CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

BLASTOCYSTE : Pourquoi, Pour qui, Comment ?

Paul BARRIERE – Thomas FREOUR

CHU NANTES

NICE – Septembre 2010

OBJECTIF de la stimulation

Obtenir et disposer :

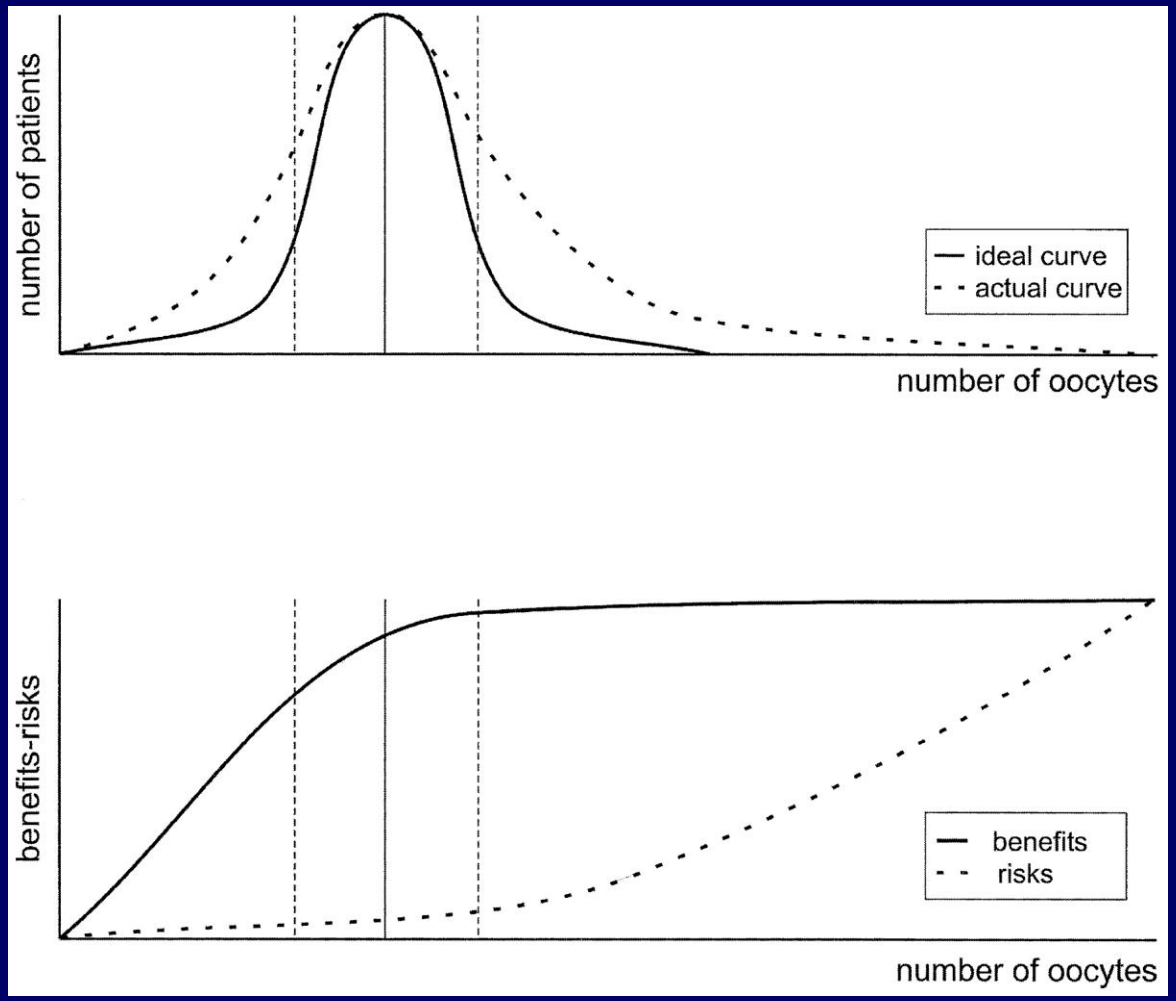
De gamètes permettant la formation d'un embryon permettant la naissance vivante d'un enfant en bonne santé

Y a-t-il un conflit d'intérêt entre le nombre et la qualité des ovocytes recueillis ?

Débat autour de la stimulation modérée :

La culture prolongée impose-t-elle un nombre élevé d'ovocytes recueillis ?

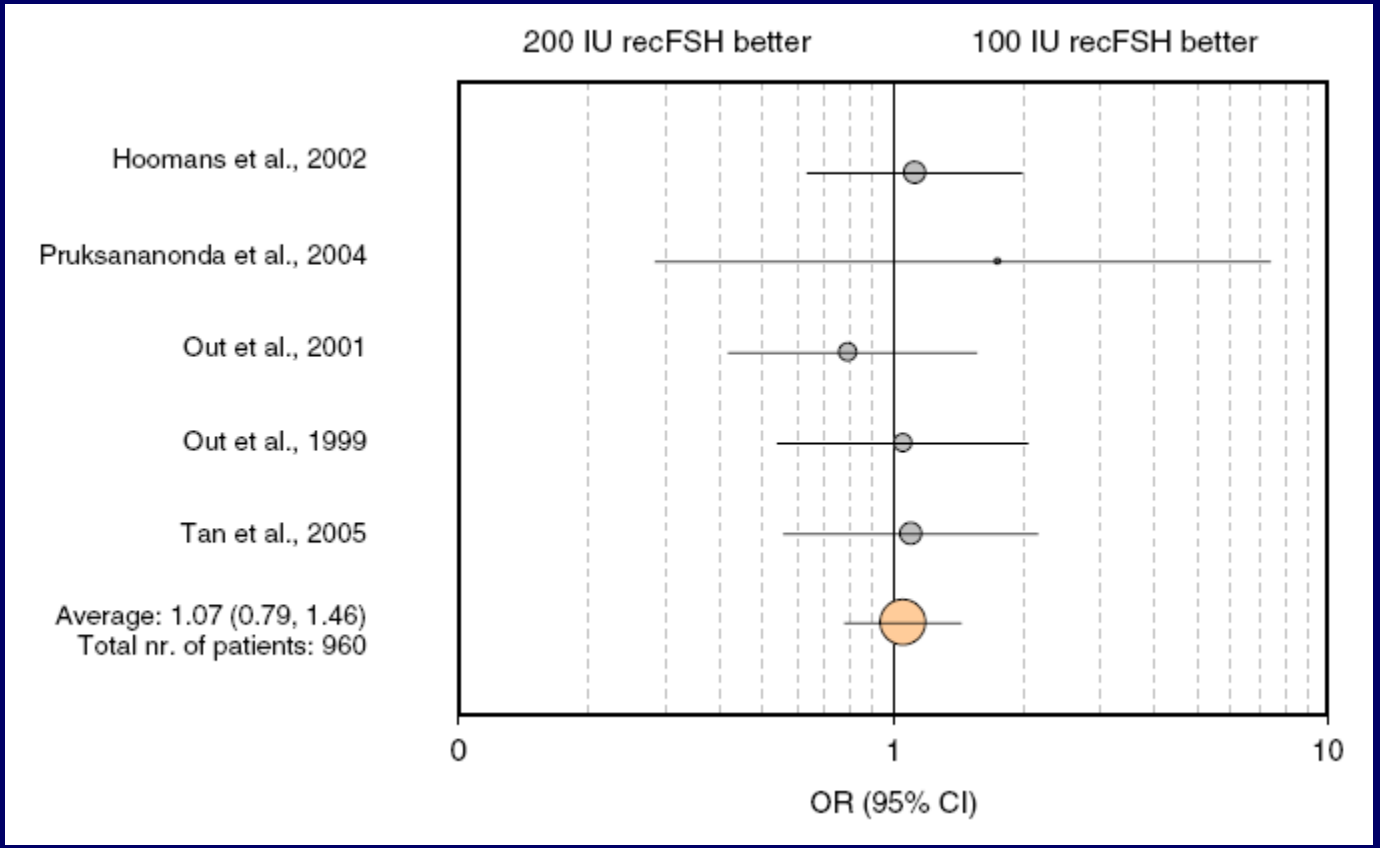
Développement multifolliculaire



Popovic-Todorovic et al. *Hum Reprod*. 2003;18:2275.

