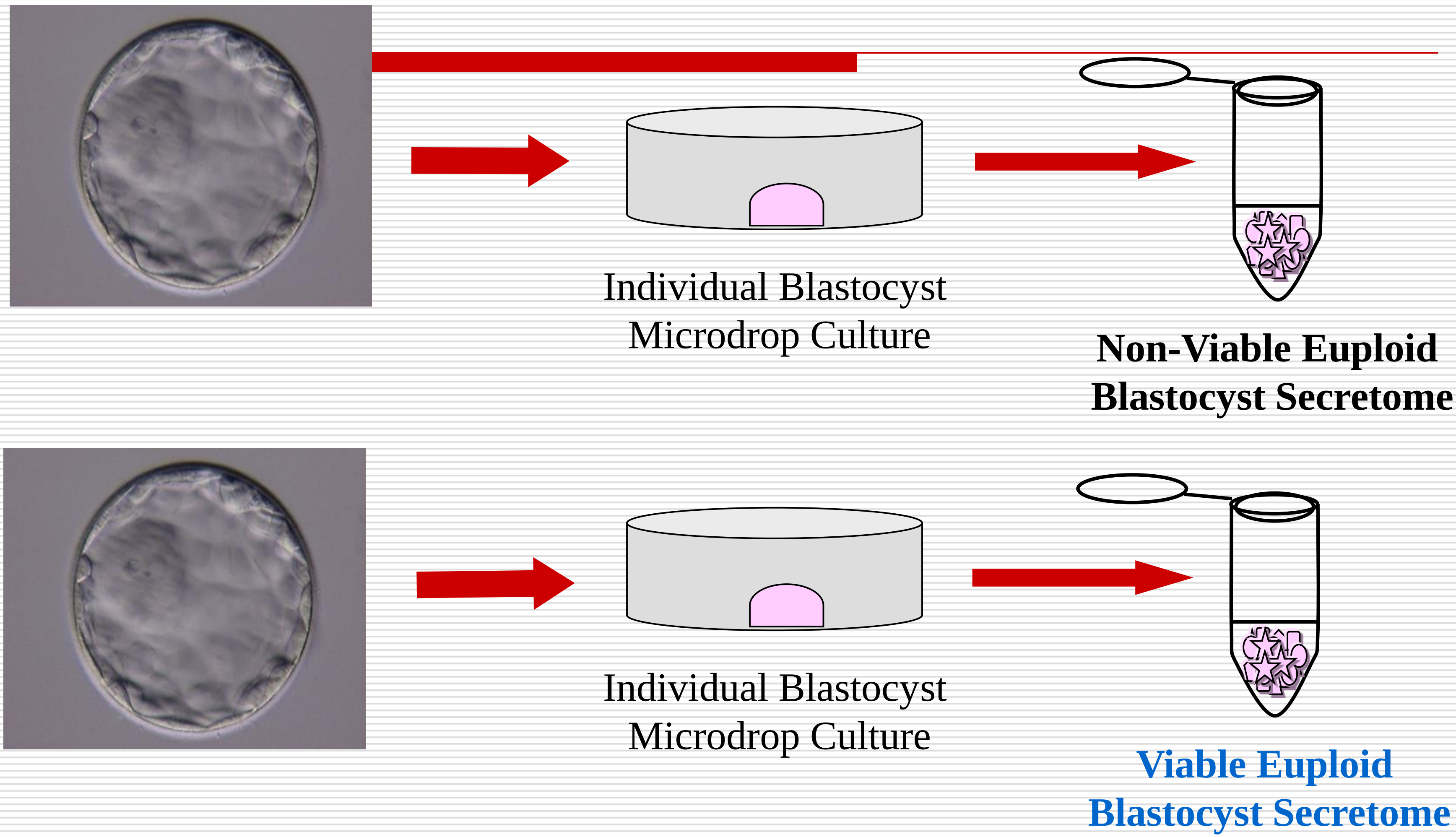
Proteomics

Proteomic Technologies





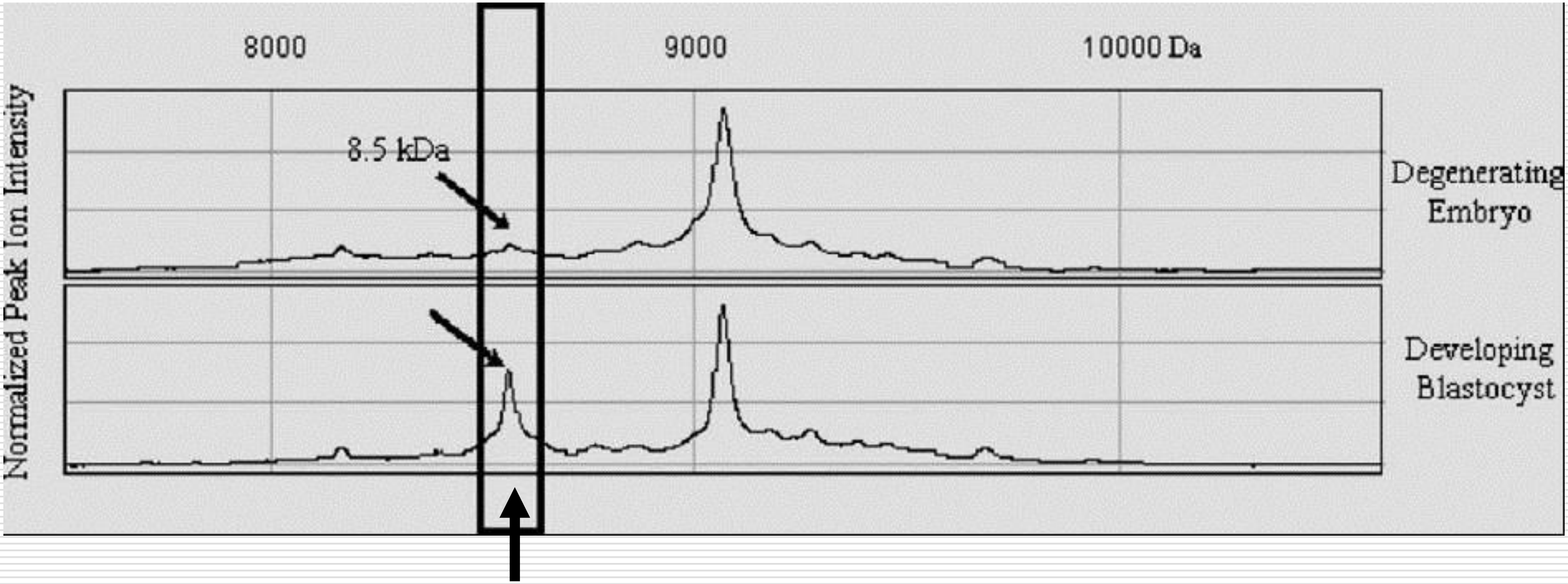
Clinical Evaluation of Viability Biomarkers



