

Sélection embryonnaire :

Le présent et l'avenir

Coup de projecteur sur le Time lapse

Dr Laurent Corcia

Médecine et Biologie de la Reproduction- CHR Orléans

Pr F. Guérif

Médecine et Biologie de la Reproduction- CHRU Tours



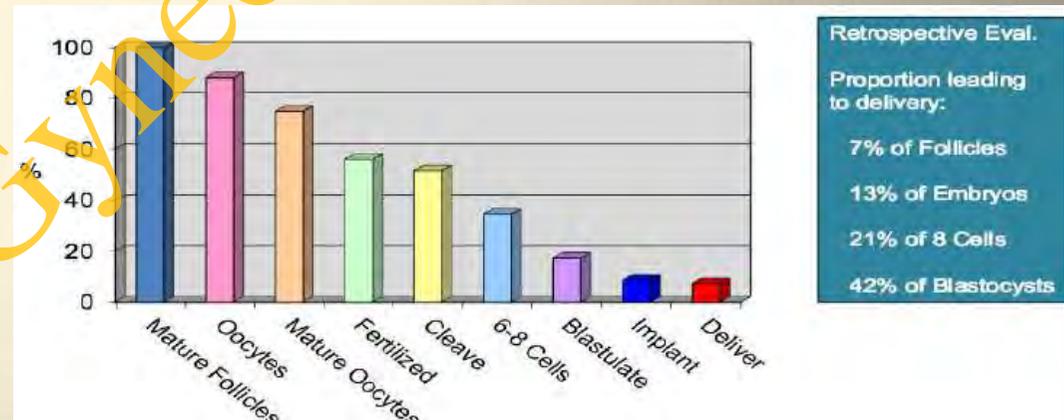
INTRODUCTION

- \approx 60 000 tentatives de FIV / an
- > 300 000 embryons produits / an

Malgré l'avancée des connaissances en FIV, les résultats restent relativement décevants :

- ❑ Taux d'implantation relativement limités (18-19%)
- ❑ 40% de transfert mono-embryonnaire
- ❑ Taux de G multiples relativement \uparrow (15,6% Gx2 en 2014, ABM)

Comment améliorer ces résultats?



EMBRYONS SANS POTENTIEL DE DEVELOPPEMENT

- | **Importance du choix de l'embryon à éliminer**
 - **Si erreur => Pertes de chances pour le couple**

EMBRYONS AVEC UN POTENTIEL DE DEVELOPPEMENT

- | **Importance du choix de l'embryon à transférer**
 - **Développement du transfert embryonnaire unique**
- | **Importance du choix de l'embryon à congeler**
 - **Si erreur => stockage massif improductif**

METHODES D'EVALUATION ET DE SELECTION DES EMBRYONS

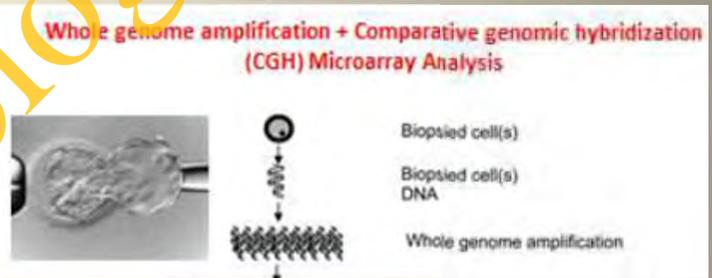
Etude morphologique



Etude morphocinétique

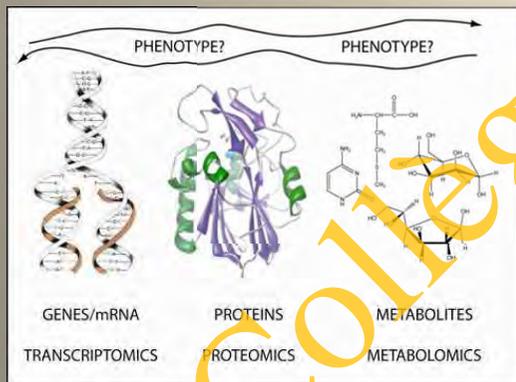


Diagnostic génétique



Existe-t-il une méthode de sélection idéale?

OMICS :
Diagnostic moléculaire



Impression 3D



La morphologie conventionnelle en routine...

- **Informations statiques isolées**
(*t0 ou t1 ou t2 ou t3 ou t5*)
- **Informations statiques combinées**
(*t0+t1+t2+t3+t5*)

Construction de scores

Taux d'implantation

Développement *in vitro*

Transfert unique / suivi individuel

EVALUATION MORPHOLOGIQUE EMBRYONNAIRE PRECOCE



Ovo
J0



2 PN



Cliv
Précoce



Emb
J2



Emb J3



0

16-20H

25-27H

42-44H

66-68H

Méthode standard d'évaluation de la qualité embryonnaire la plus répandue

QUALITE OVOCYTE

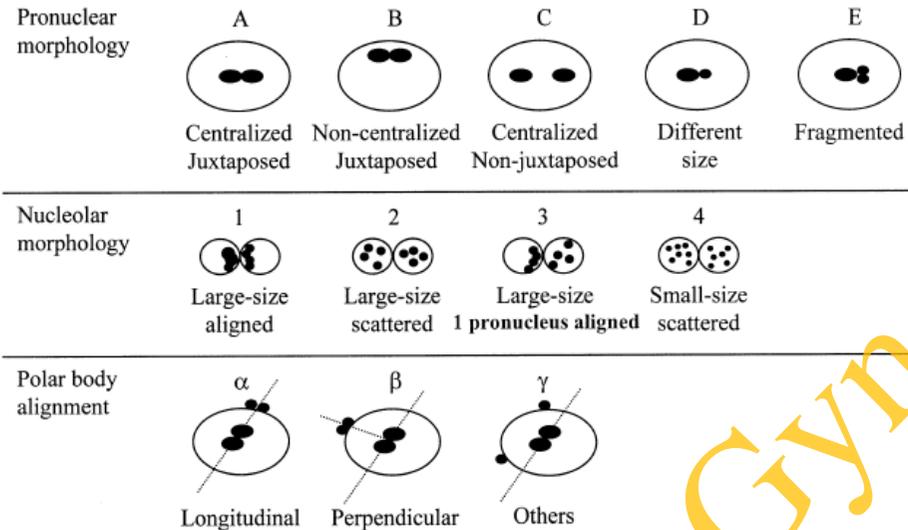


Rienzi et al, 2012

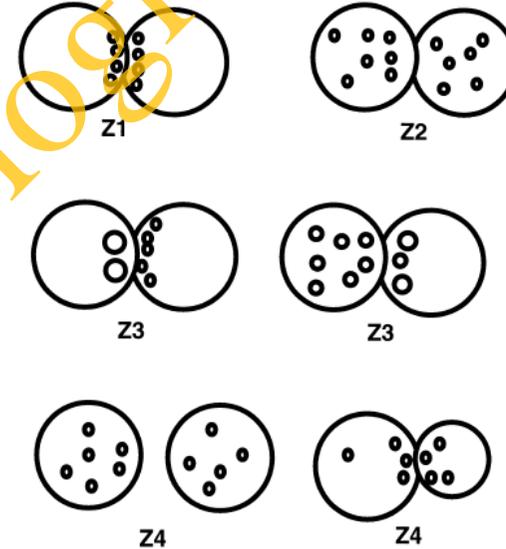
- Diam : 120-130 μm
- Cytoplasme de texture uniforme
- GP non fragmenté
- EPV limité sans débris

**Données disponibles controversées
ou restreintes à certains groupes**

SCORE ZYGOTIQUE



Gianirolli et al, Fertil Steril, 2003



Scott et al,
Hum Reprod, 2000

- | Absence de score consensuel pour évaluer les zygotes
- | La morphologie des pronuclei est un processus dynamique
- | Effet observateur dépendant lié à l'entraînement

Données controversées de la littérature

CLIVAGE PRECOCE



Présence des 2 PN



Disparition des PN



Premier clivage
2 Cell

25H post FIV
ou
27H post ICSI

Données consensuelles dans la littérature

MORPHOLOGIE EMBRYONNAIRE A J2-J3

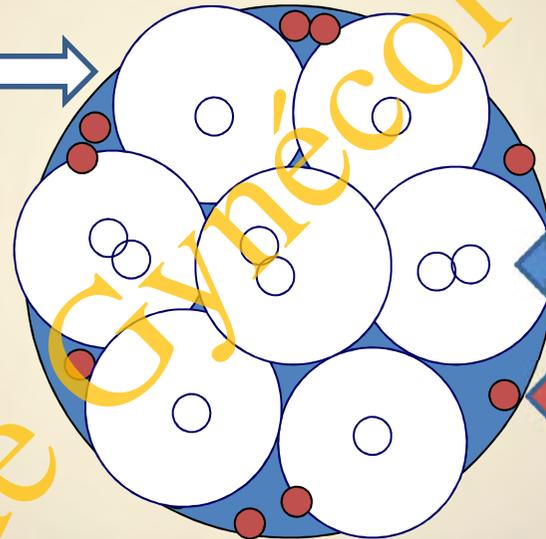
Nbre et
régularité des blastomères

Ex : 8 A

Nbre de blastomères

% de fragmentation*

Régularité des blastomères



BMN

% fragmentation

*

A : $\leq 10\%$

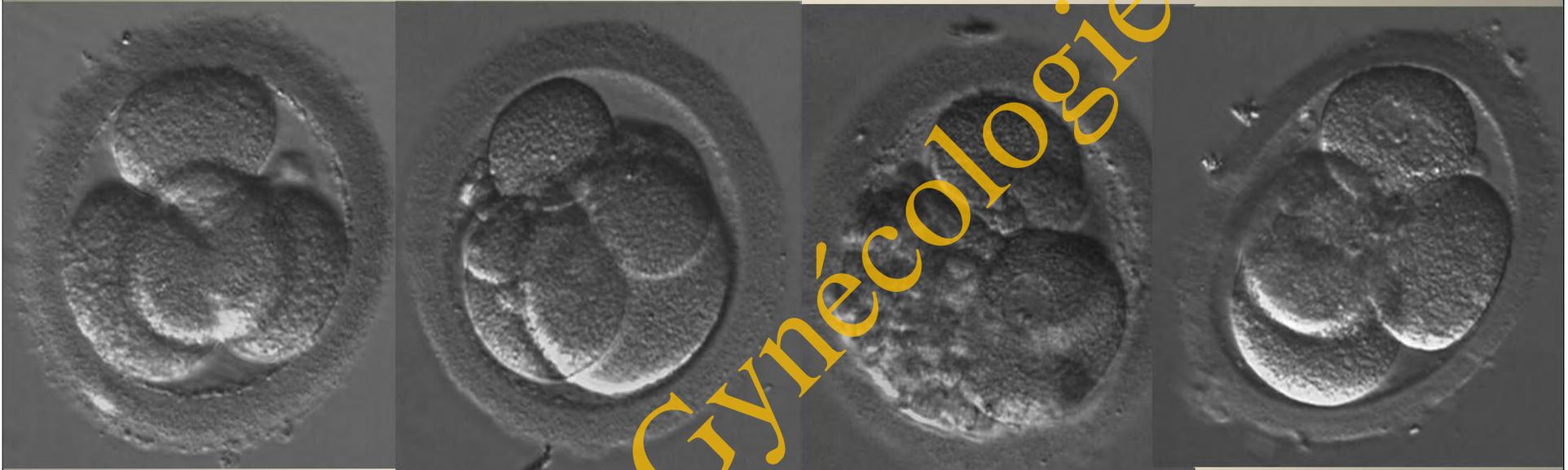
B : $> 10-30\%$

C : $> 30-50\%$

D : $> 50\%$

Morphologie embryonnaire

J2



Nombre
de blastomères

Aspect typique
des blastomères

Taux de
fragmentation

Multinucléation

Code à 3 chiffres

- Chiffre 1 : nb de cellules (2-6 à J2)
- Chiffre 2 : Aspect typique ou atypique des cellules / au nombre de cellules
- Chiffre 3 : taux de fragments
 - 1 : 0-10% du volume
 - 2 : 10-50% du volume
 - 3 : > 50% du volume

CRITERES DE SELECTION D'UN TOP EMBRYON J2-J3



- 4 Blastomères
- Taille égale
- Fragmentation $\leq 30\%$
- Absence de BMN



- 8 Blastomères
- Taille égale
- Fragmentation $\leq 30\%$
- Absence de BMN

CULTURE STADE BLASTOCYTE

Classification de Gardner

Notion de cinétique (J5 vs J6)

Stade d'expansion (1-6)

Aspect du trophoctoderme (A,B,C)

Aspect du bouton embryonnaire (A,B,C)



Gardner et al, 1998

CULTURE STADE BLASTOCYSTE

- ❑ Synchronisation embryon et utérus+++
 - ❑ Contractions utérines ↓
 - ❑ « sélection embryonnaire »
- => Tf embryonnaire unique
- ❑ ↑ Taux implantation

MAIS... INDICATIONS « LIMITEES »

**Combinaison
de paramètres individuels**

Construction de scores

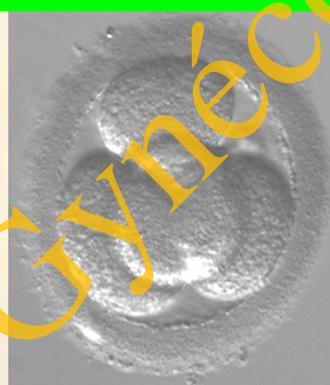
Evaluation embryonnaire séquentielle



J0



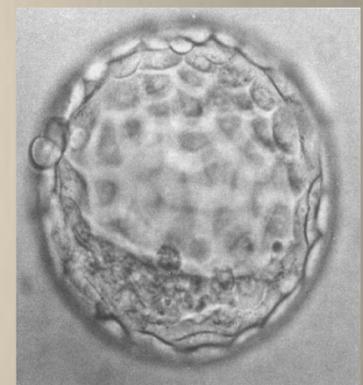
J1



J2



J3



J5/6

Collège de Gynécologie CML

The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos*

Jeffrey D.Fisch^{1,3}, Herman Rodriguez¹, Richard Ross¹, Gail Overby¹ and Geoffrey Sher^{1,2}

Table I. Graduated Embryo Scoring of cleavage-stage embryos

Evaluation	Hours after insemination	Developmental milestone	Score
1	16-18	Nucleoli aligned along pronuclear axis	20
2	25-27	Cleavage regular and symmetrical	30
		Fragmentation ^a : Absent	30
		<20%	25
		>20%	0
3	64-67	Cell number and grade ^b :	
		7, I; 8, I; 8, II; 9, I	20
		7, II; 9, II; 10, I; 11, I, compacting I	10
Total score			100

^aIf the embryo was not cleaved at 25-27 h, grading of fragmentation should occur at the 64-67 h evaluation.

