Application of OMICS technologies on Gamete and Embryo Selection

Denny Sakkas, Ph.D.

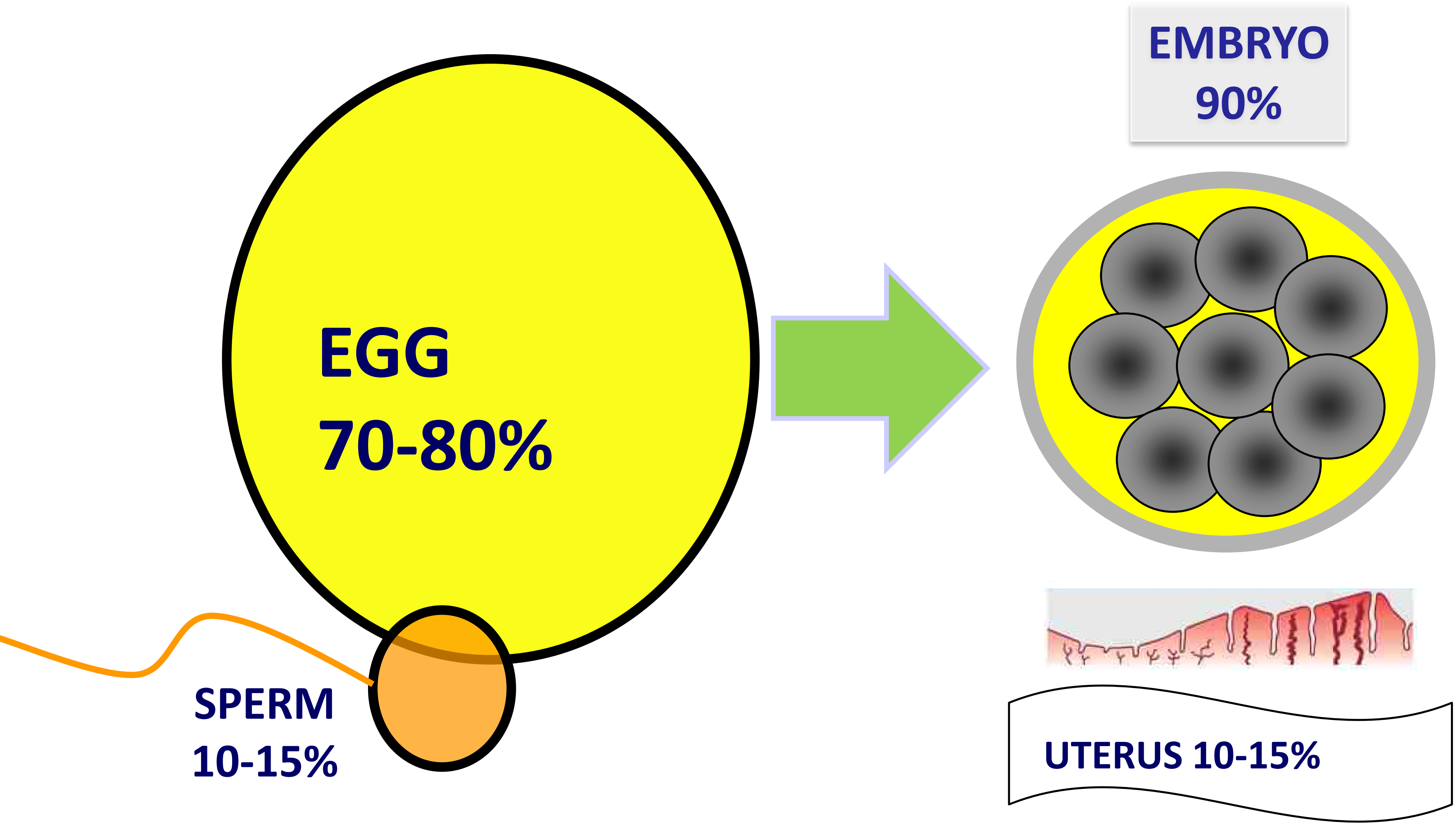
Scientific Director,
Boston IVF
Waltham, MA, USA

BOSTON IVF

THE FUTURE ROLE OF THE EMBRYOLOGIST WILL FOCUS ON PROVIDING OUR PATIENTS

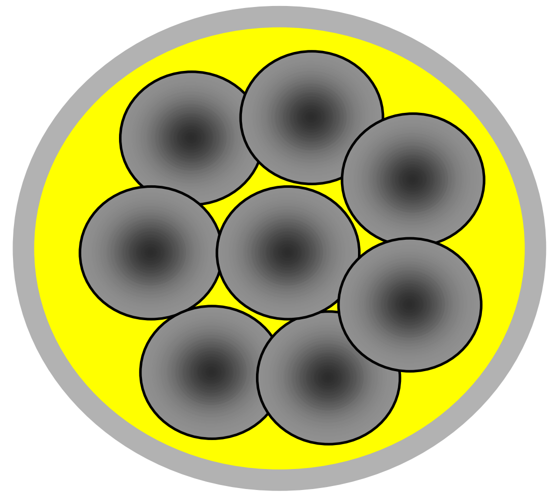
- **HIGHER SUCCESS RATES**
- **MORE PERSONALISED DIAGNOSIS AND TREATMENT**
- **AND ONE HEALTHY BABY AT A TIME**

Venn diagram of the Responsibilities of Implantation Failure



Sakkas et al., Human Reprod. Update 2015

Venn diagram of the Responsibilities of Implantation Failure

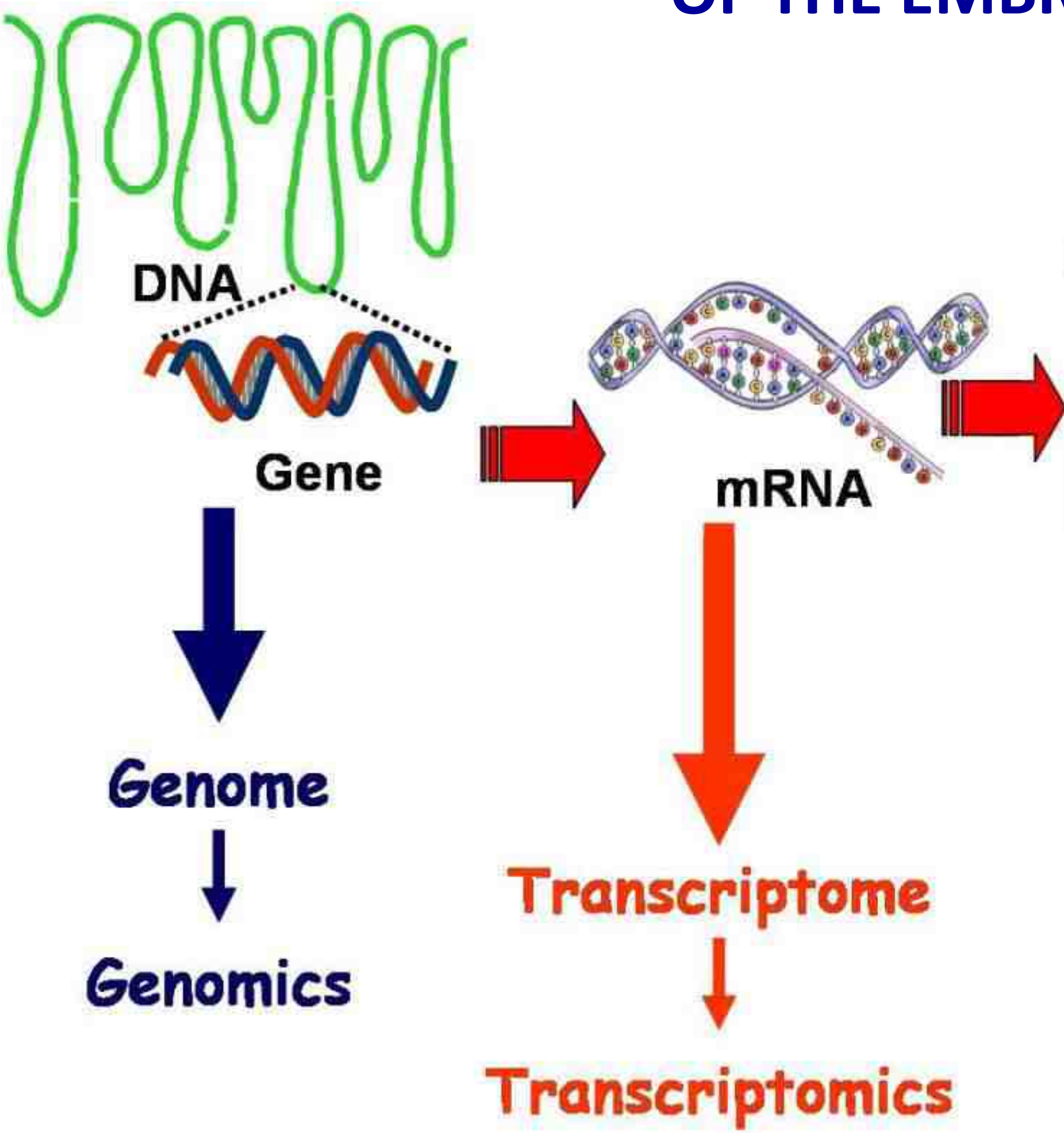


EMBRYO
90%

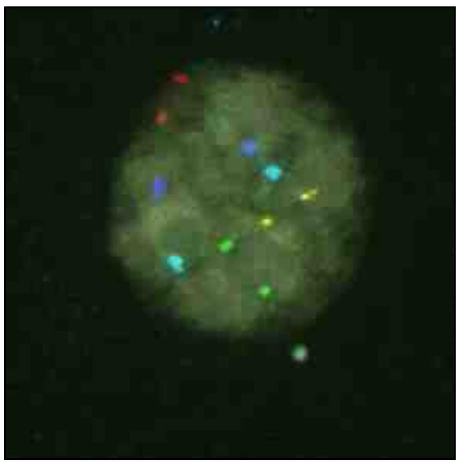
How can the egg and embryo impact the success of Reproduction?

Outcomes of ART Cycles Using Fresh Nondonor Eggs or Embryos, by Stage, 2012

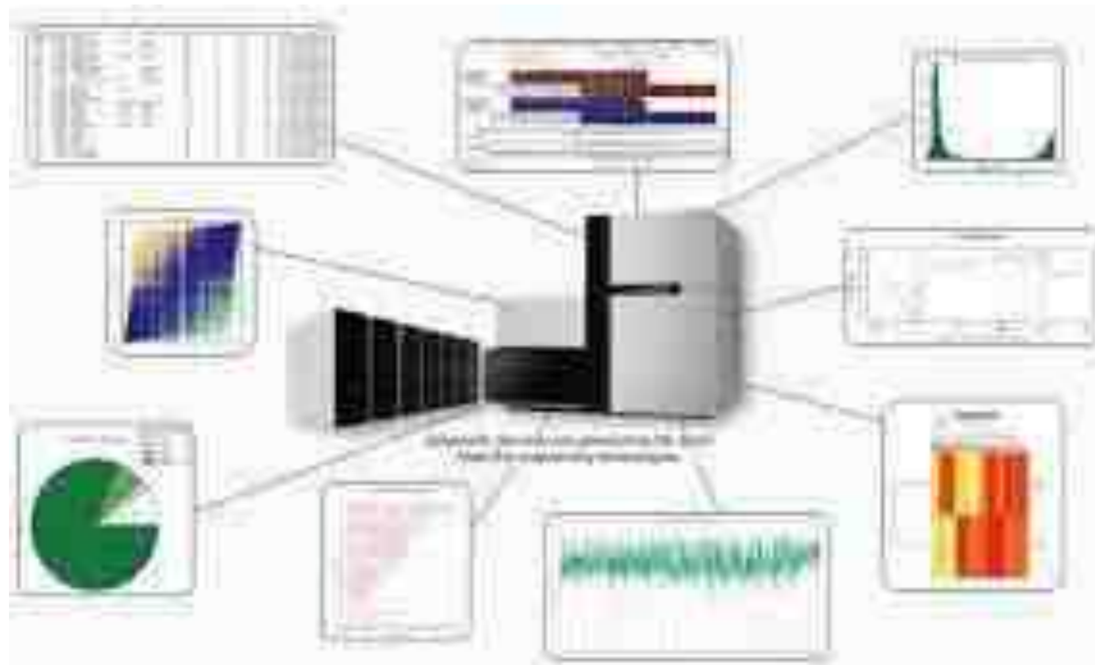
THE "OMICS" – INVASIVE ASSESSMENT OF THE EMBRYO



