OVARIAN CLUB VIII

Genes expression profile or microRNAs to explore endometrial receptivity: What is the best?

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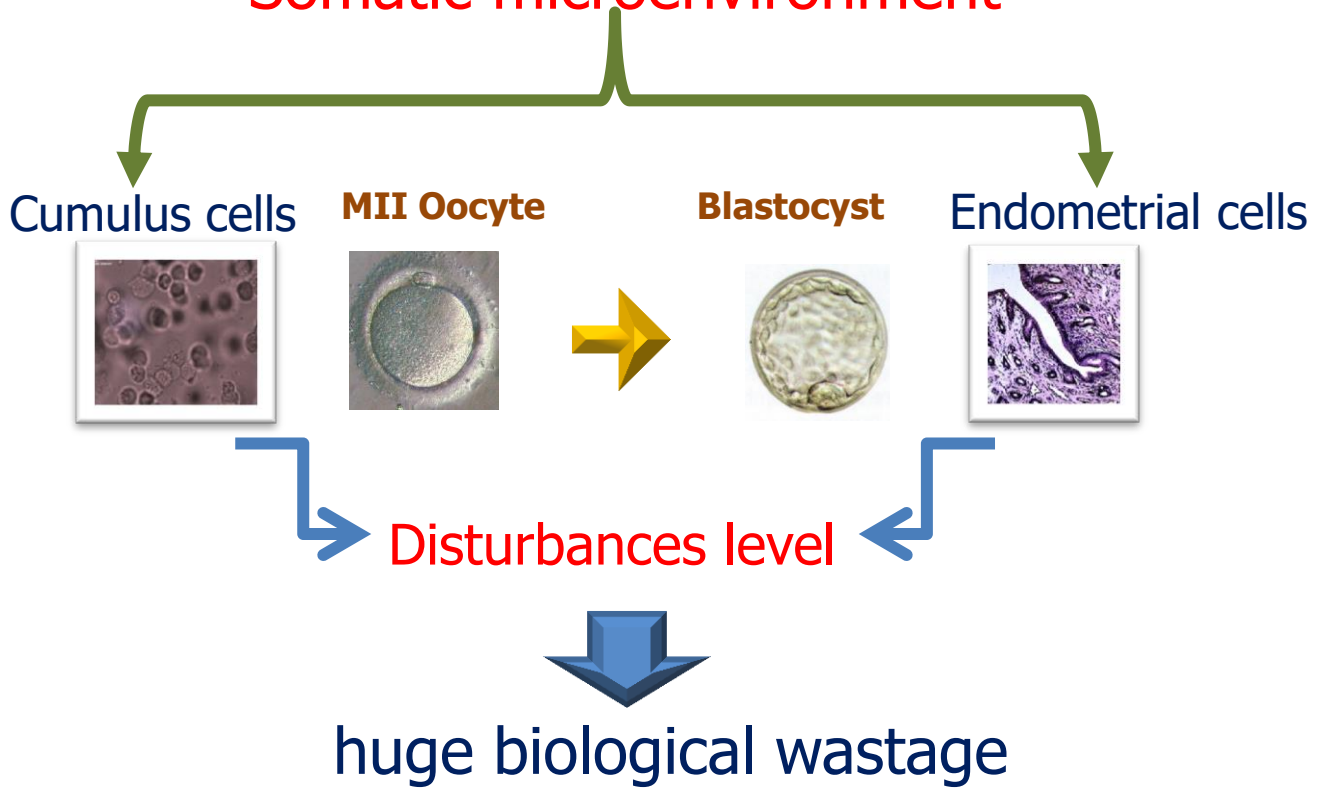
MONTPELLIER CHU CENTRE HOSPITALIER UNIVERSITAIRE

Oocytes and embryos during manipulation



huge biological wastage

. Inadequate COS

. Inadequate of Competent Embryo Selection

. Non optimum In vitro culture conditions

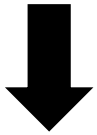
. **Inadequate endometrial receptivity**

. Fresh embryo replacement systematically should be reconsidered

Insufficient knowledge even 38 years after first IVF birth !

Context

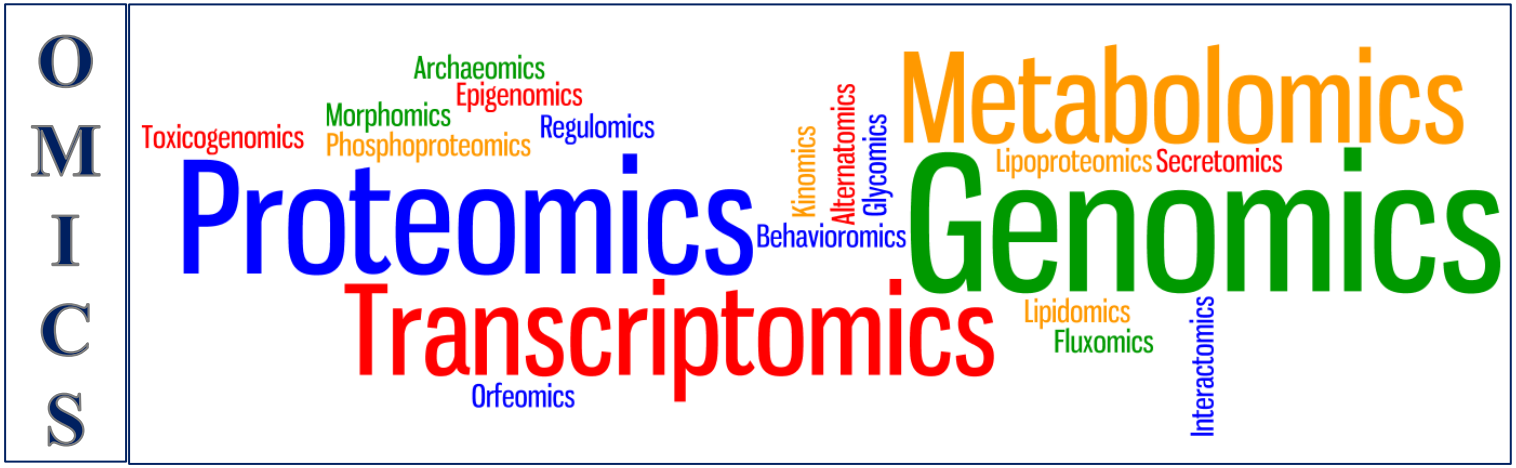
Assisted Reproductive Technology (ART)



