



## From non invasive PGD to NIPT: Analysis of the cumulus cells and beyond

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### Conflict of Interest

I declare that I have no commercial or financial interests in relation to the subject of this presentation or its content

## Why cumulus cells?

2

S.M. Hawkins, M.M. Matzuk / *Annales d'Endocrinologie xxx (2010) xxx–xxx*

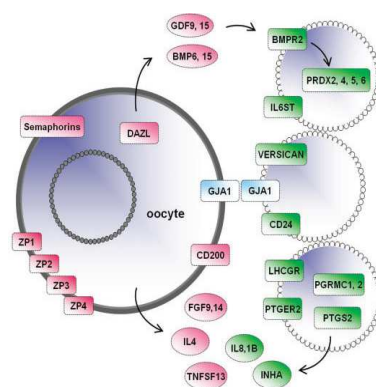
Table 1

Mouse models with defects in cumulus expansion. For more detailed information, refer to reference [4].

Gene (symbol)	Fertility status
Prostaglandin-endoperoxide synthase 2 ( <i>Ptgs2</i> ; <i>Cox2</i> )	Mostly infertile
Prostaglandin E receptor 2, subtype EP2 ( <i>Ptger2</i> )	Subfertile
Pentraxin 3 ( <i>Ptx3</i> )	Subfertile
Tumor necrosis factor $\alpha$ induced protein 6 ( <i>Tnfaip6</i> )	Infertile
Sulfotransferase family 1E, member 1 ( <i>Sult1e1</i> )	Subfertile
Alpha 1 microglobulin/bikunin ( <i>Ambp</i> )	Subfertile
Amphiregulin ( <i>Areg</i> )	Subfertile
Bone morphogenetic protein 15 ( <i>Bmp15</i> )	Subfertile
Bone morphogenetic protein receptor, type IB ( <i>Bmpr1b</i> )	Subfertile
Epiregulin ( <i>Ereg</i> <sup>wa2/wa2</sup> ; hypomorph)	Subfertile
Mitogen-activated protein kinases 3 and 1 ( <i>Mapk3</i> <sup>-/-</sup> <i>Mapk1</i> cKO)	Infertile
Nuclear receptor subfamily 5, group 2, member 1 ( <i>Nr5a1</i> ; SF1, steroidogenic factor 1) (cKO)	Infertile
Nuclear receptor subfamily 5, group 2, member 2 ( <i>Nr5a2</i> ; Lrh1, liver receptor homolog 1) (cKO)	Infertile

cKO: conditional knockout.

## What are the cumulus cells?



the human-cumulus oocyte complex  
gene expression profile



CC mirror of oocyte quality and competence

### Where ?

Surrounding oocyte cells

### When ?

During oocyte maturation and  
first embryo cleavage

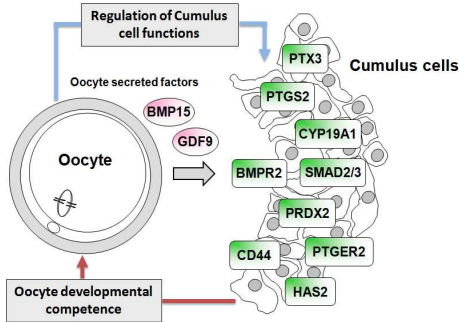
### What role ?

Bi-directional communication

Assou....Hamamah, 2006 HR

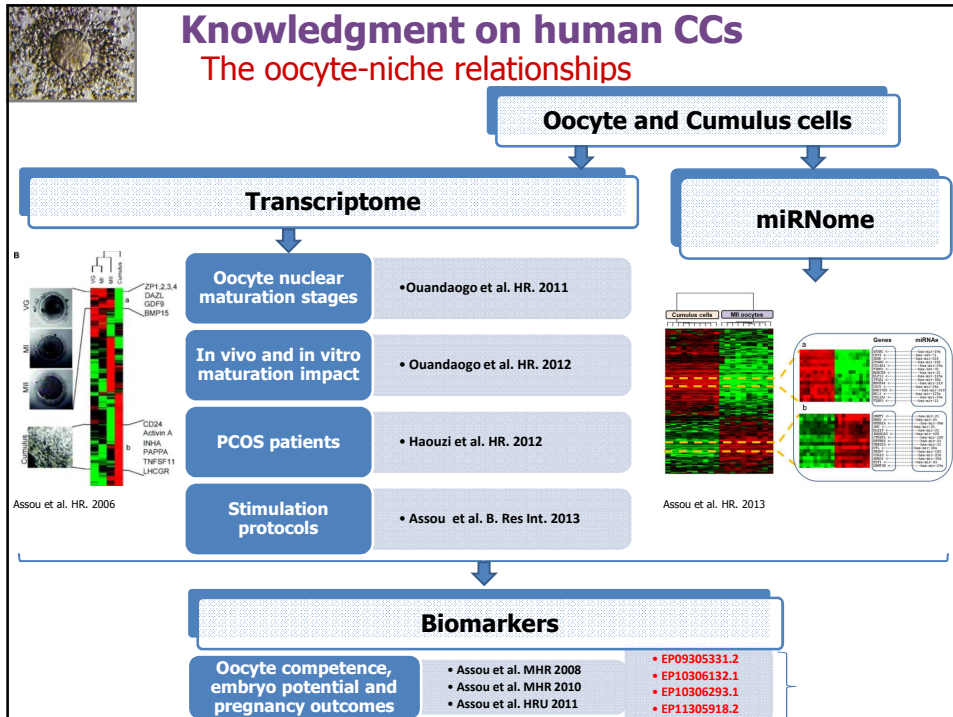
## Why cumulus cells?