The Preimplantation Genetic Screening Example:
A Strong Hypothesis Can Be A Slave To Technology

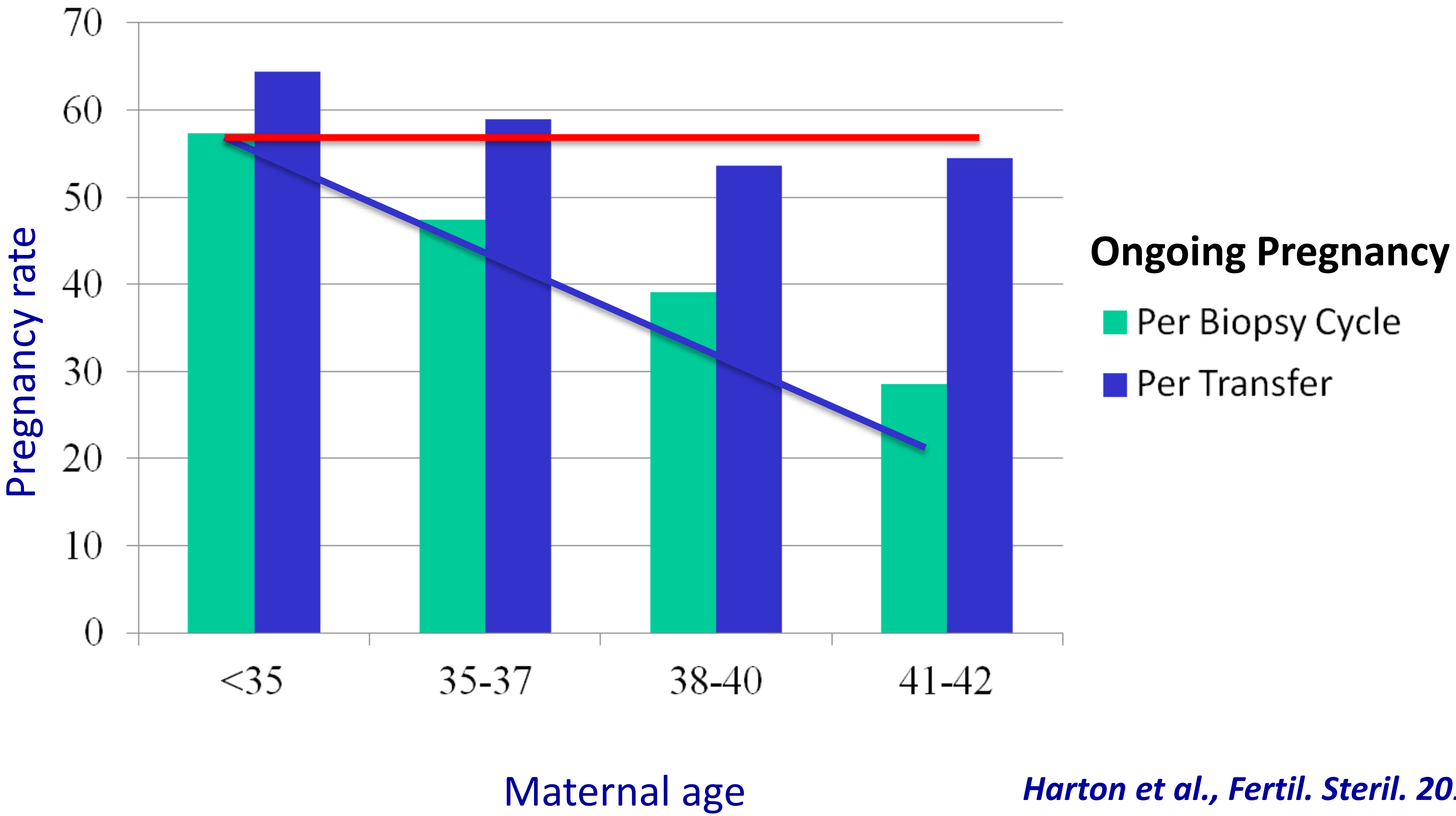


FISH
POOR RESULTS



Array CGH or NGS
GOOD RESULTS

Blastocyst Biopsy and aCGH does appear to limit the effect of age when performed on Day 5



Harton et al., Fertil. Steril. 2013

Human Reproduction, Vol.27, No.4 pp. 1217–1222, 2012
Advanced Access publication on February 16, 2012 doi:10.1093/humrep/des020

human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates

E.J. Forman[1,2,*], X. Tao[1], K.M. Ferry[1], D. Taylor[1,2,3], N.R. Treff[1,2,3], and R.T. Scott Jr[1,2]

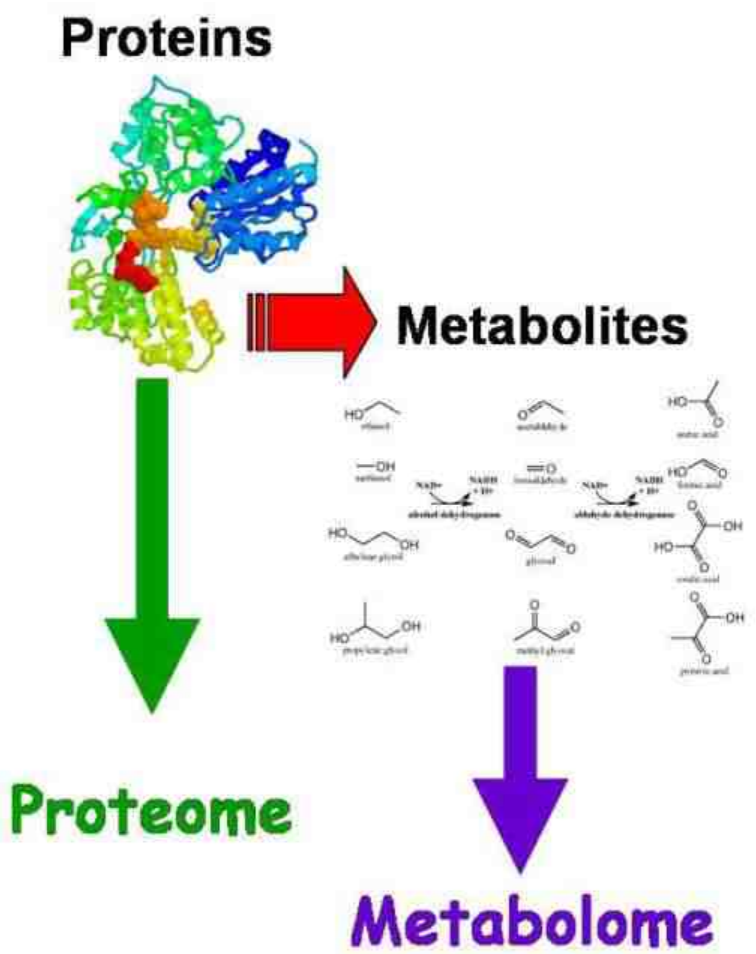
Table III Pregnancy outcomes by treatment group.

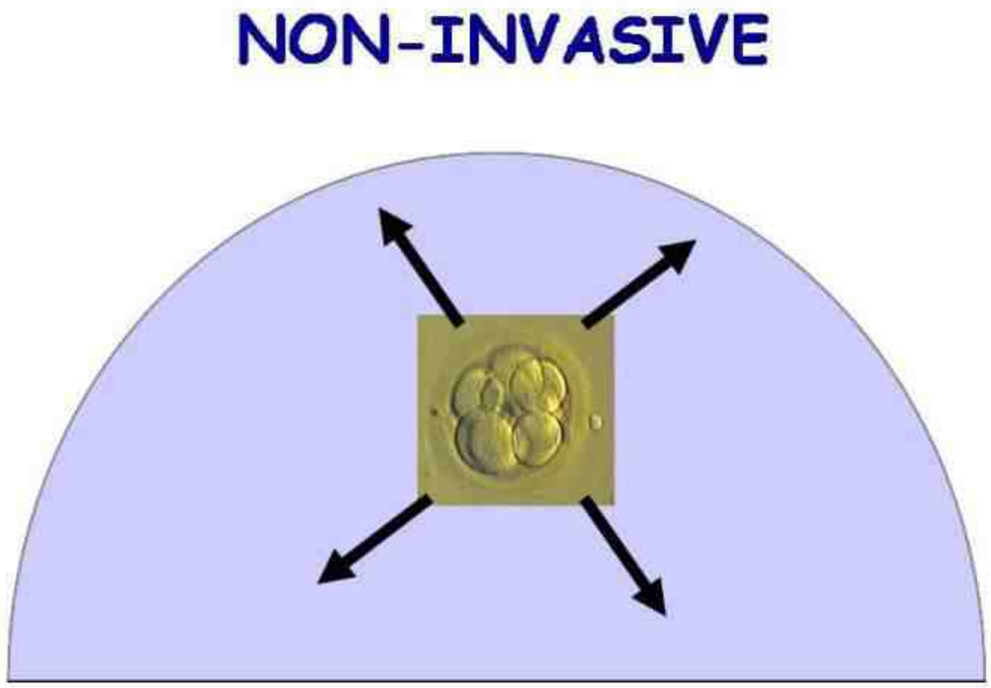
	Control SET	CCS-SET
Chemical pregnancy rate	108/182 (59.3%)	96/140 (68.6%)
Ongoing pregnancy rate	76/182 (41.8%)	77/140 (55.0%)
Clinical miscarriage rate	25/101 (24.8%)	9/86 (10.5%)
Monozygotic twin rate	0/76 (0%)	1/77 (1.3%)
Gestational age at delivery (weeks ± SD)	38.9 ± 1.4	38.6 ± 2.0
Birthweight (grams ± SD)	3286 ± 522	3281 ± 525

*Chi-square.
†Fisher's exact test.
‡t-test.