^bGrade I = symmetrical blastomeres and absent fragmentation; grade II = slightly uneven blastomeres and <20% fragmentation; grade III = uneven blastomeres and >20% fragmentation.

Table III. Comparison of Graduated Embryo Scoring (GES) with blastocyst development

GES	Total embryos for extended culture	Blastocyst development [n (%)]
70-100	269	119 (44) ^a
90-100	110	70 (64) ^b
70-85	159	49 (31) ^c
0-65	714	61 (9)
30-65	443	49 (11) ^d
0-25	271	12 (4)
Total	983	180 (18)

^a $p < 0.001$ compared with GES 0-65 and to GES 30-65.

^b $p < 0.001$ compared with GES 70-85.

^c $p < 0.001$ compared with GES 30-65.

^d $p < 0.003$ compared with GES 0-25.

Human Reproduction, Vol.24, No.9 pp. 2104–2113, 2009

Advanced Access publication on June 2, 2009 doi:10.1093/humrep/dep198

human
reproduction

ORIGINAL ARTICLE *Embryology*

Is there an advantage in scoring early embryos on more than one day?

Catherine Racowsky^{1,5}, Lucila Ohno-Machado^{2,3}, Jihoon Kim²,
and John D. Biggers⁴

Etude rétrospective

Suivi de 1257 cycles

Etablissement d'une courbe ROC et calcul de l'AUC

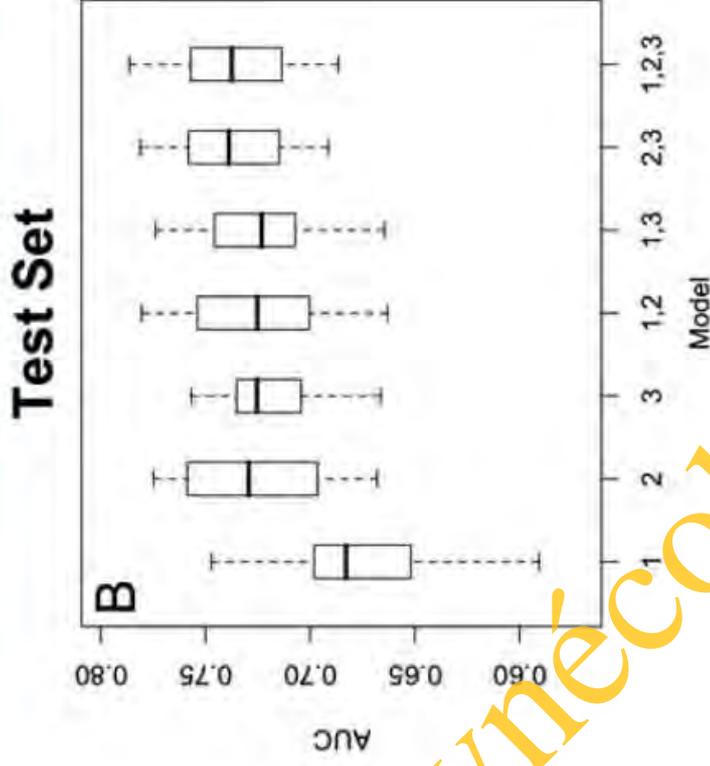
**pour déterminer si le cumul d'observations à J1 + J2 + J3
apporte une valeur ajoutée**

Point de mesure : Taux de grossesse clinique

Racowsky *et al.*, 2009

Table 1 List of embryonic features included in the study

Day	Feature	Comments (including coding of nominal data)
1	Time of evaluation	17.8–29 h after fertilization (target 25 h)
1	No. cells	Range: 1–6
1	Pronuclei	Code (day 1 stage): 0 = 1 cell + 2PN; 1 = 1 cell + 0PN; 2 = 2 cells; 3 = > 2 cells
2	Time of evaluation	37.6–50.7 h (target = 44 h)
2	No. cells	Range: 1–11
2	Fragmentation*	Code: 0 = 0%; 1 = 1–9%; 2 = 10–25%; 3 = 26–50%; 4 = > 50%
2	Symmetry	Code: 1 = No asymmetry; 2 = Moderate asymmetry; 3 = Severe asymmetry
2	Distribution of nuclei	Code: 0 = one or more blastomeres without a single nuclei; 1 = all blastomeres with a single nucleus
3	Time of evaluation	60.2–77.7 h (target = 68 h)
3	No. cells	Range: 1–14
3	Fragmentation*	Code: 0 = 0%; 1 = 1–9%; 2 = 10–25%; 3 = 26–50%; 4 = > 50%
3	Symmetry	Code: 1 = No asymmetry; 2 = Some asymmetry; 3 = Severe asymmetry



Analyse morphologique conventionnelle : les limites

- **Absence de consensus pour certains paramètres (J0, J1)**
- **Le poids décisionnel des paramètres morphologiques (J1, J2, J3) reste centre dépendant (=> variabilité des scores).**
- **Subjectivité lecture morphologiques (=> CQI + CQE)**
- **Trouver le compromis bénéfice / perte**
 - Effets issus des observations séquentielles ?
 - Effets générés par la culture prolongée ?

CRITERES METHODE IDEALE EVALUATION QUALITE EMB

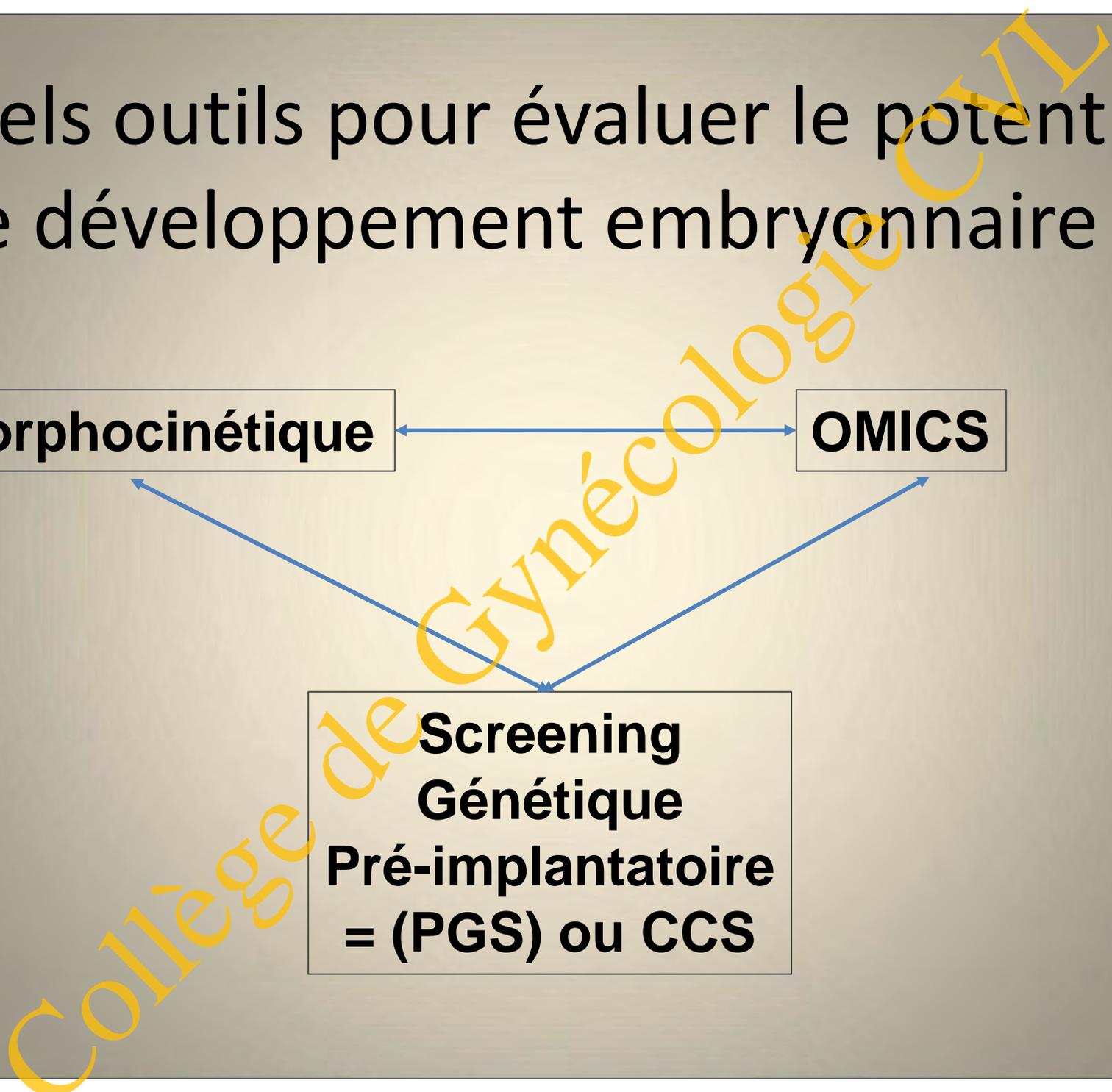
- Non invasive
- Applicable en routine
- Résultats rapides
- Facile d'emploi
- Reproductible
- Prix abordable
- Valeur prédictive pour l'obtention d'un blastocyste ↑

Quels outils pour évaluer le potentiel de développement embryonnaire ?

Morphocinétique

OMICS

**Screening
Génétique
Pré-implantatoire
= (PGS) ou CCS**

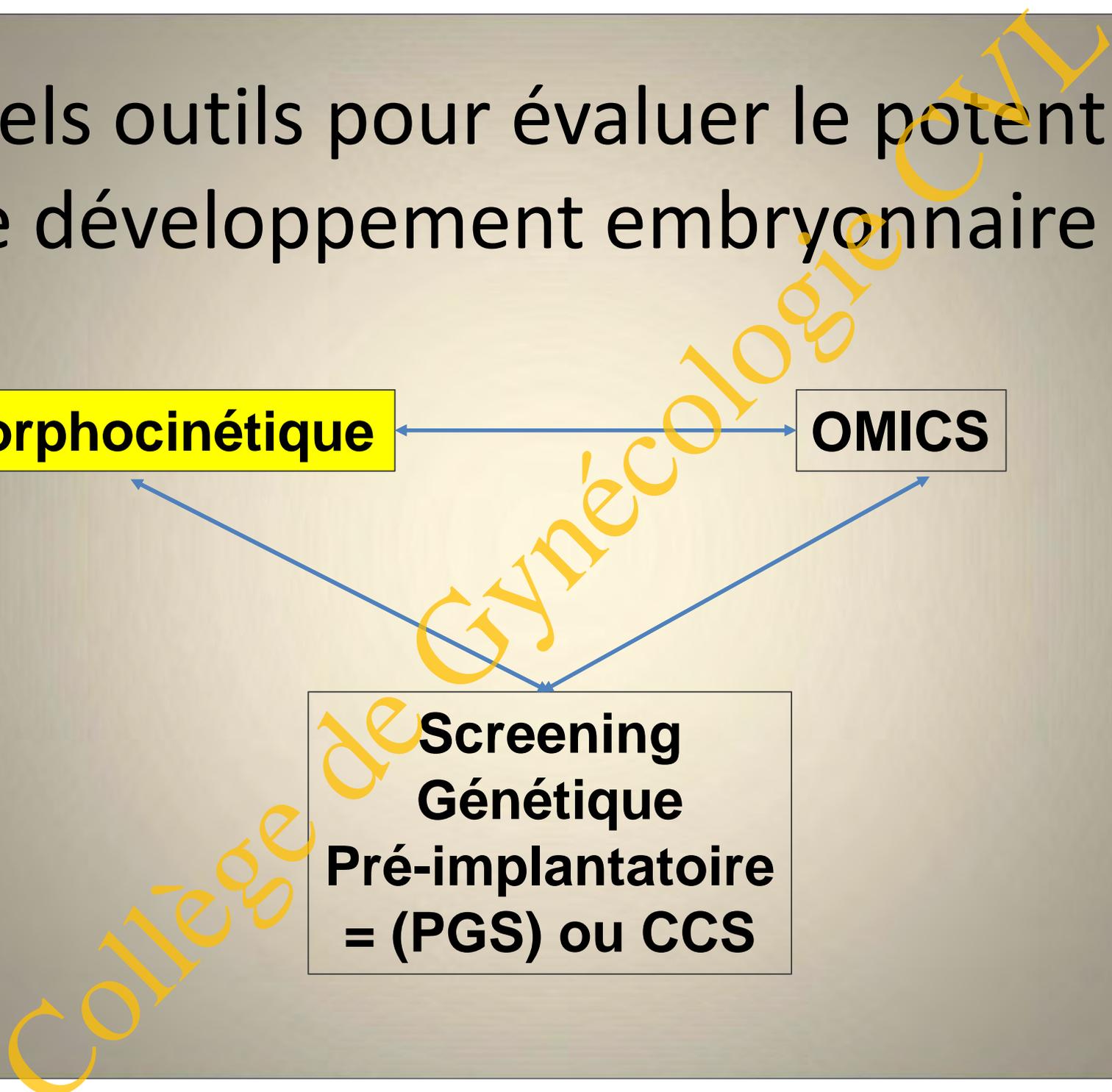


Quels outils pour évaluer le potentiel de développement embryonnaire ?