Nombre d'ovocytes et chances de grossesse

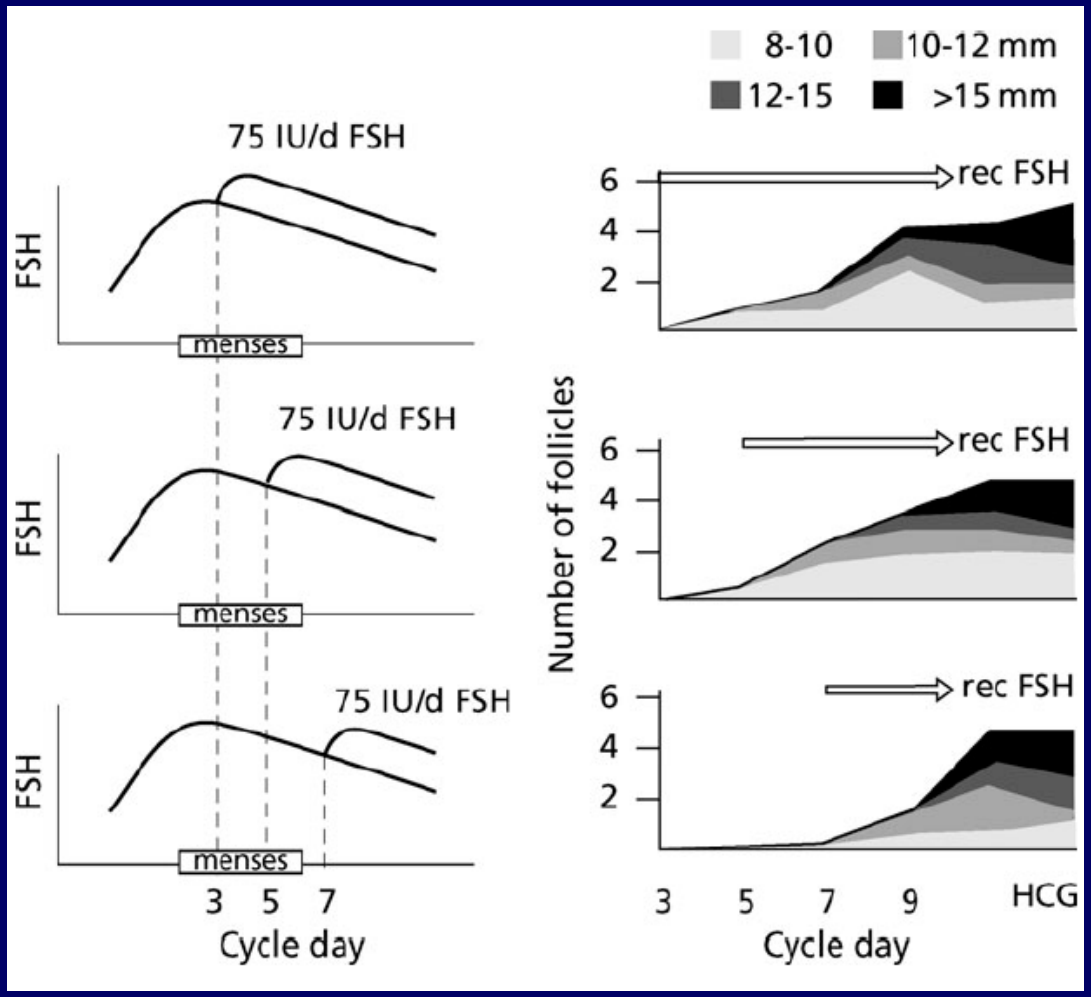
OR for Clinical Pregnancy



OR = odds ratio; CI = confidence interval.

Rombauts. *J Assist Reprod Genet*. 2007;24:343.

Mild *versus* conventionnel : *principes*



From Hohman et al., 2001 in Verberg et al., Hum Reprod Update 2009, 15:13-29

Mild *versus* conventionnel : *résultats*

Table II Included studies and number of first IVF treatment cycles (total = 592), progress to embryo transfer and treatment outcome

		Started treatment cycles (all first cycles)	Retrieval procedures	Embryo transfers	Ongoing pregnancy rate*	Live birth rate
Hohmann *et al.* (2003)	Mild	49	32	28	16%	n.a.
	Conventional	45	38	26	18%	n.a.
Heijnen *et al.* (2007)	Mild	201	147	124	13%	13%
	Conventional	193	176	160	33%	31%
Baart *et al.* (2007)	Mild	63	56	41	21%	n.a.
	Conventional	41	40	33	18%	n.a.
Total	Mild	313	235	193	15%	n.a.
	Conventional	279	254	219	29%	n.a.

*Ongoing pregnancy rate per started cycle.
n.a., not available.

25% *vs* 9%

The mild protocols are not suitable to all « presumably normal » responders !
And/Or
Cancellation criteria to refine!

TABLE 12

Pregnancy and implantation rates in preimplantation genetic diagnosis (PGD) patients and their respective controls, depending on the number of 2PN zygotes.

	Pregnancy[a]		Implantation[b]	
Groups	Control	PGD	Control	PGD
Total[c]	29% (30/103)	31% (32/103)	10% (38/367)[d]	20% (39/201)[d]
<8 zygotes	25% (14/55)	14% (6/43)	9% (17/194)	19% (10/54)
≥8 zygotes	33% (16/48)	43% (26/60)	12% (21/176)	20% (29/147)

[a] Retrieved cycles pregnant with fetal heart beat.
[b] Fetal heart beats per embryos replaced.
[c] Patients 35 and older with less than two previous failed in vitro fertilization cycles. Average maternal age in both groups was 40 years. From Munné et al. (20).
[d] $P<.005$.

Munné. Review of PGD to improve ART. Fertil Steril 2009.

Munné S, Wells D & Cohen J, Fertil Steril, 2010,

Résistance du fuseau mitotique et de la zone pellucide à la congélation lente de l'ovocyte

Meilleure résistance des ovocytes lors des cycles consommant moins de gonadotrophines

Spindle and chromosome configurations of human oocytes matured *in vitro* in two different culture media

D Christopikou *, C Karamalegos, S Doriza, M Argyrou, P Sisi, S Davies, M Mastrominas

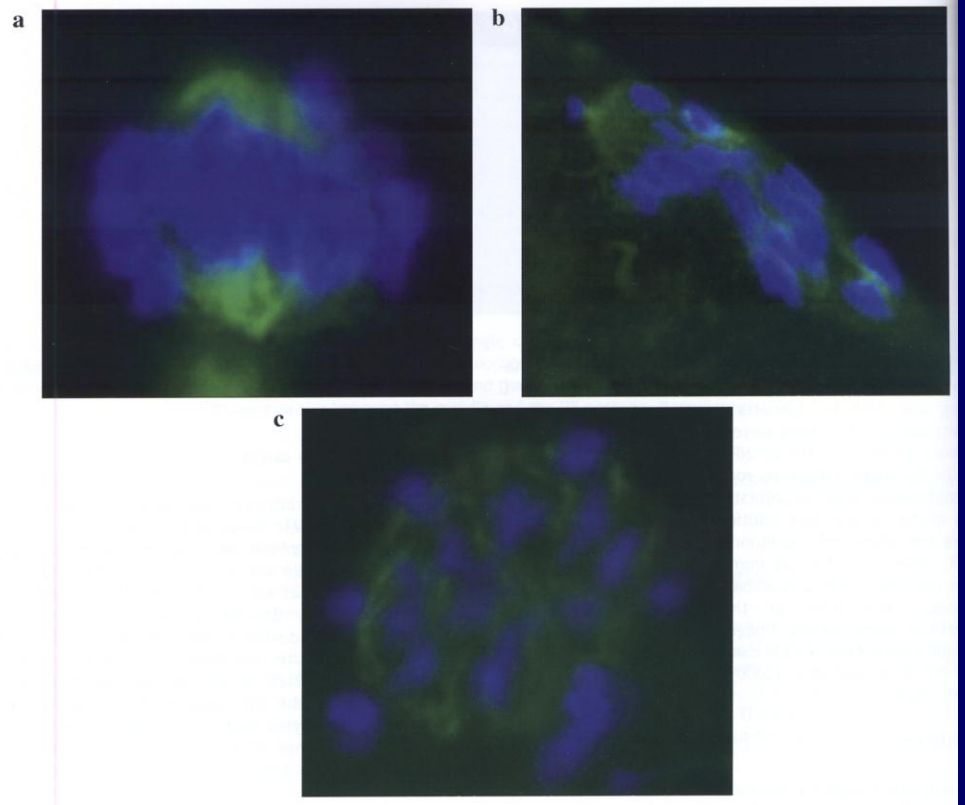


Figure 2 Epifluorescence microscopy images of spindle configurations with microtubules. **(a)** Equatorial view of normal spin configuration with chromosomes aligned along the plate of the metaphase plane displaying a barrel-shaped spindle structu magnification 100×; **(b)** Planar view of abnormal spindle configuration with chromosomal organizations associated with t disorganization of the microtubule fibres displaying an elongated spindle structure, magnification 100×; (c) Planar view abnormal spindle configuration with dispersed chromosomal organizations associated with abnormal organization of microtubule fibres displaying a round spindle structure, magnification 100×. Green = anti-α-tubulin monoclonal antibody FITC; blue = DAPI.

ANEUPLOIDIE ET NIVEAU DE STIMULATION

Résultats controversés lors des stimulations conventionnelles

Incidence augmentée : Fauser, Rotterdam

Incidence non modifiée : Pellicer, Valencia

Taux d'aneuploïdie des embryons en cycles spontané ou stimulé chez des donneurs jeunes de gamètes

LABARTA et al (IVI, Espagne)
ESHRE 2010

Résultats

- Nombre d'ovocytes en Métaphase II :

  1 vs 14.4 $\pm$ 6.5

- Nombre d'embryons analysés :

  1 vs 7.1 + - 3.4

- Aneuploïdie

  40 vs 42.2 %

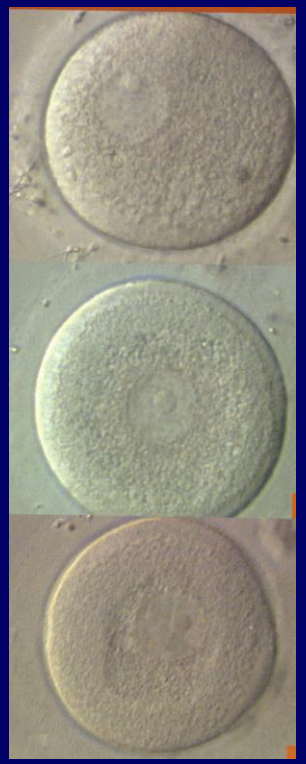
LABARTA et al (IVI, Espagne)
ESHRE 2010

OBJECTIF
Au laboratoire (1)

Identifier les gamètes et/ou les embryons capables d'installer une grossesse évolutive

Qualité des ovocytes

- Maturation nucléaire
- Homogénéité du cytoplasme
- Viscosité du cytoplasme
- Aspect de la zone pellucide
- Aspect du fuseau mitotique
- Expression génique et génétique