Cumulus cells as biomarker for oocyte quality, embryo and pregnancy outcomes



- Coordinates follicle development with oocyte maturation
- Provides energy substrate for oocyte meiotic resumption
- Regulates oocyte transcription
- Promotes nuclear and molecular maturation of the oocyte

Assou...Hamamah et al. 2010, MHR



## MicroRNAs: new candidates for the regulation of the human cumulus–oocyte complex

S. Assou<sup>1,2</sup>, T. Al-edani<sup>1,2</sup>, D. Haouzi<sup>2</sup>, N. Philippe<sup>2</sup>, C.-H. Lecellier<sup>3</sup>,  
D. Piquemal<sup>4</sup>, T. Commes<sup>2,4</sup>, O. Ait-Ahmed<sup>2</sup>, H. Dechaud<sup>1,2,5</sup>,  
and S. Hamamah<sup>1,2,5,\*</sup>

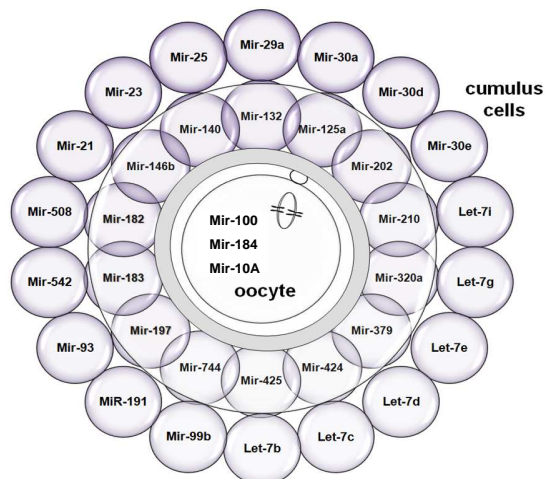
## MicroRNA expression in CCs and oocyte Important facts

### 3 miRNAs in MII oocytes

MIR184 (1988 reads) } most abundant  
MIR10A (555 reads) }

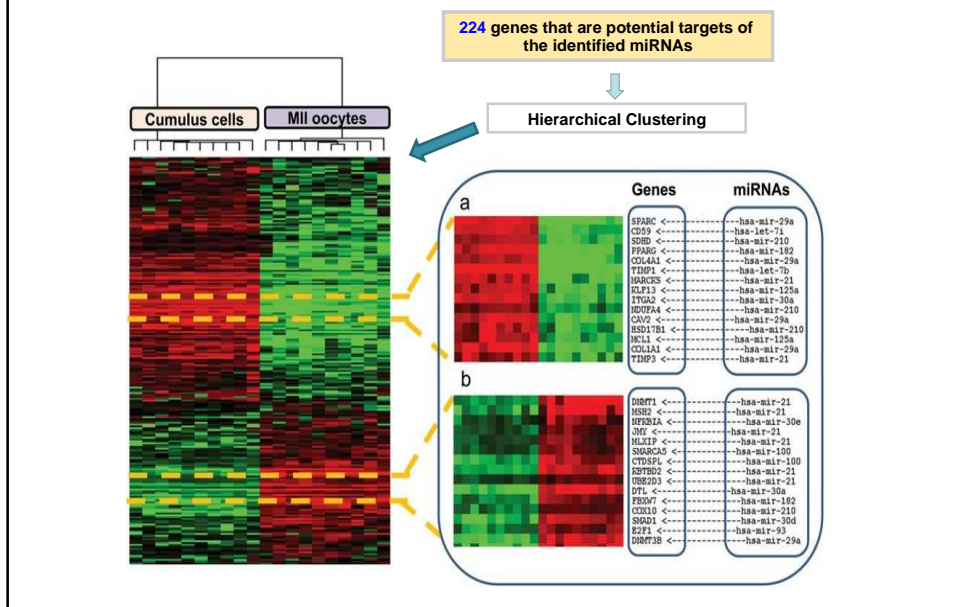
### 32 miRNAs in cumulus cells

LET7b (51 reads) } most abundant  
MIR21 (28 reads) }

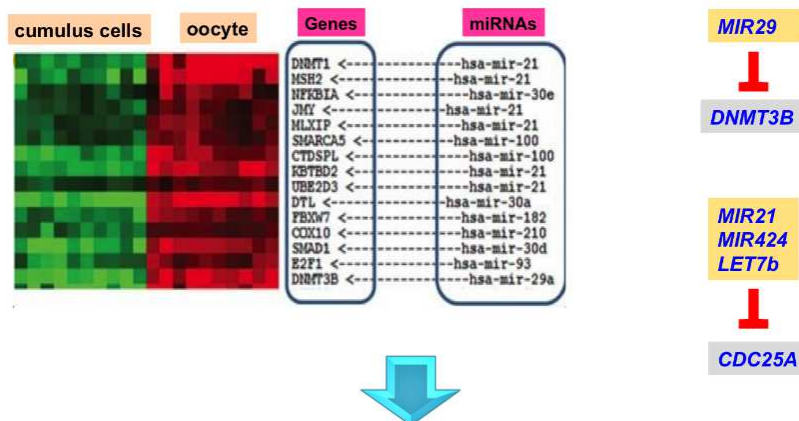


Assou...Hamamah et al. 2013, HR

## Differentially expressed genes in oocyte and CCs are predicted targets of oocyte and CC-miRNAs

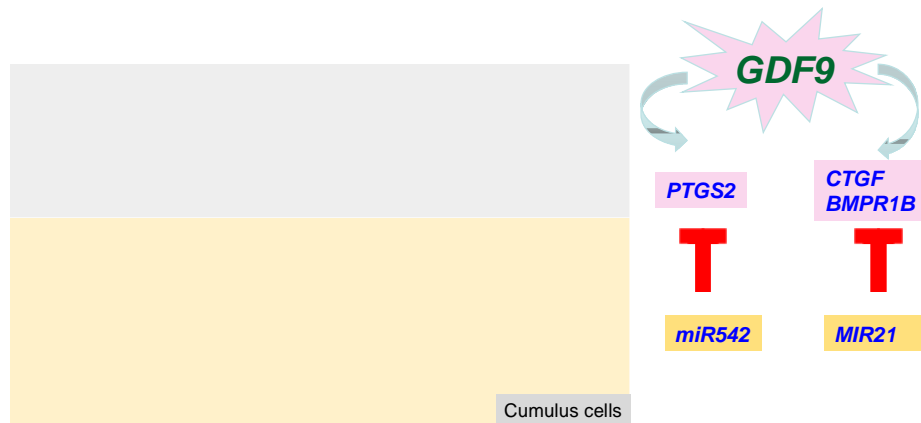


## Important genes for chromosome or chromatin functions are up-regulated in the MII oocyte and are predicted targets of CC-miRNAs



this suggests a dialogue between the cumulus cells and the oocyte through microRNA action