Morphocinétique

OMICS

**Screening
Génétique
Pré-implantatoire
= (PGS) ou CCS**



Time-Lapse : le futur de la morphologie ?

- Suivi morphologique dynamique semi-continu grâce à une caméra.
- Avantages :
 - * Absence de sortie des boîtes de culture de l'incubateur
⇒ Environnement STABLE
 - * SUIVI de la cinétique sur le plan :
 - quantitatif :
 - qualitatif : anomalies division, réab. fragments, collapse blastocyste...

Principaux systèmes commercialisés

- Embryoscope[®] :
 - Incubateur 3 gaz complet équipé de caméras
 - Boîtes de culture spécifiques
 - Image toutes les 10 minutes

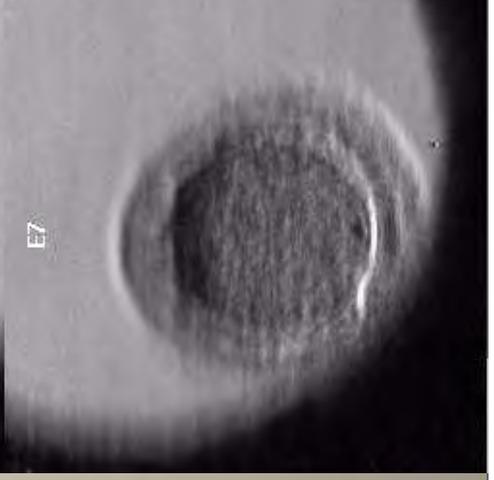
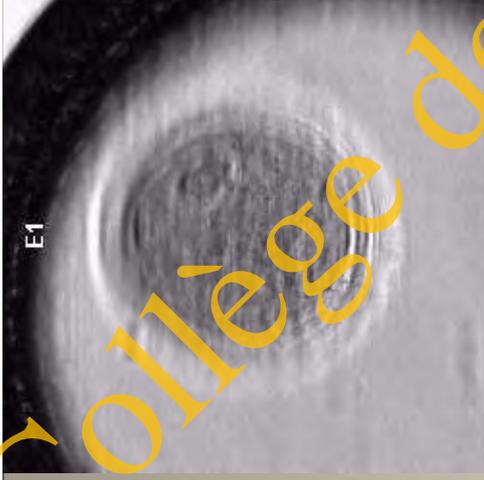
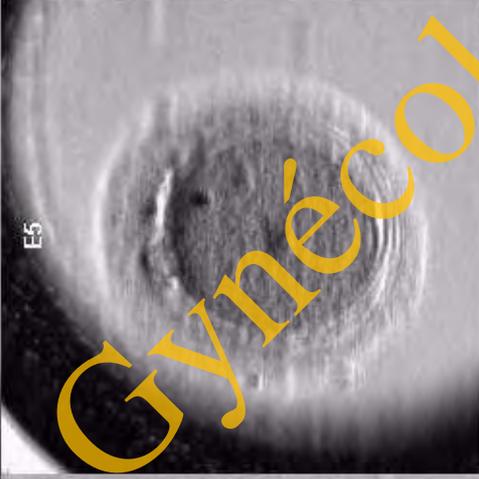
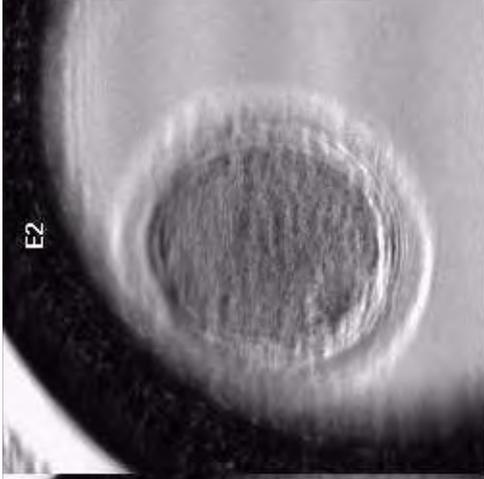
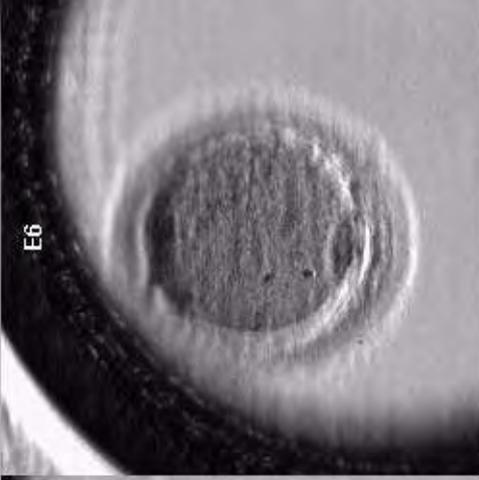
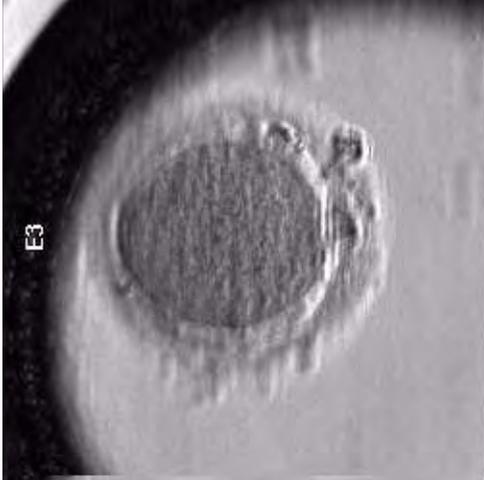


Embryoscope[®]

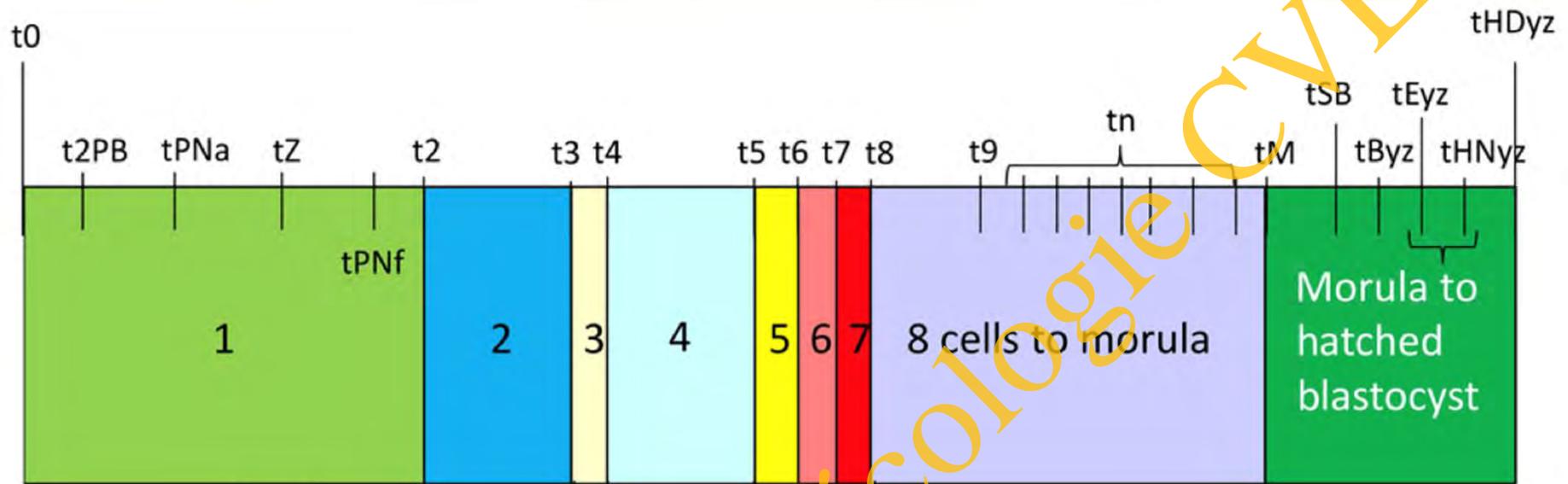
- Primo Vision[®] :
 - Equipement individuel pour chaque boîte de culture (support avec microscope)
 - Usage dans des incubateurs usuels
 - Boîtes de culture spécifiques
 - Image toutes les 10 minutes



Primo Vision[®]

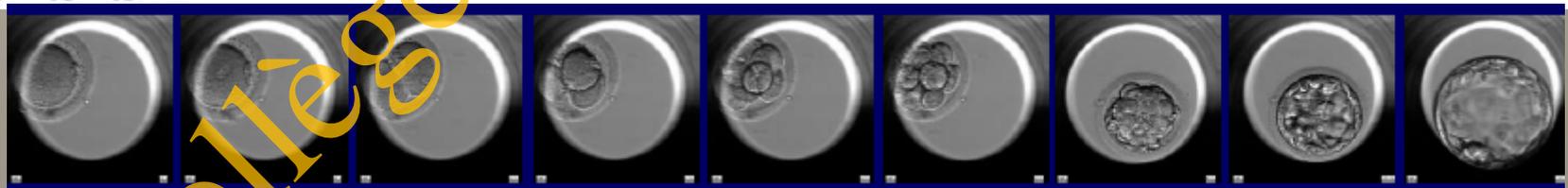


Collège de Gynécologie CHL



$VP = tPNf - tPNa$
 $ECC1 = t2 - tPB2$
 $ECC2 = t4 - t2$
 $ECC3 = t8 - t4$
 $s2 = t4 - t3$
 $s3 = t8 - t5$

Compaction = $tM - tSC$
Blastulation = $tB - tSB$



The use of morphokinetics as a predictor of embryo implantation†

Marcos Meseguer^{1,*}, Javier Herrero¹, Alberto Tejera¹,
Karen Marie Hilligsøe², Niels Binger Ramsing², and Jose Remohí¹

¹Instituto Valenciano de Infertilidad, Universidad de Valencia, Plaza de la Policía Local, 3, Valencia 46015, Spain ²Unisense FertiTech A/S, Aarhus, Denmark

**Etude rétrospective
285 couples (ICSI)
264 embryons avec implantation connue**

Evaluation of potential selection parameters based on a logistic regression analysis

A logistic regression analysis was used to select and organize which observed timing events (expressed as binary variables inside or outside the optimal range as defined earlier) should be used together with the morphological exclusion criteria. The model identified the time of division to 5 cells, $t5 \text{ OR} = 3.31$ (95% CI 1.65–6.66) followed by synchrony of divisions after the 2 cell stage, $s2 \text{ OR} = 2.04$ (95% CI 1.07–4.07) and the duration of the 2 cell cycle, $cc2 \text{ OR} = 1.84$ (95% CI 0.95–3.58) as the most promising variables characterizing implanting embryos.

By using exclusion variables plus $t5$, $s2$ and $cc2$, we defined a logistic regression model. An ROC curve analysis to determine the predictive properties of this model with respect to probability of implantation gave an $\text{AUC value of } 0.720$ (95% CI 0.645–0.795).