Ongoing IRB Approved Clinical Study

Proteomics

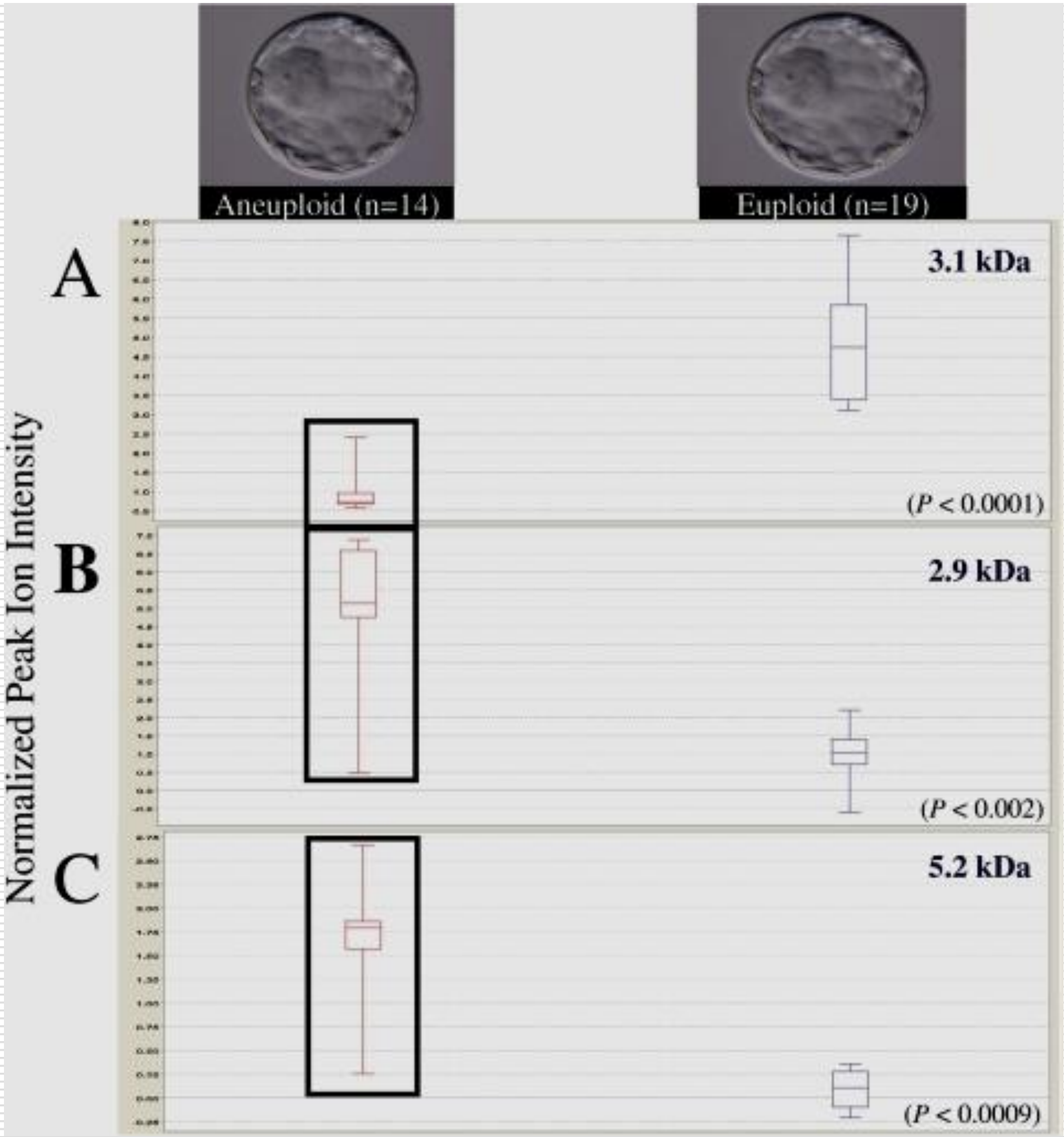
The expression of an 8.5-kDa protein biomarker appears to be directly associated with ongoing human blastocyst development.



Significant difference in expression

[Katz-Jaffe et al. Fertil Steril 2006]

Proteomics And Aneuploidy



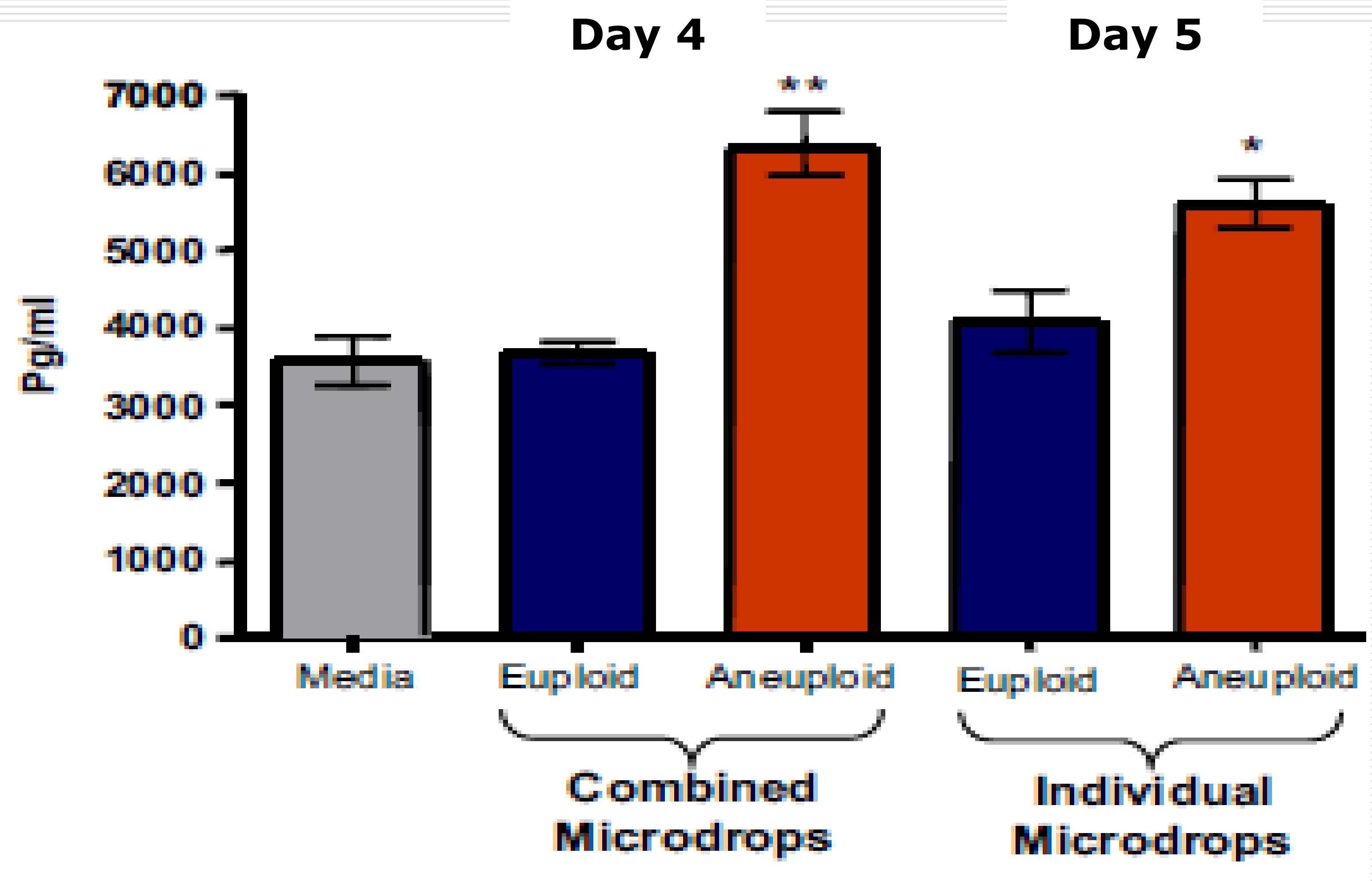
Aneuploid **Euploid**

Examples of biomarkers that were differentially expressed in the secretome signatures of euploid blastocysts (*n* = 19) compared with the secretome signature of aneuploid blastocysts (*n* = 14) (*P* < 0.05).

[Katz-Jaffe et al. Mol Hum Rep 2009]