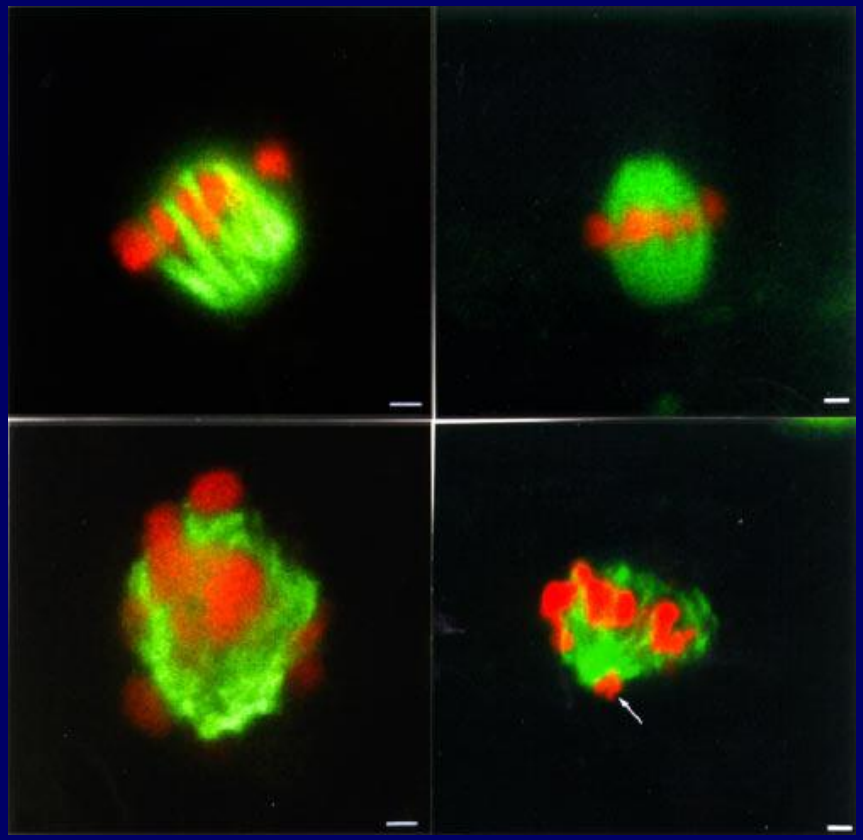


FIG. 11. Composite micrographs of confocal images of the meiotic spindles in oocytes obtained from naturally cycling women in two age groups, 20–25 yr (*top*) and 40–45 yr (*bottom*). Chromatin is represented in *red* (propidium iodide) and microtubules in *green* (fluorescein isothiocyanate). *Top*, Oocytes obtained from the younger age group at metaphase II exhibit a uniform matrix of microtubules in the spindle and a distinct, uniform chromosome distribution within the metaphase plate. *Bottom*, Oocytes obtained from the older age group with microtubules in disarray compared with the oocytes in the *top panels*. A distinct metaphase plate of chromosomes is not visible because most chromosomes were not well aligned or dislocated from the metaphase plate.

Qualité ovocytaire

Le modèle de « l'egg sharing »

Faddy et al, RBMonline, in press

- 2 cliniques :
  - 129 donneuses vs 129 receveuses
  - 156 donneuses vs 159 receveuses
- Age : 31.1 vs 41.2 et 30.5 vs 42.4
- Proportion d'embryon par ovocyte : 0.67 vs 0.65 et 0.68 vs 0.66

Qualité ovocytaire

Le modèle de « l'egg sharing »

Faddy et al, RBMonline, in press

Probabilité de grossesse de la receveuse :

- Si la donneuse est enceinte : OR 2.15 (95% CI 1.33-3.46) p=0.002
- 0.53 si donneuse enceinte vs 0.34 si donneuse non enceinte

Analyse du globule polaire

J Assist Reprod Genet
DOI 10.1007/s10815-010-9422-7

TECHNICAL INNOVATIONS

Are zona pellucida laser drilling and polar body biopsy safe for *in vitro* matured oocytes?

Ibrahim Hammoud · Denise Molina-Gomes · Martine Albert · Marianne Bergere · Marc Bailly · Robert Wainer · Jacqueline Selva · Francois Vialard

Hammoud et al 2010

JARG

TABLE 1

Overall outcome of 85 patients undergoing chromosomal analysis of first polar body for the chromosomes 13, 15, 16, 18, 21, and 22 (first panel).

	Pregnant	Nonpregnant	Total
No. biopsied oocytes	303	235	538
No. FISH-diagnosed oocytes (%)	277 (91)	213 (91)	490 (91)
No. euploid oocytes (%) (mean ± SD)	158 (57) (3.6 ± 1.4)	131 (61) (3.1 ± 1.8)	289 (59)
No. inseminated oocytes	125	109	234
No. fertilized oocytes (%)	104 (83)	84 (77)	188 (80)
No. embryos (%)	97 (93)	81 (96)	178 (95)
No. transferred embryos (mean ± SD)	93 (2.1 ± 0.8)	69 (1.6 ± 0.8)	162 (1.9 ± 0.8)
No. clinical pregnancies	43	0	43
No. abortions	11	0	11

Note: Values are number (percentage) unless otherwise noted.

Magli. Chromosome 1, 4, and 6 aneuploidies and implantation. Fertil Steril 2010.

Magli et al, Fertil Steril, 2010

OBJECTIF
Au laboratoire (2)

- Ne pas altérer les gamètes confiés
- Optimaliser leurs chances de développement

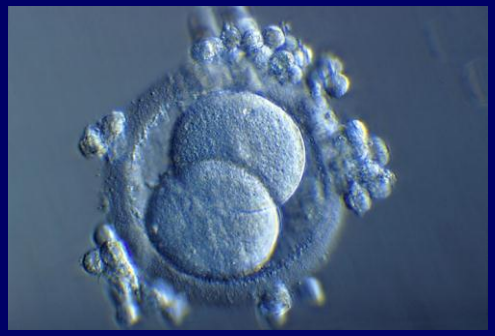
QUESTION :

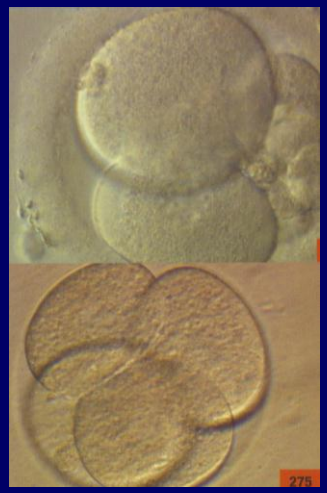
CHOIX DE L'EMBRYON
à transférer

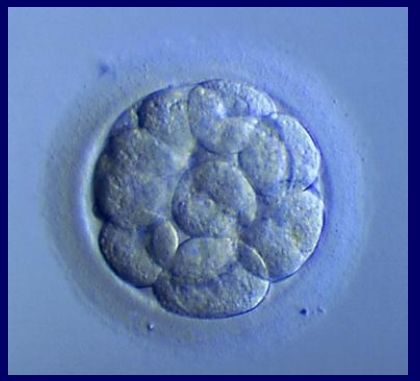
vs

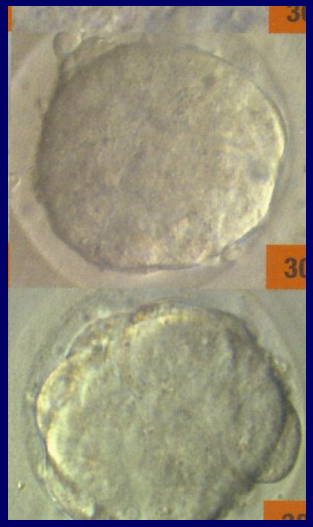
CHOIX du stade de l'embryon
à transférer

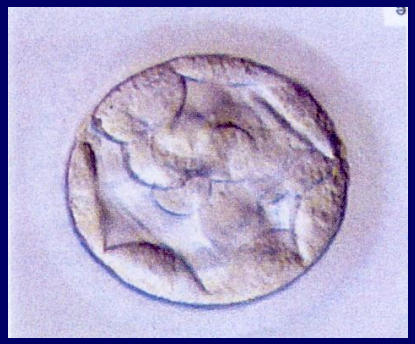
QUALITE DES EMBRYONS

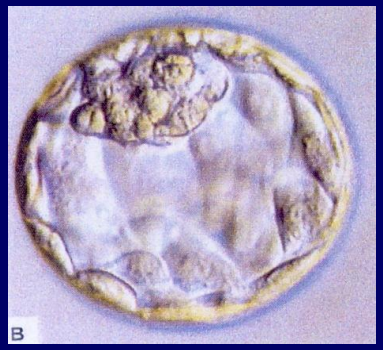


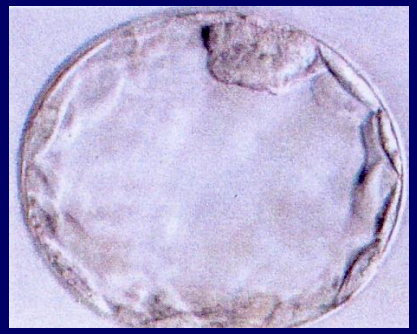












Influence of oocytes and spermatozoa on early embryonic development

Andres Salumets, M.Sc.,[a] *Anne-Maria Suikkari, M.D., Ph.D.,*[a] *Tonu Möls, Ph.D.,*[b] *Viveca Söderström-Anttila, M.D., Ph.D.,*[a] *and Timo Tuuri, Ph.D.*[a]

The Family Federation of Finland, Helsinki, Finland

Objective: To evaluate the effect of oocytes and spermatozoa on early embryonic development.

Design: Retrospective study.

Setting: Infertility Clinic, the Family Federation of Finland.

Patient(s): Fifty-nine oocyte donation cycles with oocytes shared among 118 recipient couples.

Intervention(s): Culture of all fertilized oocytes.

Main Outcome Measure(s): Standard sperm (concentration, progressive motility, and morphology according to Tygerberg strict criteria) and embryo (morphology and cleavage stage) characteristics.

Result(s): A marked effect of the oocyte on both embryo morphology and blastomere cleavage rate was demonstrated. In addition, a significant sperm effect on blastomere cleavage rate was found. Sperm morphology as determined according to strict criteria rather than sperm count or progressive motility was positively associated with the blastomere cleavage rate. None of the measured sperm characteristics influenced embryo morphology.