## Genes involved in a crosstalk between CCs and oocyte are predicted targets of CC-miRNAs



**GDF9** is known as a regulator of **PTGS2**, **CTGF**, **BMPR1B** and to be essential in oocyte-cumulus cells crosstalk

- We have reported the first sequencing data of small non coding RNAs in the human cumulus cells and oocyte.
- Our results illustrate the cellular specificity of the microRNAs. Some miRNAs are highly abundant in the oocyte and not even detected in the cumulus cells.
- CC-miRNA may be regulators of mRNAs over-expressed in the oocyte, illustrating the dialogue between cumulus cells and oocyte through miRNA action.



### The impact of female aging on gene expression in human CCs

- Molecular signature according to female age
- Pathways significantly affected by female aging
- Predicted miRNAs that target genes impacted by female age

Al-Edani ....Hamamah et al, 2014

### Impact of female aging on oocyte quality

Decrease of proteins stored in the oocyte

mtDNA damage, reduction of ATP production

Reduction of oocyte metabolic function

Increase of oxidative stress and apoptosis

Increase of oocyte aneuploidy

these modifications affect oocyte competence and quality

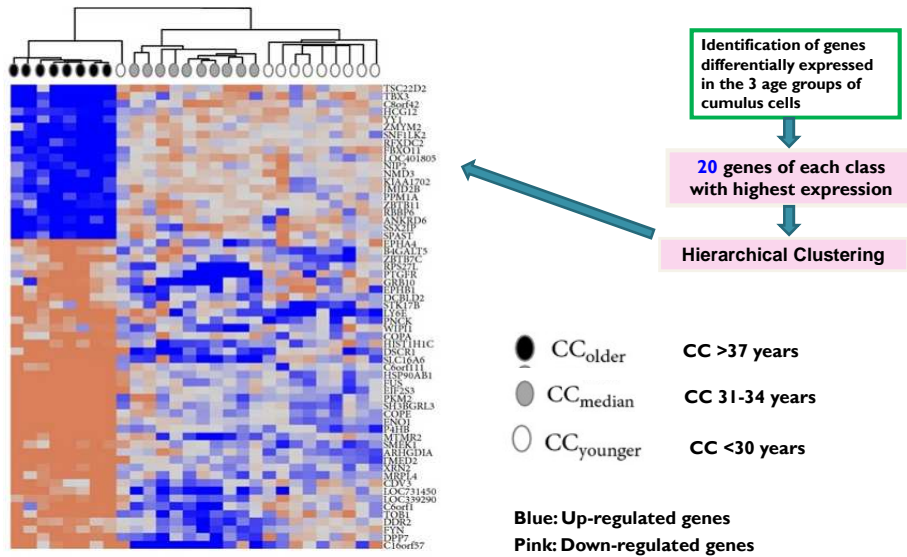
Bentov et al., 2011; Fragouli et al., 2010



Question: which molecular changes account for these physiological changes ?

Impact of aging on gene expression in the CCs

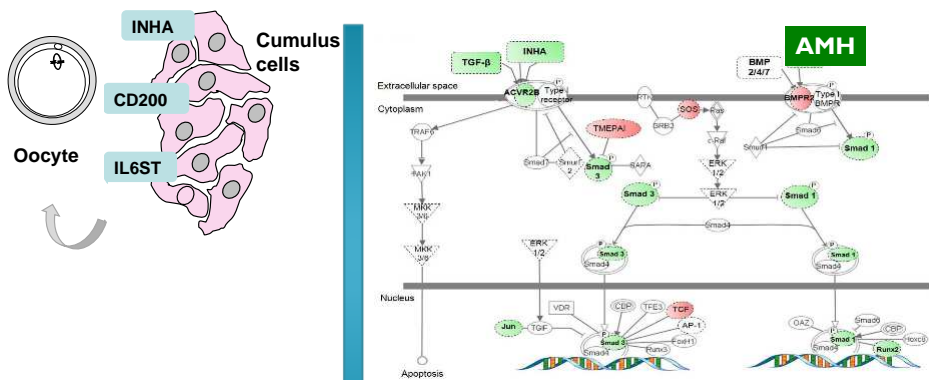
### Molecular signature of cumulus cells according to female age



Al-Edani ....Hamamah et al, 2014

Molecular signatures reveal that CC >37 years are distantly located from the other groups

### Genes that play an essential role in the CCs-oocyte dialogue and oocyte quality are down-regulated in older CCs

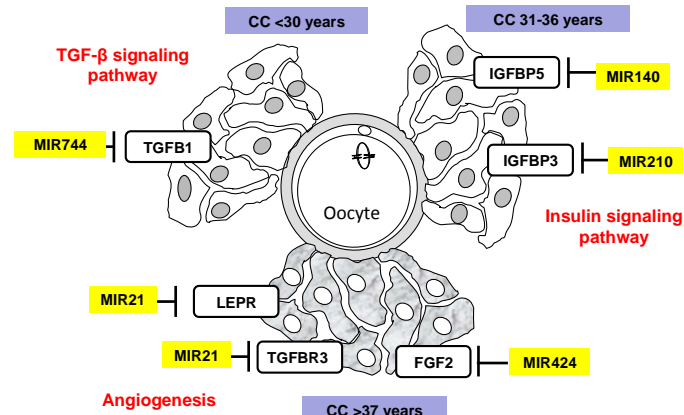


Many genes of the TGF-b pathway are down-regulated (green), a few of them are up-regulated (red)