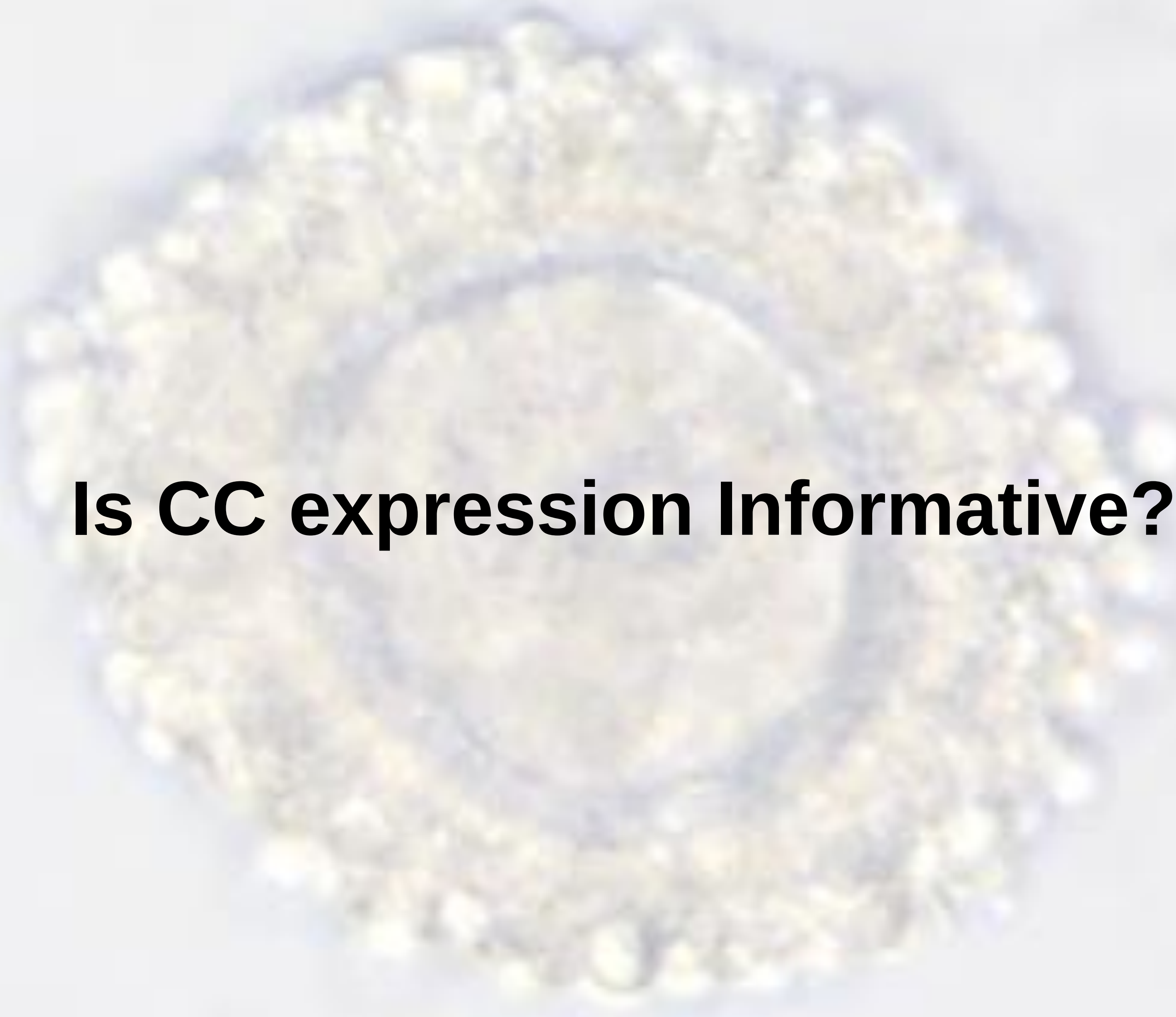
Proteomics And Aneuploidy

Lipocalin-1: **Was expressed significantly higher in the culture media of aneuploid embryos (McReynolds et al. Fertil. Steril. 2011)**



Cumulus cell gene expression

Is CC expression Informative?



Goal and Experimental Method

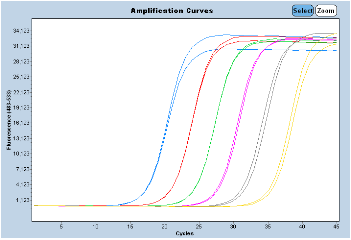
Extraction

total-RNA

Reverse
Transcription

cDNA

PCR
for
specific genes



Score for the most
competent Embryo

The strongest pregnancy predictive genes in 3 consecutive QPCR studies

23 genes were evaluated until now in 122 patients *

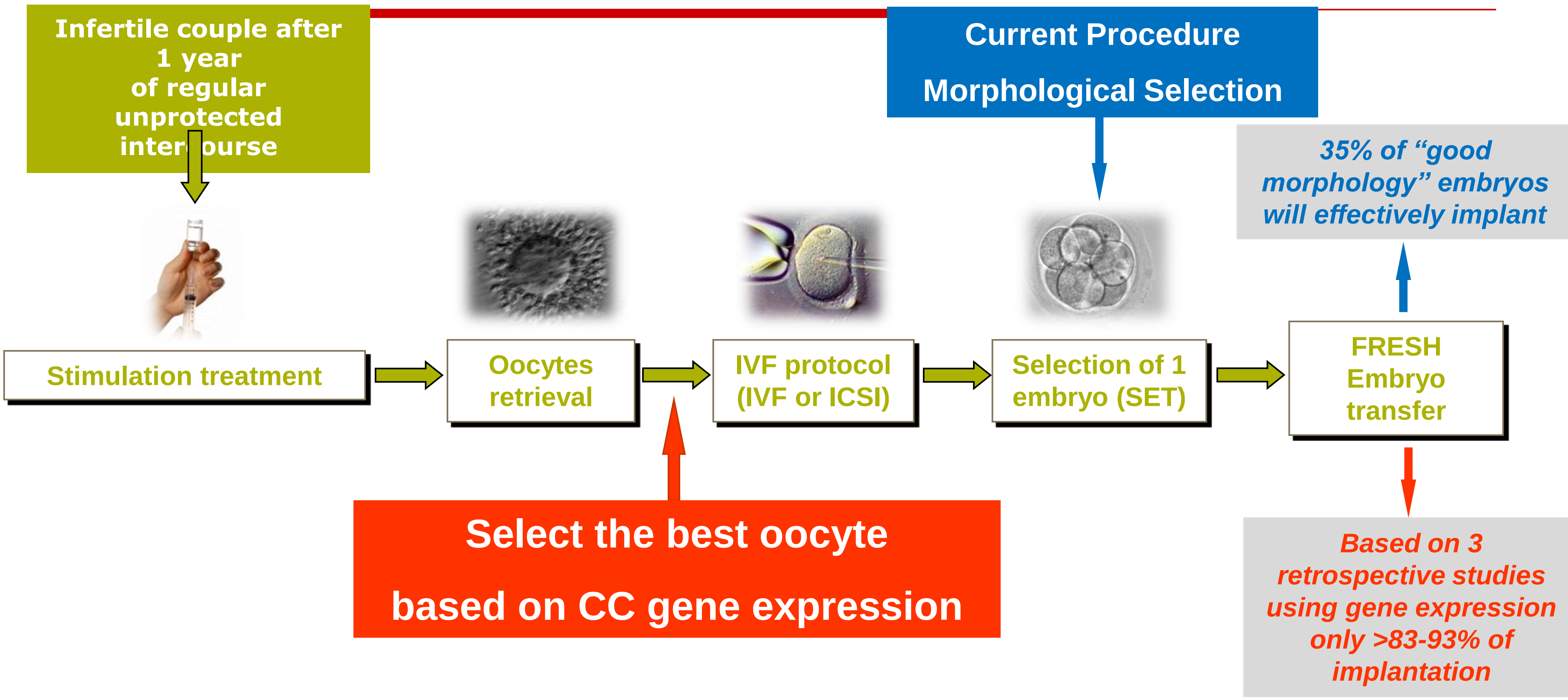
Study	Total n	% pregnant	Genes retained for pregnancy	PPV	NPV	Accuracy
1st	42	45%	SDC4 and VCAN	88	81	83
2nd	33	48%	EFNB2, CAMK1D and STC1	80	78	79
3rd	47	40%	EFNB2, GSTA3, GSTA4, PGR and GPX3	93	93	93

Example of a multiparametric pregnancy prediction model

Chance on pregnancy = -2.25846 + 0.79256 x EFNB2 + 0.09491 x GSTA4 - 0.09632 x PGR