- More than 7/10 transferred embryos fail to implant
- Birth live rate < 20%

>30% of implantation failures are thought to result from abnormal endometrial receptivity and/or to defects in the embryo-endometrium dialogue

Interests of the Omics technologies



Physiology

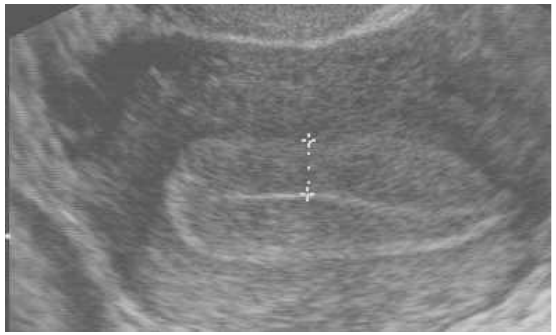
↓

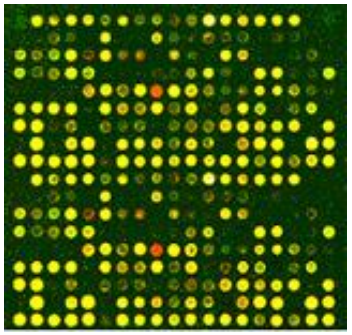
Biomarkers

↓

Diagnostic tools

Endometrial receptivity and COS

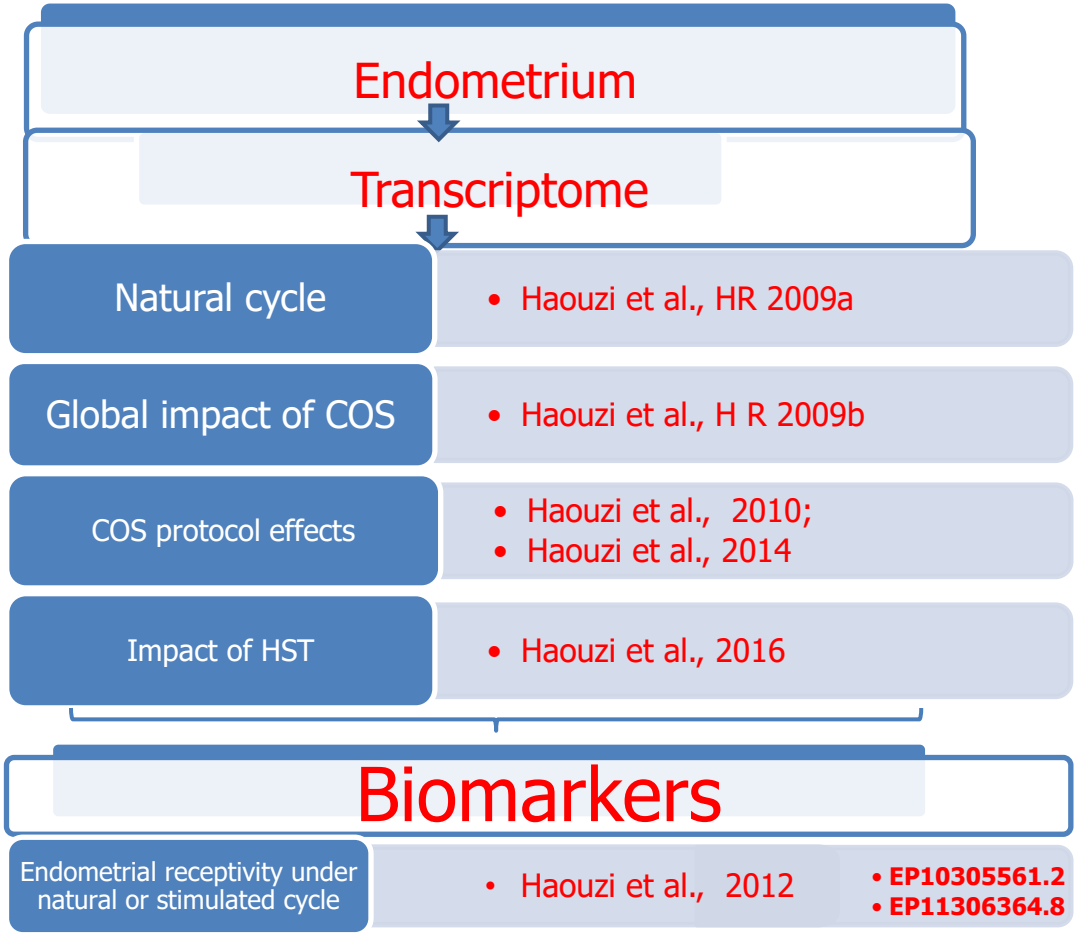




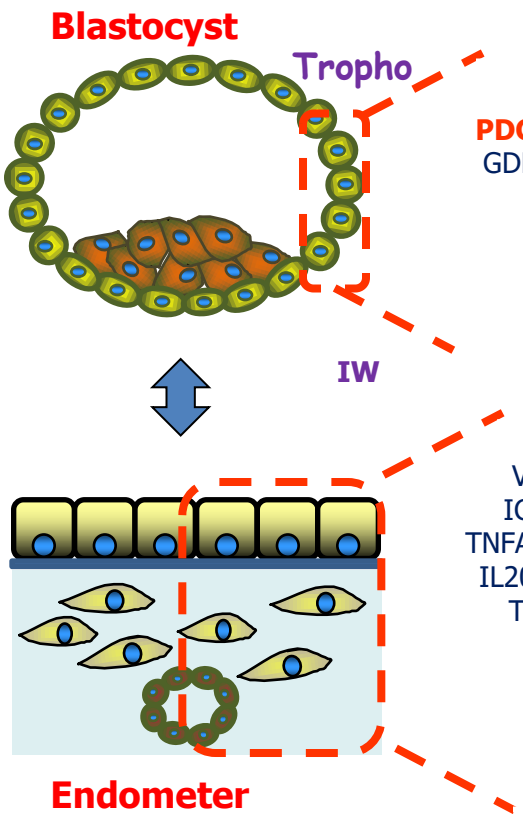
- Advanced endometrial maturation
- Supraphysiological levels of steroids
- Morphological and biochemical alterations