Al-Edani ....Hamamah et al, 2014



## Genes impacted by aging in relation to CC-miRNAs



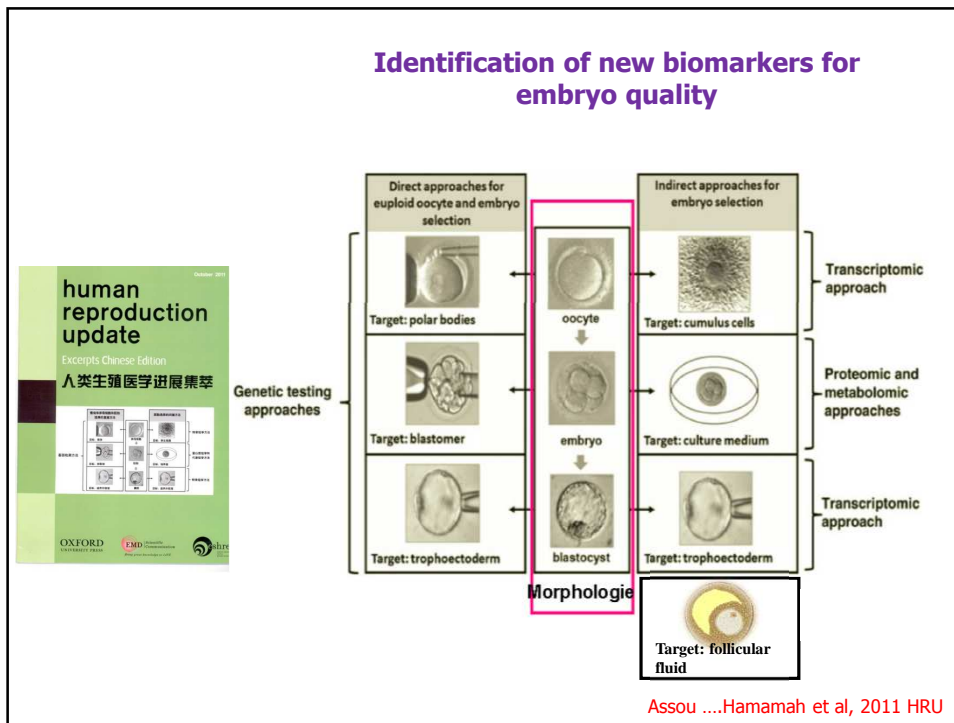
CC-miRNAs are validated regulators of the genes involved in pathways altered with aging  
 These miRNAs are potential biomarker candidates of follicle aging

Al-Edani ....Hamamah et al, 2014

## Transcriptomic data show

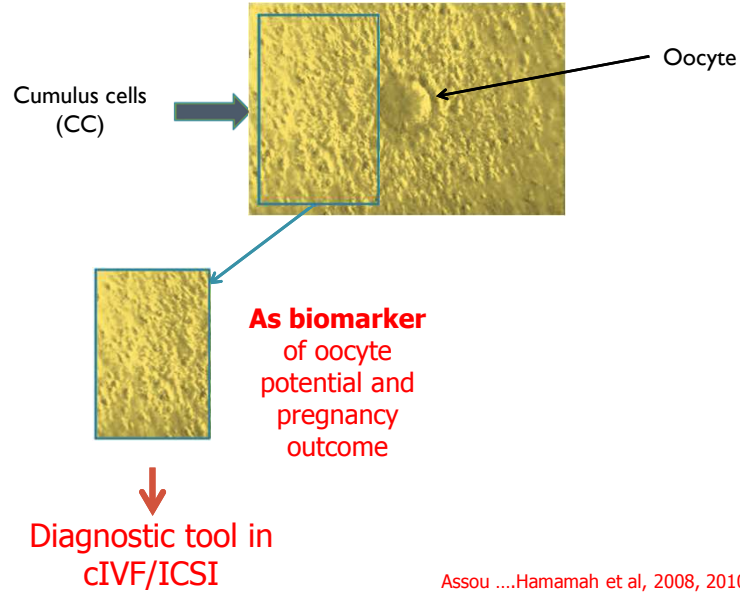
- aging widely impacts gene expression
- the decrease in fertility that occurs at 37 is supported by a dramatic molecular change after the age of 36
- the physiological impact of aging is underlied by an alteration of expression of genes and pathways that are critical for oocyte quality and competence (insulin,TGF-beta etc)
- Genes that are essential to buffer the effect of hypoxia (angiogenic genes), which is linked with aging, are up-regulated in older CCs

## Identification of new biomarkers for embryo quality



**CCs Gene expression profile as non invasive test to predict embryo as well pregnancy outcomes**

## Cumulus cells predictive value



A meta analysis was performed on 27 studies  
7 genes stand out as common biomarkers through different studies  
(HAS2, SERPIN2, PTGS2, PTX3, GREM1, VCAN, RGS2)