→ Gene only models perform well

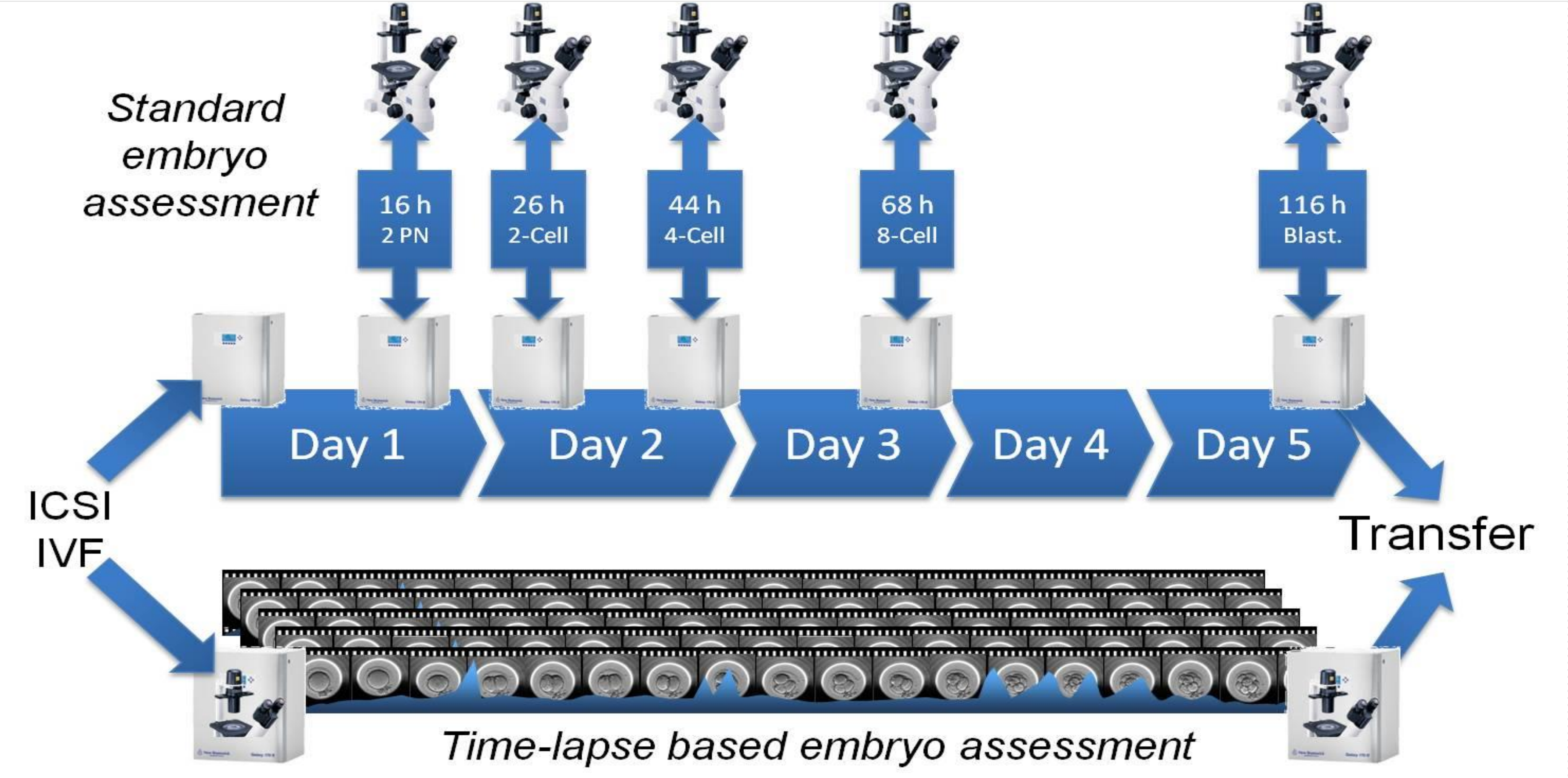
Objective = Select the best oocyte and not the best embryo



Time-lapse

Superior amount of information with time-lapse

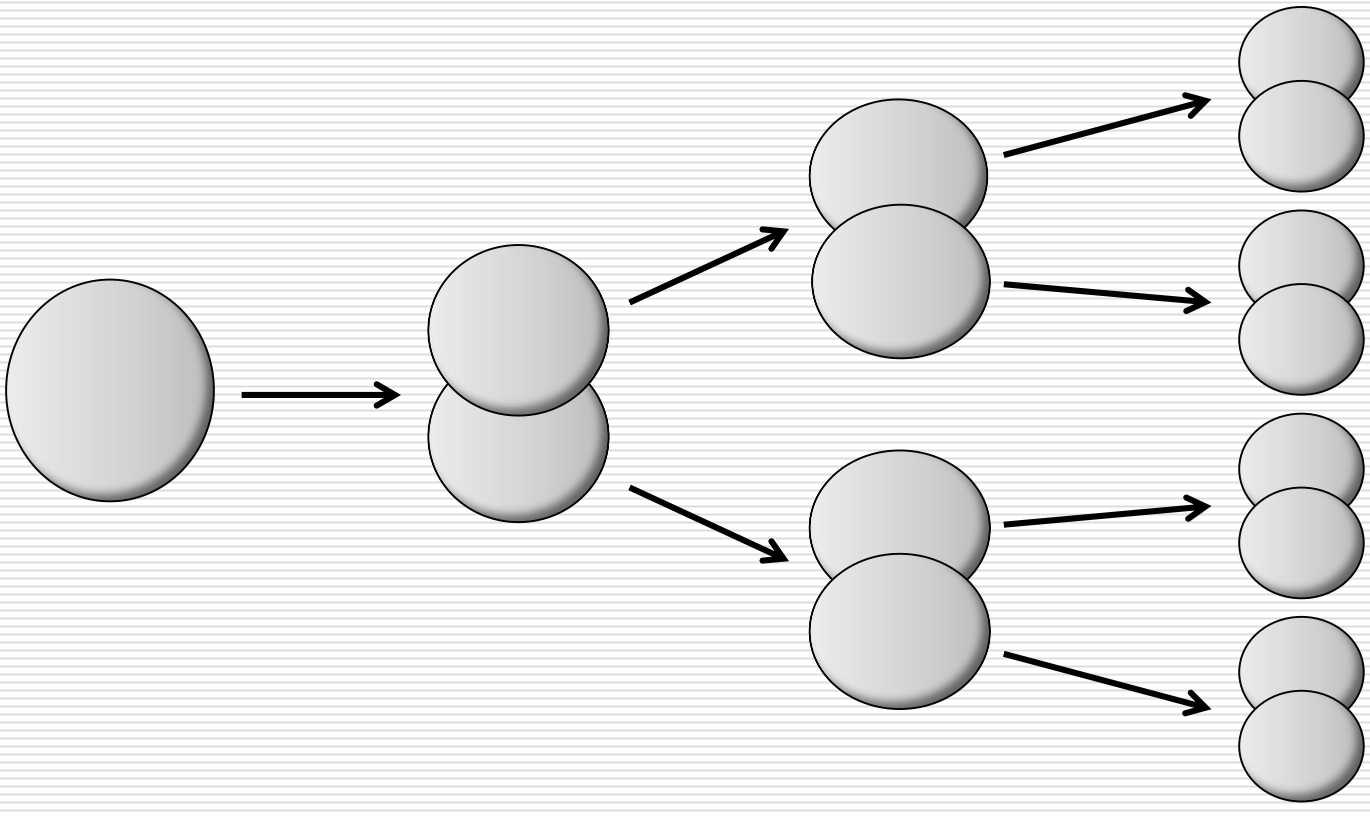
- The difference is not only "quantity"



Over 5 days per embryo: approx. 5000 images (700 time values / 7 focal planes)