These data were used to generate the hierarchical selection model described later.

$$s2=t4-t3 \quad cc2=t3-t2$$

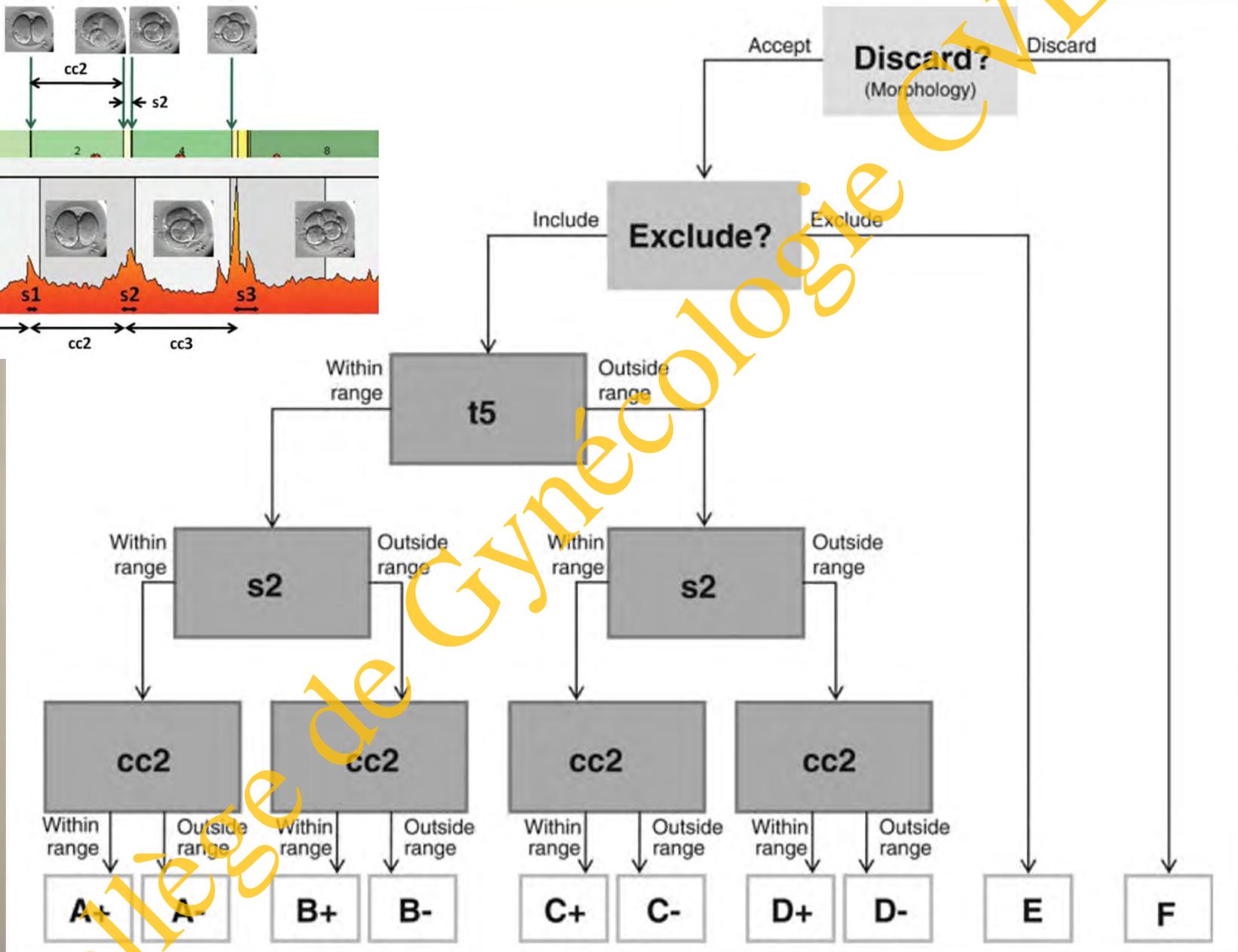
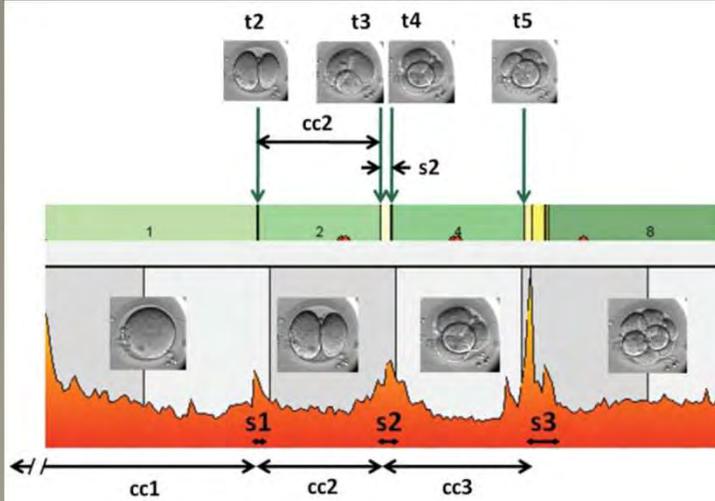


Table III Implantation in the embryo categories of the hierarchical classification tree model.

Embryo category	<i>n</i> total	<i>n</i> implanted	Implantation (%)	Embryo category	Implantation (%)
A+	29	19	66	A	52
A-	25	9	36	B	27
B+	24	7	29	C	19
B-	25	6	24	D	14
C+	32	8	25	E	8
C-	21	2	10		
D+	10	1	10		
D-	33	5	15		
E	48	4	8		

The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection

N. Basile¹, P. Vime², M. Florensa³, B. Aparicio Ruiz⁴, J.A. García Velasco¹, J. Remohí⁴, and M. Meseguer^{4,*}

¹IVI Madrid, Madrid, Spain ²IVI Sevilla, Sevilla, Spain ³IVI Barcelona, Barcelona, Spain ⁴IVI Valencia, Valencia, Spain

**Etude rétrospective
1664 couples (ICSI)
754 embryons avec implantation connue**

Logistic regression analysis, elaboration of the algorithm and embryo scoring

A logistic regression analysis was performed to determinate the most relevant variables related to implantation. The model identified being in the optimal ranges for $t3$ OR = 1.542 (95% CI 1.094–2.173) followed by $cc2$ OR = 1.425 (95% CI 1.025–1.981) and $t5$ OR = 1.210 (95% CI 0.845–1.731) as the most relevant variables related to implantation. As observed in Fig. 2 the optimal ranges defined for the first two variables in the algorithm ($t3$ and $cc2$) have a higher proportion of implanting embryos (green circles) than non-implanting embryos. With this information, a hierarchical classification model similar to our previous one was generated. The model represented in Fig. 3 combines morphology and kinetic criteria subdividing embryos into ten categories (A–/A+; B–/B+; C–/C+; D–/D+, E and F) according to implantation potential.

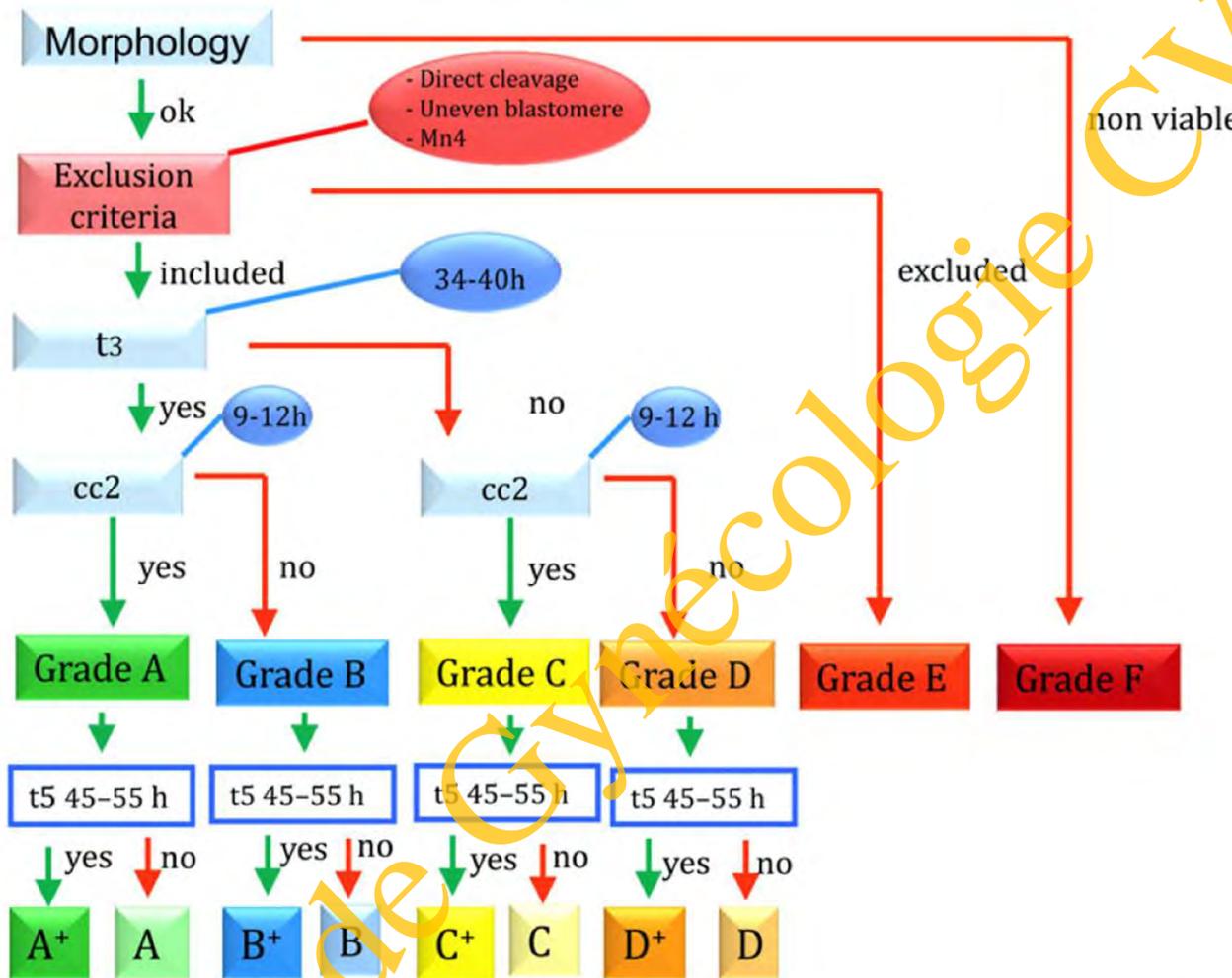


Figure 3 Hierarchical classification of embryos based on: (i) Morphological screening; (ii) the new morphological criteria; (iii) timing of cell division to three cells (t3); (iv) duration of second cell cycle, cc2, i.e. the time from division to a two blastomere until division to a three blastomere embryo; (v) timing of cell division to five cells. The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

Table IV Implantation in the embryo categories of the hierarchical classification tree model (see Fig. 3).

Embryo category	Sub-category	No. of embryos	Embryos implanted	Implantation rate % (95% CI)
A	+	333	106	32 (26.9–37.1)
	–	74	23	31 (20.8–42.9)
	Total	407	129	32 (27.1–36.5)
B	+	124	40	32 (23.6–40.4)
	–	61	12	20 (9.8–30.2)
	Total	185	52	28 (21.4–34.6)
C	+	70	23	33 (22–44)
	–	70	14	20 (10.5–30.5)
	Total	140	37	26 (14.8–37.2)
D	+	38	8	21 (8.1–33.9)
	–	155	30	19 (12.7–25.3)
	Total	193	38	20 (14.3–25.7)
E	Total	197	34	17 (11.7–22.3)

Algorithme : application universelle ou dépendance des conditions de culture ?

Human Reproduction, Vol.28, No.3 pp. 634–641, 2013

Advanced Access publication on January 12, 2013 doi:10.1093/humrep/des462

human reproduction

ORIGINAL ARTICLE *Embryology*

Type of culture media does not affect embryo kinetics: a time-lapse analysis of sibling oocytes

Natalia Basile^{1,*}, Dean Morbeck², Juan García-Velasco¹, Fernando Bronet¹, and Marcos Meseguer³

¹Instituto Valenciano de Infertilidad, Av. Del Talgo 68 (28023), Madrid, Spain ²Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN, USA ³Instituto Valenciano de Infertilidad, Universidad de Valencia, Valencia, Spain

Table III Percentage of embryos falling within optimal ranges for t5, s2 and cc2 according to the media utilized.

Culture media	t5			s2			cc2		
	N	Out of range (%)	48.8–56.6 h (%)	N	Out of range (%)	<0.75 h	N	Out of range (%)	<12 h
Global	242	62.0 (150/242)	38.0 (92/242)	263	49.0 (129/263)	51.0 (134/263)	269	46.8 (126/269)	53.2 (143/269)
Sage	239	59.8 (143/239)	40.2 (96/239)	269	49.4 (133/269)	50.6 (136/269)	274	47.8 (131/274)	52.2 (143/274)
P			NS			NS			NS

t5 (time to 2 cells), cc2 (t3 – t2), s2 (t4 – t3).

Basile, 2012

Algorithme : application universelle ou dépendance des conditions de culture ?