Conclusion(s): Embryo morphology, i.e., fragmentation and blastomere uniformity, are predominantly determined by oocyte quality, whereas both the oocyte and spermatozoa influence the blastomere cleavage rate. (Fertil Steril® 2002;78:1082–7. ©2002 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, embryo quality, oocyte, spermatozoa

La qualité embryonnaire

Différentes mesures :

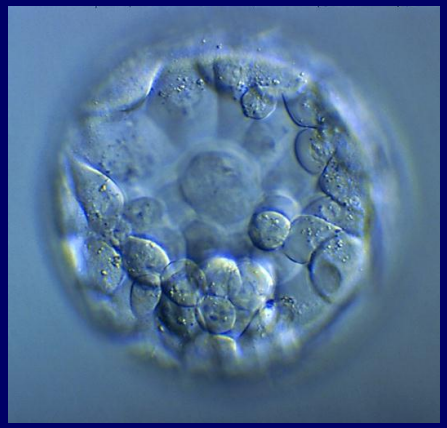
- Morphologiques
- Génomiques
- Fonctionnelles

Qualité embryonnaire

Analyses morphologiques

Scores embryonnaires

Évaluations morphologiques diverses



CRITERES MORPHOLOGIQUES

- Clivage précoce
- Taux de fragmentation
- Pattern des pronuclei
- Halo cytoplasmique
- Taux de blastulation

Les limites des évaluations morphologiques

- Pas d'études prospectives randomisées exploitables sur la morphologie embryonnaire
- Ne dépiste pas l'altération qualitative liée à l'âge (STENSEN et al – 2010)
- Influence des conditions de culture de chaque laboratoire
- Variations intra – et inter observateurs (Arce et al 2006, Paternot et al 2009)
- Risques d'invasivité des observations

- Aspect des pronuclei
- Clivage précoce
- Nombre de blastomères à J2
- Taux de fragmentation

Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: A prospective study based on 4042 embryos

F.Guerif[1], A.Le Gouge[2], B.Giraudeau[2], J.Poindron[1], R.Bidault[1], O.Gasnier[1] and D.Royere[1,3]

Guérif *et al*
Hum.Reprod
2007 & 2009

4042 embryons – 1762 blastocystes

4 paramètres corrélés au taux de blastulation

Clivage précoce et nombre de blastomères prédictifs d'une bonne morphologie du blastocyste à J5

Bonne morphologie du blastocyste corrélée au taux d'implantation

Analyse multivariée peu prédictive pour les critères précoces (AUC/ROC = 0.688)

Table 5 Cumulative deliveries including fresh and frozen-thawed cycles according to number of TQE available on day 2

		Group 1 ≥ 2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Number of couples		165	125	166
Number of deliveries	Fresh blastocyst transfer	72	42	54
	Frozen-thawed blastocyst transfer	7	6	4
	Overall results	79	48	58
	Rate per couple	47.9%[a]	38.4%	34.9%[a]
	Overall twin	2	1	1
	Rate per couple	2.5%	2.1%	1.7%

Values with the same superscript are significantly different ($p<0.05$)

TQE top quality embryo

Guérif F et al, JARG, 2009

Qualité embryonnaire

Aspects fonctionnels

ASPECTS FONCTIONNELS

- Sécrétion de HLA-G soluble pour les embryons

  Controverse technologique

Journal of Reproductive Immunology 75 (2007) 128–132

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

www.elsevier.com/locate/jreprimm

sHLA-G production by human IVF embryos: Can it be measured reliably?

Ian Sargent[a,*], Anna Swales[a], Nathalie Ledee[b], Noemi Kozma[c], Julie Tabiasco[c], Philippe Le Bouteiller[c]

Table 7. Influence of the culture medium on sHLA-G detection in embryo culture supernatants.

Parameter	*Liège (595 embryo supernatants)*		P-*value*[a]
	Culture medium 1 (Irvine Scientific)	*Culture medium 2 (Cook)*	
sHLA-G-positive ES (%)	77/328 (23.5)	187/267 (70.0)	<0.0001
sHLA-G-positive ES among embryos which were transferred (%)	34/122 (27.9)	33/54 (61.1)	<0.0001
Implantation rate[b] (%)	15.6	23.1	NS

[a]Fisher's exact test.
[b]Only mean implantation rate of the group observed (HLA-G positive or negative, implantation rate of each reproductive unit) is reported; for explanation, see footnote b in Table 1.
ES = embryo culture supernatant; sHLA-G = soluble human leukocyte antigen G; NS = not statistically significant.

Tabiasco et al, RBMonline, 2009

Fertil Steril. 2010 Apr 16. [Epub ahead of print]

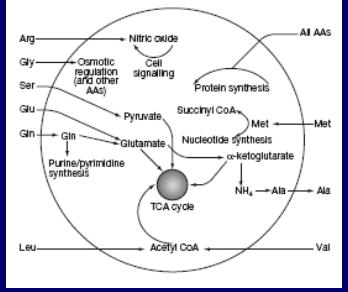
Levels of follicular G-CSF and interleukin-15 appear as noninvasive biomarkers of subsequent successful birth in modified natural in vitro fertilization/intracytoplasmic sperm injection cycles.

Lédée N, Frydman R, Osipova A, Taieb J, Gallot V, Lombardelli L, Logiodice F, Petitbarat M, Fanchin R, Chaouat G, Achour-Frydman N, Piccinni MP.

INSERM U782, Univ Paris-Sud, UMR-S0782, Hôpital Antoine Béclère, Clamart, France; Centre DENOTHE of the University of Florence, Department of Internal Medicine-Immunoallergology Unit, Florence, Italy.

CONCLUSION(S): Combined follicular G-CSF and IL-15 quantification appears as an efficient and noninvasive method to define oocyte competence for subsequent successful conception in modified natural IVF/ICSI cycles.

Embryo metabolism (1) Aminoacids



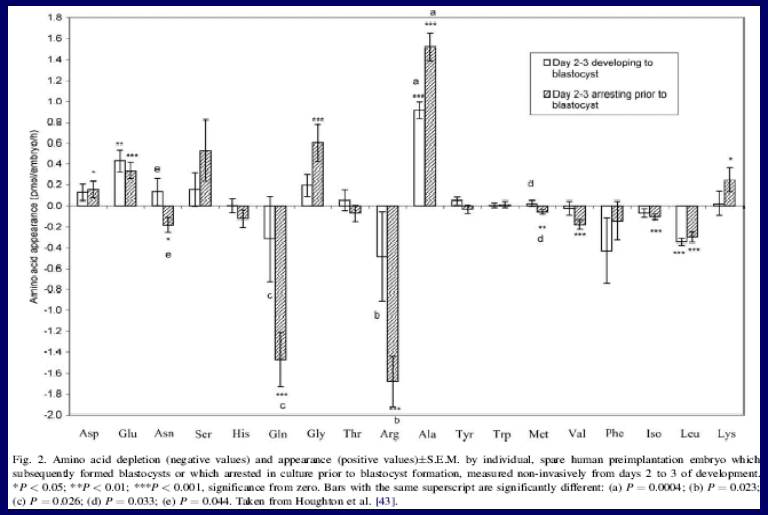


Fig. 2. Amino acid depletion (negative values) and appearance (positive values)±S.E.M. by individual, spare human preimplantation embryo which subsequently formed blastocysts or which arrested in culture prior to blastocyst formation, measured non-invasively from days 2 to 3 of development. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, significance from zero. Bars with the same superscript are significantly different: (a) $P = 0.0004$; (b) $P = 0.023$; (c) $P = 0.026$; (d) $P = 0.033$; (e) $P = 0.044$. Taken from Houghton et al. [43].

Houghton et al, 2002

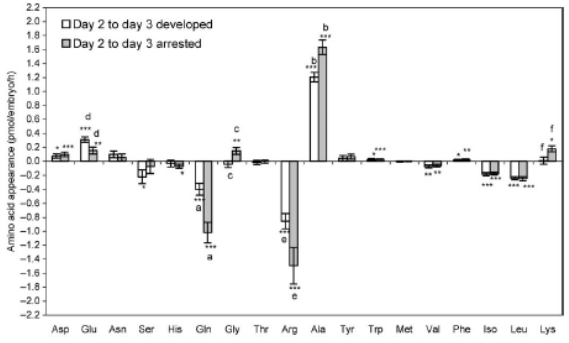


Figure 1. Amino acid depletion and appearance by frozen–thawed human embryos from day 2 to day 3 of development. For embryos which developed to the blastocyst stage, $n = 21$ and for embryos which arrested prior to the blastocyst stage, $n = 25$. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ significance from zero. Bars with the same superscript are significantly different; a and b, $P = 0.001$; c, $P = 0.0025$; d, $P = 0.016$; e, $P = 0.032$; f, $P = 0.0448$.

Stokes et al, 2007

Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover

D.R.Brison[1,5], F.D.Houghton[2], D.Falconer[3], S.A.Roberts[4], J.Hawkhead[2], P.G.Humpherson[2], B.A.Lieberman[1,3] and H.J.Leese[2]

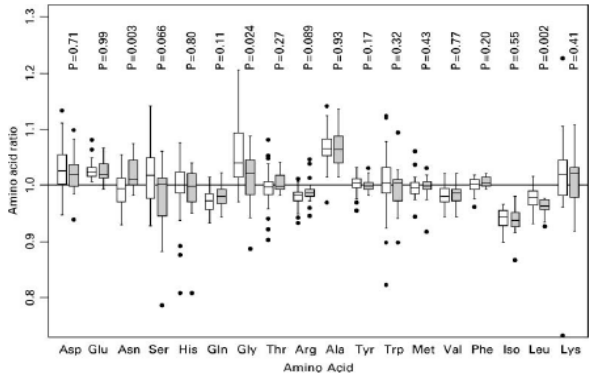


Figure 1. Box plots of the 18 individual amino acids showing the medians (thicker lines), inter-quartile ranges (boxes), ranges (whiskers; excluding outliers) and outlying observations (open circles) for amino acid appearance in the culture medium. Open boxes are the cycles that did not achieve a pregnancy and shaded boxes those yielding a pregnancy. *P*-values are for a Mann–Whitney test comparing the two groups for each amino acid.