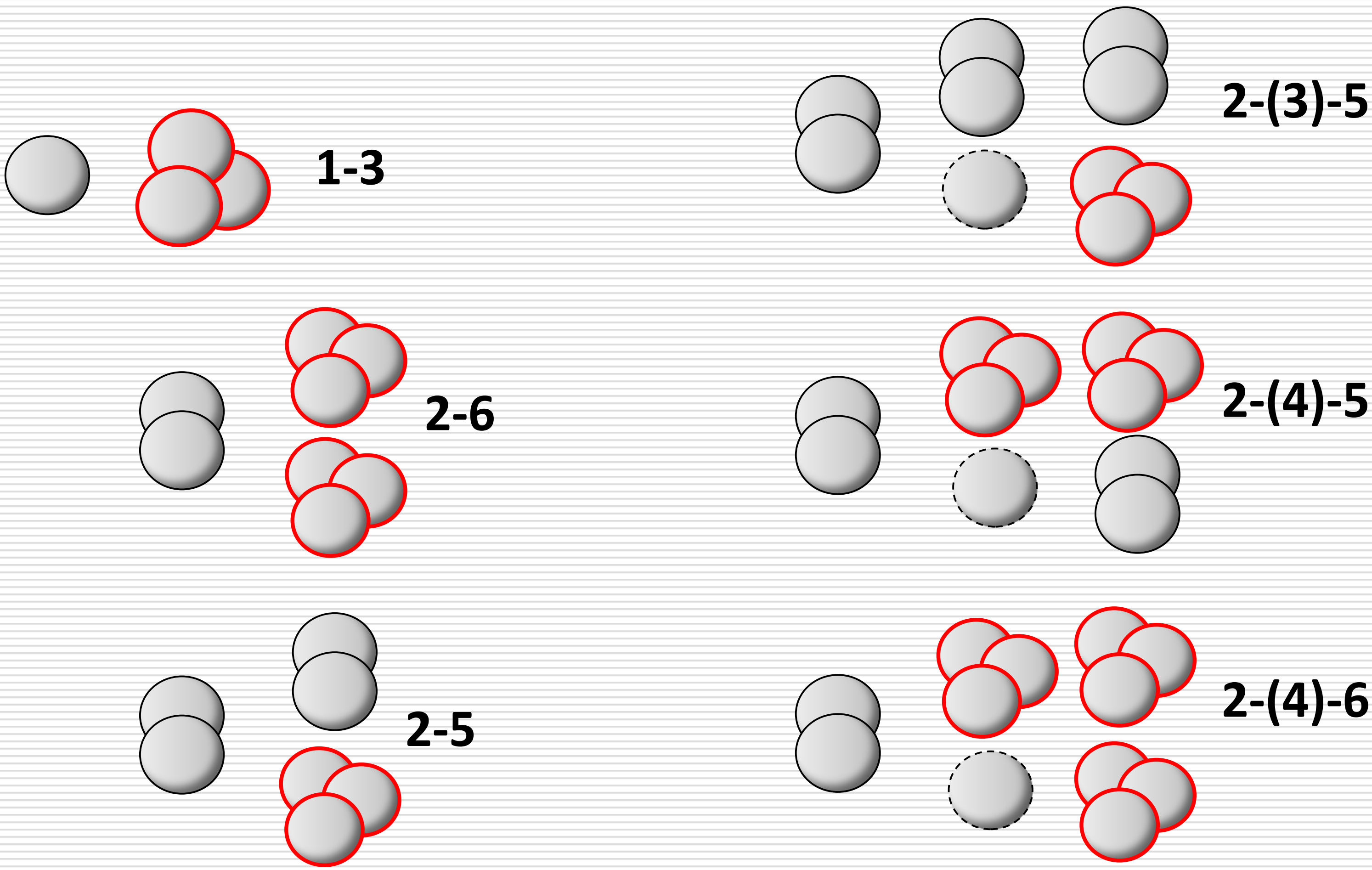
Cleavage patterns

”Regular” cleavage pattern ⇨ Establish ”positive selection” criteria

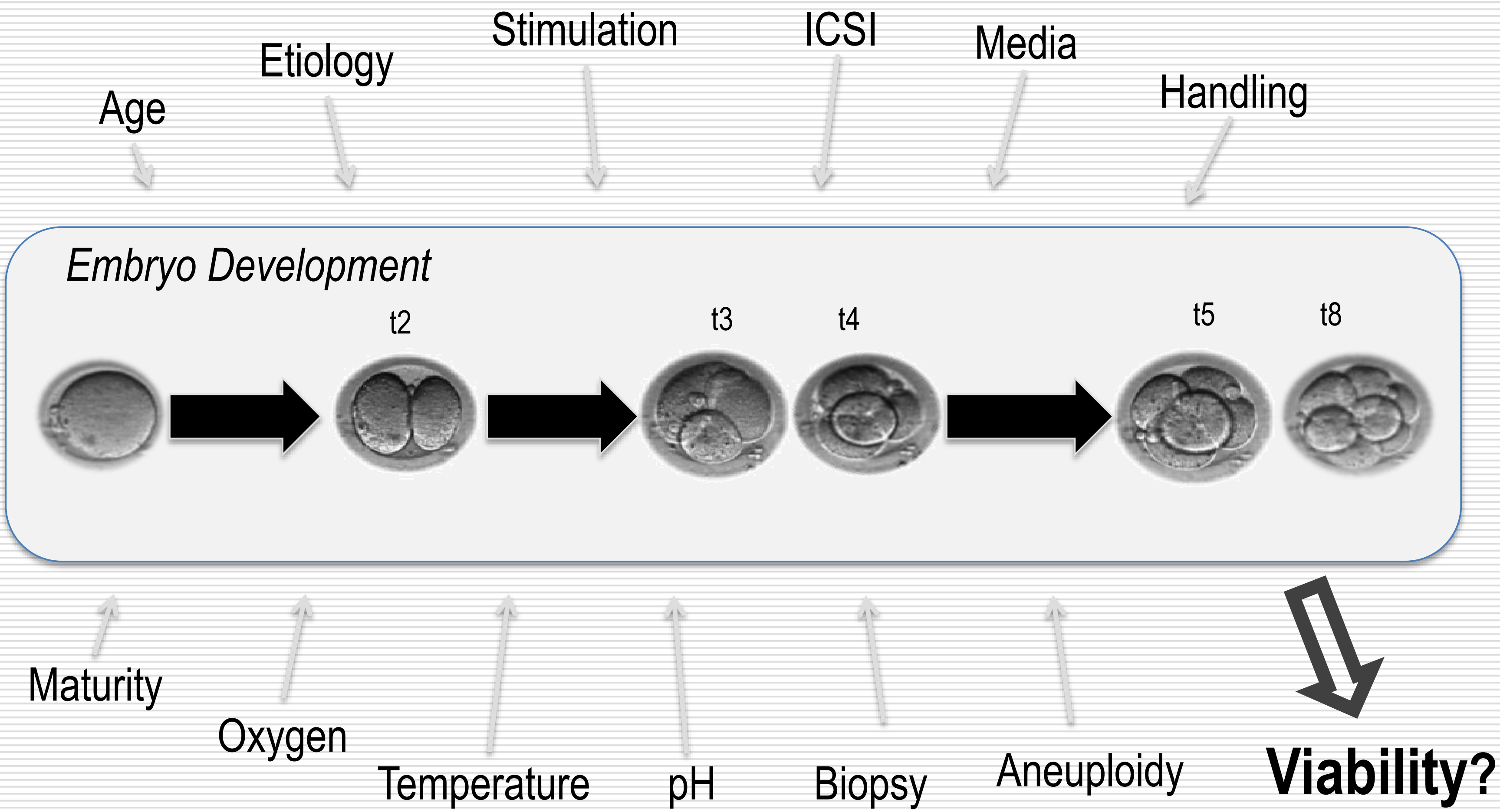


Cleavage patterns

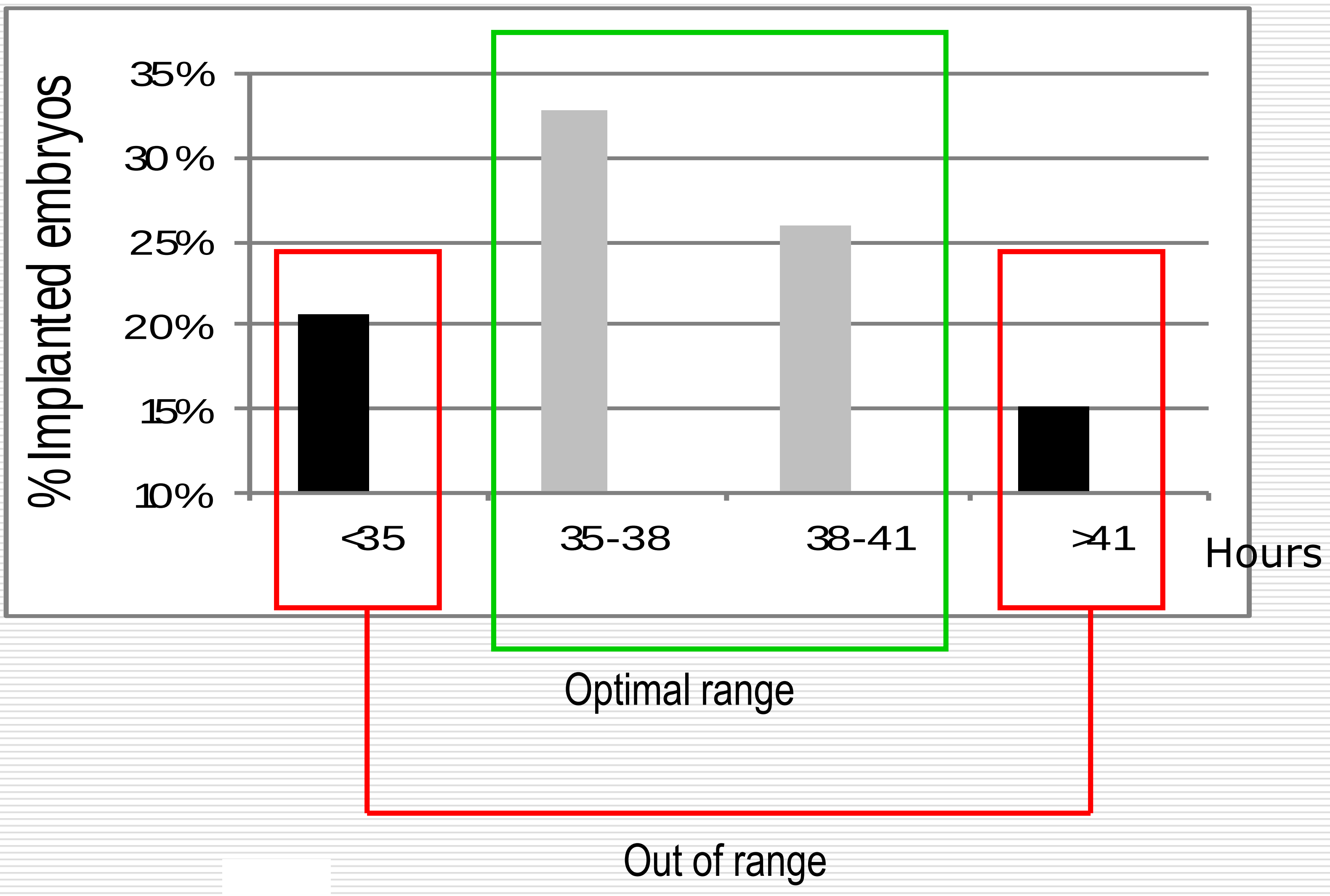
Unusual cleavage patterns ⇨ Establish ”negative selection” criteria