Time-lapse evaluation of human embryo development in single versus sequential culture media—a sibling oocyte study

Haydar Nadir Ciray • Turan Aksoy • Cihan Goktas
Bilgen Ozturk • Mustafa Bahceci

Table 1 Morphokinetic parameters obtained from all (ALL) and transferred/frozen (GOOD) embryos

		dp	t2	t3	t4	t5	cc2	s2
All	Single	25.12±5.21 (170)	27.36±4.12 (167)	37.75±6.64 (164)	40.07±5.98 (160)	48.77±9.49 (159)	10.52±5.99 (164)	2.54±5.26 (160)
	Sequential	25.92±4.99 (149)	29.09±4.86 (148)	39.53±6.15 (144)	41.45±6.07 (138)	52.22±9.34 (138)	10.63±4.64 (144)	1.88±3.52 (138)
	<i>P</i> ^a	0.03	0.001	0.01	0.02	0.001	0.24	0.78
Good	Single	23.70±3.28 (75)	26.19±3.36 (75)	37.25±5.46 (75)	38.85±4.13 (75)	49.19±8.40 (75)	11.06±3.34 (75)	1.60±2.97 (75)
	Sequential	24.75±3.13 (68)	27.56±3.21 (68)	39.16±3.93 (68)	40.33±3.77 (68)	53.34±6.34 (68)	11.60±2.64 (68)	1.17±2.02 (68)
	<i>P</i> ^a	0.04	0.009	0.02	0.02	0.002	0.40	0.86

Milieux Irvine : unique vs séquentiel

Cirray, 2012

Algorithme : application universelle ou dépendance des conditions de culture ?

Effect of oxygen concentration on human embryo development evaluated by time-lapse monitoring

Kirstine Kirkegaard, M.D., Johnny Juhl Hindkjaer, M.Sc., and Hans Jakob Ingerslev, D.M.Sc.
The Fertility Clinic, Aarhus University Hospital, Aarhus, Denmark

Time points of embryonic stages.

Stage	n	O ₂ 20%	n	Group 2	n	O ₂ 5%	P value
2-cell	120	28.0 (27.1–28.9)	123	28.9 (28.0–29.9)	120	27.9 (26.9–28.8)	.24 ^a
3-cell	114	38.3 (37.1–39.6)	118	39.0 (37.8–40.2)	118	37.8 (36.6–38.9)	.12 ^b
4-cell	110	41.0 (39.7–42.4)	114	41.0 (39.7–42.4)	116	40.5 (39.2–41.9)	.41 ^b
5-cell	102	53.0 (51.2–54.9)	110	51.1 (49.4–52.8)	106	50.3 (48.6–52.0)	.09 ^b
6-cell	90	55.9 (54.0–57.8)	106	53.4 (51.8–55.1)	102	52.6 (51.0–54.3)	.03 ^b
7-cell	69	59.2 (57.2–61.2)	96	55.6 (54.0–57.2)	86	53.7 (52.1–55.4)	<.001 ^b
8-cell	55	61.2 (59.0–63.4)	75	57.5 (55.7–59.3)	58	54.5 (52.6–56.5)	<.001 ^a
Early blastocyst	57	96.5 (94.5–98.5)	85	96.7 (95.1–98.4)	87	97.2 (95.6–98.8)	.84 ^a
Full blastocyst	46	102.2 (100.4–104.0)	69	104.5 (103.0–106.0)	61	104.8 (103.2–106.4)	.08 ^a

Note: All data followed a normal distribution after log transformation and are displayed as medians with 95% confidence intervals. P value tests the hypothesis of no difference between the groups. The number of 8-cell embryos is smaller than the number of early blastocysts, because some embryos compacted earlier than the 8-cell stage and those embryos were therefore not included in the number of 8-cell embryos.

^a Analysis of variance

^b Kruskal-Wallis test.

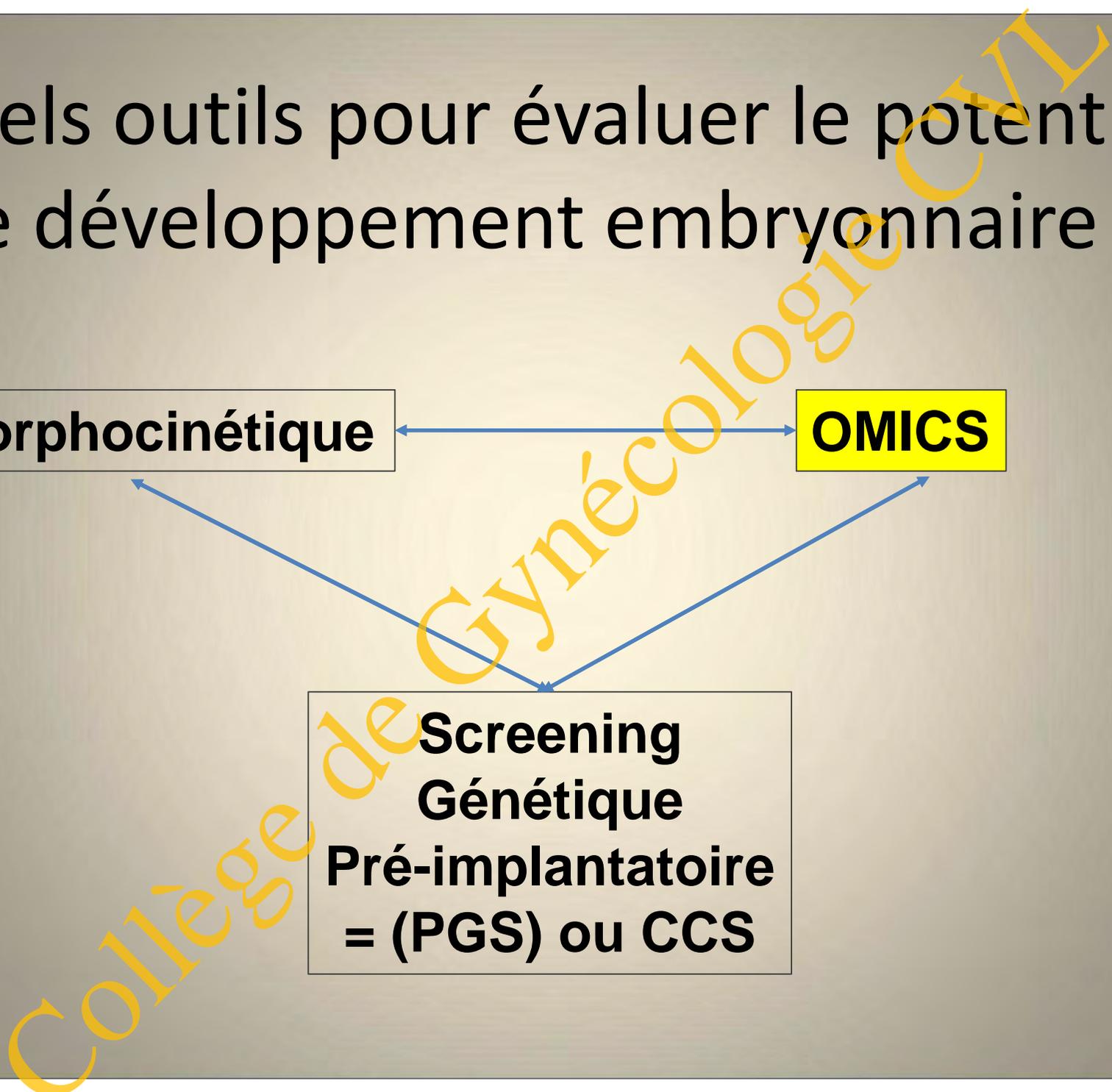
Kirkegaard, 2013

Quels outils pour évaluer le potentiel de développement embryonnaire ?

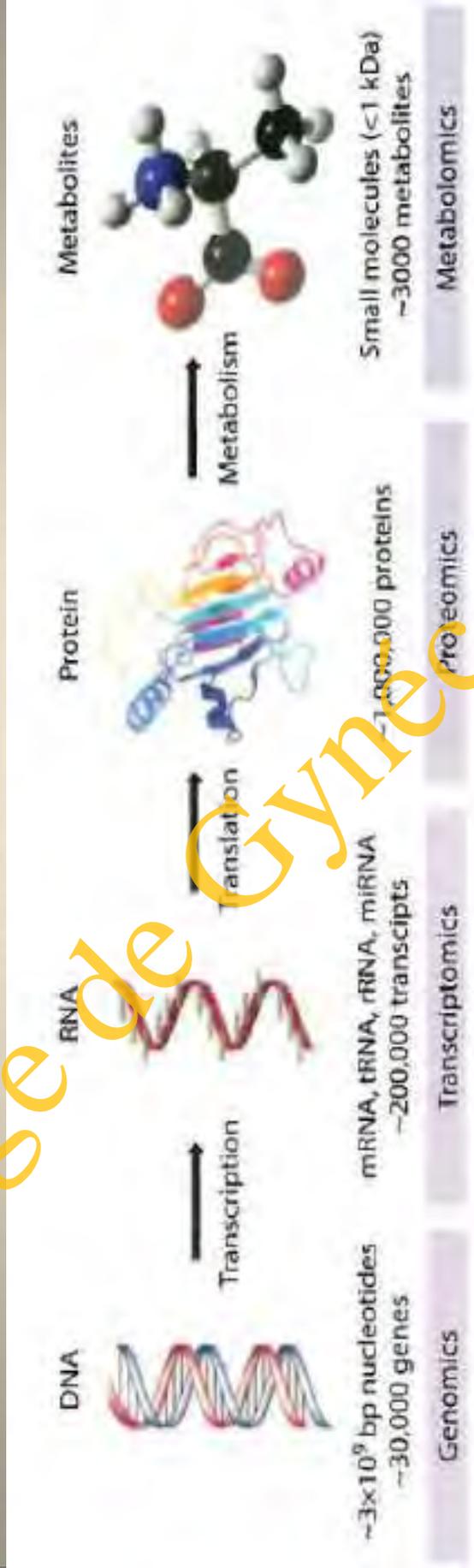
Morphocinétique

OMICS

**Screening
Génétique
Pré-implantatoire
= (PGS) ou CCS**



Collège de Gynecologie CHU



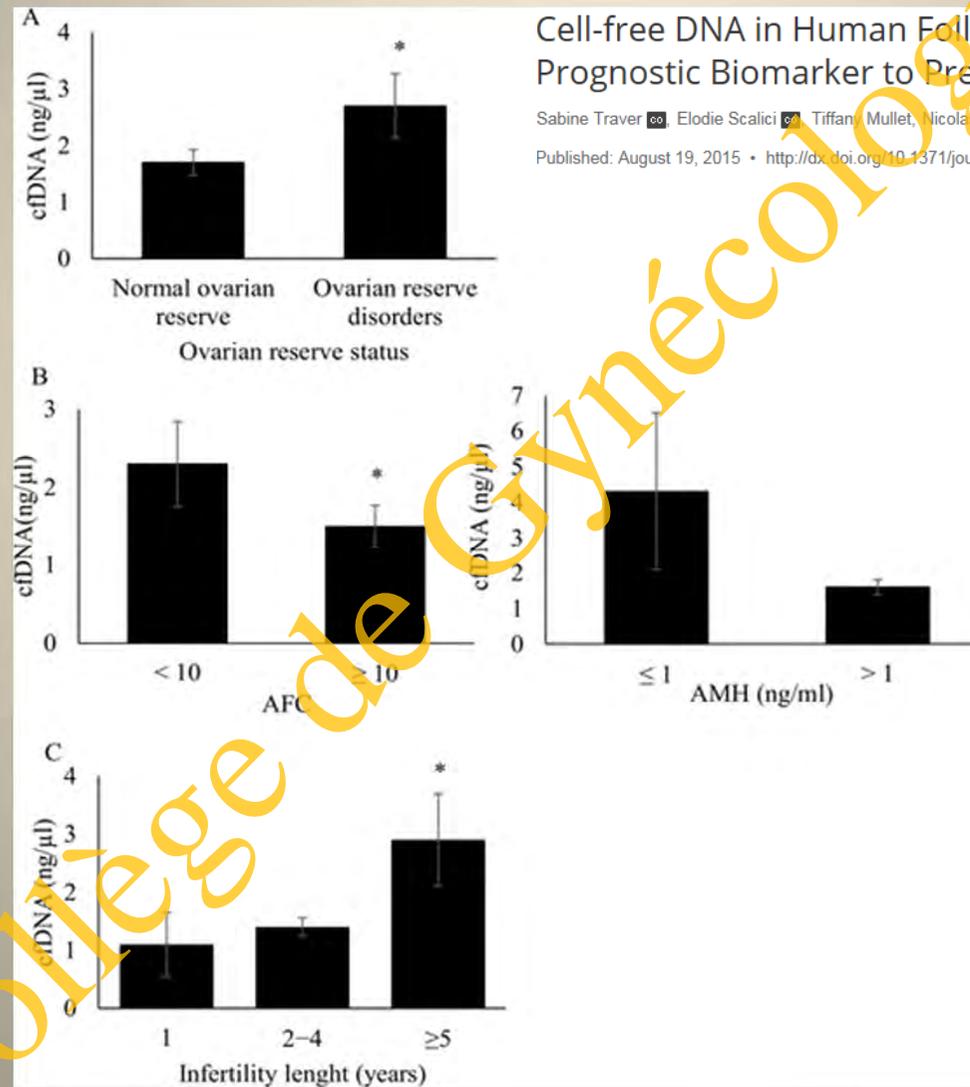
Génomique ADN libre

PCR ADN quantitative

Cell-free DNA in Human Follicular Microenvironment: New Prognostic Biomarker to Predict *in vitro* Fertilization Outcomes