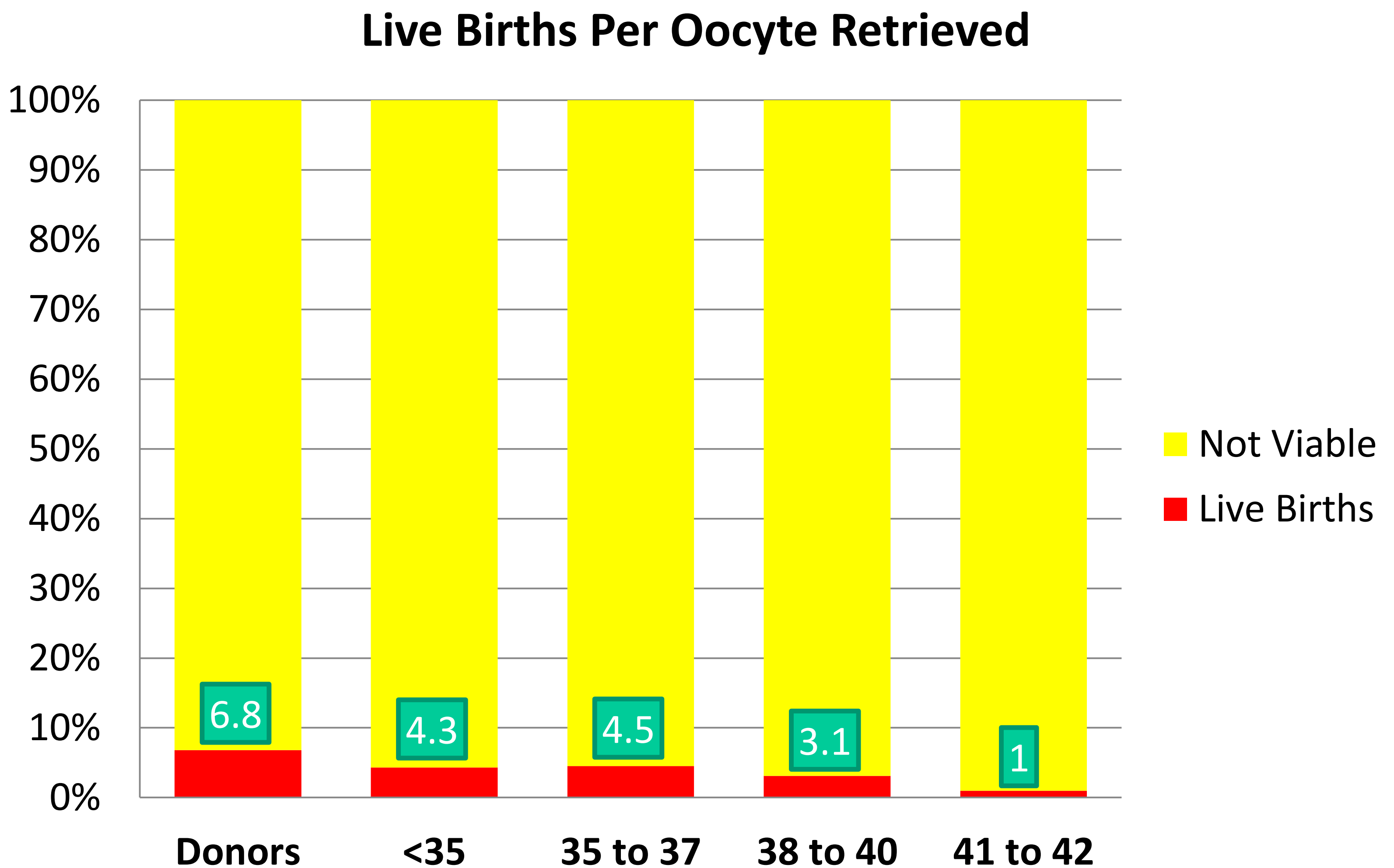
BOSTON IVF

THE OMICS – NON-INVASIVE ASSESSMENT OF THE EMBRYO





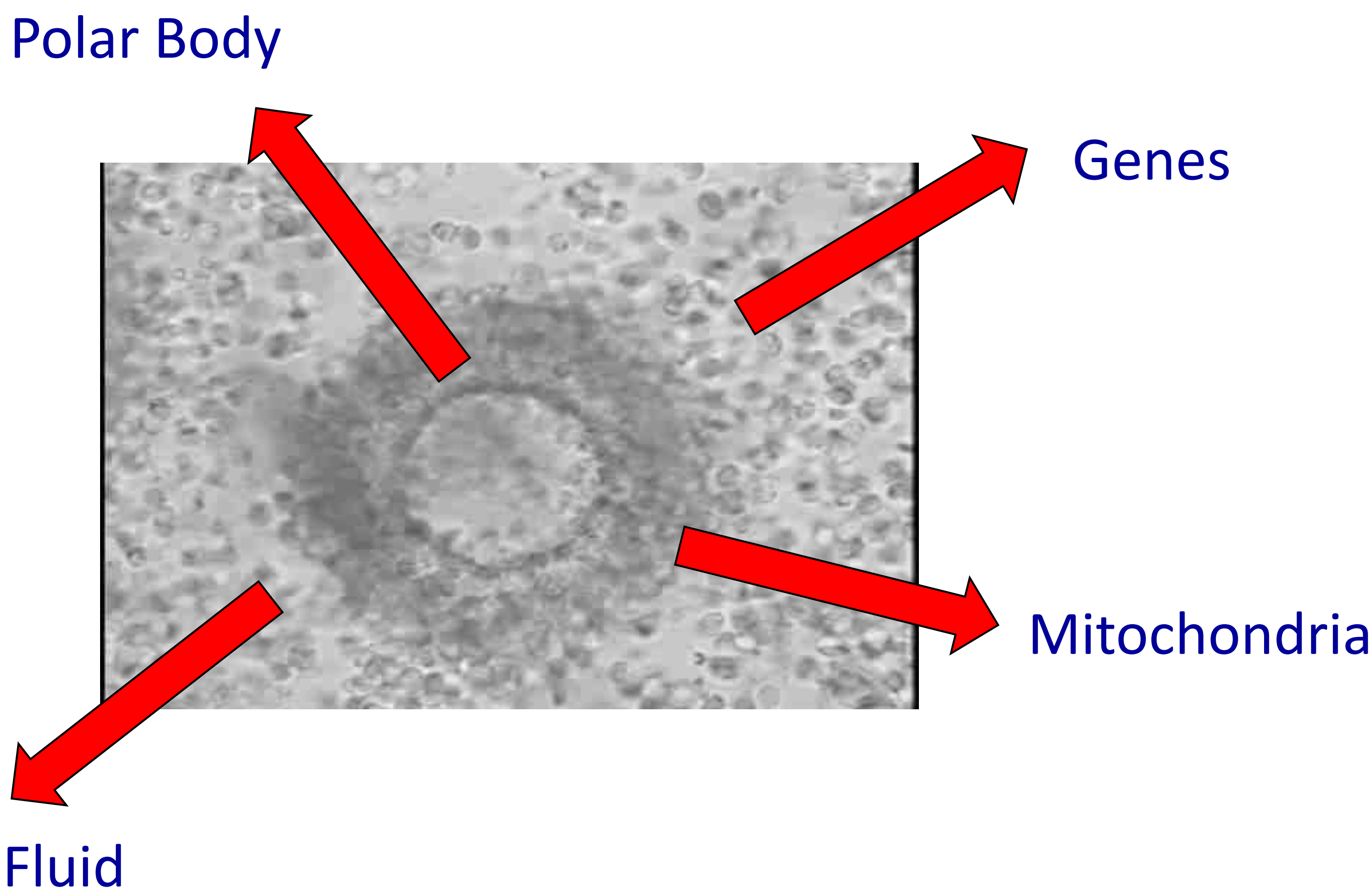
How many live births occur per 100 oocytes retrieved?



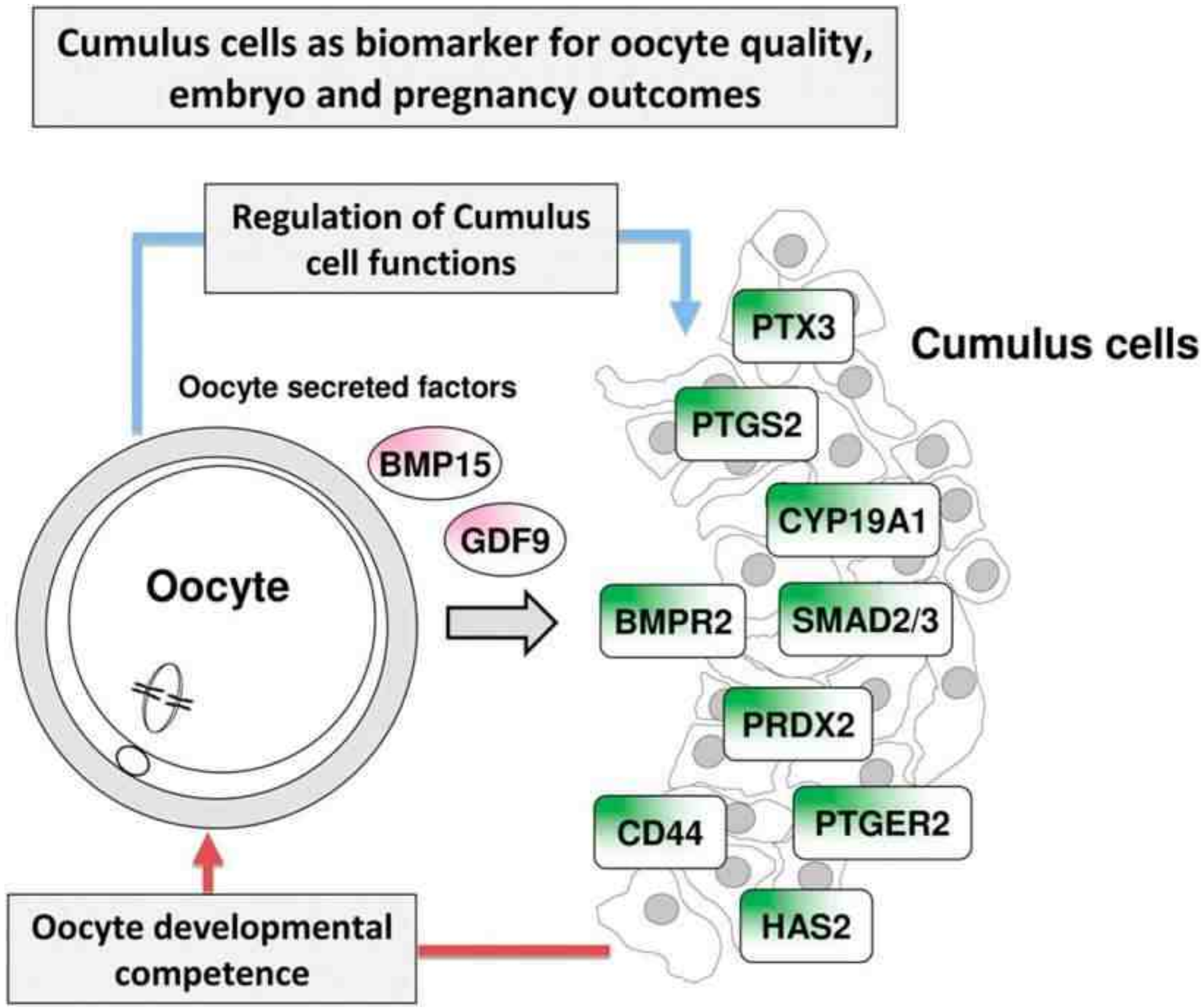
BOSTON IVF

Patrizio and Sakkas, Fert Stert, 2008

Cumulus or Follicular cells as surrogate markers of viability



Potential role of CCs as a regulator of oocyte competence and as biomarkers for oocyte/embryo quality or pregnancy outcome.



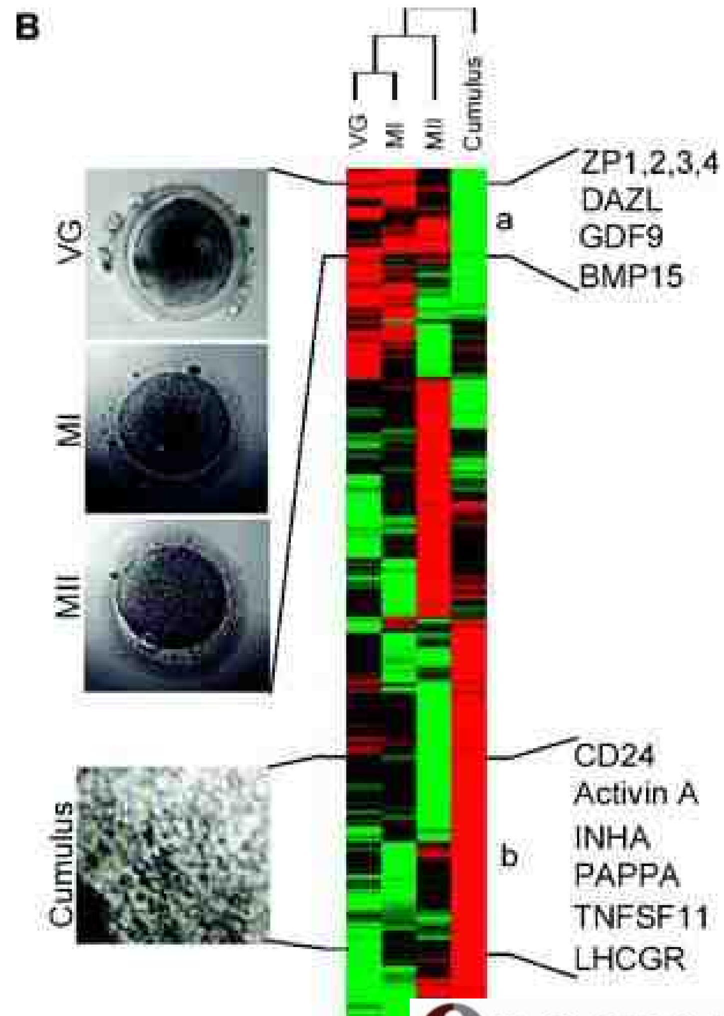
Assou S et al. Mol. Hum. Reprod. 2010;16:531-538

Cumulus or Follicular cells as surrogate markers of viability

Hamamah et al. Comparative protein expression profiling in human cumulus cells in relation to oocyte fertilization and ovarian stimulation protocol. Reprod Biomed Online. 2006 13:807-14.