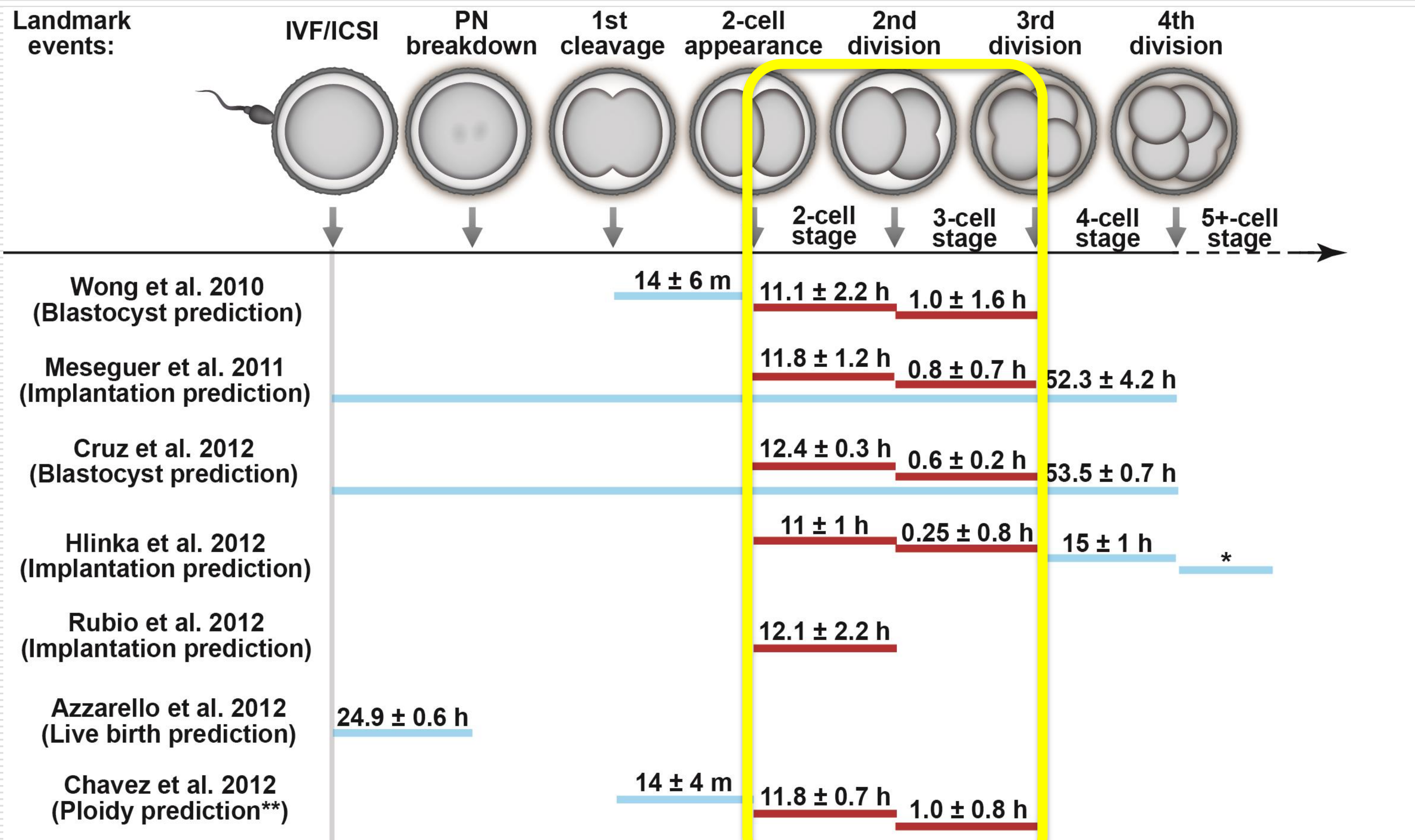
Factors affecting cleavage patterns



Establishing optimal ranges



Time-lapse markers described by different studies



* 5-8 cell stage: 40 ± 10 m; 8 cell stage: 23 ± 1 h; 9-16 cell stage: 55 ± 15 m.

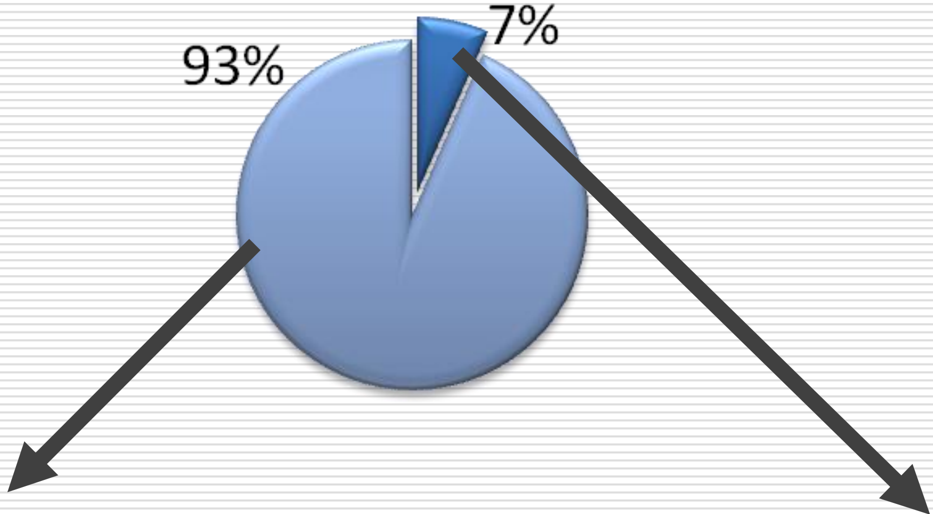
** dynamic assessment of fragmentation was also included in the study

Chen et al. *Fertility & Sterility*, (2013)