Sabine Traver, Elodie Scalici, Tiffani Mullet, Nicolas Molinari, Claire Vincens, Tal Anahory, Samir Hamamah

Published: August 19, 2015 • <http://dx.doi.org/10.1371/journal.pone.0136172>

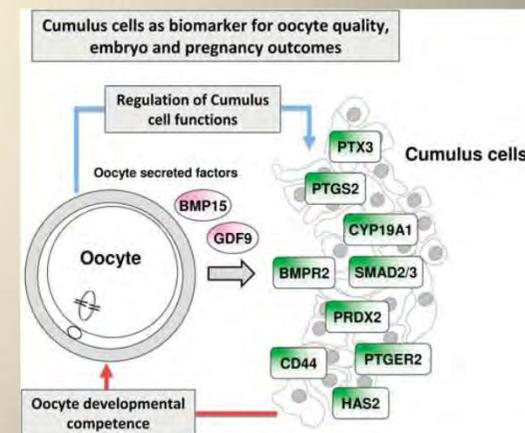
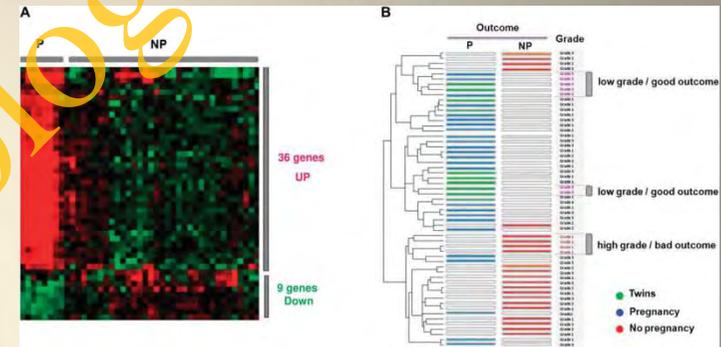


2015

Transcriptomique / protéomique

Le dialogue cumulo-ovocytaire

- Interactions entre les Cellules du Cumulus et l'ovocyte indispensables pour la **maturation ovocytaire**.
- Le profil transcriptomique des cellules du cumulus a montré son lien avec la **compétence au développement**.
- Des **gènes cibles** ont été identifiés, les candidats biomarqueurs potentiels sont en cours de validation (*Assou 2010; Wathlet 2011; Assidi 2011*)



Confronter les résultats des équipes

Analyse du secrétome embryonnaire

Hypothèse : les protéines produites par l'embryon et secrétées dans l'environnement du milieu de culture reflètent des différences de potentiel de développement

- Techniques : spectrométrie de masse (SELDI TOF)
- Katz Jaffe 2005, 2006, 2007, 2008, 2009, 2010, 2011
- Analyse comparées de spectres et identification de protéine marqueurs de développement (ex: ubiquitine)

Confronter les résultats des équipes

Pyruvate et Glucose

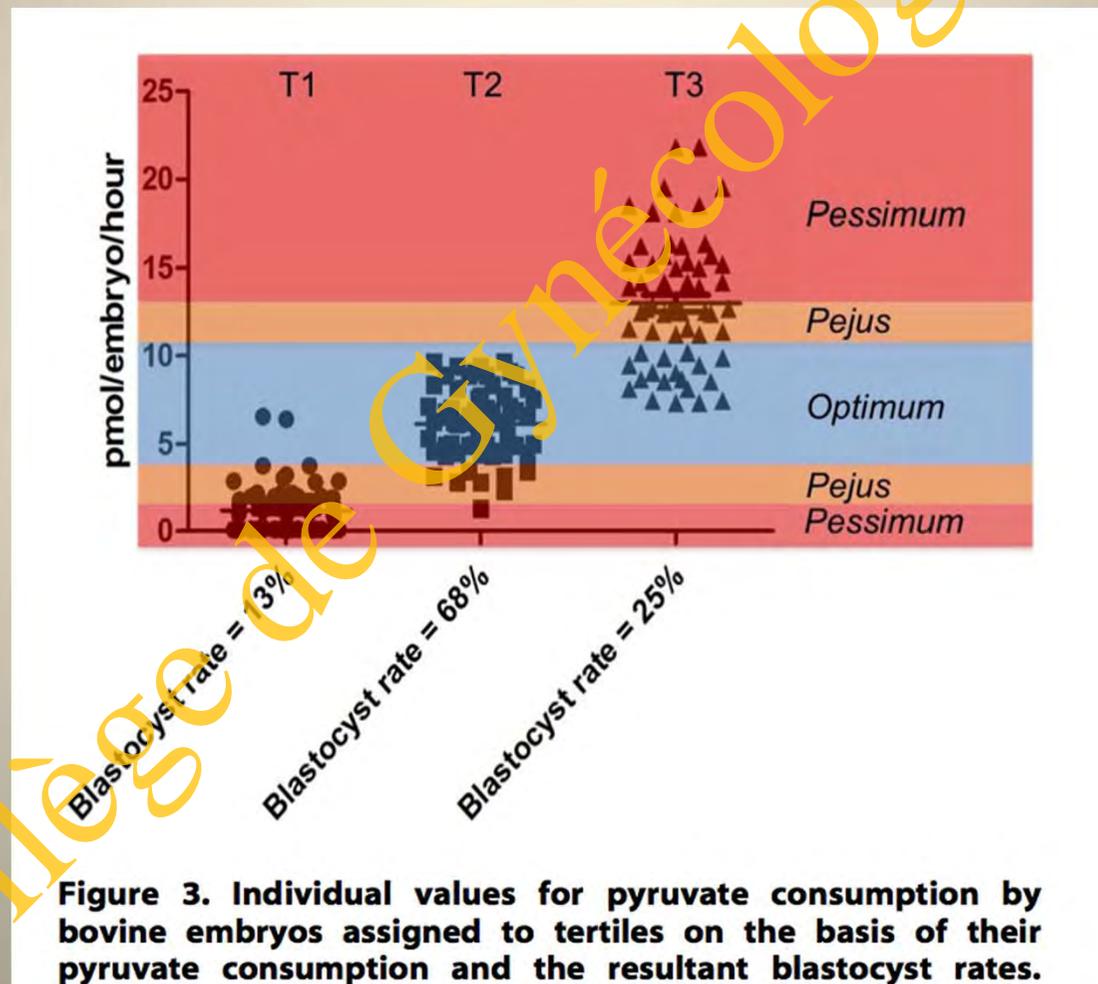
- Modèles murin et bovin (études cinétiques)
- Technique ultramicrofluorescence (Leese et Barton, 1984)
- Culture en microgouttes (4 à 10 μ l)
- Culture pendant 3 à 24 heures
- Milieux d'analyse souvent modifiés

- Pyruvate : consommation avant compaction
- Glucose : consommation croissante post compaction

**Problème actuel : sensibilité des techniques de mesure
Milieux de culture commerciaux**

A Simple Approach for CONsumption and RElease (CORE) Analysis of Metabolic Activity in Single Mammalian Embryos

Fabrice Guerif^{1,2,3,4}, Paul McKeegan¹, Henry J. Leese¹, Roger G. Sturme^{1*}

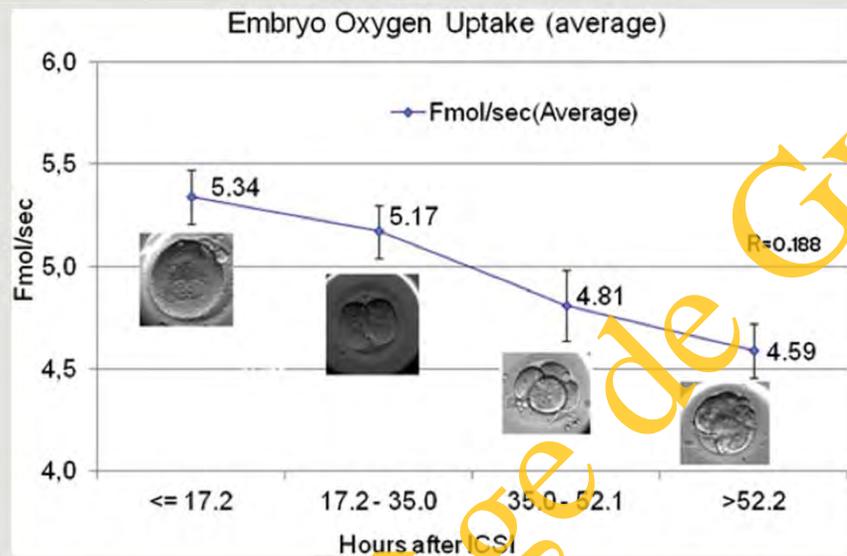


Guérif, 2013

Consommation d'oxygène

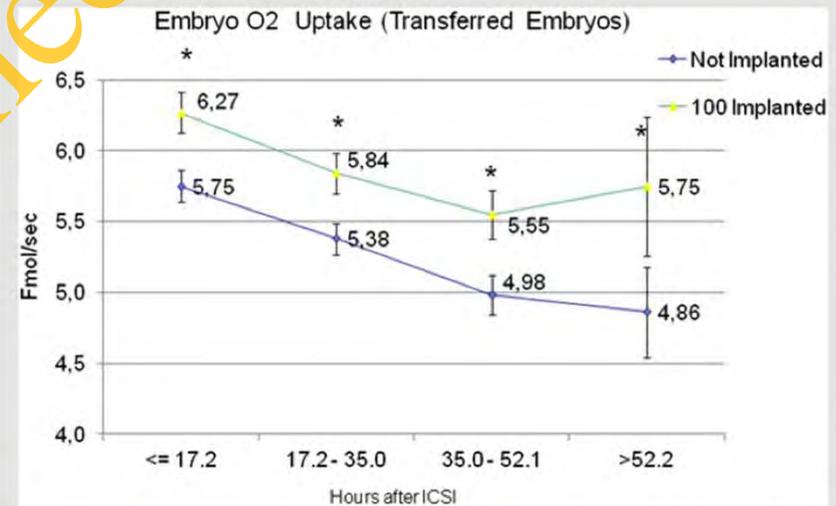
Time-dependent O₂ consumption patterns determined optimal time ranges for selecting viable human embryos

Alberto Tejera, Ph.D., Javier Herrero, Ph.D., Tamara Vilorio, Ph.D., Josep Lluís Romero, M.D., Pilar Gamiz, Ph.D., and Marcos Meseguer, Ph.D.



Time-dependent embryo O₂ consumption. Averages in each of four time ranges.

Tejera. O₂ consumption changes depending on embryo stage. Fertil Steril 2012.



Time-dependent embryo O₂ consumption. Averages from transferred embryos in each of the four time ranges depending on implantation success. *Significant difference ($P < .05$) between implanted embryos and nonimplanted embryos.

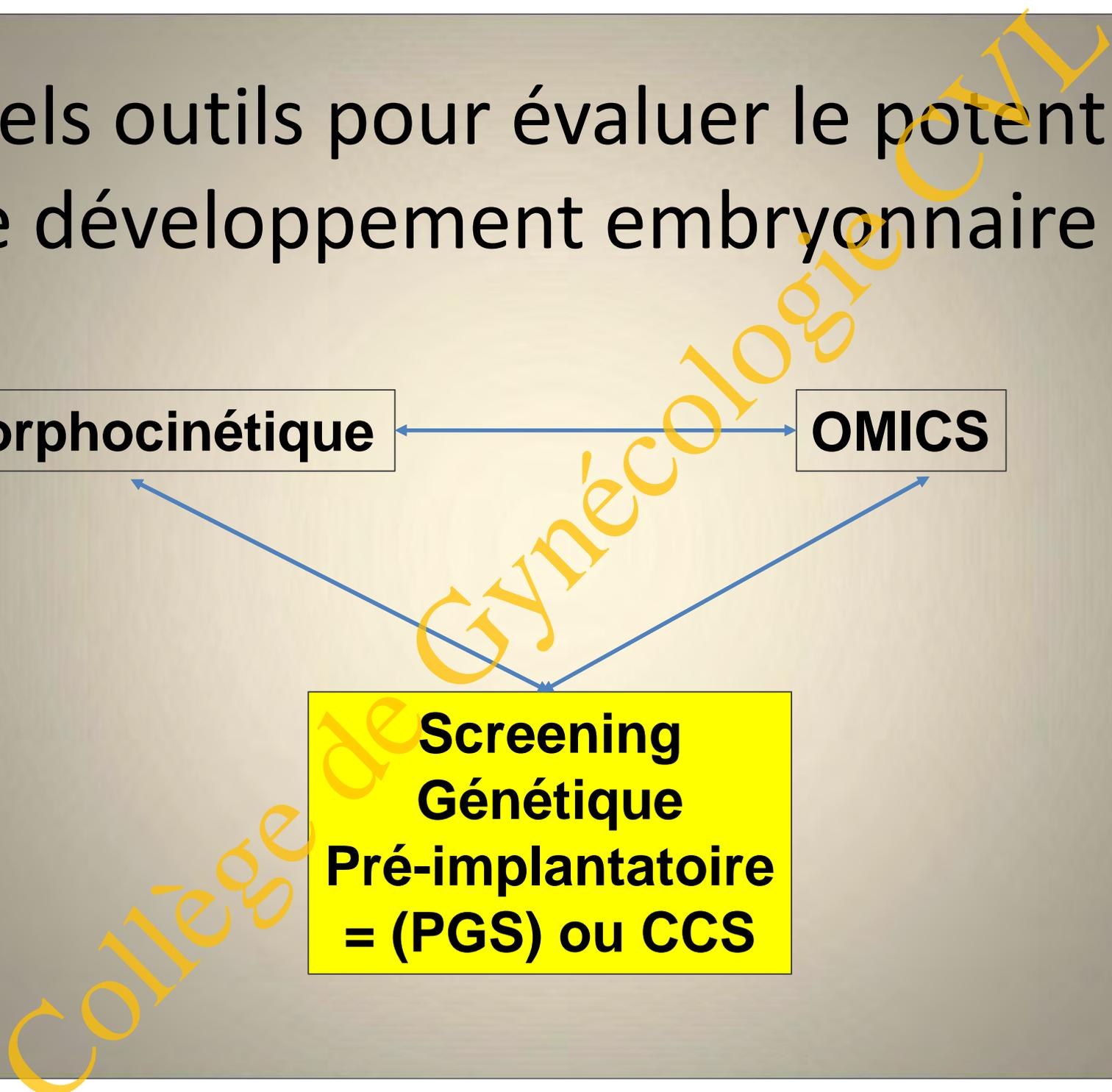
Tejera. O₂ consumption changes depending on embryo stage. Fertil Steril 2012.