	Oocyte maturation			Embryo quality and competence				Pregnancy		Live birth		
	MII oocyte	Fertilization	expanded Cumulus	top quality embryo	embryo outcome D2/3	Embryo outcome D5/6	Early cleavage	Fragmentation	Pregnancy	Implantation	Live birth	Weight at birth
McKenzie et al. 2004	*HAS2	*PTGS2, GREM1		HAS2, PTGS2, GREM1 ↑								
Zhang et al. 2005		PTX3 ↑								PTX3 ↑		
Cillo et al. 2007		HAS2, GREM1 ↑		HAS2, GREM1 ↑								
Feuerstein et al. 2007	STAR, PTGS2, AREG, SCD1, SCD5 ↓ CX43 ↓					STAR, AREG, PTGS2, CX43, SCD1, SCD5 ↓						
Assou et al. 2008										BCL2L11, PCK1 ↑ et NFIB ↓		
Hamel et al. 2008										FDX1, 3betaHSD, CYP19A1, SERPIN2, CDC42 ↑		
Van Montfoort et al. 2008							CCND2, CTNND1, CXCR4, DHCR7, DVL3,					

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							GPX3, HSPB1, TRIM28 ↓					
Anderson et al. 2009	PTGS2 ↑	BDNF ↓	BDNF, TNFAIP6, PTX3 ↓	BDNF ↓ et PTGS2 ↑	GREM1 ↑ et BDNF ↓							
Assidi et al. 2011									DPP8, HIST1H4C, UBQLN1, CALM1, NRP1, PSMC6 ↑ et TOM1 ↓			
Hamel et al. 2010									PGK1, RGS2 ↑			
Hamel et al. 2010									UGP2, PHLDA1 ↑			
Adriaenssens et al. 2010	VCAN ↓				ALCAM, GREM1 ↑			RPS6KA2 ↑				
Yung et al. 2010		ADAMTS-1 ↑										
Gebhardt et al. 2011											VCAN, PTGS2 ↑	PFKP ↑
Wathlet et al. 2011	VCAN ↓ PTGS2 ↑				TRMP7, ITPKA ↑	SDC4, ITPKA ↑			SDC4, VCAN ↑			
Ouadaogo et al. 2011	CBX3, LIMS1, F2RL1, ANKRD5, THBS1, QKI, SLC38A2, SUMF1, THDF2 ↓, C14ORF											

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	MII oocyte	Fertilization	expanded Cumulus	top quality embryo	embryo outcome D2/3	Embryo outcome D5/6	Early cleavage	Fragmentation	Pregnancy	Implantation	Live birth	Weight at birth
	4,PTGES, COX17, C5ORF2, 5,UBR3, BRD3 ↑											
Wathlet et al. 2012					TRMP7	CYP11A1			ITPKA, EFN2 ↑		EFNB2, CAMK1D, STC2	
Feuerstein et al. 2012						PLIN2, RGS2, ANG ↑			RGS2 ↑			
Chung-Hao Lu et al. 2013	SERPIN2 ↓		SERPIN2 ↓ et PLAU ↑ (CC souris)									
Ekart et al. 2013	VCAN, HAS2 ↓					HAS2, VCAN et PR ↑					VCAN ↑	
Assou et al. 2013					PTX3, et STC2 ↑	TRIM65, GSTM2 ↓						
Iager et al. 2013									FGF12, GPR137B, SLC2A9, ARID1B, NR2F6, ZNF132, FAM36A ↑ et ZNF93, RHBDL2, DNAJC15, M TUS1, NUP133 ↓			
Wathlet et al. 2013									EFNB2 ↑			
Papler et al. 2014									EFNB2, RGS2, VCAN			
Sheng-Hsiang Li et al. 2015	GJAL, SERPIN1	PRSS35 ↑										

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	MI I oocyte	Fertilization	expanded Cumulus	top quality embryo	embryo outcome D2/3	Embryo outcome D5/6	Early cleavage	Fragmentation	Pregnancy	Implantation	Live birth	Weight at birth
Papler et al. 2015		No differentially expressed gene identified								No differentially expressed gene identified		
Assidi et al. 2015				NRP1, CALM1, DPP8, UBQLN1, PSMD6 ↑								

## STATE OF THE ART

**OOCYTE MATURATION** → 13 STUDIES

**EMBRYO OUTCOME** → 12 STUDIES

**PREGNANCY** → 11 STUDIES

**LIVE BIRTH** → 3 STUDIES

# LIMITS

## META-ANALYSIS

Comparison of 26 studies

No common gene  
in microarray  
analysis  
!!

9 genes in common  
in RT-qPCR analysis

7 genes

Oocyte maturation

VCAN

HAS2

Live birth

1 gene

Embryo competence

2 genes

+

+

Pregnancy

1 gene

Common genes  
between  
different  
end  
point

**2014**

Human Reproduction Update Advance Access published June 27, 2014  
 Human Reproduction Update, Vol.6, No.3, pp.1-18, 2014  
 doi:10.1093/humupd/dnu031

human reproduction update

**Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy**

S. Traver<sup>1</sup>, S. Assou<sup>1,2</sup>, E. Scalici<sup>1,3</sup>, D. Haouzi<sup>1</sup>, T. Al-Edani<sup>1,2</sup>, S. Belloc<sup>2</sup>, and S. Hamamah<sup>1,3,4\*</sup>

www.nature.com/scientificreports

**2016**

SCIENTIFIC REPORTS

OPEN

**Circulating microRNAs in follicular fluid, powerful tools to explore *in vitro* fertilization process**

Received: 24 July 2015  
 Accepted: 08 April 2016  
 E. Scalici<sup>1,2,3</sup>, S. Traver<sup>1</sup>, T. Mullet<sup>1,2</sup>, N. Molinari<sup>1</sup>, A. Fenières<sup>1</sup>, C. Brunet<sup>1</sup>, S. Belloc<sup>2</sup> & S. Hamamah<sup>1,2,3\*</sup>