Ubaldi F et al. Fertil Steril 1997;
Lass A et al. Hum Reprod 1998;
Haouzi et al Hum Reprod, 2009a, b;
Cha J et al. Nature Med 2012

Endometrial transcriptome profile during the IW



Embryo-Endometrial tissue dialogue during IW



Growth factors

PDGFA, PGF, GFER, NENF, IGF2BP1, IGF2BP3, GDF15, INHBC, TNFSF9, F11R, FGFR3, FGFR4, IL27RA

VEGFC, HDGFRP3, IGF1, IGFBP2, IGFBP4, IGFBP5, IGFBP7, MEGF9, PDGFC, TGFB1/1, TNFAIP3, IL10RB, IL13RA1, IL15, IL17RD, IL1R1, IL20RA, IL6ST, ILDR1, LIFR, **PDGFRA**, TGFR1, TGFR2, PTGFRN, FGFR10P2, EDNRA, **KDR**

Adhesion molecules and ECM

ITGAE
LAMA1, LAMC1
MCAM
COLEC12
HAPLN1, HMMR

ITGA9, ITGAV, ITGB1, ITGB8
LAMA2, FN1, DCN, FBN1, TIMP2,
TIMP3, COL1/3/4/5/14/15/18
ALCAM, PECAM1, CEACAM1
LGALS1, LGALS3, LGALS8
CD44, SDC2

Haouzi *et* al. HR 2011

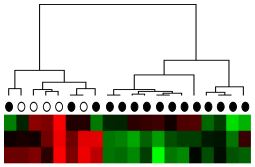
Understand the molecular mechanisms governing the human endometrial receptivity

Physiology

FROM PRE-SCREENING TO FUNCTIONAL ANALYSES

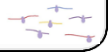
OMICS

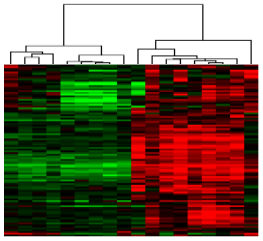
miRNome
microRNAs



Affymetrix® miRNA 4.1 Array Strips

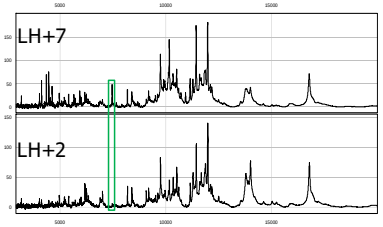
Transcriptome
mRNAs





GeneChip® Human Genome U133 Plus 2.0 Array
(Haouzi et al., 2009a, b; 2010; 2011; 2012; 2014; 2015)

Proteome
Proteins

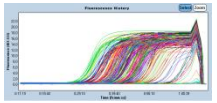


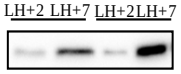
Seldi-Tof + LC/MS/MS
(anion exchange, pH9)

(Bissonnette et al., 2016)