Embryo metabolism (2)
Pyruvate and Glucose

- switch from lactate and pyruvate to glucose metabolism around compaction
- **Pyruvate** (<Day 4) and **glucose** uptake (>Day 4) :
  - largely studied in the 90's
  - Animal models (Gardner DK, 2000)
  - not proved to be relevant in humans up to now
- Association of both could be correlated with blastocystis formation (Gardner et al, 2000, 2001).
- *Limits : technical aspects, assay method, correlation with implantation ?*

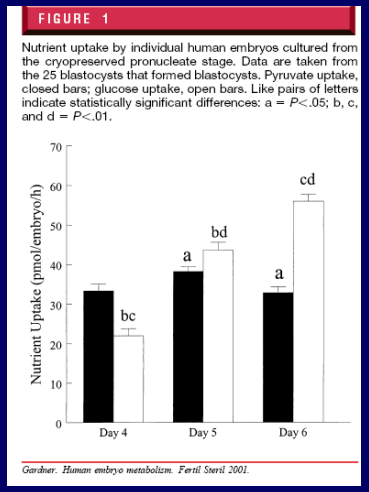


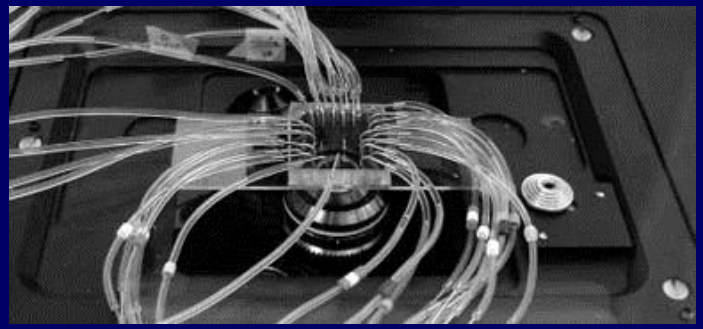
FIGURE 1

Nutrient uptake by individual human embryos cultured from the cryopreserved pronucleate stage. Data are taken from the 25 blastocysts that formed blastocysts. Pyruvate uptake, closed bars; glucose uptake, open bars. Like pairs of letters indicate statistically significant differences: a = $P<.05$; b, c, and d = $P<.01$.

Gardner. Human embryo metabolism. Fertil Steril 2001.

- In the future : technical improvements

such as microfluidic chips (Urbanski, 2008)



Embryo metabolism (3)
Metabolomic analysis

- Metabolome : reflects individual phenotype
- Near InfraRed or RAMAN Spectroscopy
- Prediction of embryo viability (and subsequent implanation) according to spectra-based algorithm
- Currently under definitive validation by RCT

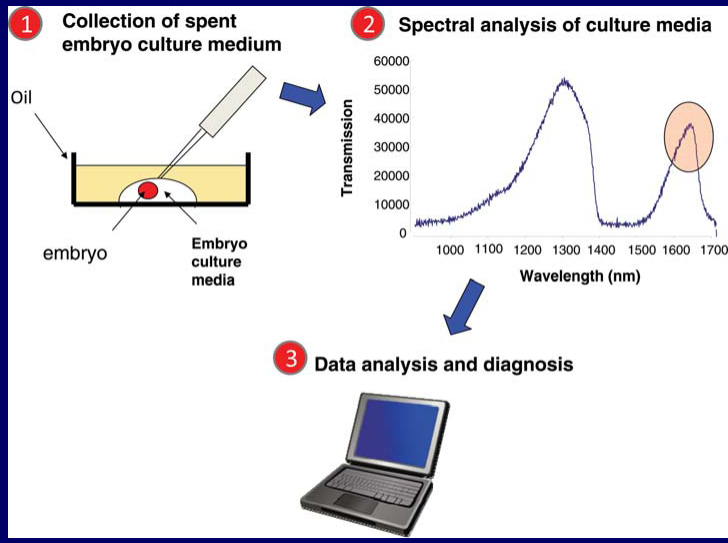


Table IV. Studies of non-invasive metabolomic profiling of embryo culture media to assess embryo viability in IVF.

Study	Study design	*n*	Day of transfer	Number of embryos transferred	Analytical technique	Center	Findings
Seli *et al.* (2007)	Algorithm development	36	Day 3	MET	Raman	YFC	A
Scott *et al.* (2008)	Blinded analysis	41	Day 3 and 5	MET	Raman	RMANJ	B
Seli *et al.* (2007)	Algorithm development	33	Day 3	MET	NIR	RMANJ	A
Seli *et al.* (2007)	Blinded analysis	16	Day 3	MET	NIR	YFC	B
Seli *et al.* (2008b)	Algorithm development	121	Day 2	SET	NIR	KLC	A, C
Seli *et al.* (2008b)	Blinded analysis	60	Day 2	SET	NIR	KLC	B, D
Vergouw *et al.* (2008)	Algorithm development	29	Day 2	SET	NIR	VUMC	A, C
Vergouw *et al.* (2008) Seli *et al.* (2008b)	Algorithm development	304	Day 3	SET	NIR	VUMC	A, C
Hardarson *et al.* (2008)	Algorithm development	137	Day 5	SET	NIR	FCG, SG	A, C, D

SET, single embryo transfer; MET, multiple embryo transfer; YFC, Yale Fertility Center, New Haven, CT, USA; RMANJ, Reproductive Medicine Associates, Morristown, New Jersey, USA; VUMC, Vrije Universiteit Medical Center, Amsterdam, The Netherlands; KLC, Kato Ladies Clinic, Tokyo, Japan; FCG, Fertilitets centrum, Göteborg, Sweden; SG, Shady Grove Fertility Reproductive Science Center, Rockville, Maryland, USA.
A, the mean viability score of embryos that implanted and resulted in fetal cardiac activity or live birth was significantly higher compared with the mean viability score of embryos that failed to implant; B, spectroscopic analysis by an observer blinded to pregnancy outcome, using a previously established regression algorithm demonstrated that the mean viability score of embryos that resulted in a pregnancy was higher compared with embryos that failed to implant; C, study showed the metabolomic profile of embryo culture media to be independent of morphology; D, a positive correlation was detected between increasing viability scores and the potential of individual embryos to result in a pregnancy.

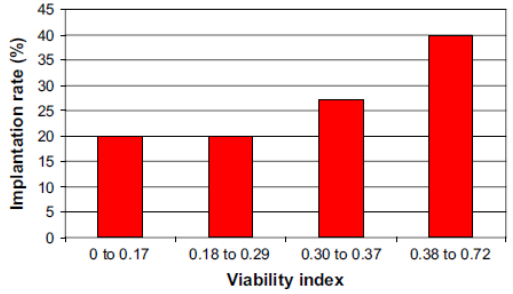
Botros et al, Mol Hum Rep, 2008

Embryo metabolism (3)

Metabolomic analysis : Viability scores / index

FIGURE 1

Viability indices determined in the blinded analysis of day 2 embryo culture media shows a positive correlation with pregnancy. Viability indices determined by blinded analysis of the spent culture media of 60 embryos transferred on day 2 were analyzed in quartiles (15 patients in each group). A positive correlation between increasing viability index value and the reproductive potential of individual embryos was observed (Pearson correlation coefficient = 0.5241, $P<.001$).

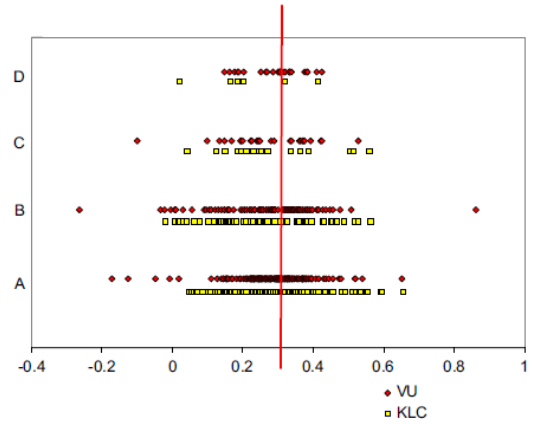


Seli. Metabolomic profiling of human embryos. Fertil Steril 2009.

Correlation with implantation

FIGURE 2

The relationship between the relative viability index and morphological grade for day 3 and day 2 embryos. Viability indices of day 3 (n = 304, *diamonds*) and day 2 (n = 181, *squares*) embryos is shown for each morphological grade. The Pearson correlation coefficients for day 3 and day 2 embryos were 0.0002 and −0.1223, respectively. The null hypothesis of no association is accepted for both day 3 ($P=.9975$) and day 2 ($P=.1009$) samples. The red line indicates the cutoff predicted by receiver operating characteristic analysis.

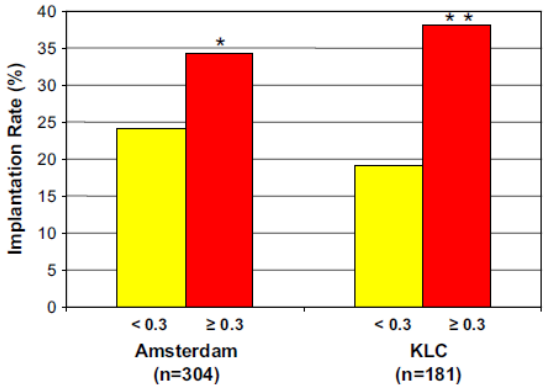


Seli. Metabolomic profiling of human embryos. Fertil Steril 2009.

Independant of morphology

FIGURE 3

Implantation rates of day 2 and day 3 embryos with viability indices below (*yellow*) and above (*red*) the cutoff value (0.3) determined by receiver operating characteristic analysis. Both day 3 and day 2 embryos with a high viability index (≥0.3) demonstrated a higher implantation rate compared with those with a lower viability index (<0.3) (*$P<.05$; **$P<.001$).



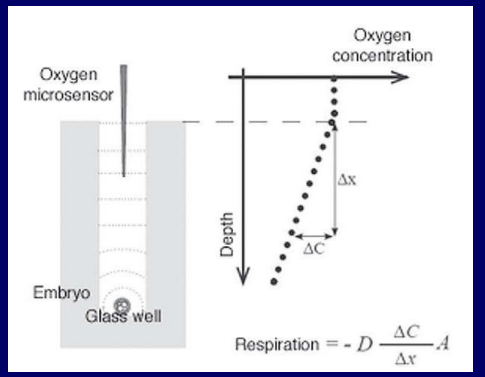
Seli. Metabolomic profiling of human embryos. Fertil Steril 2009.