Hum. Reprod. Advance Access published July 30, 2013  
 Human Reproduction, Vol.6, No.3, pp.1-12, 2013  
 doi:10.1093/humrep/dnt229

human reproduction ORIGINAL ARTICLE Reproductive biology

**MicroRNAs: new candidates for the regulation of the human cumulus-oocyte complex**

S. Assou<sup>1,2</sup>, T. Al-Edani<sup>1,2</sup>, D. Haouzi<sup>1</sup>, N. Philippe<sup>1</sup>, C.-H. Lecellier<sup>2</sup>, D. Piquemal<sup>1</sup>, T. Commes<sup>1,3</sup>, O. Ait-Ahmed<sup>1</sup>, H. Dechaud<sup>1,3</sup>, and S. Hamamah<sup>1,3,4\*</sup>

Hum. Reprod. Advance Access published September 29, 2014  
 Human Reproduction, Vol.6, No.9, pp.1-9, 2014  
 doi:10.1093/humrep/dnu229

human reproduction ORIGINAL ARTICLE Embryology

**2013**

**2014**

**Cell-free DNA in human follicular fluid as a biomarker of embryo quality**

E. Scalici<sup>1,2,3\*</sup>, S. Traver<sup>1,2</sup>, N. Molinari<sup>1</sup>, T. Mullet<sup>1,2</sup>, M. Monforte<sup>2</sup>, E. Vintejeoux<sup>1</sup>, and S. Hamamah<sup>1,2,3\*</sup>

**2015**

PLOS ONE

RESEARCH ARTICLE

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

Sabine Traver, Elodie Scalici, Tiffany Mullet, Nicolas Molinari, Claire Vincens, Samir Hamamah

### Cell-free DNA

- Double-stranded molecules
- Short or long fragments
- Apoptotic or necrotic origine
- No identified physiological role

### Circulating microRNAs

- Single-stranded molecules
- 19-25 nucleotides
- Two mechanisms of release
- Degradation of target mRNA and translation inhibition

Schwarzenbach et al., Nature Reviews Clinical Oncology, 2014

Scalici et al,  
2014

## Cell-free DNA in human follicular fluid as a biomarker of embryo quality

E. Scalici<sup>1,2,3,†</sup>, S. Traver<sup>1,†</sup>, N. Molinari<sup>4</sup>, T. Mullet<sup>2,3</sup>, M. Monforte<sup>3</sup>,  
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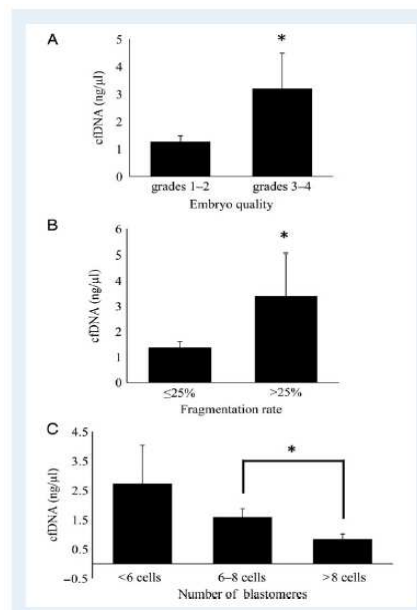