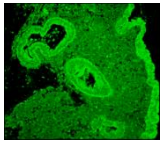
BIOLOGY

qRT-PCR/western blot
in fertile and RIF patients

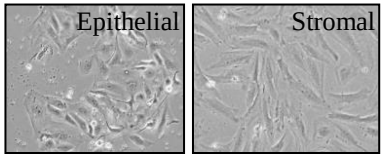




Immunofluorescences of endometrium sections



Purification and primary culture of Human endometrial cells

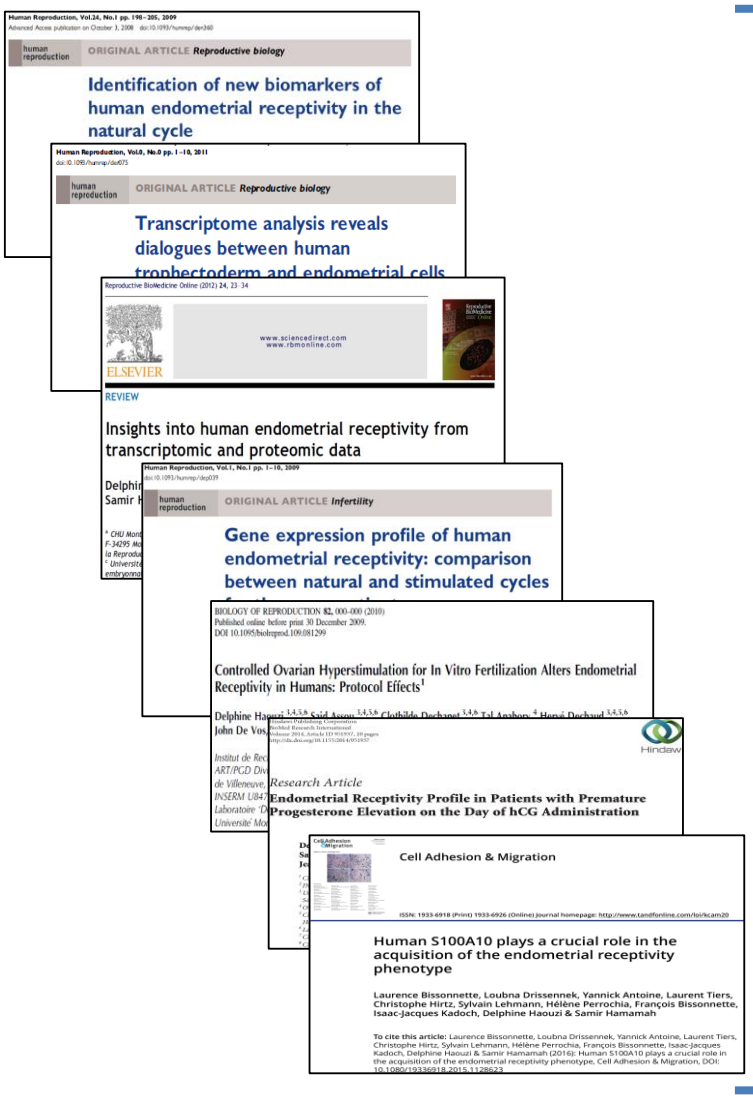


shRNA

Complete overview to select/identify relevant biomarkers of endometrial receptivity

Assessing the human endometrial receptivity: the Win-Test®, Window Implantation Test

Diagnostic tool

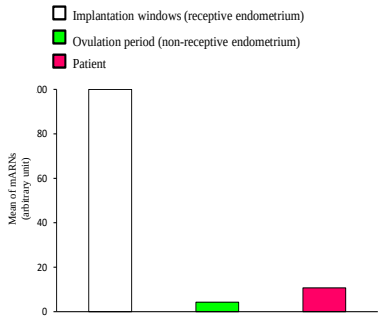


Biomarkers's selection

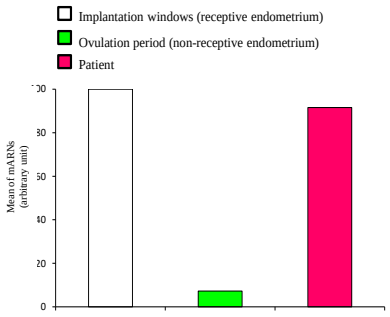
Patent n°EP10305561.2

Quantification by RT-qPCR

Non-receptive



Receptive

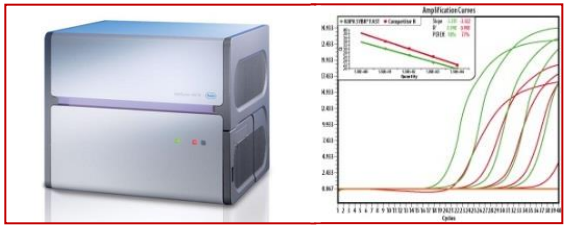


Principle of the Win-Test

Biopsy → **qRT-PCR** → **Results**

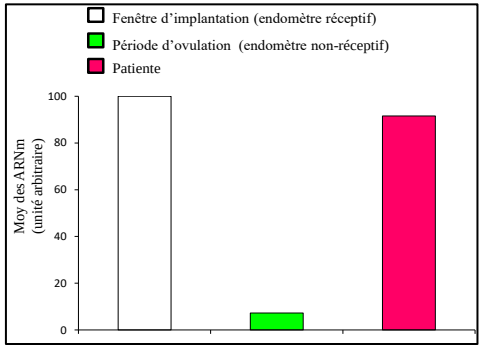
Implantation window

RNA extraction and quantification by qRT-PCR

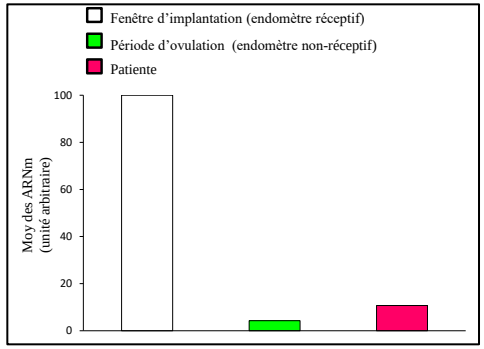


Analysis et interpretation

Receptive endometrium



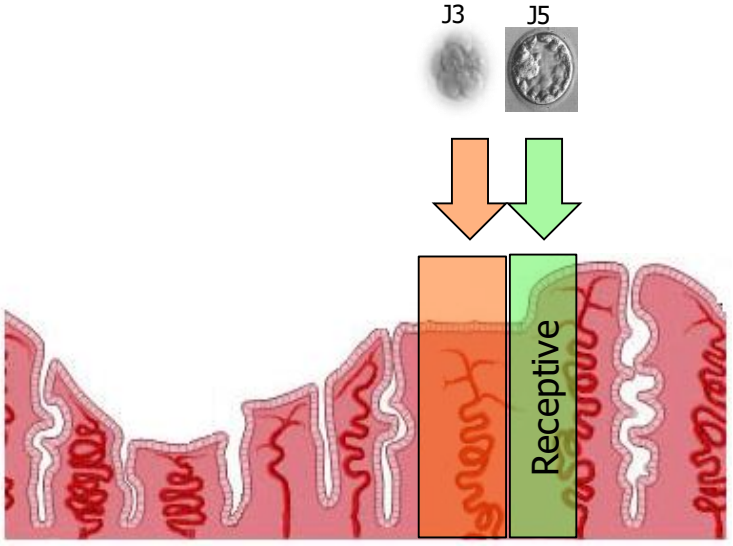
Non-receptive endometrium



Personalized embryo transfers according to the Win-Test® results

To detect the IW under natural cycle or hormone replacement therapy (HRT)

To perform personalized embryo transfer in the respect of the synchronization of the fœto-maternal dialogue

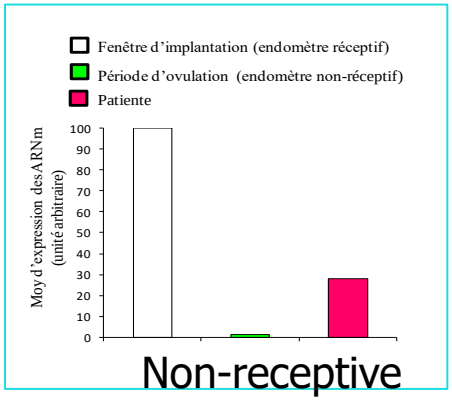


Receptive endometrium	⟷	Blastocysts
72h/48h before the endometrium become receptive	⟷	Day 2/3 embryos