Feuerstein et al. Gene expression in human cumulus cells: one approach to oocyte competence. Hum Reprod. 2007 22:3069-77.

Hamel et al. Identification of differentially expressed markers in human follicular cells associated with competent oocytes. Hum Reprod. 2008 23:1118-27.

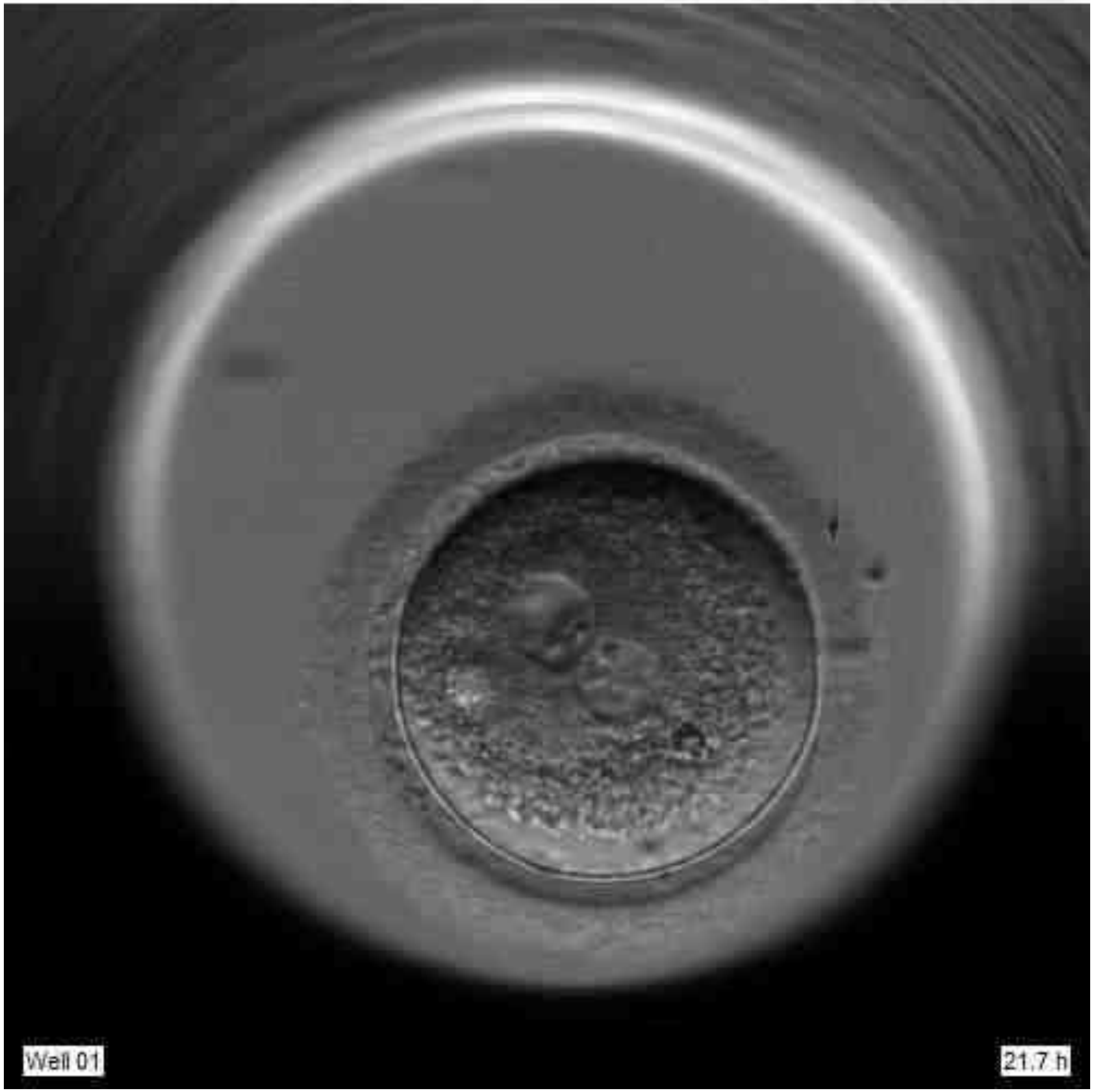


BOSTON IVF

Real Time Morphology

BOSTON IVF

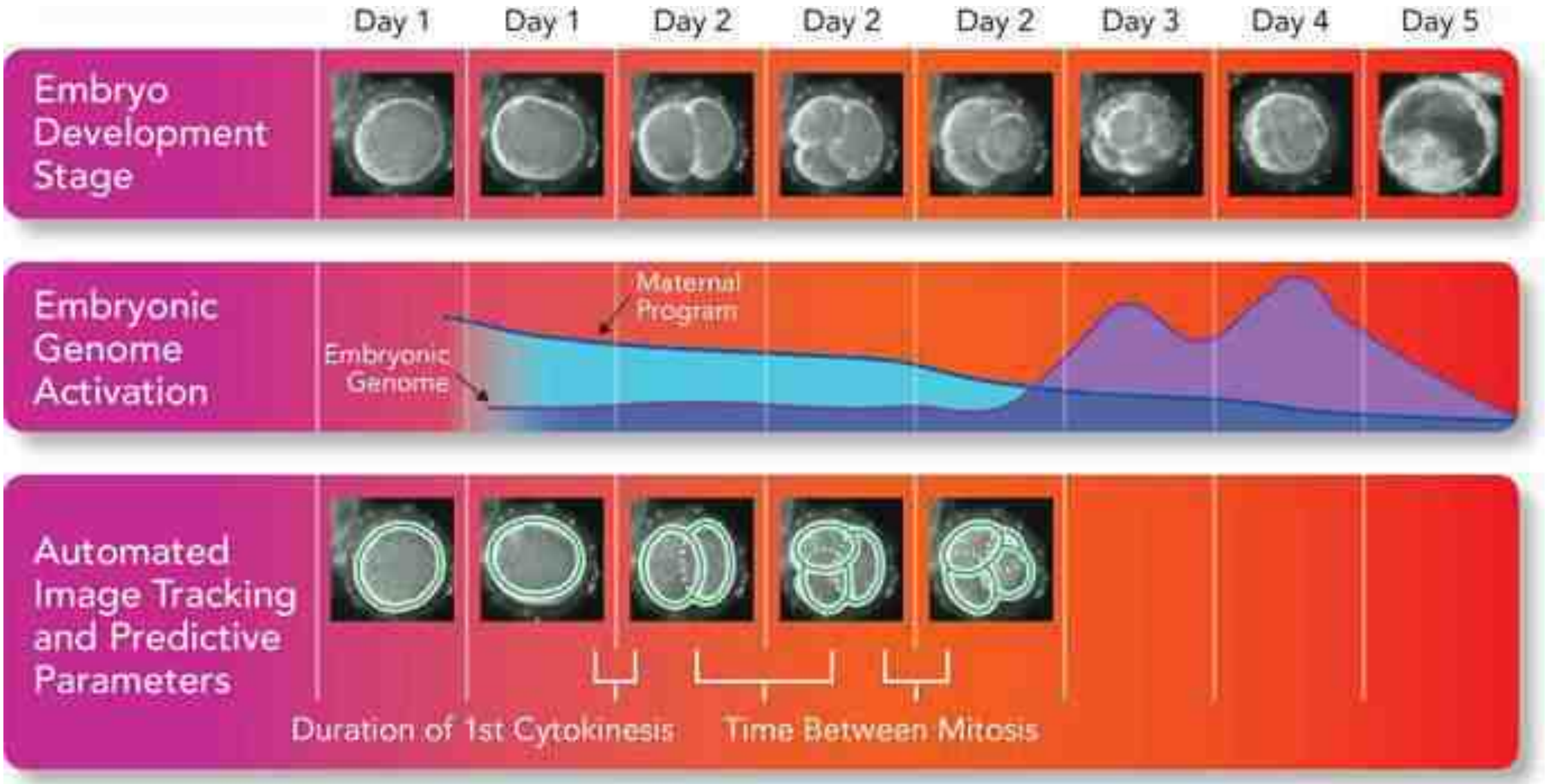
BOSTON IVF



BOSTON IVF

Auxogyn

- Using a Day 2 algorithm they can predict with over 90% efficiency which day 2 embryo will develop to a blastocyst
- Wong et al. Nat Biotechnol. 2010



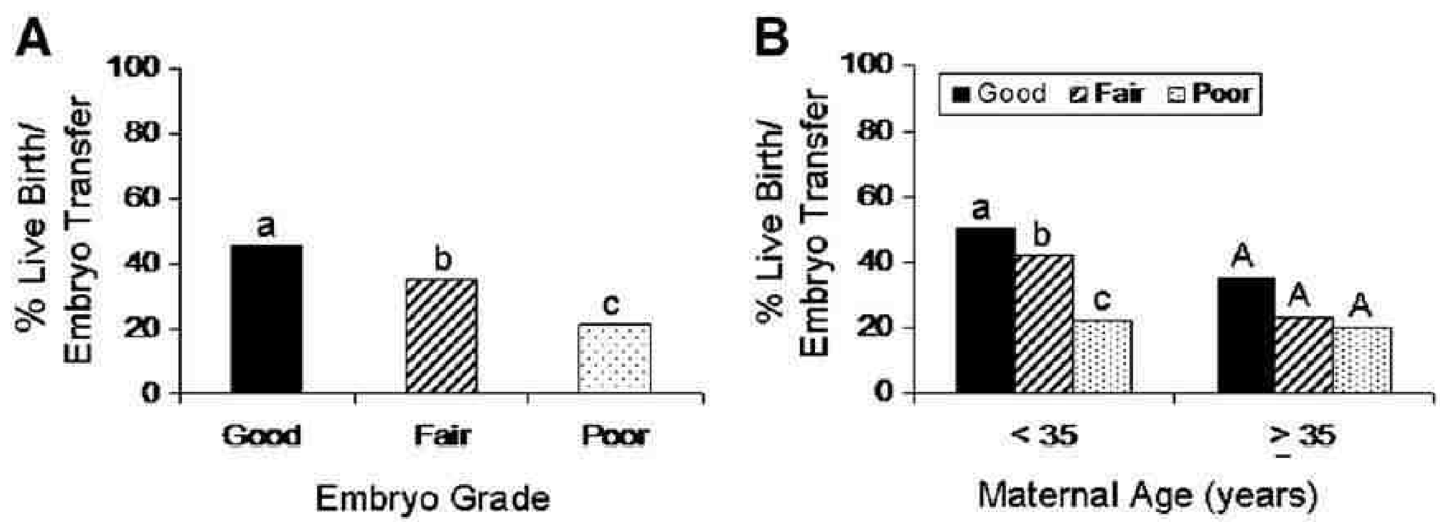
BOSTON IVF

Is there still a place for morphology and will real time imaging replace it?

Utility of the national embryo morphology data collection by the Society for Assisted Reproductive Technologies (SART): correlation between day-3 morphology grade and live-birth outcome

FIGURE 1

(**A**) Relationship between embryo quality (grade) and the percentage of live births/embryo transfers in patients receiving two day-3 embryos of the same grade. Embryo quality (grade) was positively correlated with the percentage of live births/embryo transfer. Groups with different letters indicate statistical difference ($P < .0001$). (**B**) Relationship between embryo quality (grade) and maternal age in patients in whom two day-3 embryos of the same grade were transferred. Embryo quality (grade) was positively correlated with the percentage of live births/embryo transfer for the <35 year olds ($P = .011$), with a positive trend (not statistically significant) with the >35 year olds ($P = .299$). Groups with different letters are statistically significantly different from one another.



Vernon. Correspondence. Fertil Steril 2011.

Human Reproduction, Vol.26, No.12 pp. 3289–3296, 2011
Advanced Access publication on October 3, 2011 doi:10.1093/humrep/der325

human reproduction ORIGINAL ARTICLE *Embryology*

Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer

A. Ahlström*, C. Westin, E. Reismer, M. Wikland, and T. Hardarson

Table III Effect of Expansion, ICM and TE separately analysed and adjusted for known and significant confounders (age, GQE, FSH total dose and number of earlier cycles).