Direct cleavage from 1-3 cells

Static observation will miss 63%

1659 transferred embryos



Regular cleavage from 1 to 2

Direct cleavage from 1-3 cells

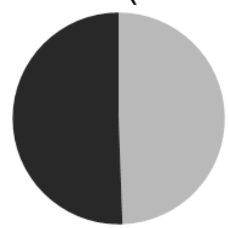
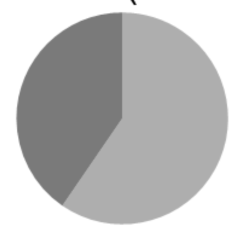
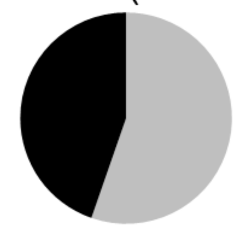
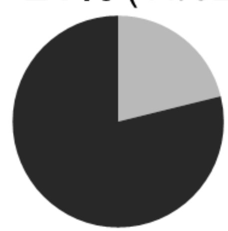
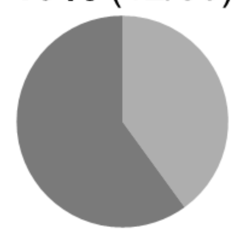
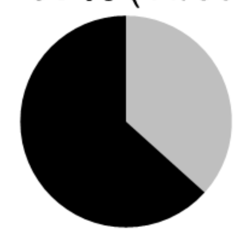
Implantation rate < 2%
(n=109)

Implantation rate >13%
(n=1550)

Rubio I et al. 2012 Fertility and Sterility

Morphokinetic markers correlate with implantation

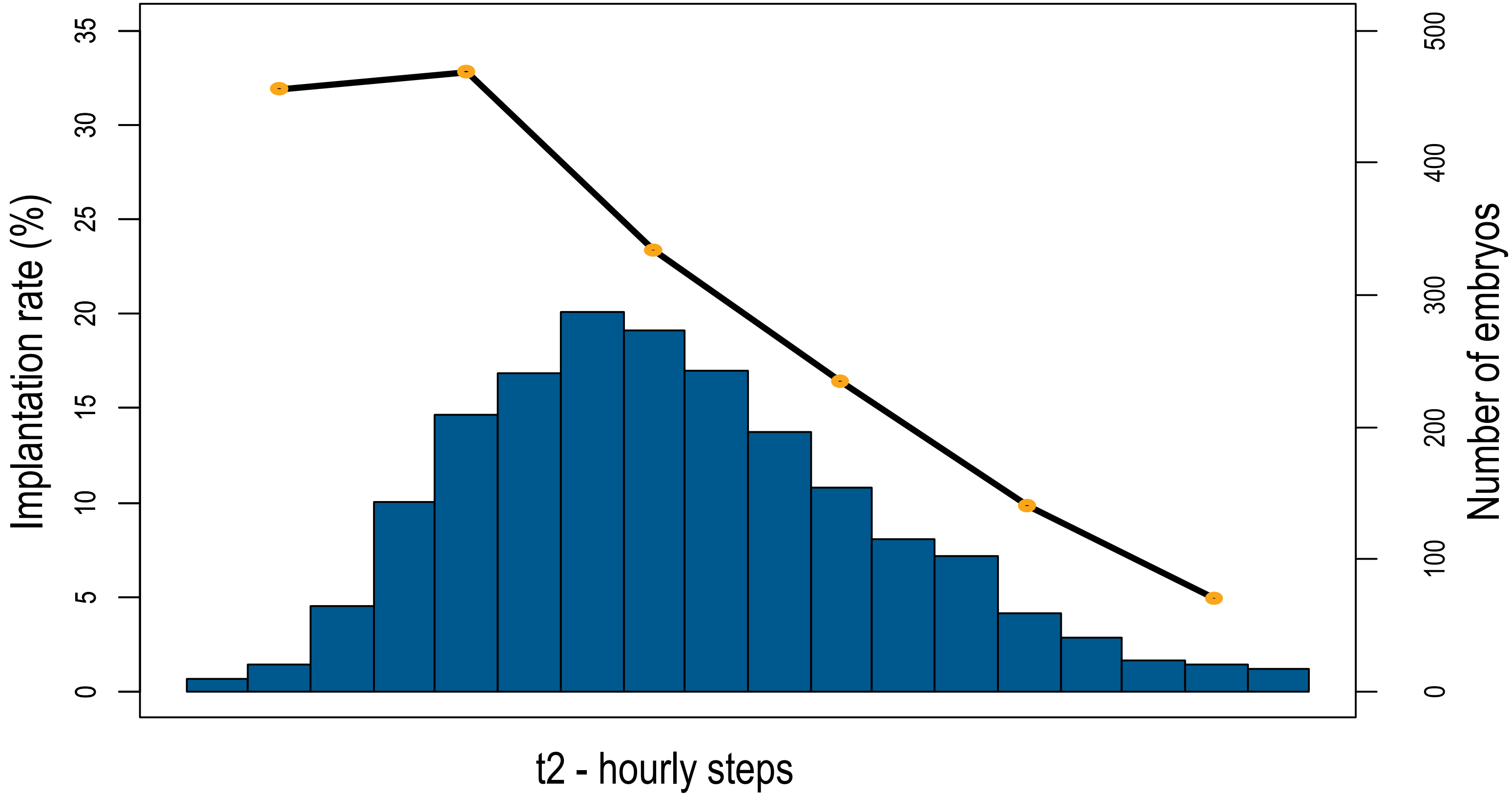
Retrospective analyzes

Patient Population	# Pts	# Embs	Avg Age (years)	Implantation Rate	Clinical Pregnancy Rate	Ongoing Pregnancy Rate
At least 1 Eeva High transferred	47	89	32.1 ± 5.2	**49%** (44/89) 	**60%** (28/47) 	**55%** (26/47) 
Only Eeva Lows transferred	30	52	32.2 ± 5.1	**21%** (11/52) 	**40%** (12/30) 	**37%** (11/30) 
p-value			$p=0.9$	$p<0.001$	$p=0.09$	$p=0.11$

Chen et al. *Fertility & Sterility* (2013)

Slide is courtesy of: Auxygen

t2 and implantation rates

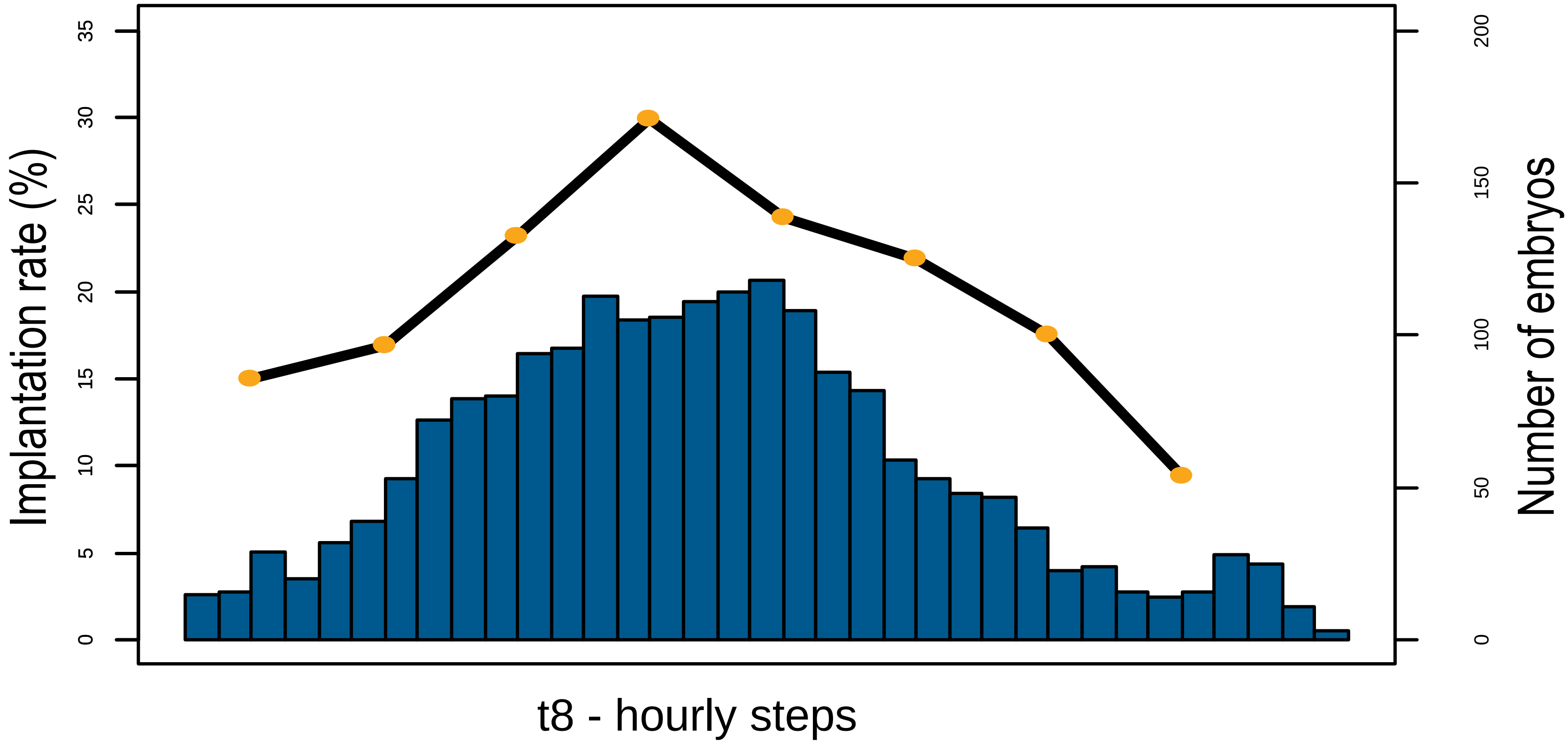


Early cleavage is an important parameter

But the exact definition of „early“ depends on the individual laboratory and the conditions