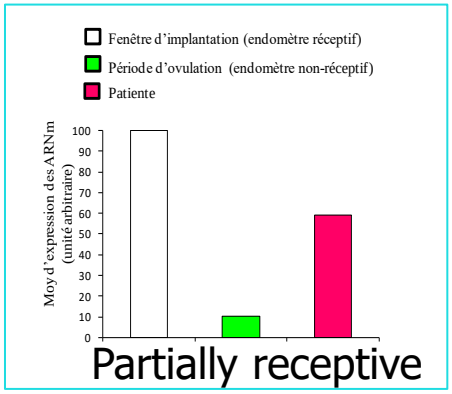
Detection of the implantation window

Patient:
33 years, male infertility

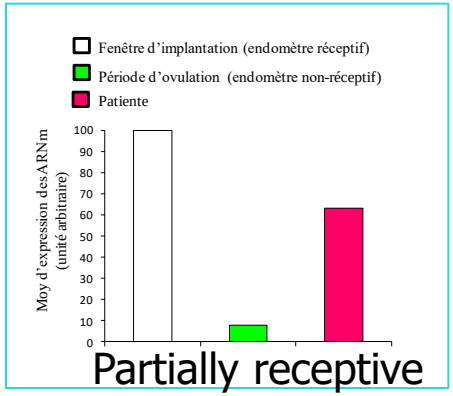
1st evaluation:
Pg+6



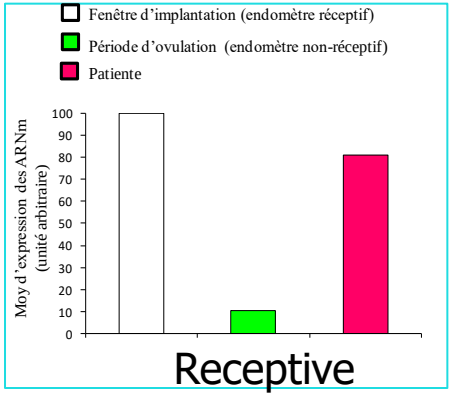
2nd evaluation
Pg+7



3rd evaluation
Pg+8

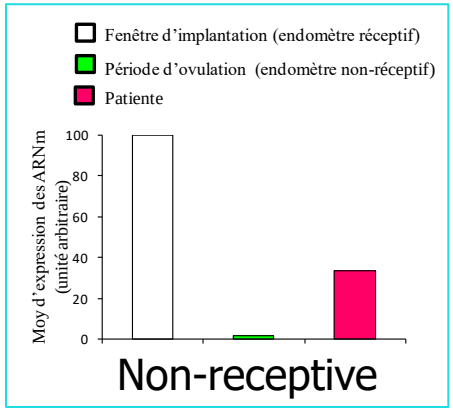


4th evaluation
Pg+9

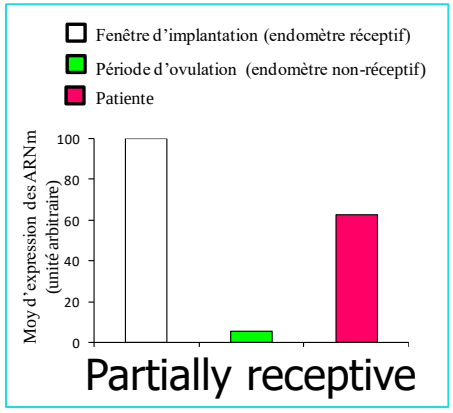


Patient:
37 years, unexplained infertility

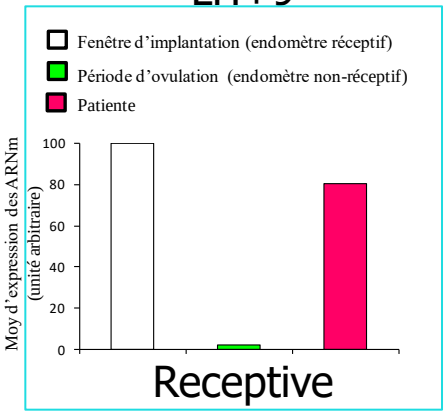
1st evaluation
LH+7



2nd évaluation
LH+8



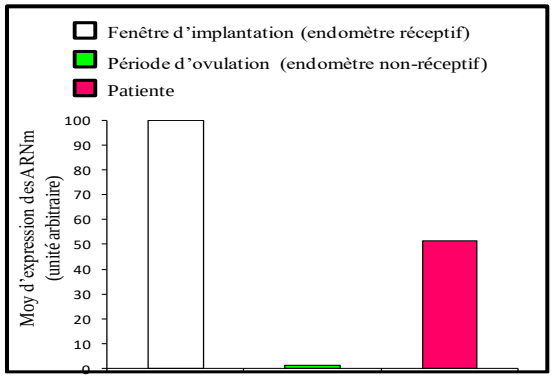
3rd evaluation:
LH+9



Des exemples de transferts personnalisés

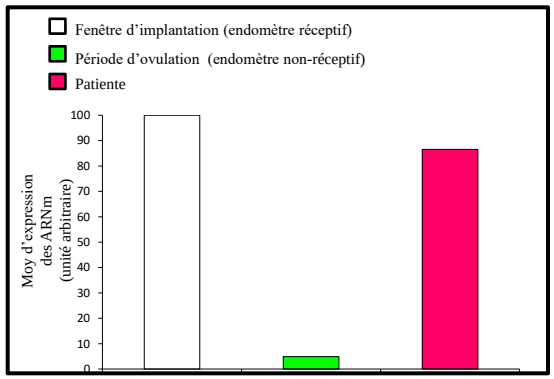
Patient:
32 years
3 IVF attempts ↔ 6 fresh embryos transfered: failures
3 egg donation (Spain, Czech republic) ↔ 7 fresh transfered embryos+ 2 frozen embryos transfered : failures