Variable	Value	Live birth, % (*n*)	OR (95% CI)	*P*-value
Expansion	0 + 1 + 2	23.7 (58)		
	3	36.4 (148)	1.57 (1.07–2.29)	0.0196
	4	46.5 (216)	1.38 (1.04–1.83)	0.0259
Inner cell mass	A	45.3 (301)		
	B	31.0 (62)	0.62 (0.44–0.89)	0.0082
	C	14.3 (1)	0.38 (0.05–3.05)	NS
Trophectoderm cells	A	49.9 (234)		
	B	33.9 (128)	0.56 (0.41–0.74)	0.0001
	C	8.0 (2)	0.17 (0.04–0.76)	0.0198

GEE models have been used as they allow for adjustment of within-individual correlation.
For Expansion OR for 3 versus 0 + 1 + 2 and 4 + 5 versus 3 are presented.
For ICM and TE cells OR for 1 versus 0 and 2 versus 1 are presented.
Expansion: 0 was categorized together with 1 due to very few observations with 0 category and no occurrence of event in 0 category. 2 was categorized together with 0 and 1 due to no significant difference in the step 0 + 1 versus 2 in the analysis.

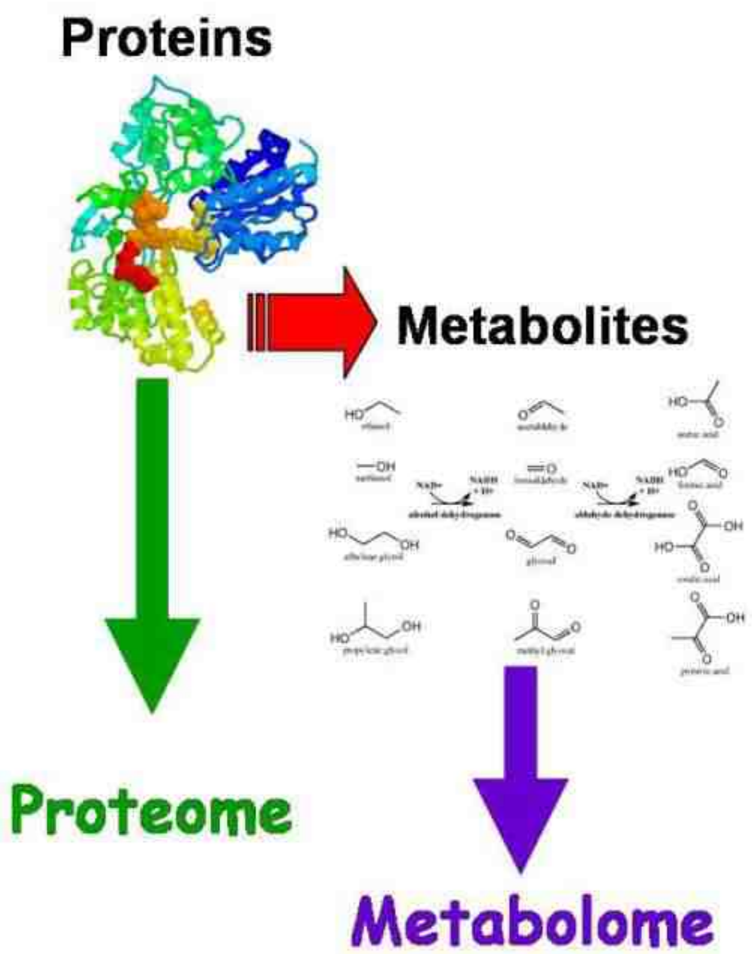
Table 1 Patient and morphology characteristics of transferred embryos divided by live birth outcome.

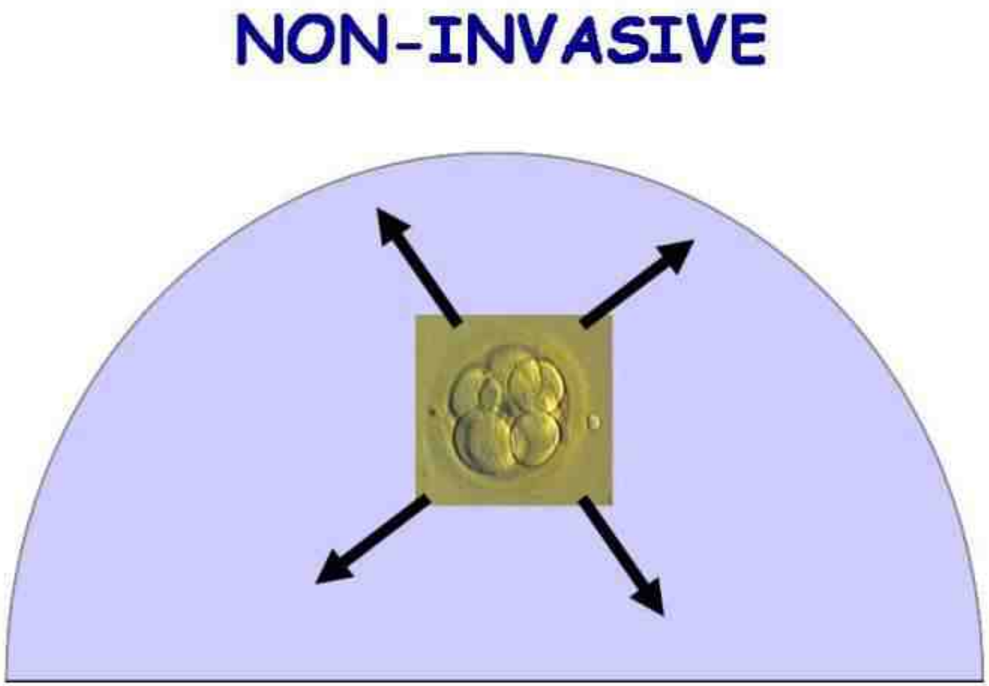
Variable	No live birth (n = 695)	Live birth (n = 422)
Expansion[a], (*n*) %		
0	3.0 (21)	0.0 (0)
1	6.6 (46)	5.7 (24)
2	17.3 (120)	8.1 (34)
3	37.3 (259)	35.1 (148)
4	35.8 (249)	51.2 (216)
Inner cell mass[a], (*n*) %		
A	71.7 (364)	82.7 (301)
B	27.2 (138)	17.0 (62)
C	1.2 (6)	0.3 (1)
Trophectoderm cells[a], (*n*) %		
A	46.3 (235)	64.3 (234)
B	49.2 (250)	35.2 (128)
C	4.5 (23)	0.5 (2)
No score for ICM and TE[a]	187	58

Morphology and Real Time Imaging

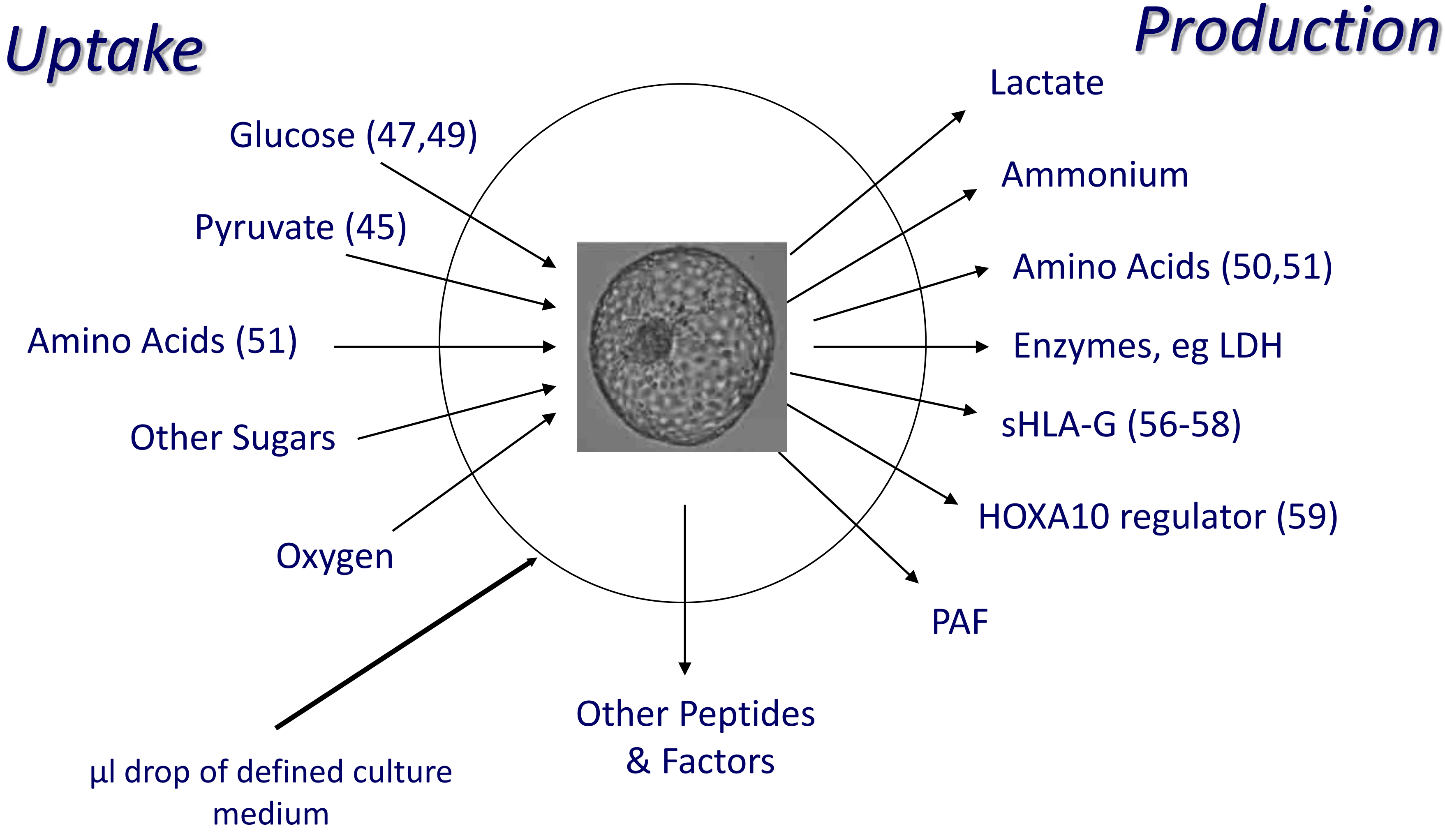
- Morphology has been assisting embryologists for many years to improve embryo selection.
  - It may be logical that by providing more detailed information concerning cleavage patterns and times that morphokinetics has the potential to improve the chance of live birth
  - No large RCT has yet compared MORPHOKINETICS against SET with blastocyst