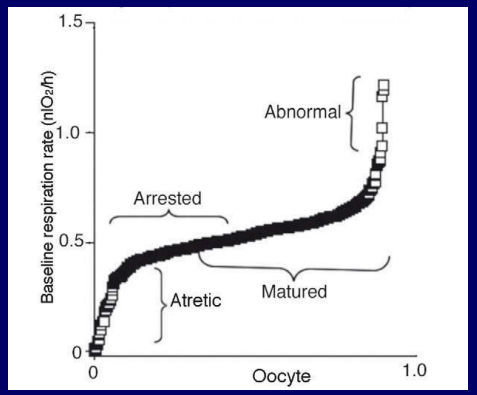
Independant of the day of assessment

Commercially available

Embryo metabolism (4) Respirometry

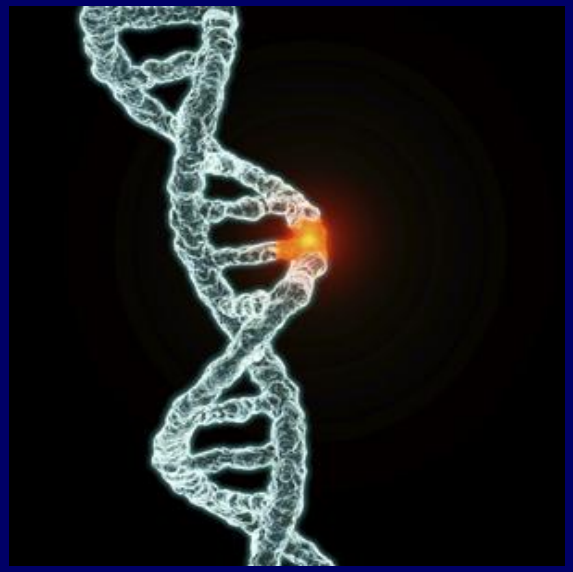
- Oxygen consumption of preimplantation embryos has been shown to reflect developmental competence and subsequent implantation in animal models (Lopes AS et al, Theriogenelogy, 2007).
- Preliminary work on human oocyte with the Embryoscope® (Scott et al, RBMonline, 2008)
- No proof of efficiency on human embryos up to now