1st evaluation: Win-Test at Pg+6



Partially receptive

2nd evaluation: Win-Test at Pg+8



Receptive

Suggestion :Day-5 embryo ransfert at Pg+8 or a day-3 at Pg+6

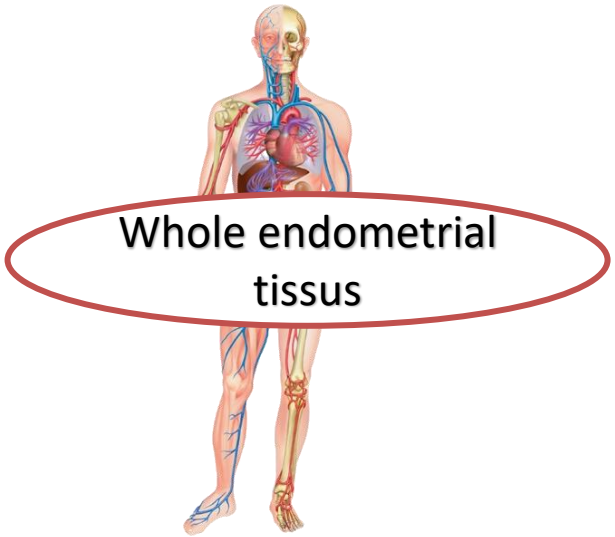
2 day-3 frozen embryos transferred, subsequent cycle — Pg+6

Birth
(2 boys)

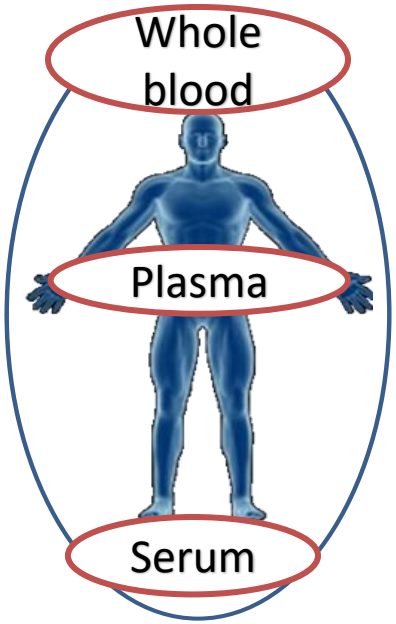
Toward a new generation of the Win-Test: non-invasive endometrial receptivity test

Objective: avoid to perform an endometrial biopsy

Tissue/cells

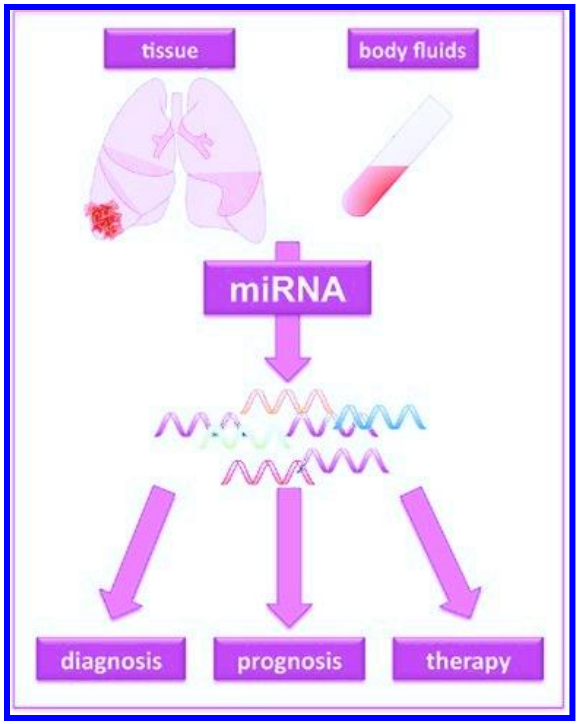


Bloodstream ?



Circulating microRNAs as biomarkers of human endometrial receptivity: myth or reality ?

Circulating microRNAs: diagnostic, pronostic tools and therapeutic target in Oncology



Lindinger et al., Cancer Genetics, 2012

- Circulating miRNAs:
diagnostic and pronostic tools for certain cancers

- OncomiRs, over-expressed in tumour

  - Therapeutic targets

  - Anti-miR drug

Circulating microRNAs:
powerful tools in oncology and in other patholgies,
including gynecological and obstetric disorders

Overview of studies on miRNAs and Human endometrial receptivity

Study	Patients	Number of patients	Biological samples	Approach	Compared samples (number, cycle day)	Number of microRNAs	
						up-regulated	down-regulated
Kuokkanen *et al*., 2010	Fertile volunteers	8	Endometrial tissue	miRNAs microarrays	Purified epithelial cells from biopsies obtained during the late proliferative (n=4) *vs.* Mid-secretory phase (n=4) (day 12±1) (day 19-23)	12	12
Sha *et al*., 2011	Infertile patients	5	Endometrial tissue	Deep sequencing	Prereceptive (1 pool of 5) *vs*. Receptive (1 pool of 5) (LH+2) (LH+7)	8	12
Altmäe *et al*., 2013	Fertile patients	7	Endometrial tissue	miRNAs microarrays	Prereceptive (n=4) *vs*. receptive (n=3) (LH+2) (LH+7)	2	2
Kresowik *et al*., 2014	Fertile patients	12	Endometrial tissue	RTqPCR of 8 selected miRNAs	Proliferative phase (n=12) *vs*. Secretory phase (n=12) (day 7-10) (day 20-24)	4	2
			Serum			1	0
Vilella *et al*., 2015	Fertile patients	20	Endometrial fluid	miRNAs microarrays	Early proliferative phase (n=4) *vs*. implantation windows (n=4) (day 6-8) (day 19-23)	9	0
					Late proliferative phase (n=4) *vs*. implantation windows (n=4) (day 9-14) (day 19-23)	8	0
					Early secretory phase (n=4) *vs*. implantation windows (n=4) (day 15-18) (day 19-23)	1	5
					Late secretory phase (n=4) *vs*. implantation windows (n=4) (day 24-28) (day 19-23)	0	4
Revel *et al*., 2011	Fertile and RIF patients	16	Endometrial tissue	TaqMan miRNA arrays	Fertile patients (n=5) vs. RIF patients (n=11) day 20-24	10	3
Qin *et al*., 2016	Healthy and infertile patients	6	Plasma	miRNAs microarrays	Unexplained recurrent spontaneous abortion (n=3) *vs*. normal early pregnancies (n=3)_6–10 weeks of gestation	9	16