Non-Invasive: Part 2



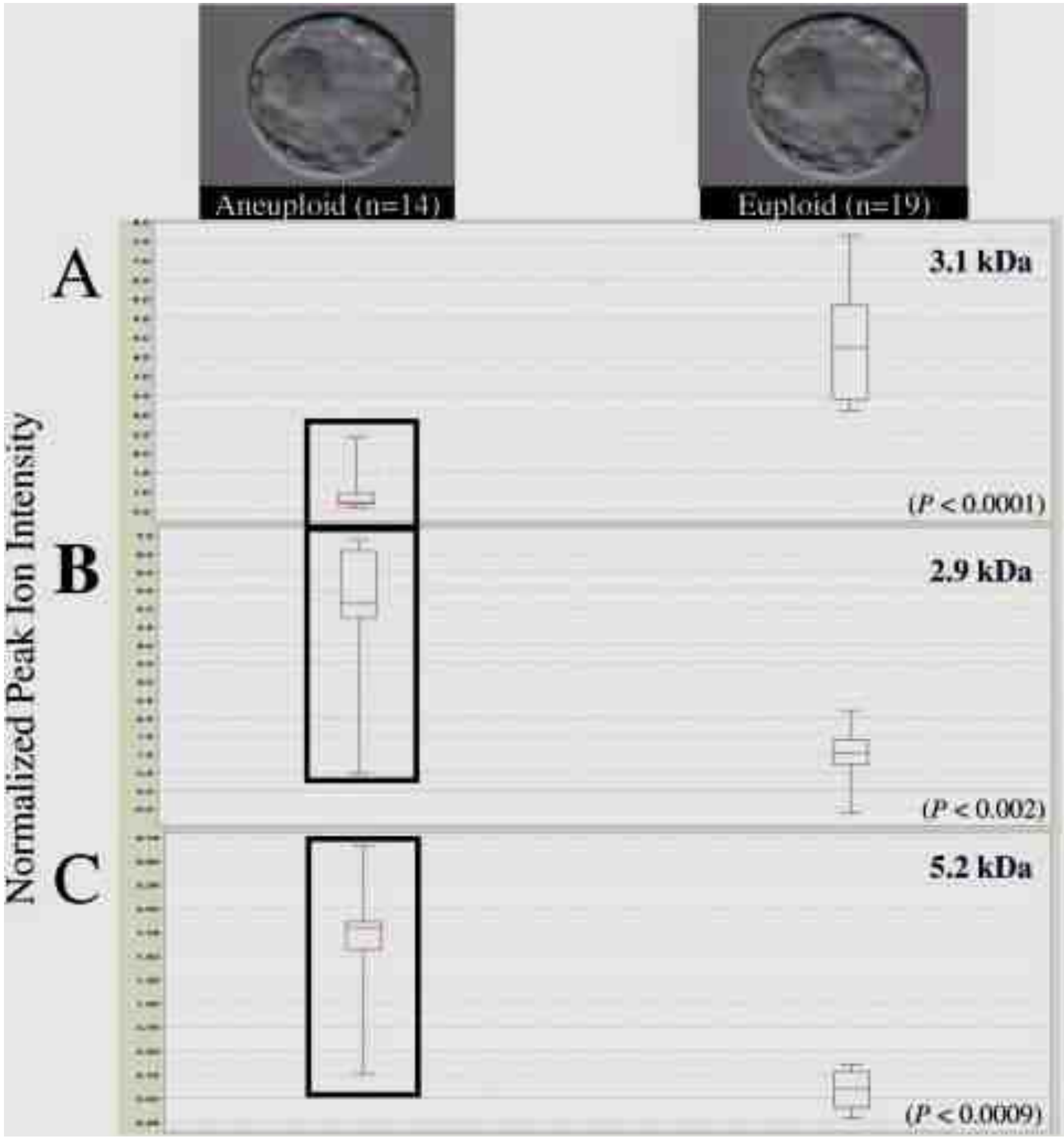


CHANGES IN THE CULTURE MEDIA INDICATING THE VIABILITY OF EMBRYOS



[Gardner and Leese, Handbook of IVF. 2000]
[Sakkas and Gardner, Curr. Opin. Obstet. Gynecol. 2005]

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]

Other Candidates

The effect of the biochemical marker soluble human leukocyte antigen G on pregnancy outcome in assisted reproductive technology—a multicenter study

Dirk Kotze, Ph.D.,[a] Thinus F. Kruger, M.D., Ph.D., D.Sc.,[b] Carl Lombard, Ph.D.,[c] Trishanta Padayachee, M.Sc.,[c] Levent Keskintepe, D.V.M., Ph.D.,[d] and Geoffrey Sher, M.B.Ch.B.[d,e]

TABLE 1

Mean age, mean number of embryos returned, number of chemical pregnancies, clinical pregnancies, and ongoing pregnancies by site and sHLA-G test result for individuals with day-3 transfers only.

Site	sHLA-G	Mean age (y)	Mean no. of embryos[a]	Chemical pregnancy[b]	Clinical implantation[b]	Multiple clinical[b]	Ongoing pregnancy[b]	Multiple ongoing[b]	Total (n)
A		35.1	1.8	248 (79.7)	222 (71.4)	103 (53.1)	220 (70.7)	101 (32.5)	311
	Negative	34.7	2.0	55 (70.5)	46 (59.0)	22 (28.2)	44 (56.4)	22 (28.2)	78
	Positive	35.3	1.7	193 (82.8)	176 (75.5)	81 (34.8)	176 (75.5)	79 (33.9)	233
B		34.6	3.7	87 (55.1)	80 (50.6)	47 (29.7)	72 (45.6)	38 (24.1)	158
	Negative	33.9	3.8	33 (50.8)	31 (47.7)	15 (23.1)	27 (41.5)	9 (13.8)	65
	Positive	35.1	3.6	54 (58.1)	49 (52.7)	32 (34.4)	45 (48.4)	29 (31.2)	93
C		33.7	3.0	238 (61.5)	225 (58.1)	57 (14.7)	202 (52.2)	44 (11.4)	387
	Negative	33.1	3.1	57 (38.0)	49 (32.7)	14 (9.3)	44 (29.3)	7 (4.7)	150
	Positive	34.0	2.9	181 (76.4)	176 (74.3)	43 (18.1)	158 (66.7)	37 (15.6)	237
D		36.6	1.7	53 (38.4)	48 (34.8)	21 (15.2)	47 (34.1)	19 (13.8)	138
	Negative	39.0	2.2	8 (23.5)	8 (23.5)	1 (2.9)	7 (20.6)	0 (0.0)	34
	Positive	35.8	1.6	45 (43.3)	40 (38.5)	20 (19.2)	40 (38.5)	19 (18.3)	104
E		37.5	3.3	282 (47.9)	248 (42.1)	53 (9.0)	206 (35.0)	26 (4.4)	589
	Negative	38.0	3.0	119 (39.3)	101 (33.3)	13 (4.3)	65 (21.5)	4 (1.3)	303
	Positive	37.0	3.6	163 (57.0)	147 (51.4)	40 (14.0)	141 (49.3)	22 (7.7)	286
F		35.2	3.6	102 (47.7)	88 (41.1)	31 (14.5)	72 (33.6)	26 (12.1)	214
	Negative	35.1	3.7	30 (31.9)	23 (24.5)	5 (5.3)	8 (8.5)	3 (3.2)	94
	Positive	35.2	3.5	72 (60.0)	65 (54.2)	26 (21.7)	64 (53.3)	23 (19.2)	120
Total		35.7	2.9	1,010 (56.2)	911 (50.7)	312 (17.4)	819 (45.6)	254 (14.1)	1,797

[a] Mean number of embryos transferred.
[b] Count (percentage of total).

Kotze. sHLA-G: noninvasive predictor of pregnancy. Fertil Steril 2013.

Other Candidates

Stamatkin *et al. Reproductive Biology and Endocrinology* 2011, 9:63
http://www.rbej.com/content/9/1/63

REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

PreImplantation Factor (PIF) correlates with early mammalian embryo development-bovine and murine models

Christopher W Stamatkin[1], Roumen G Roussev[1], Mike Stout[2], Victor Absalon-Medina[3], Sivakumar Ramu[1], Chelsi Goodman[1], Carolyn B Coulam[1], Robert O Gilbert[3], Robert A Godke[2] and Eytan R Barnea[4,5*]

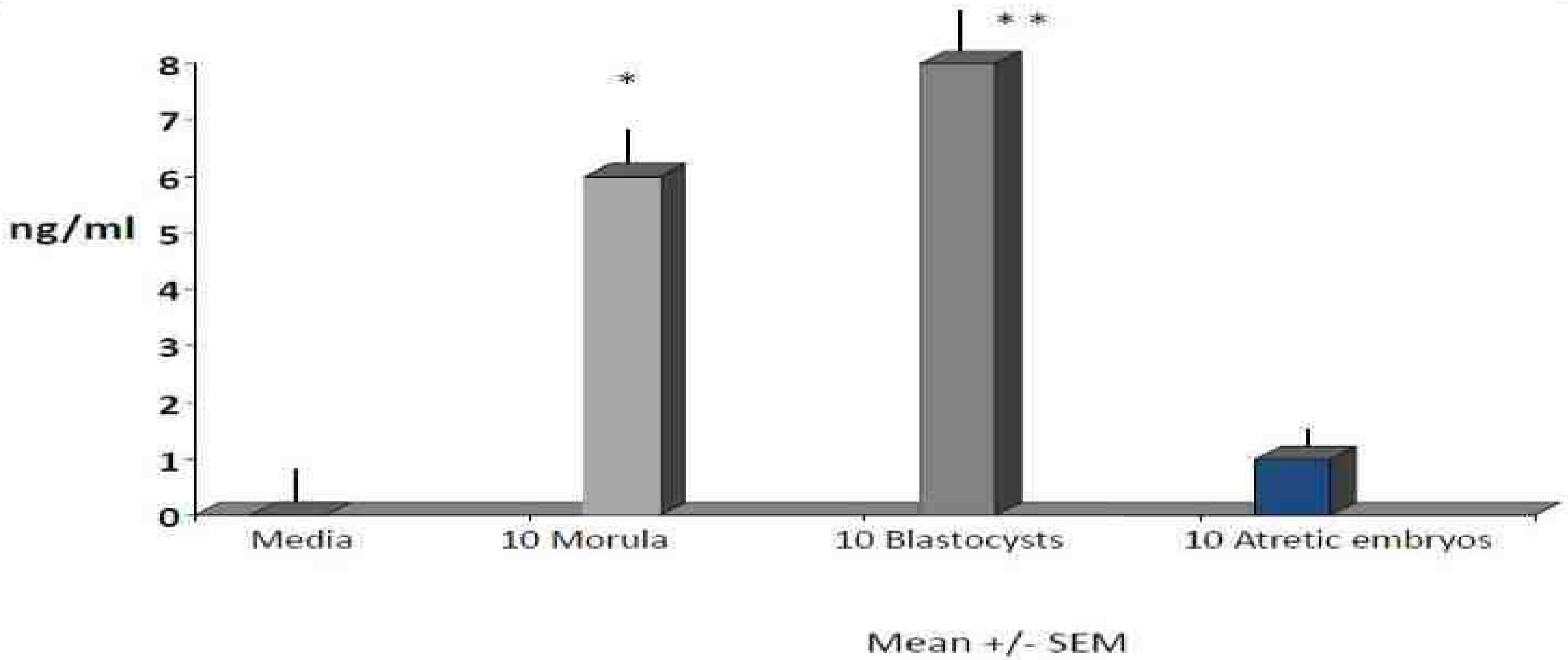


Figure 2 PIF detection in culture medium was associated with mouse embryo viability. Mouse embryos were cultured in groups up to the morula stage medium collected and further cultured up the blastocyst stage collecting the medium. PIF levels were compared with cultured atretic embryos and medium control. Data are expressed mean+/-SEM. PIF levels were significantly higher in both morulae and blastocysts ($p = 0.05$ and $p = 0.01$) respectively, as compared to similar levels obtained in atretic and medium alone used as control.