Quels outils pour évaluer le potentiel de développement embryonnaire ?

Morphocinétique

OMICS

**Screening
Génétique
Pré-implantatoire
= (PGS) ou CCS**



Screening génétique pré-implantatoire

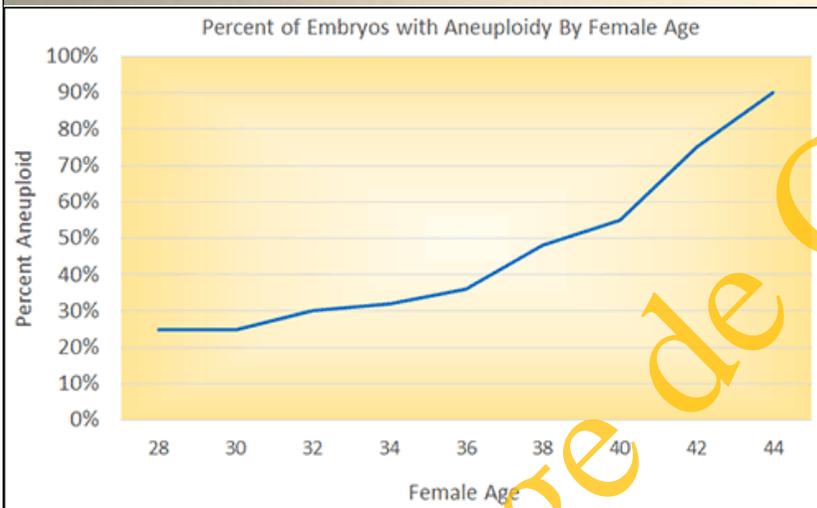
- Aneuploïdie
 - Fréquence élevée pour l'embryon précoce humain

Aneuploidy in humans: estimated levels at different stages

Population	Methodology*	Timeframe of studies	Incidence of aneuploidy‡	Most common aneuploidies
Newborns	Karyotyping	1960s–1970s	0.3%	+13; +18; +21; XXX; XXY; XYY
Stillbirths	Karyotyping	1970s–1980s	4%	45,X; +13; +18; +21; XXX; XXY
Spontaneous abortions	Karyotyping	1970s–1980s	>35%	45,X; +15; +16; +21; +22
Preimplantation embryos	Karyotyping	1990s	20–40%	+16; +17; +18
	FISH	1990s–present	25–>70%	Various
	CGH, SNP array, CGH array	2000–present	30–60%	+15; +16; +21; +22

Screening génétique pré-implantatoire

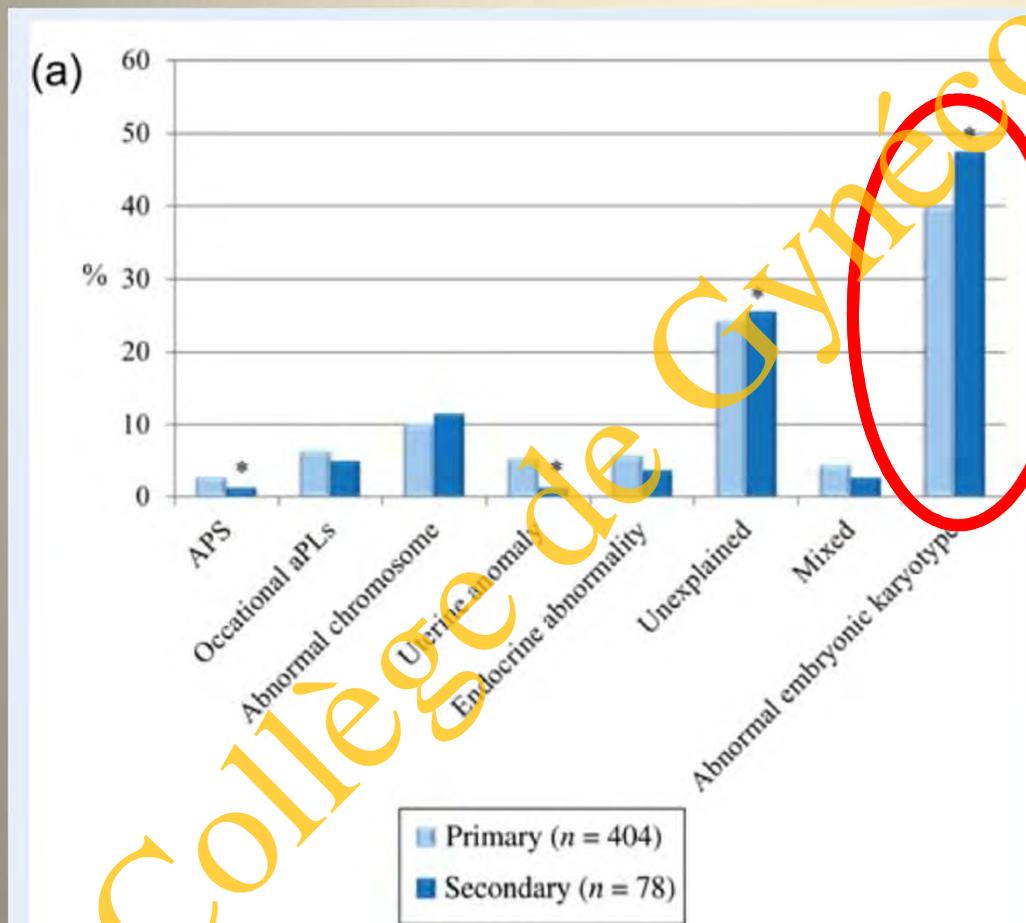
- Aneuploïdie
 - Fréquence accrue avec l'âge



Day 3 biopsy		
Age group (y)	Implan. ^{a,b} (+Sac)	Aneuploidy rate ^{c,d}
<35	40.6% (73/180)	53.1% (530/999)
35-37	43.6% (44/101)	68.2% (420/616)
38-40	42.1% (59/140)	73.7% (659/894)
41-42	31.6% (18/57)	85.8% (460/536)
>42	7/30	92.6% (340/367)
P value	NS	< .001
Total	39.6% (201/508)	70.6% (2409/3412)

Screening génétique pré-implantatoire

- Aneuploïdie
 - Cause la plus fréquence de FCS à répétition



Screening génétique pré-implantatoire

- Aneuploïdie
 - Cause d'échecs répétés d'implantation

Table I. Assumed aetiologies for repeated implantation failure (RIF)

Decreased endometrial receptivity

- Uterine cavity abnormalities
- Thin endometrium
- Altered expression of adhesive molecules
- Immunological factors
- Thrombophilias

Defective embryonic development

- Genetic abnormalities (male/female/gametes/embryos)
- Zona hardening
- Suboptimal culture conditions

Multifactorial effectors

- Endometriosis
- Hydrosalpinges
- Suboptimal ovarian stimulation

Screening génétique pré-implantatoire

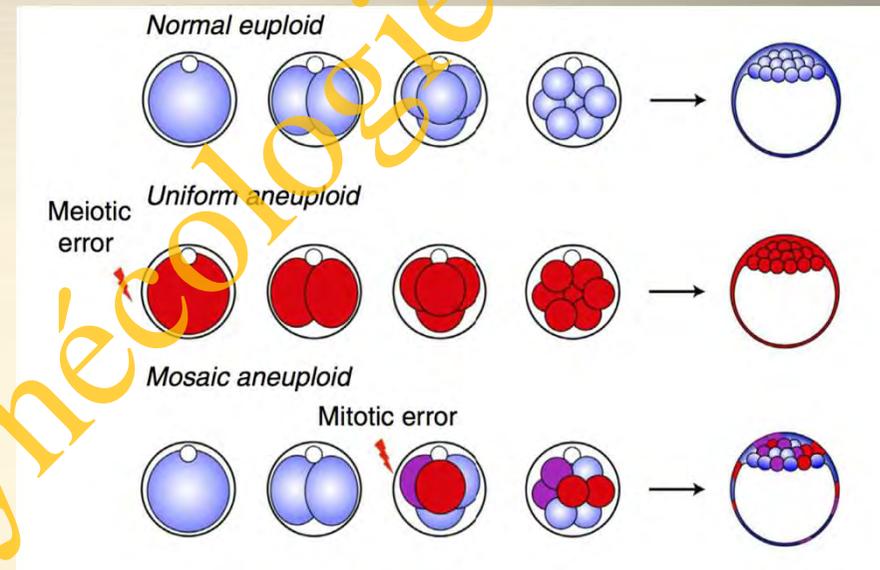
- **Initiation du PGS**

- Embryons J3

- FISH

- Verlinsky 1995

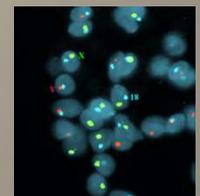
- ...



- **Essais randomisés (≥ 2004) => débats**

- Conclusion : Absence de gain apporté par le PGS de « première génération » (Staessen, 2004 ; Mastenbroek, 2007)

- => Déclin du PGS

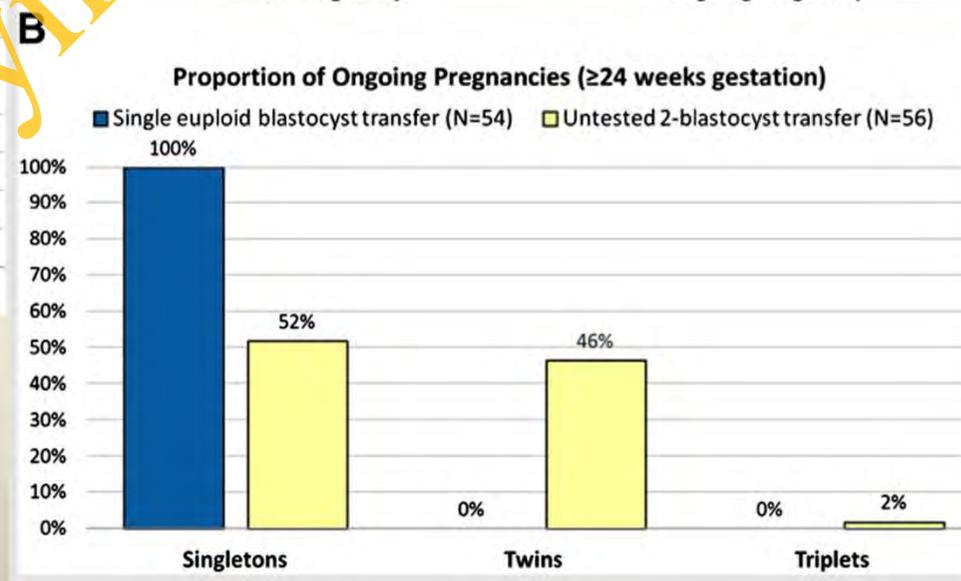
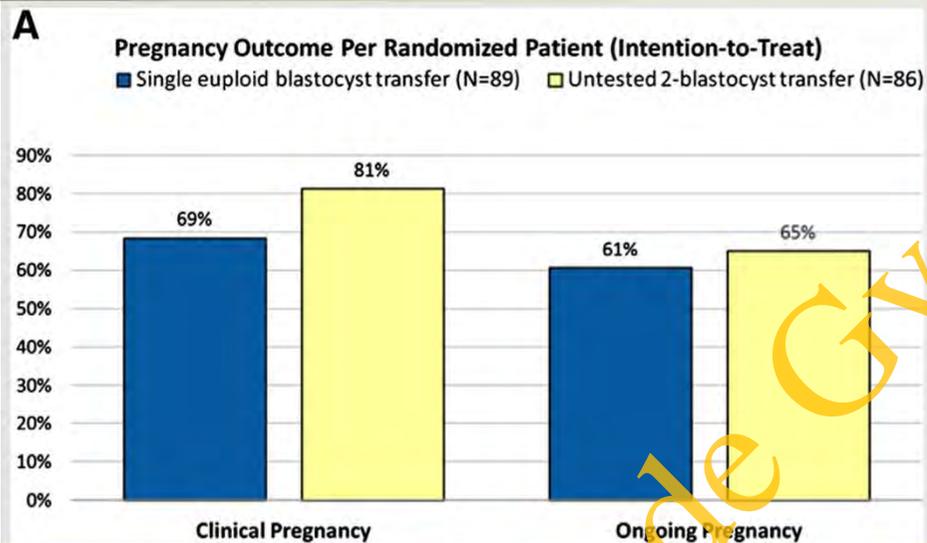