Based on n > 2000 treatment cycles from different clinics
Courtesy of: FertiliTech

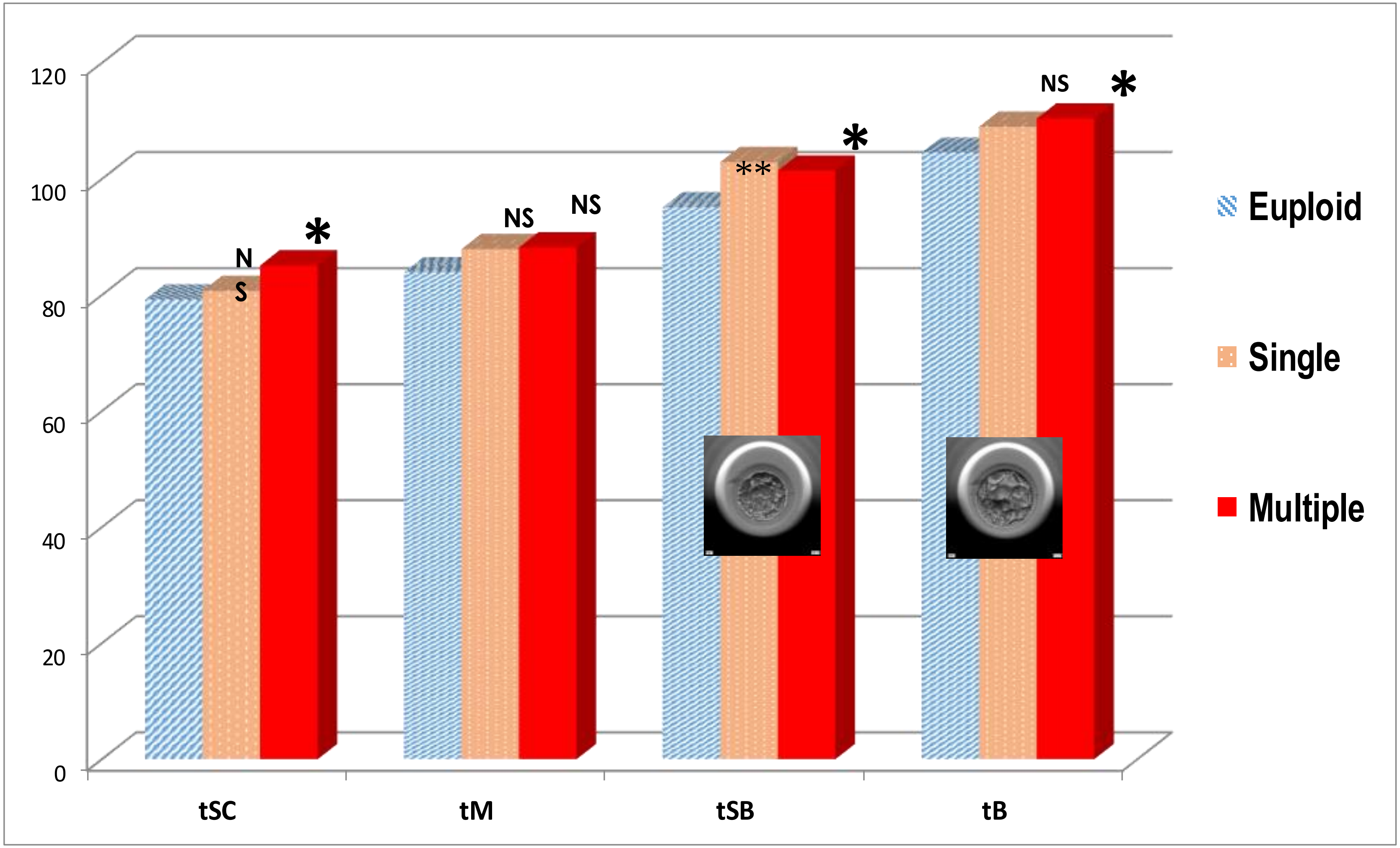
t8 and implantation rates



- Embryos being 8-cell too early or too late have a much lower implantation potential
- Chosing day 3 embryos with cell numbers that are much higher than 8 as standard is not beneficial

Based on n > 2000 treatment cycles from different clinics
Courtesy of: FertiliTech

tSB / tB and euploidy/aneuploidy



* P<0.05 ** P<0.01 (MWW test)

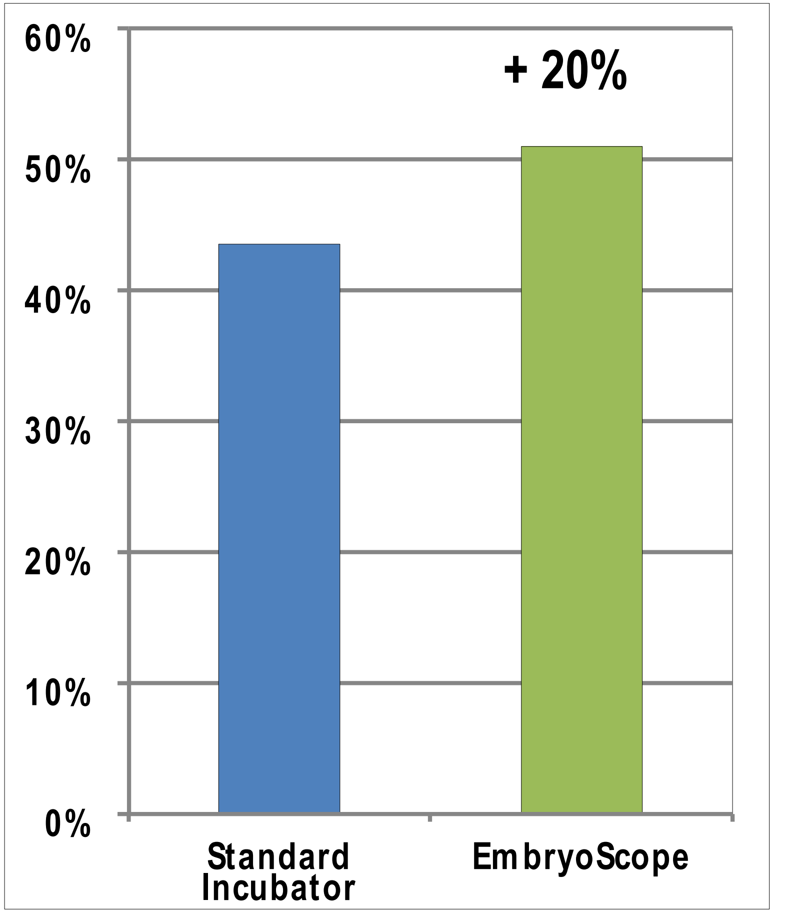
Courtesy of: FertiliTech

tSB time from insemination to start of blastulation (h)
tB time from insemination to reach 'full' blastocyst (h)

The benefits of morphokinetics

Less Disturbance = Better Development

More Observations = Better Selection



Meseguer et al 2012, Fertil & Steril

Conclusions

- Embryo assessment is one of the most critical procedures that play a role in the success of IVF/ART

- Traditional embryo assessment is challenged by different factors, ie, subjectivity, low efficiency

- New “non-invasive” techniques may provide valuable additional information to optimize embryo assessment and maximize the chances of IVF success

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