PLoS ONE 10(8): e0136172. doi:10.1371/journal.pone.0136172

Traver et al,  
2015

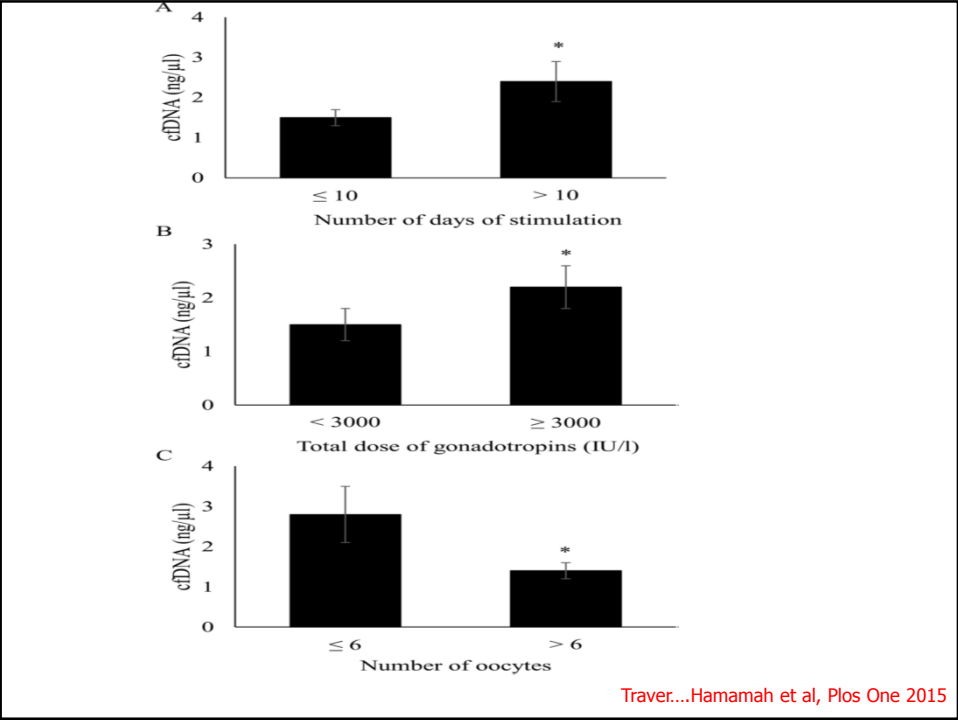
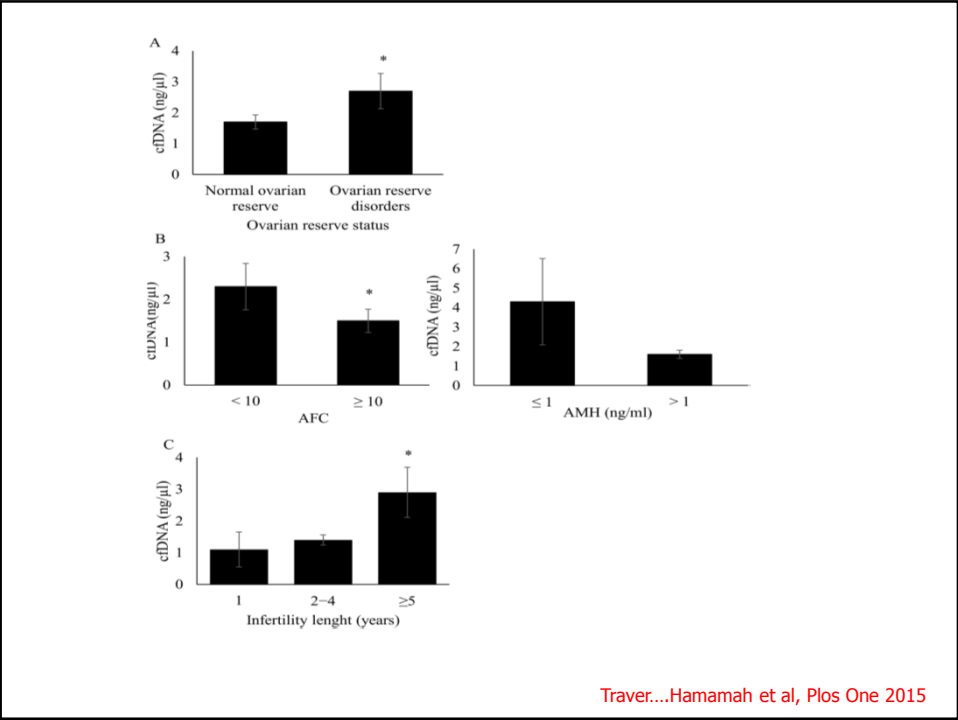
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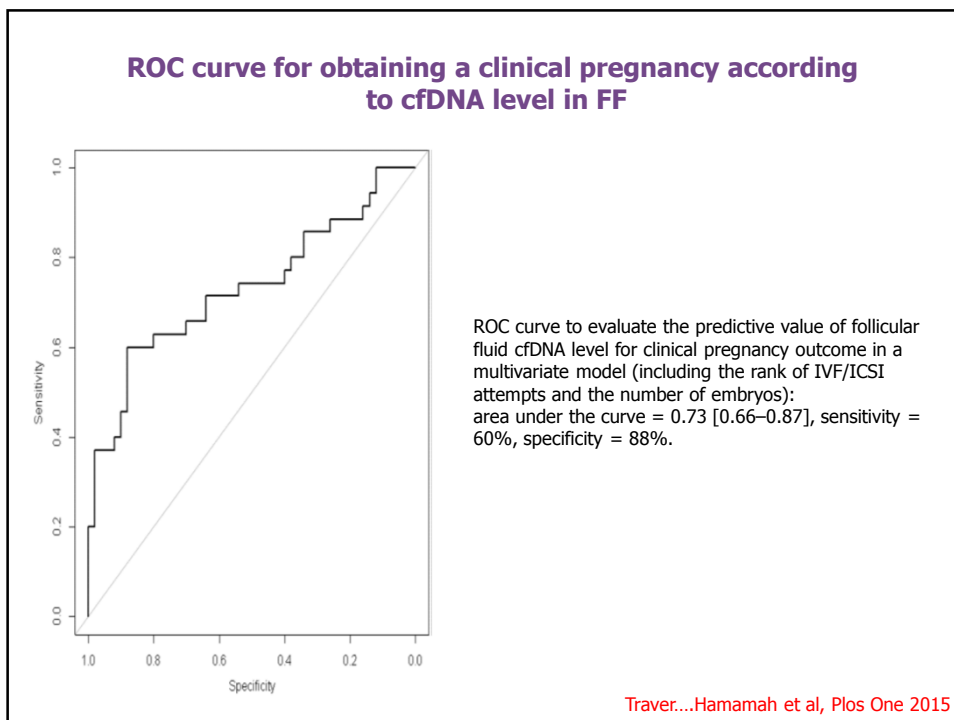
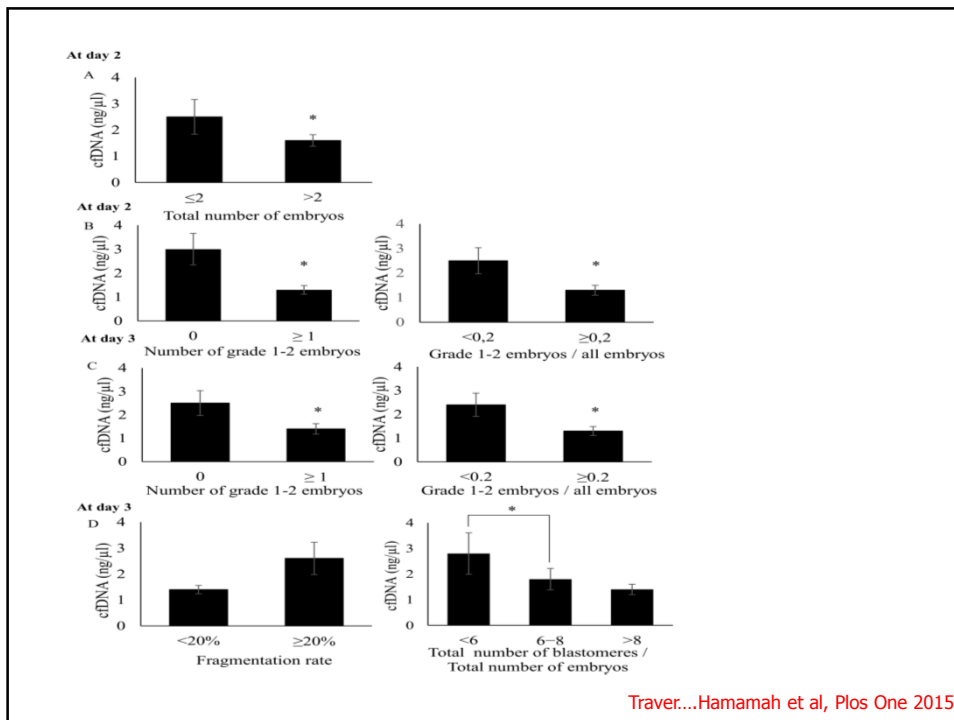
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Claire Vincens<sup>3</sup>, Tal Anahory<sup>3</sup>, Samir Hamamah<sup>1,2,3\*</sup>