Qualité embryonnaire

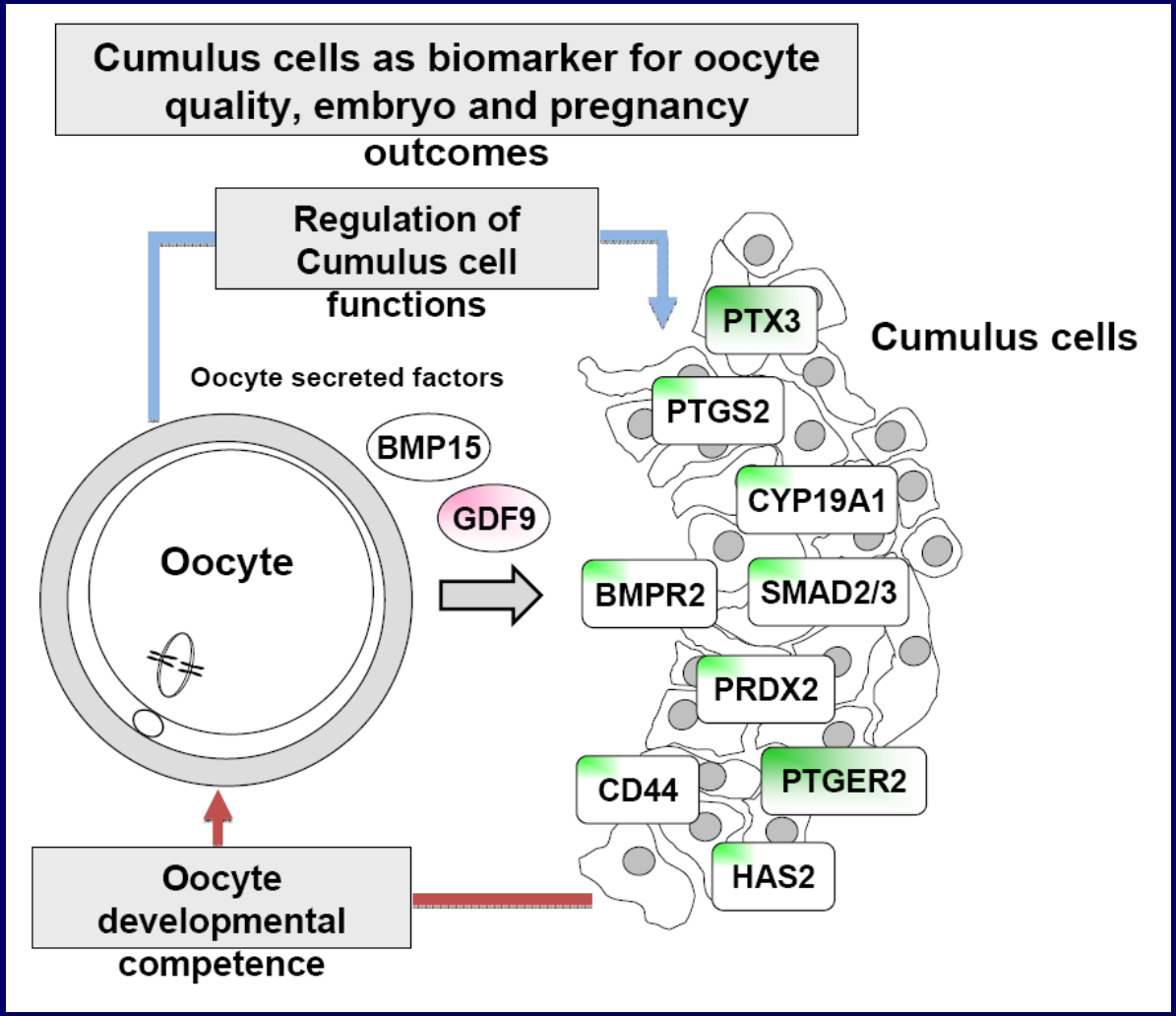
Génomique



- Expression génique

  Cellules du cumulus

  Hamamah et al 2007; 2008; 2009; 2010

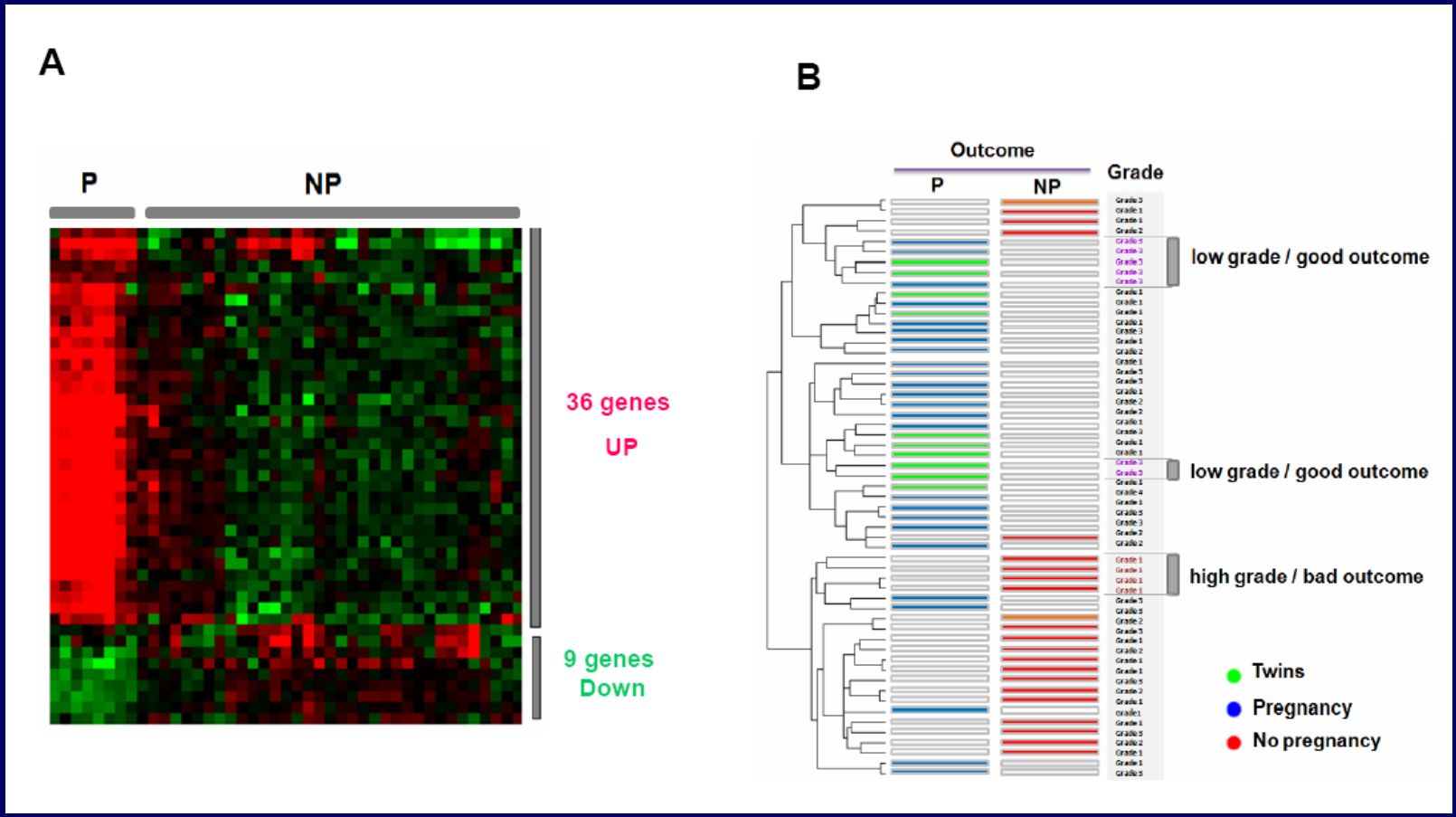


ASSOU S et al, Mol Hum Reprod, 2010, in press

Pas de corrélation entre le grade embryonnaire et le potentiel d'implantation

Score génomique différent du score embryonnaire

Hamamah et al, 2009 / 2010



ASSOU S et al, Mol Hum Reprod, 2010

Expression génique des cellules du cumulus

Différences observables entre stimulations par hMG ou rFSH

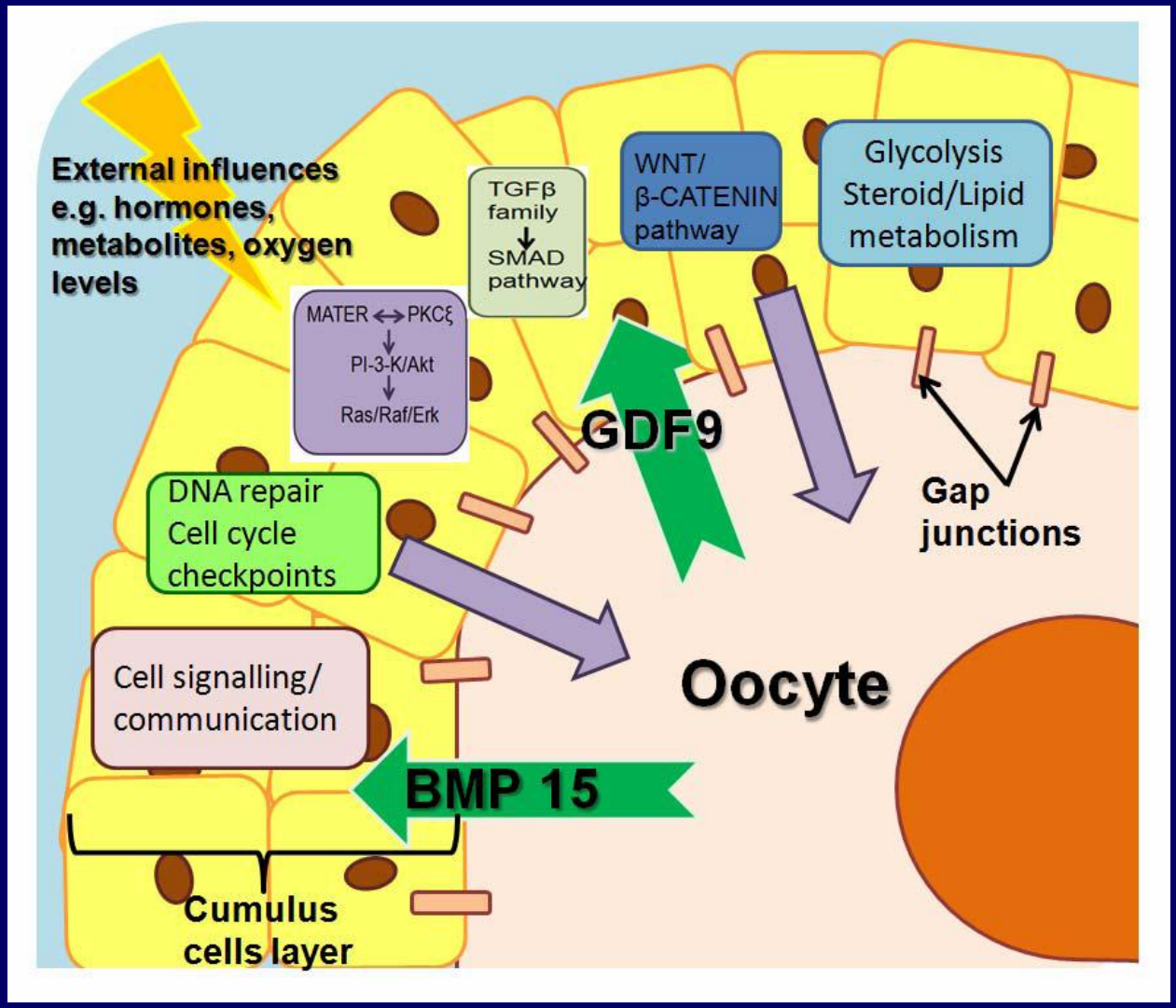
Adriaenssens et al

Human Reproduction 2010

TRANSCRIPTOME DES CELLULES DU CUMULUS

Huang & Wells

Mol Hum Reprod, 2010



Huang & Wells, Mol Hum Reprod, 2010

Table 1 Summary of studies on the analyses of human CCs

Recent human cumulus cell studies aimed at understanding follicular influences on oocyte competence			
Samples used	*Factor(s) studied*	*Outcome measures*	*Reference*
Pooled samples of CCs from individual patients	8-hydroxy-2′-deoxyguanine, a by-product of oxidative stress-induced digestion	Oocyte fertilisation rates Embryo quality	Seino et al, 2002
Pooled samples of CCs from individual patients	Superoxide dismutases	Maternal age Female fertility diagnoses ART outcome	Matos et al, 2009
Pooled samples of GCs /CCs from individual patients Human granulosa tumour cell line , KGN	Glutathione S transferase theta 1	Maternal age Oocyte quality	Ito et al, 2008
Pooled samples of CCs	WNT/β-catenin pathway	Expression levels of WNTs, FZD receptors and pathway components of WNT/β-catenin pathway	Wang et al, 2009
Pooled samples of CCs	PKCα–MATER pathway	PKCα–MATER interactions	Maraldi et al, 2009
Pooled samples of CCs from patients	CCs apoptosis rate	Zona pellucida thickness variation Maturation stage of oocytes Fertilisation rate Embryo score	Host et al, 2002
Individual follow up of each oocyte collected, CCs associated with the oocyte and subsequent embryo development Single embryo transfer and double embryos transfer performed and pooled together for analysis	CCs apoptosis rate	Blastocyst formation rate	Corn et al, 2005

Huang & Wells, Mol Hum Reprod, 2010

Recent human cumulus cell studies aimed at identifying new markers of oocyte viability			
Samples used	*Genes of interest*	*Outcome measure*	*Reference*
Pooled samples of CCs and oocytes	BARD1, RBL2, RBBP7, BUB3 and BUB1B	Oocyte regulator and maturation markers	Gasca et al, 2007
Individual follow up of each oocyte and associated CCs until embryo development Double embryo transfer performed	BCL2L11, PCK1, NFIB	Embryo score Clinical pregnancy	Assou et al, 2008
Individual follow up of each oocyte and associated CCs until embryo/blastocyst development	Cx43, STAR, COX2, AREG, SCD1 and SCD5	Oocyte maturity Embryo quality Blastocyst development	Feuerstein et al, 2007
Individual follow up of each oocyte and associated CCs until embryo	PTGS2, HAS2 and GREM1	Oocyte maturity Embryo quality	McKenzie et al, 2004

development			
Individual follow up of each oocyte and associated CCs until embryo development	GREM1 and HAS2	Oocyte fertilisation rate Embryo quality	Cillo et al, 2007
Individual follow up of CCs associated with oocytes which give rise to early cleavage and non early cleavage embryos	CCND2, CXCR4, GPX3, CTNND1, DHCR7, DVL3, HSPB1 and TRIM28	Oocyte quality Embryo quality	Van Montfoort et al 2008
Individual follow up of each oocyte and associated CCs until embryo development Single and double embryo transfers were performed and pooled together for analysis	CDC42, 3βHSD, SERPINE2, FDX1 and CYPA191	Clinical pregnancy outcome	Hamel et al, 2008

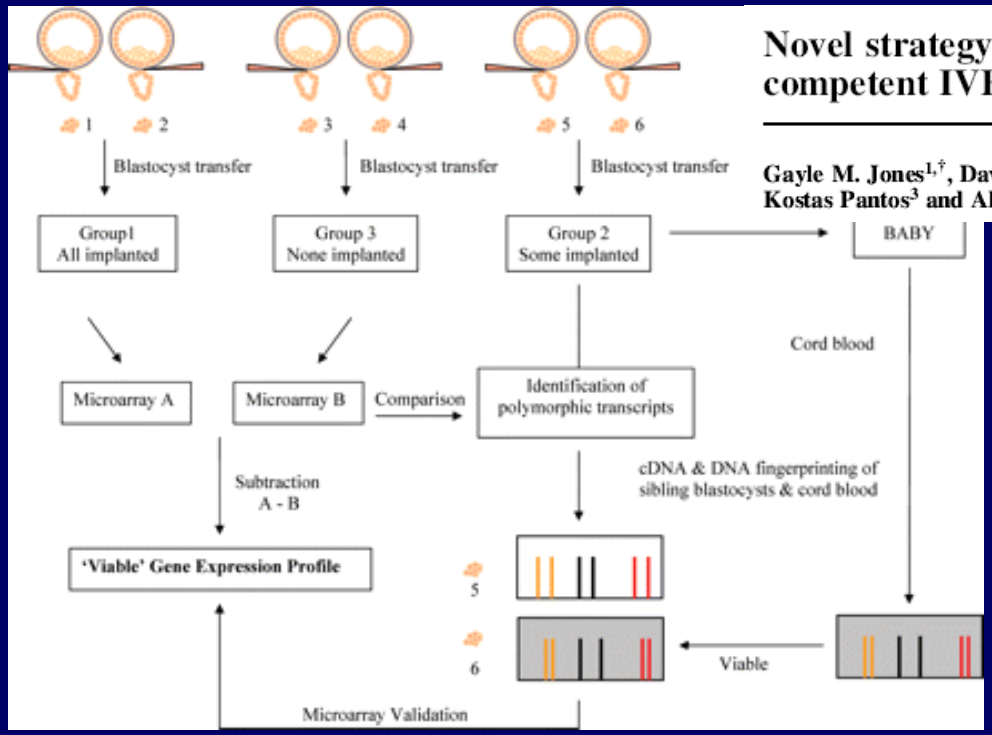
Huang & Wells, Mol Hum Reprod, 2010

Expression génique

Prélèvement de trophoblaste initial du blastocyste

-Microarray gene expression profiling

-DNA fingerprinting



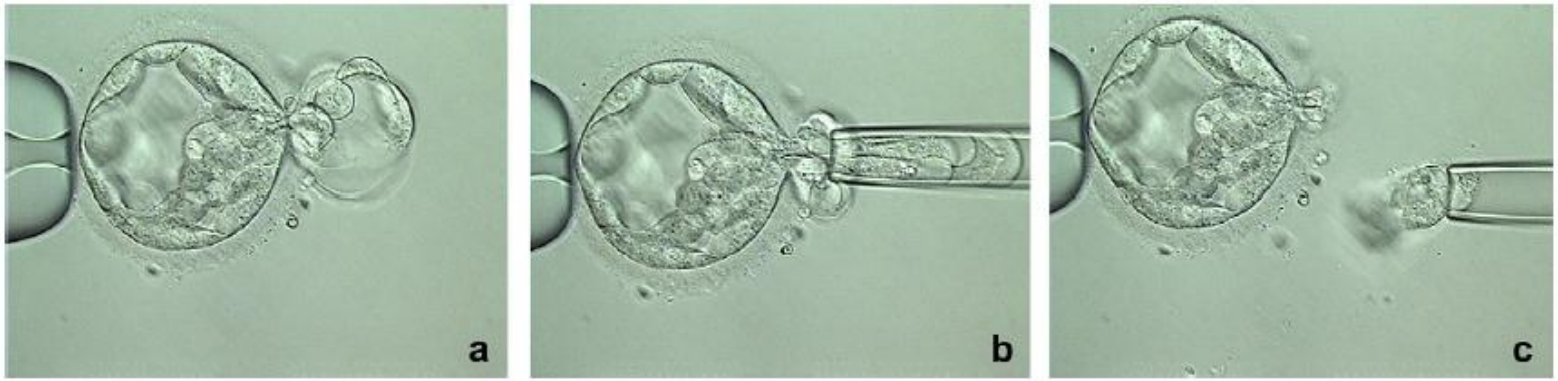
Novel strategy with potential to identify developmentally competent IVF blastocysts