RIF, repeated implantation failure

To date, there are very few studies on miRNAs profiles during the menstrual cycle

miRNAs throughout the menstrual cycle

	Kuokkanen *et al* ., 2010	Kresowik *et al* ., 2014	Sha *et al* ., 2011	Altmäe *et al* ., 2013	Vilella *et al* ., 2015
MIR30D	2.2	2.74	6.92	3.29	2.62 (/ES), 2.98 (/EP), 3.04 (/LP)
MIR30B	2.6	2.96	2.99	4.23	-
MIR31	2.1	1.49	3.32	-	-
MIR203	2.5	2.42	2.01	-	-
MIR503	-3.6	-2.01	-4	-	-
MIR193A-3P	5.2	-	2.27	-	-
MIR455-3P	-	-	-2.23	-	-3.14 (/ES)
MIR455-5P	-	-	-2.53	-	-1.95 (/ES)
MIR424	-	-	-3.18	-	-5.02 (/ES)
MIR29B	2.8	-	-	-	4 (/EP), 3.67 (/LP)
MIR29C	2.6	-	-	-	2.22 (/LP)
MIR200C	2.1	-	-	-	2.51 (/EP), 2.46 (/LP)
MIR210	7.1	-	-	-	2.11 (/LP), 2.16 (/EP)

Fold changes during the implantation window are indicated

ES, early secretory
EP, early proliferative
LP, late proliferative

And few miRNAs are in common between these studies

Study design

Patientes under hormone replacement therapy (HRT)
Biopsies during IW (Pg+6 to Pg+9)

WIN TEST

Endometrial receptivity status

Non-receptive *vs.* Receptive
(n=5) (n=15)
Affymetrix' miRNA 4.1 Array Strips

miRNAs associated with endometrial receptivity ?

Personalized embryo transfers
in **receptive** patients

Outcome

Negative β-hCG *vs.* Positive β-hCG
(n=5) (n=5)
Affymetrix' miRNA 4.1 Array Strips

miRNAs associated with IF ?

Miscarriage* *vs.* Live birth
(n=5) (n=5)
Affymetrix' miRNA 4.1 Array Strips

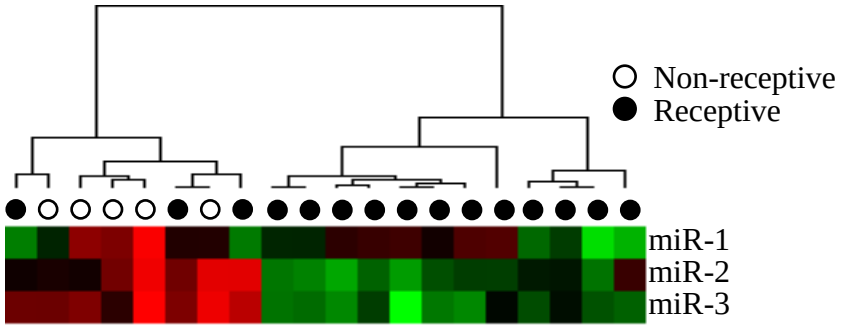
*8-12 weeks post-amenorrhoea

miRNAs associated with miscarriage ?

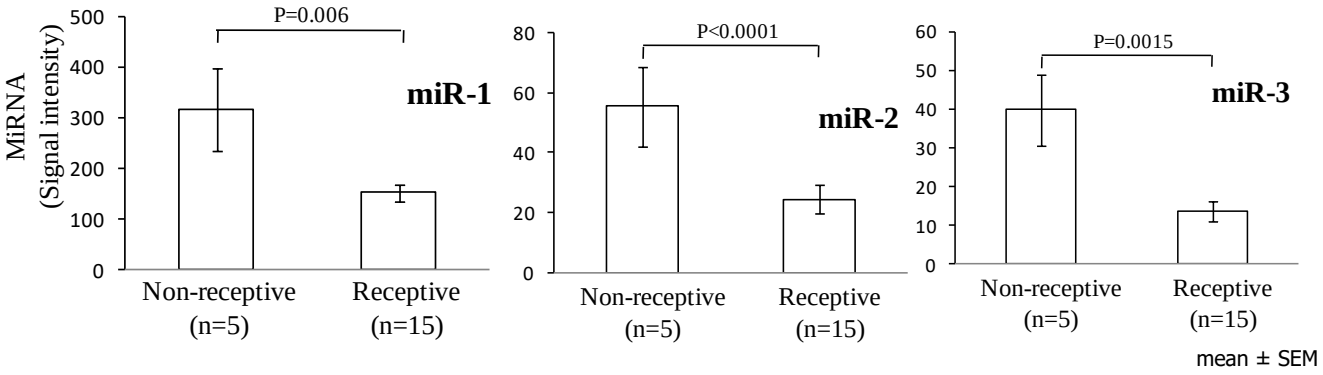
Endometrial miRNAs associated with endometrial receptivity

Supervised classification

Hierarchical clustering of 20 endometrium samples diagnosed as receptive or non-receptive during the theoretical implantation window