Screening génétique pré-implantatoire

- **Biopsie**
 - A J5 avec vitrification du blastocyste et transfert différé
 - **Développement de nouvelles techniques**
 - CGR arrays (Comparative Genomic Hybridization)
 - SNP arrays (Single Nucleotide Polymorphism)
 - qPCR
 - NGS
- ⇒ Nouvelles études prospectives randomisées (RCT)
- ⇒ Conclusion : Gain apporté par le PGS de « nouvelle génération » MAIS études critiquées (méthodologie)

In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial

Eric J. Forman, M.D.,^{a,b} Kathleen H. Hong, M.D.,^{a,b} Kathleen M. Ferry, B.Sc.,^a Xin Tao, M.Sc.,^a Deanne Taylor, Ph.D.,^a Brynn Levy, Ph.D.,^{a,c} Nathan R. Treff, Ph.D.,^{a,b} and Richard T. Scott Jr., M.D.^{a,b}



Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization

Gary L. Harton, B.S.,^a Santiago Munné, Ph.D.,^b Mark Surrey, M.D.,^c Jamie Grifo, M.D., Ph.D.,^d Brian Kaplan, M.D.,^e David H. McCulloh, Ph.D., H.C.L.D.,^d Darren K. Griffin, Ph.D.,^f and Dagan Wells, Ph.D.,^{g,h} for the PGD Practitioners Groupⁱ

Comparison of implantation rate and aneuploidy rate between biopsy at day 3 or day 5/6 by SART age group.

Age group (y)	Day 3 biopsy		Age group (y)	Day 5/6 biopsy	
	Implan. ^{a,b} (+Sac)	Aneuploidy rate ^{c,d}		Implan. ^{a,b} (+Sac)	Aneuploidy rate ^{c,d}
<35	40.6% (73/180)	53.1% (530/999)	<35	51.1% (119/233)	31.7% (306/966)
35–37	43.6% (44/101)	68.2% (420/616)	35–37	54.2% (65/120)	44.2% (237/536)
38–40	42.1% (59/140)	73.7% (659/894)	38–40	47.2% (59/125)	43.1% (324/751)
41–42	31.6% (18/57)	85.8% (460/536)	41–42	40.4% (19/47)	76.3% (200/262)
>42	7/30	92.6% (340/367)	>42	5/18	84.8% (112/132)
<i>P</i> value	NS	<.001	<i>P</i> value	NS	<.001
Total	39.6% (201/508)	70.6% (2409/3412)	Total	49.2% (267/543)	47.8% (1179/2467)

Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos

E. Fragouli^{1,2,*}, S. Alfarawati², K. Spath¹, and D. Wells^{1,2}

Table III The chromosome constitution of embryos in relation to quality at the cleavage stage.

Chromosome constitution	Good quality (%)	Average quality (%)	Poor quality (%)
Normal	48 (17.6)	8 (13)	2 (9.5)
Aneuploid	225 (82.4)	53 (87)	19 (90.5)

Table VI The chromosome constitution of embryos in relation to expansion at the blastocyst stage.

Chromosome constitution	Grades 5 and 6 (%)	Grades 3 and 4 (%)	Early (%)
Normal	62 (51)	280 (44)	31 (33)
Aneuploid	60 (49)	362 (56)	63 (67)

Table VIII The chromosome constitution of embryos in relation to TE quality.

Chromosome constitution	TE Grade A (%)	TE Grade B (%)	TE Grade C (%)	Total
Normal	137 (41)	169 (50)	31 (9)	337 (100)
Aneuploid	115 (28)	242 (60)	49 (12)	406 (100)

Table VII The chromosome constitution of embryos in relation to ICM quality.

Chromosome constitution	ICM Grade A (%)	ICM Grade B (%)	ICM Grade C (%)	Total
Normal	172 (51)	149 (44)	16 (5)	337 (100)
Aneuploid	181 (45)	202 (50)	23 (5)	406 (100)

Age moyen des femmes : 38.6 ans

Fragouli, 2014

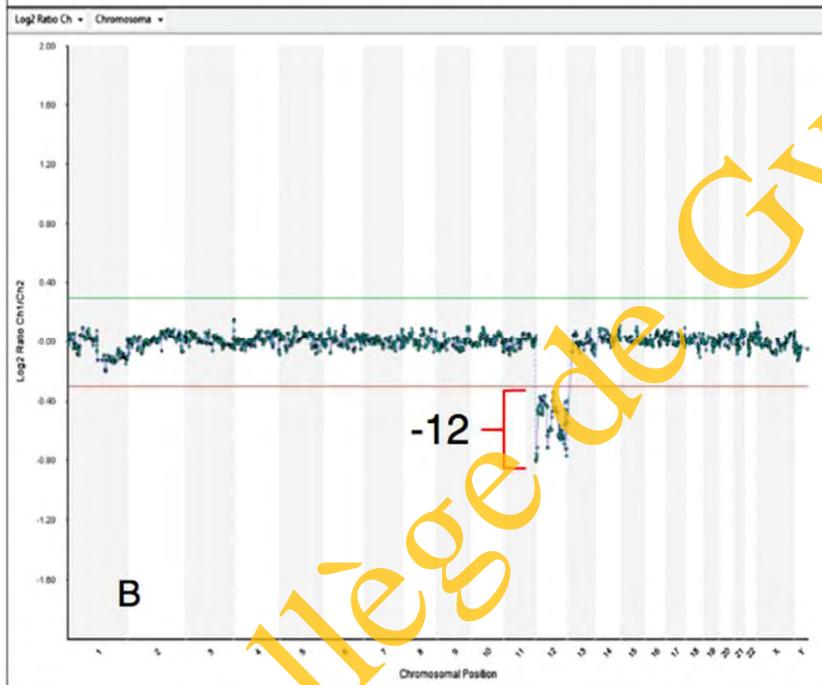
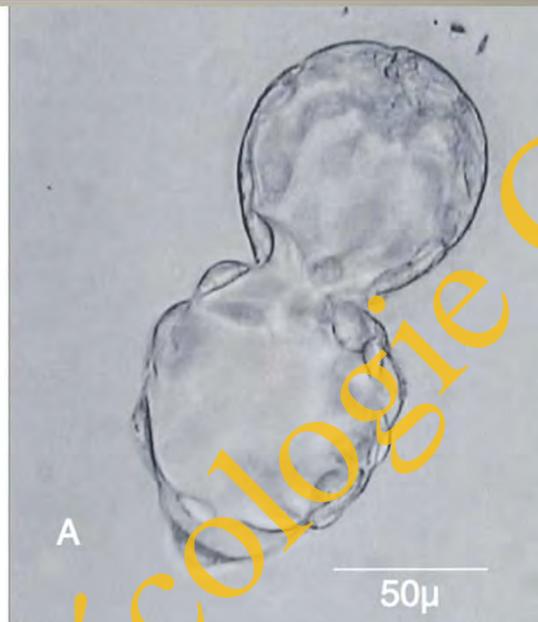
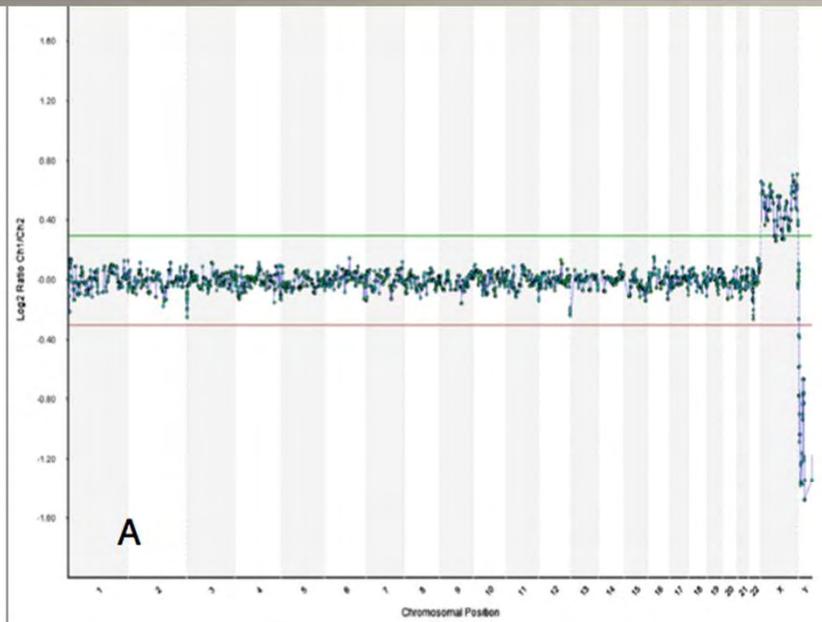


Figure 2 Representative aCGH data obtained from human blastocysts via trophoctoderm biopsy performed on post-fertilization day 5. While standard microscopy confirmed good morphology (Grade 5AA) for both blastocysts, ploidy status was not uniform. Using aCGH to screen embryos before fresh transfer, normal chromosomal status (46,XX) was verified in A, but not in B (45,XY,-12).

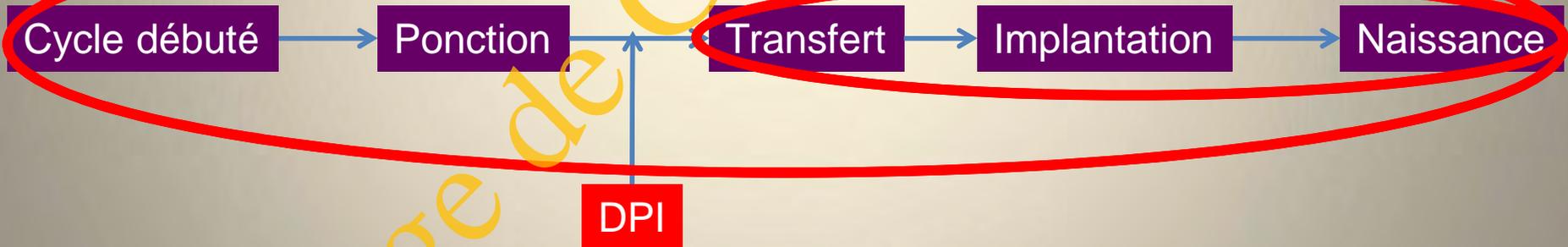
human
reproduction

OPINION

Preimplantation genetic screening: back to the future

Sebastiaan Mastenbroek Sjoerd Repping

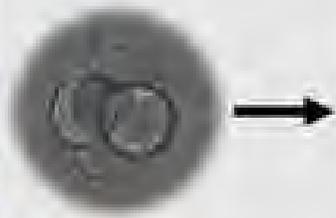
INTERDIT EN FRANCE



OBSERVATION EMBRYON 3D



1. Visualisation des embryons
au microscope optique
Acquisition d'images



2. Reconstitution 3D
à l'aide d'un logiciel informatique



3. Impression 3D

Meilleure évaluation % + localisation fragments

Conclusions

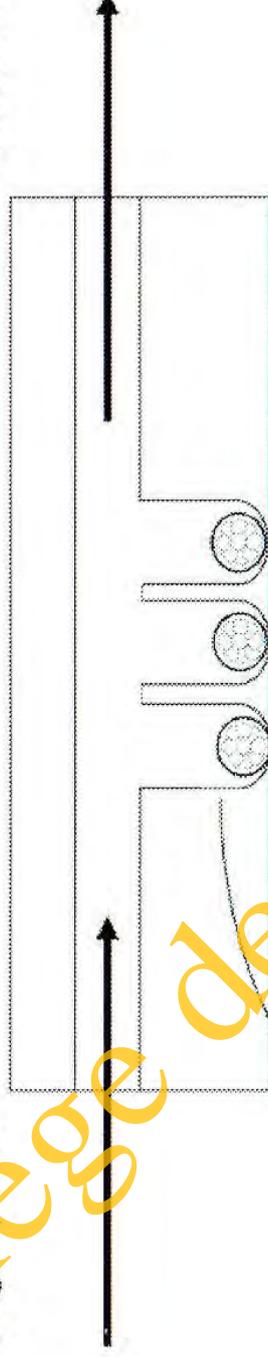
Collège de Gynécologie CVL

Medium Introduced:

Changing metabolite pool, introduction of stage-specific factors etc.

Medium Expelled:

Analysis of metabolites/biomarkers



Embryos cultured individually or in groups in volumes ranging from nano to microlitres

Channel or microchamber created in biologically compatible, gas permeable and transparent material, such as PDMS

Embryos imaged within a time-lapse incubation system

College de Gynécologie CHL

Dans le futur...
Appréhender le dialogue
endomètre-embryon ?