The Metabolism of Embryos

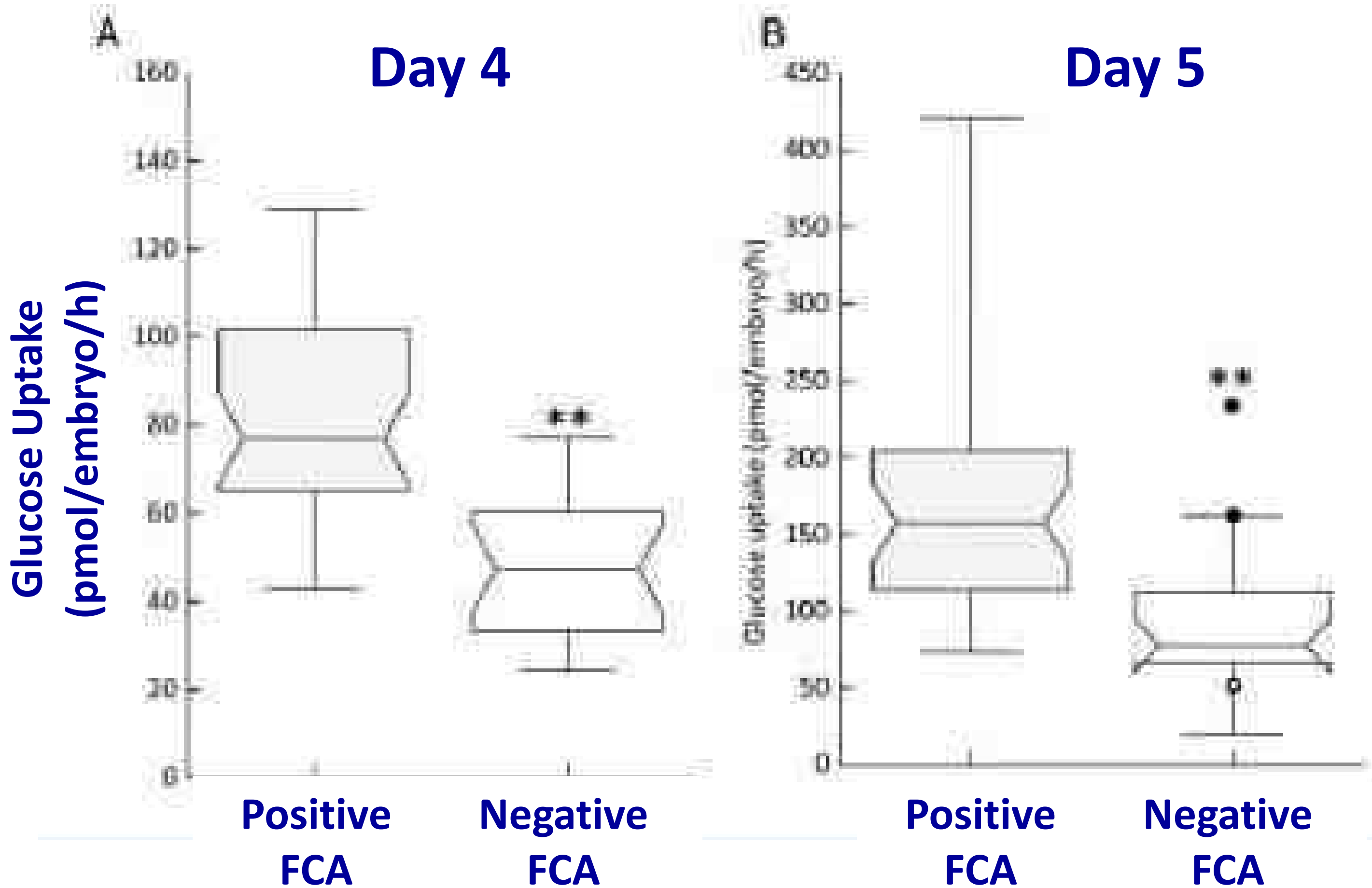
- Assessment of specific metabolic pathways, their relative activities and their regulation in relation to embryo viability

- Example:
  - Glucose, Lactate, Pyruvate
  - TCA Cycle functions before 8-cell stage and
  - Glycolysis functions after the 8-cell stage

It was first shown in 1980 that Glucose metabolism is linked with the viability of embryos

BOSTON IVF

Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. (Gardner et al., Human Reproduction 2012)



BOSTON IVF

Clinical Performance Summary of Metabolomics by Near Infra Red

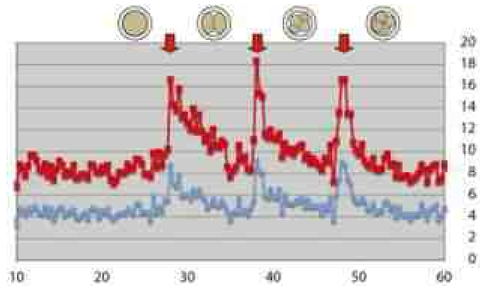
TYPE OF NIR INSTRUMENT	STUDY TYPE		MORPHOLOGY	MORPHOLOGY PLUS VIAMETRICS (NIR)	BENEFIT
Prototype *Hardarson et al. (Human Reprod, 2012)*	Single Embryo Transfer	Live Birth Rate	Day 2: 22/83 (26.5%) Day 5: 36/80 (45.0%)	Day 2:27/ 87 (31.0%) Day 5: 30/77 (39.0%)	YES NO
Prototype *Vergouw et al. (Human Reprod, 2012, 2014)*	Single Embryo Transfer	Live Birth Rate	Day 3: 68/163 (41.7%)	Day 3:61/146 (41.8%)	NO
Commercial *Economou et al. (ESHRE)*	Double Embryo Transfer	Clinical Pregnancy Rate	8/28 (29%)	16/28 (57%)	YES
Commercial *Sfontouris et al. (J Reprod Fertil 2013)*	Multiple Embryo Transfer	Clinical Pregnancy and Implantation Rate	41/86 (47.7%) 66/257 (25.7%)	21/39 (53.9%) 35/102 (34.3%)	YES

The Non- invasive Proteomics/ Metabolomics Example:
A Strong Hypothesis Can Be A Slave To Technology

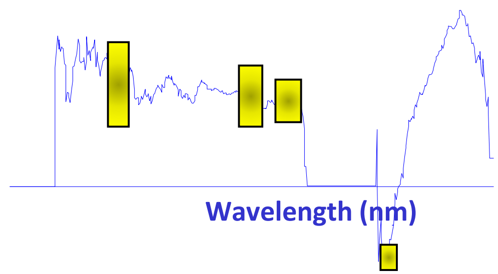
?

INCONSISTENT
RESULTS

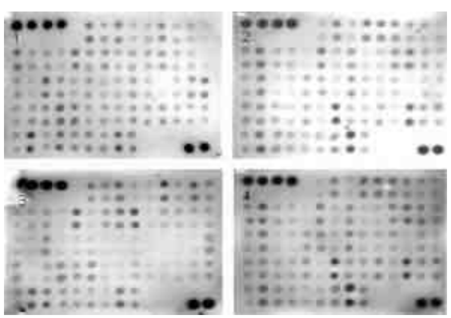
EMBRYO ASSESSMENT FOR SINGLE EMBRYO TRANSFER



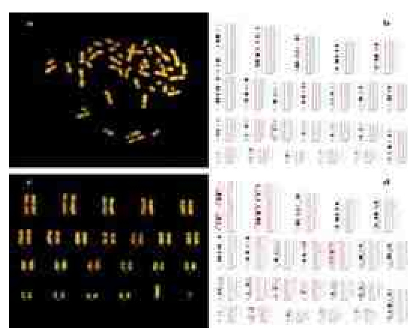
Real Time Morphology

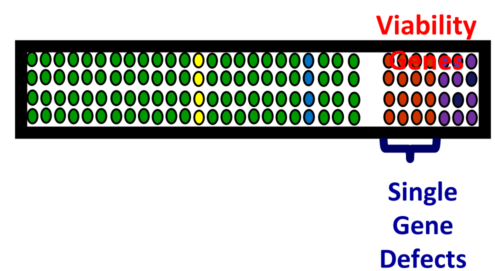


Metabolic assessment or imaging



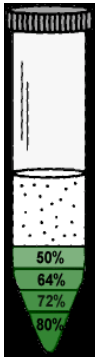
Proteomics





Transcriptomics and Genomics

Selection Methods to isolate the best sperm Proteomics



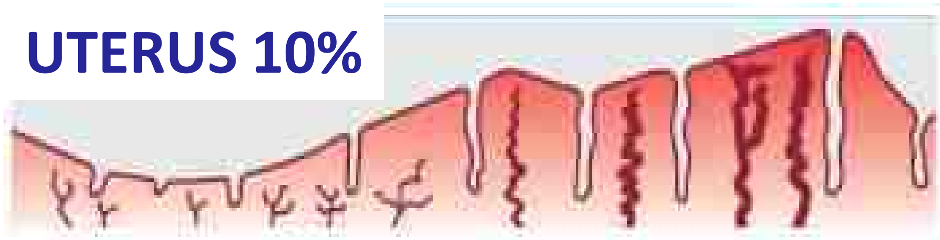
- Density – *Gradient Separation*
- Surface Charge – *Electrophoresis, Zeta Method*
- Morphological Characteristics – *IMSI*

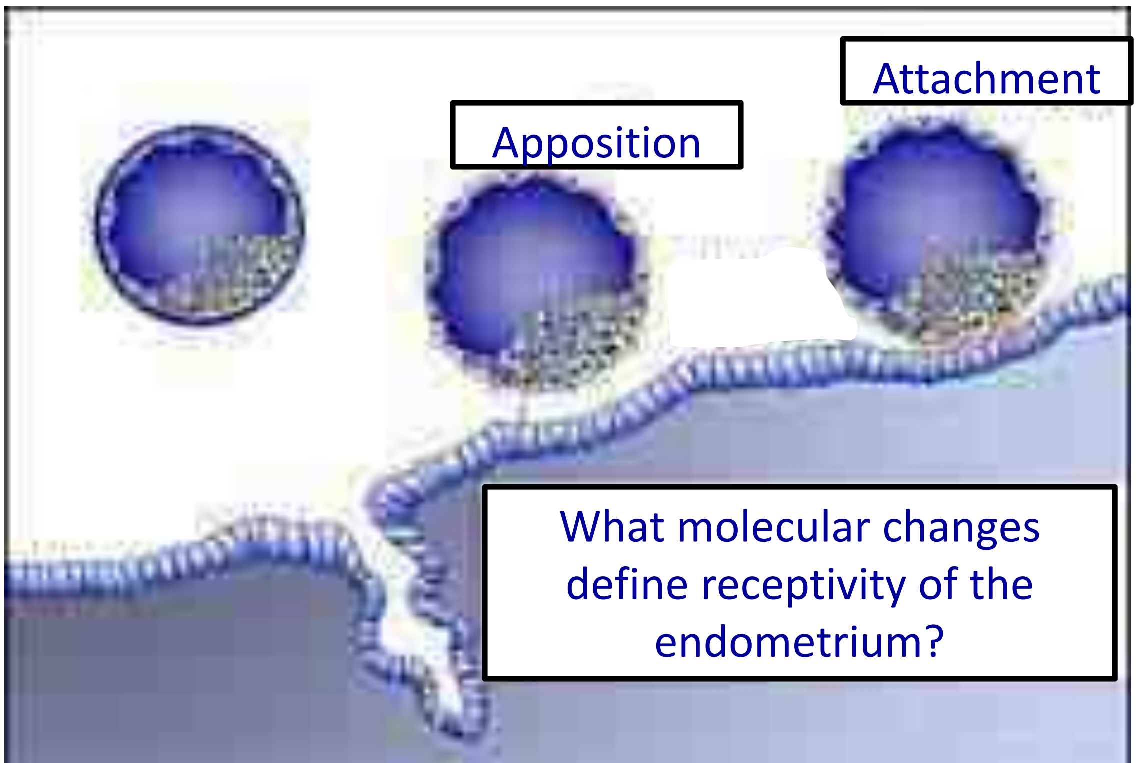
- Motility Characteristics – *Zech Selector, Microfluidics*
- Membrane Integrity – *Hyaluronan Binding, HOST*

- Surgical – *Testicular Surgery*

Venn diagram of the responsibilities of Reproduction Failure:

UTERUS 10%



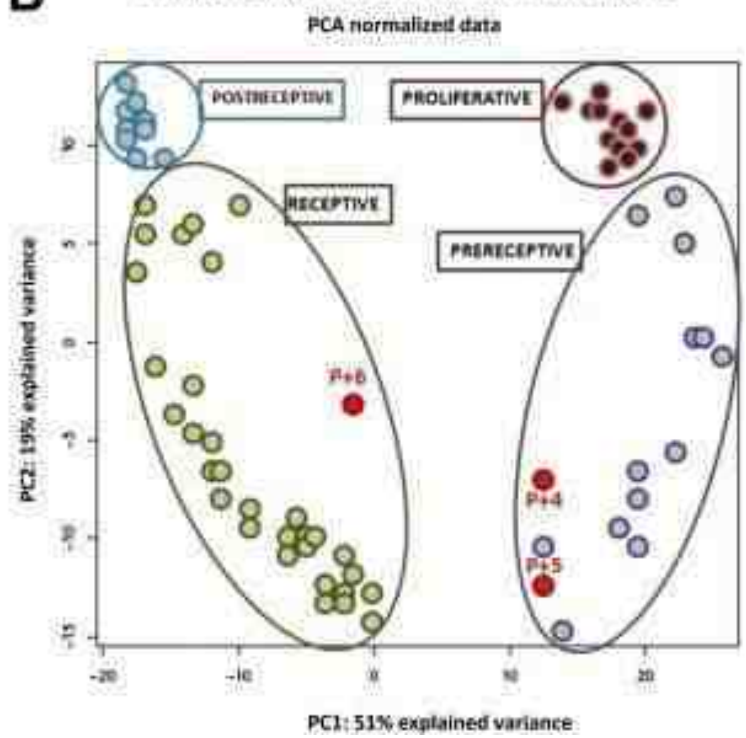


The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

A Clinical outcome of non receptive RIF and control patients that underwent pET

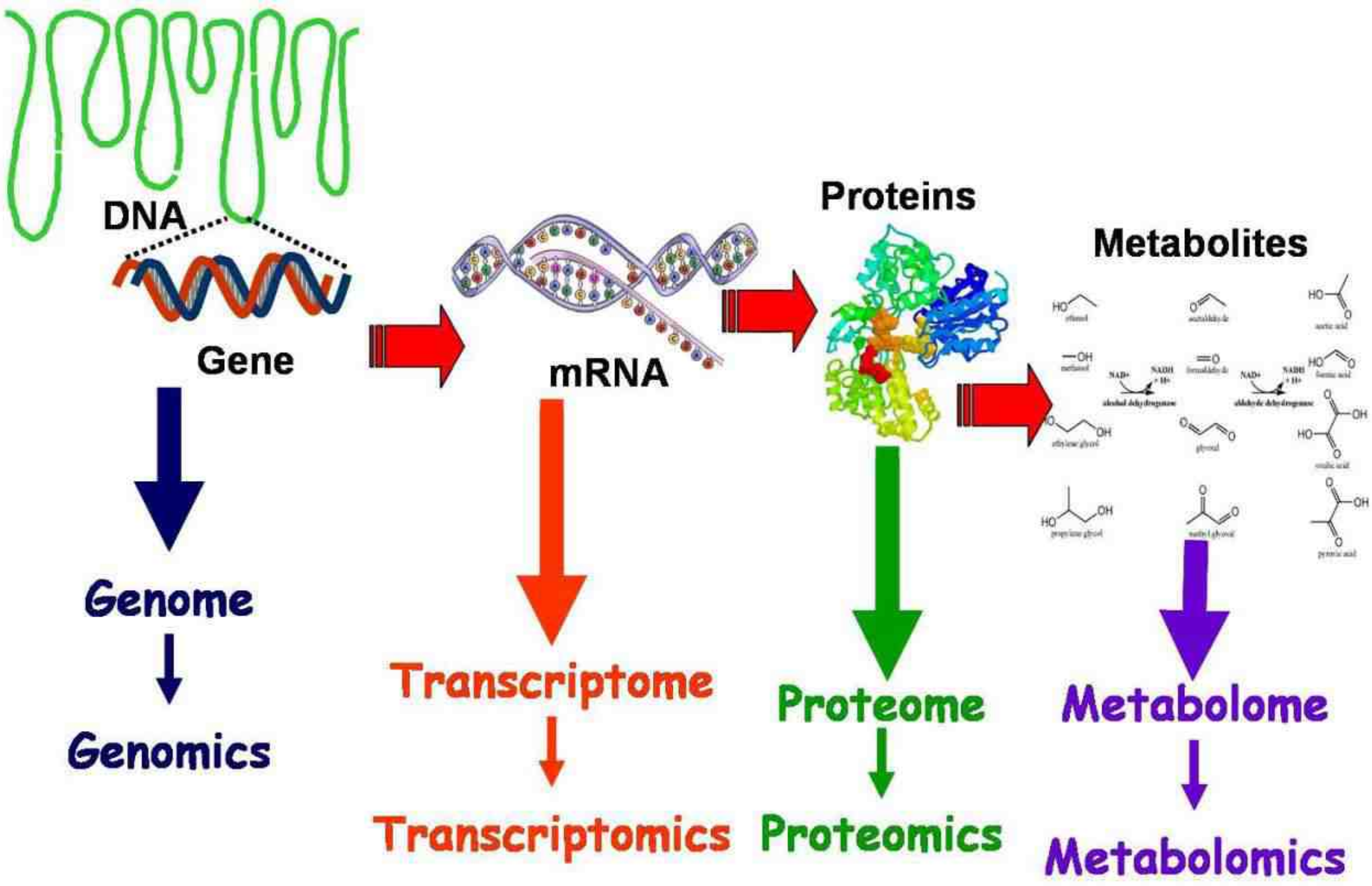
	Non Receptive
No. of patients	25
No. of previous failed cycle RIF Patients	5.0±1.8
No. of previous failed cycle Control Patients	0.3±0.6
ERA Prediction	
Pre-receptive	21/25 (84.0)
Post-receptive	4/25 (16.0)
2nd ERA at the specified day (P+4;P+6;P+7;LH+9)[a]	18
Months between 1st and 2nd ERA	2.6±2.8
2nd ERA Receptive at the specified day	15
Patients with pET[b] after 2nd RECEPTIVE ERA	8
Months between 2nd RECEPTIVE ERA and pET	1.8±0.7
Implantation rate using pET	5/13 (38.5)
Pregnancy rate using pET	4/8 (50.0)
Biochemical pregnancies (%)	0/4 (0.0)
Clinical abortions (%)	0/4 (0.0)

B Principal component analysis with ERA samples

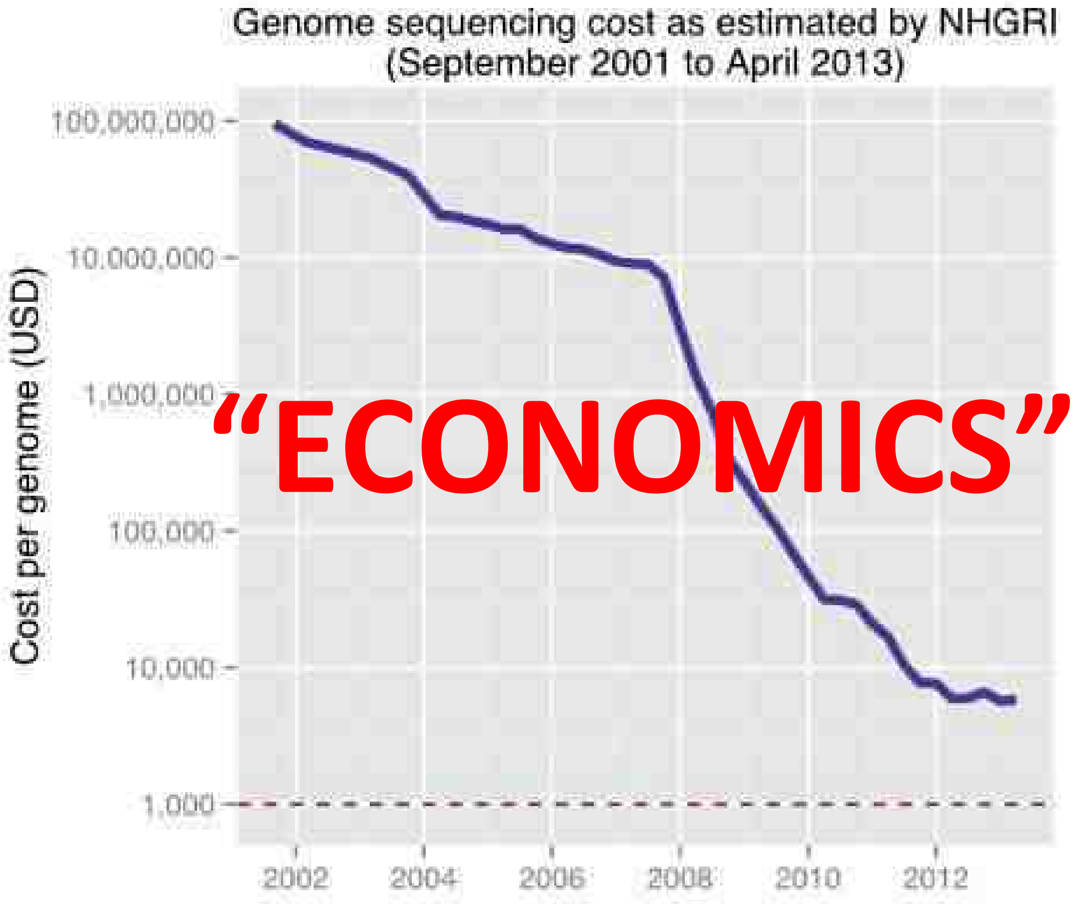


Ruiz-Alonso et al. Fertility and Sterility, Volume 100, Issue 3, 2013, 818 - 824

The “Omes and Omics”

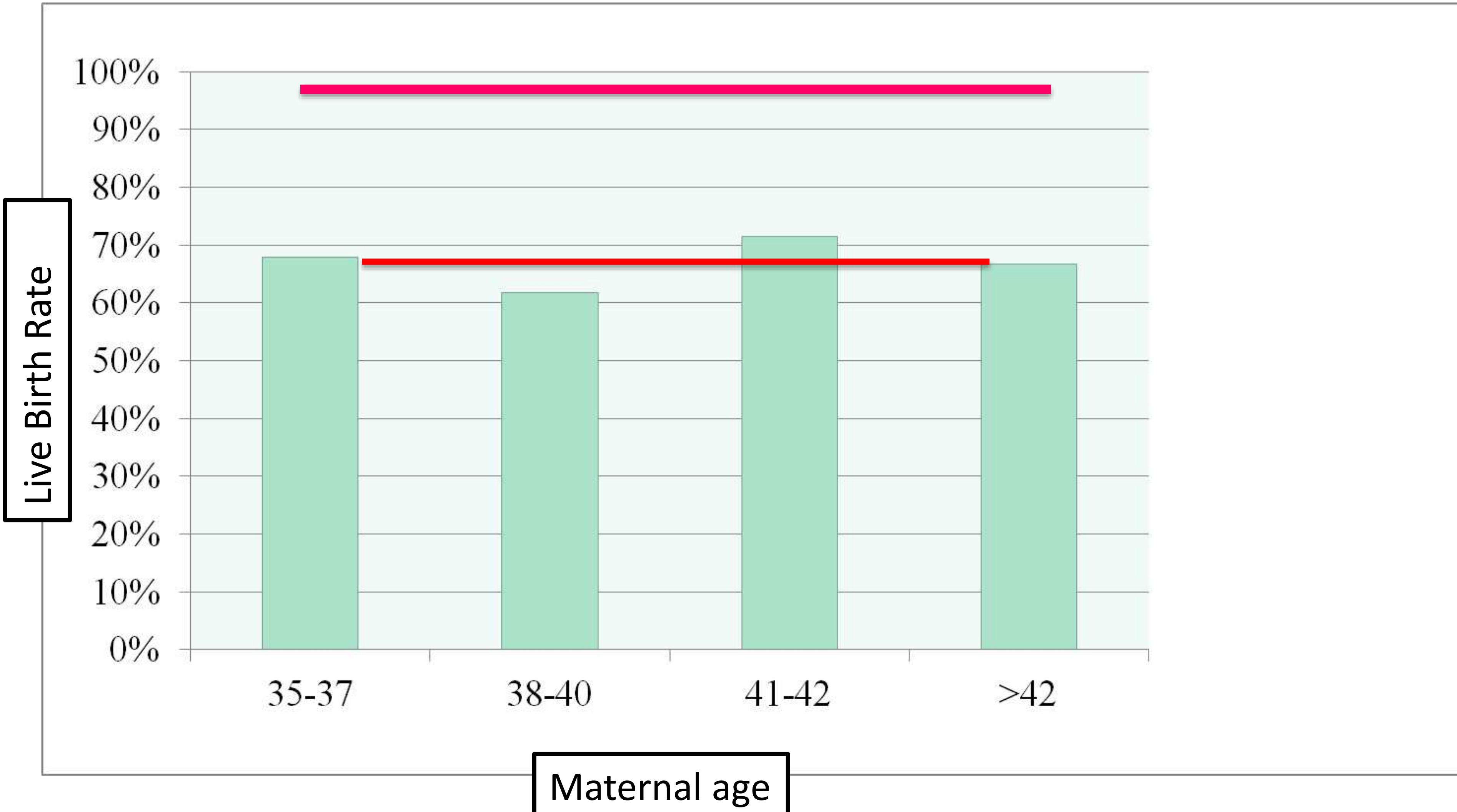


THE FINAL AND THE BIGGEST "OMICS"



"ECONOMICS"

OMICS technologies used to improve Singleton Live Birth Rates



Thank you

BOSTON IVF