Scalici....Hamamah et al, HR 2014







2016

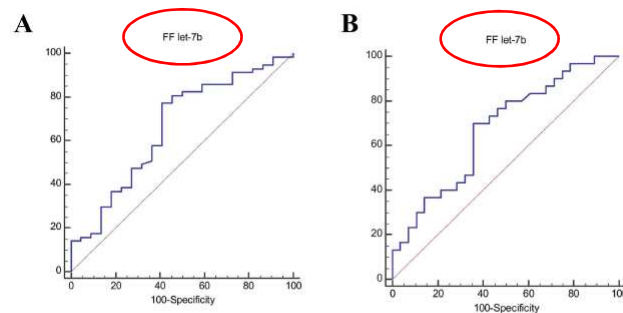
www.nature.com/scientificreports

# SCIENTIFIC REPORTS

**OPEN** Circulating microRNAs in follicular fluid, powerful tools to explore *in vitro* fertilization process

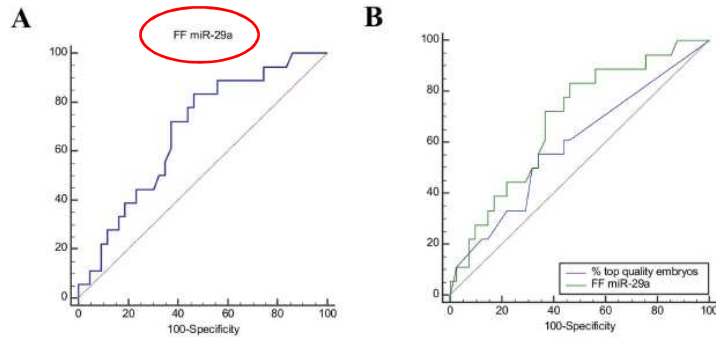
Received: 24 July 2015 Accepted: 08 April 2016  
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Traver...Hamamah et al, Plos One 2015



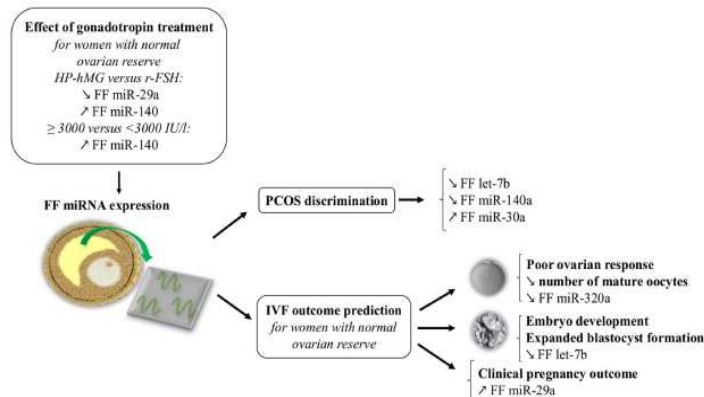
**Figure 4.** (A) ROC analysis to evaluate FF let-7b expression predictive value for blastocyst formation. (B) ROC analysis to evaluate FF let-7b expression predictive value for expanded blastocyst development. These analyses included only the group of women with normal ovarian reserve (n = 91).

Scalici, ...Hamamah et al, 2016



**Figure 5.** (A) ROC analysis to evaluate FF miR-29a expression predictive value for clinical pregnancy outcome. (B) Comparison of the ROC curves showing the predictive value of FF miR-29a expression and top quality embryo proportion for clinical pregnancy outcome. These analyses included only the group of women with normal ovarian reserve (n = 91).

Scalici, ...Hamamah et al, 2016



**Figure 6.** Schematic model showing that miRNA expression profiling in FF samples provides powerful tools to efficiently discriminate women with polycystic ovary syndrome (PCOS) and to predict IVF outcomes. The expression of some FF miRNAs varies according to the gonadotropin treatment. HP-hMG, highly purified human menopausal gonadotropin; r-FSH, recombinant follicle-stimulating hormone.

Scalici, ...Hamamah et al, 2016

## Conclusions

miRNA expression profiling in human FF might provide biomarkers to efficiently discriminate women with PCOS and to predict blastocyst development and clinical pregnancy outcomes.

However, their specific role in the oocyte micro-environment, their regulation by gonadotropins and their involvement in female infertility should be further investigated.



These new potential biomarkers could be used in the daily practice to improve personalized IVF strategies and to identify new therapeutic targets in female infertility management.

## Conclusions

Omics provides opportunity to analyse human oocytes and CCs expression profiles on a genome scale and permitted significant progress in the understanding of the molecular events involved in the processes governing oocyte maturation.

Many of the genes described here are biomarkers to monitor health, viability and competence of oocytes.

Analysis of CC surrounding the oocyte can be a non-invasive approach for oocyte, embryo selection and pregnancy outcome

This is a novel concept, providing a new potential strategy for competent oocyte and embryo selection



Alice Ferrières



Ounissa Aït Ahmed



Samir Hamamah



Delphine Haouzi



Elodie Scalici



Nicolas Nafati

Développement embryonnaire précoce  
humain et pluripotence



Laurence Bissonnette



Emilie Paurret

Inserm U1203



Rafii Arash



Yannick Antoine



Anna Gala



Hervé Dechaud



Tamadir Al-Edani



Loubna Drissennek