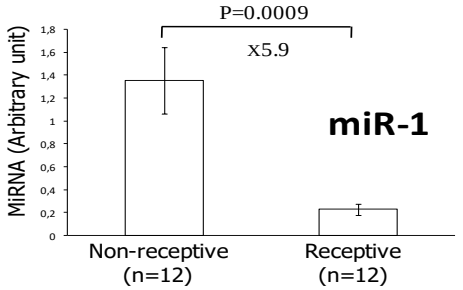


The microarray signals of each candidate

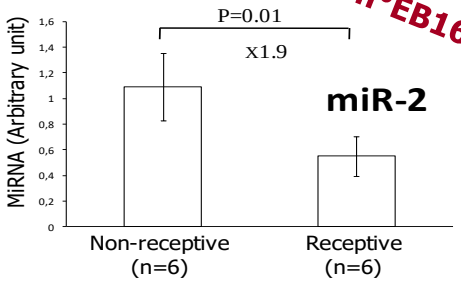


Validation by RT-qPCR in an independent cohorte

TaqMan miRNA assay



Patent n°EB16391



For the miR-3, ongoing validation

Identification of 3 miRNAs over-expressed in non-receptive endometrium

Endometrial miRNAs associated with implantation failure

Positive β-hCG *vs.* Negative β-hCG

Number of miRNAs differentially expressed

Expression console (Affymetrix)

Significant analysis of microarrays

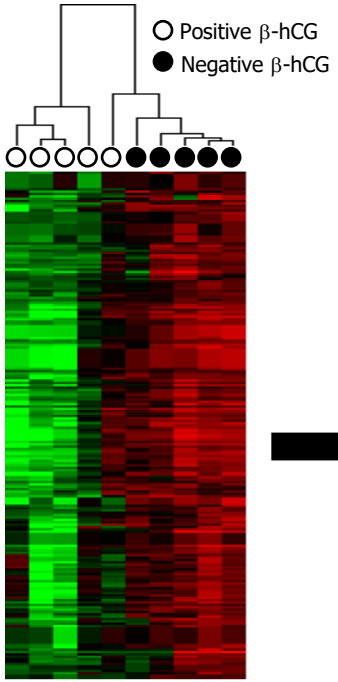
ANOVA	T-test	Wilcoxon
240	242	257

Fold change ≥2, FDR ≤ 5%

215 miRNAs
in commun to the 3 statistical analyses

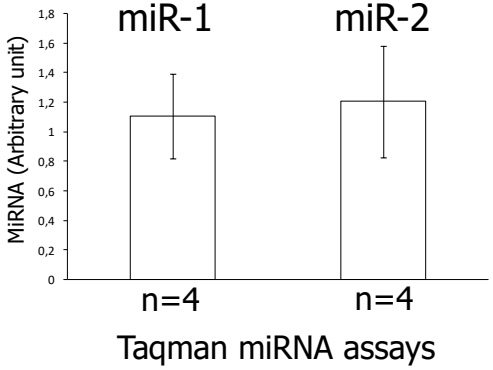
All over-expressed in the endometrium from the 'negative β-hCG' group

Supervised cluster



Selection of two miRNAs for quantification in serum samples

Patent n°EB16392



Identification of endometrial miRNAs associated with implantation failure that can be detected in serum samples

Endometrial miRNAs associated with miscarriage

Miscarriage *vs.* Live birth

Number of miRNAs differentially expressed

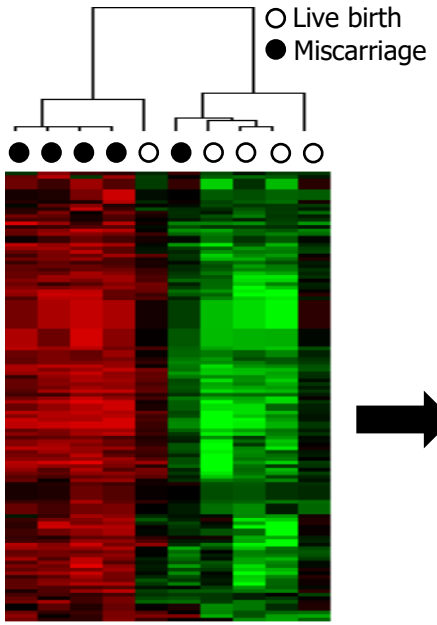
Expression console (Affymetrix)	Significant analysis of microarrays	
ANOVA	T-test	Wilcoxon
146	206	208

Fold change ≥2, FDR ≤ 5%

126 miRNAs
in commun to the 3 statistical analyses

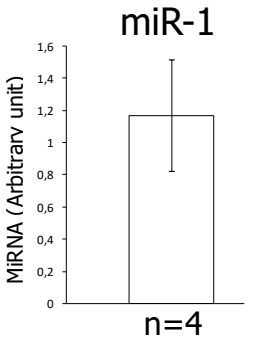
All over-expressed in the endometrium from the 'miscarriage' group

Supervised cluster



Selection of five miRNAs for quantification in serum samples

Patent n°EB16393



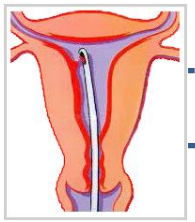
Taqman miRNA assays

Ongoing validation…

Identification of endometrial miRNAs associated with miscarriage that can be detected in serum samples

Conclusion and perspectives

Endometrial tissue



miRNA associated With: Endometrial receptivity, Implantation failure & miscarriages

Bloodstream

Quantification in the bloodstream ?

Develop a non-invasive diagnostic/pronostic tool to limit the use of invasive endometrial biopsies for the evaluation of endometrial receptivity AND predict attempt outcomes

This circulating miRNA-based test might become a rapid, easy and cheaper clinical diagnostic tool to allow performing personalized embryo transfer.

It would be possible to select strategies by which miRNA technologies might be utilized in novel, non-hormonal therapeutic approaches to avoid miscarriages and consequently, to increase the pregnancy rate.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