Gayle M. Jones[1,†], David S. Cram[1,2,5,†], Bi Song[1], Georgia Kokkali[3,4], Kostas Pantos[3] and Alan O. Trounson[1,2]

Human Reproduction 2009

FIGURE 1

Blastocyst biopsy. On day 3, a small "channel" in the zona pellucida approximately 5 μm wide was created using a laser (Hamilton Thorne). Embryos were then transferred to blastocyst-stage medium and incubated for a further 48 hours (day 5 of development). Day 5 and 6 blastocysts graded as grade 3 or higher (19) with herniating trophectoderm cells were identified for biopsy (**A**). Using an inverted Nikon microscope with Narishige manipulators and injectors, the herniating trophectoderm cells protruding through the day 3 hole in the zona pellucida were aspirated into a 30 μm biopsy tool (**B**), and the laser was fired at the constricted area of cells to gently separate them from the rest of the blastocyst (**C**).



Schoolcraft. Clinical application of CGH on blastocysts. Fertil Steril 2009.

Schoolcraft et al, Fertil Steril, 2010

TABLE 2

Comparison of patient characteristics and treatment cycle outcome for patients with aneuploidy screening and a set of contemporary cycles from the same clinic.

	Contemporary comparison group (n = 113)	Comprehensive chromosome screening group (n = 45)
Average maternal age (yrs)	37.1	37.7
Average no. of previous failed IVF treatments	1.24	2.42
Day 3 FSH (IU)	7.6	7.3
Average no. of oocytes retrieved per cycle	19.4	18.6
Average no. of blastocysts transferred per cycle	2.7 (299 transfrred in 113 cycles)	2.0 (90 transferred in 45 cycles)
Biochemical pregnancy (positive pregnancy test) per cycle[a]	84.0% (95/113)	82.2% (37/45)[a]
Implantation rate (proportion of transferred embryos producing a fetal sac)	46.5% (139/299)	72.2% (65/90)[b]
Implantation rate (proportion of transferred embryos producing a fetus with heartbeat)	44.8% (134/299)	68.9% (62/90)[c]

[a] Only one patient had no euploid embryos available for transfer. The pregnancy rate per cycle with an embryo transfer was 84.1% (37/44).
[b] $P < .0001$ (chi-squared test with Yates correction).
[c] $P < .0001$ (chi-squared test with Yates correction).

Schoolcraft. Clinical application of CGH on blastocysts. Fertil Steril 2009.

Schoolcraft et al, Fertil Steril, 2010

Ploïdie des ovocytes et des embryons

	20-34	***35-39***	***40-45 ans***
aneuploïdie des ovocytes (%)	15	25	55
taux d'implantation (%)	35	28	7
Taux d'implantation des blastocystes sélectionnés par CGH (%)	75	75	65

Wells et al, 2010

Origine de l'aneuploïdie

Aberrations métaboliques dans les cellules du cumulus associées à l'aneuploïdie des ovocytes : 261 gènes exprimés différemment

Wells et al, 2010

Nombre de blastomères à J3 et risque de fausse-couche *(Hourvitz et al)*

RBM Online - Vol 13. No 4. 2006 504-509 Reproductive BioMedicine Online; www.rbmonline.com/Article/2156 on web 1 August 2006

Article

Role of embryo quality in predicting early pregnancy loss following assisted reproductive technology

No. of cells in the leading embryo transferred			
4–5	89	40 (44.9)	
6–8	710	218 (30.7)	
9–10	17	1 (5.9)	0.005

Choix de la stratégie de transfert

- Critères cliniques et biologiques
- Contre-indications à la grossesse gémellaire
- Politique de centres
- Efficience du programme de congélation

Comment évaluer a priori la compétence embryonnaire

1- choix de l'embryon à transférer

2- choix de la culture prolongée ou non

Intérêts de la culture prolongée

- Augmentation du taux d'implantation ?
- Sélection d'embryons euploïdes ?
- Facilitation de l'acceptation du single embryo transfer (SET) ?

Risques de la culture prolongée

- Vécu de l'absence de transfert ?
- Perte de chance ?
- Augmentation de la fréquence des grossesses multiples monozygotiques ?

Absence de transfert après culture prolongée

8 études randomisées : ***de 0 à 28 %***

Papanikolaou et al 2008

Embryo *[transfer strategy]* selection

- Extended culture to the blastocyst stage is thought to result in better embryo selection and higher implantation rates.
- BUT … is it suitable for every patients ? For every cycles ?
- Nomogram = statistical tool to generate graphical representation of a predictive model
- Could help in reducing the incidence of cancelled Day 5 transfers
- Currently undergoing external multicenter validation

human reproduction ORIGINAL ARTICLE *Embryology*

A cycle-based model to predict blastocyst transfer cancellation

Lionel Dessolle[1,2,4], Thomas Fréour[1], Paul Barrière[1], Emile Daraï[2], Célia Ravel[3], Miguel Jean[1], and Charles Coutant[2]

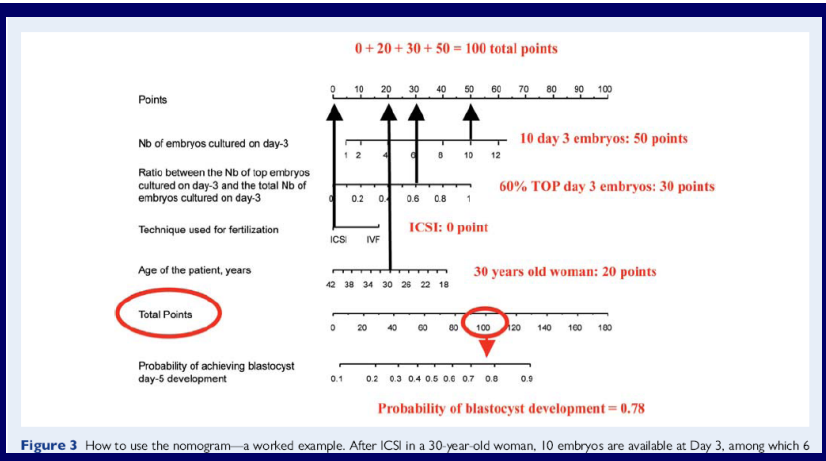


Figure 3 How to use the nomogram—a worked example. After ICSI in a 30-year-old woman, 10 embryos are available at Day 3, among which 6

Nomogramme : Modèle prédictif

Facteurs indépendants associés à l'obtention de blastocystes :

Age, nombre et qualité à J3, technique de fécondation

Cut-off de 55 % (NPV : 80.5 % FP 17 %)

Dessolle et al 2010

Transferts ultra-précoces non validés par des études méthodologiquement satisfaisantes

Essai de transferts à J1 en cas de fragmentation répétée

ETUDE PROSPECTIVE NON RANDOMISEE
Guerif et al 2009

SET VS SBT

Inclusion à J2 sans critères embryonnaires

	SET n=243	***SBT*** n=218	
Taux d'accouchement Par ponction (transfert frais)	25.1 %	34 %	*(p<0.05)*
Taux d'accouchement Cumulatif/couple	34.2 %	37.9 %	NS
Nombre cycles d'AMP/ Accouchement	4.65	3.49	*(p<0.04)*

ETUDE RANDOMISEE PROSPECTIVE J2 vs J5 en SET

Brugnon et al ESHRE 2010 (0-155)

- Patientes incluses : Plus de 5 ovocytes matures
  3 Top embryons à J2
- 52 SET à J2 VS 55 SBT à J5
- Taux cumulatif de grossesses incluant les embryons congelés 51.92 % vs 49.09 %

	SET J2	SBT J5
TG après transfert « frais »	46.15 %	41.82 %
Taux de congélation	98.07 %	76.36 %
Nombre embryons congelés	3.31 ± 0.28	2.33 ± 0.33
Taux de survie	71.87 %	53.19 %
Taux de SET après décongélation	78.94 %	91.30 %
Taux de grossesse après TEC	9.76 %	11.11 %
Taux d'implantation en TEC	8.69 %	20 %

Risque de grossesse multiple monozygotique après transfert de blastocyste

Dessolle et al RBM 2010

2.3 % des SET

3.4 % des SBT

Modalités de la culture prolongée

- Milieu global ?
- Milieu séquentiels ?
- Teneur en oxygène des milieux ?
- Coculture sur cellules endometriales : Endocell ?

CONCLUSIONS (1)

Avantages probables :

- taux de grossesse
- Facilitation du SET

Mais :

- conditions de culture optimales nécessaires
- Apport de la coculture à valider
- Attente de la vitrification

CONCLUSIONS (2)
CHOIX de l'EMBRYON à transférer

- CRITERES MORPHOLOGIQUES INSUFFISANTS
- METABOLOMIQUE NON VALIDEE
- FONCTIONNELLE (GSF) : TARDIVE
- GENOMIQUE : PRECOCE (GP1, cumulus)
  RAPIDE