RegioneEmilia-Romagna

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale di Ferrara

Ordine dei Medici Chirurghi e degli Odontoiatri della provincia di FERRARA

Convegno Nazionale

Fertilità di Coppia: "Ri"Parliamone

Palazzo Bellini, Comacchio (FE)
18 novembre 2019

MiRNA, biomarkers embrionale e cross talk endometrio embrione

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Conflitto di interesse

Gli studi sono stati sostenuti da
MERCK con Grant diretti e GFI2018

Fattori maggiormente responsabili del fallimento dell'impianto:

Trasferimento embrioni di scarsa qualità

Endometrio non recettivo

Sviluppo della blastocisti

Metodi di valutazione della qualità della blastocisti

Studio morfologico
E morfocinetico

Grado di espansione

Trofoectoderma

ICM (Inner Cell Mass)

Varie tipologie di grading

GENOMICA

METABOLOMICA

Nano HPLC MS

Gli attuali metodi non sono accurati,
una promettente possibilità potrebbe essere
l'utilizzo di marcatori molecolari non invasivi

Studi di Metabolomica

Molecular BioSystems

Dynamic Article Links

Cite this: DOI: 10.1039/c1mb05358b

www.rsc.org/molecularbiosystems

METHOD

A mass spectrometry-based targeted metabolomics strategy of human blastocoele fluid: a promising tool in fertility research†

Angelo D'Alessandro,[a] Gevi Federica,[a] Simone Palini,[b] Carlo Bulletti[b] and Lello Zolla*[a]

Received 2nd September 2011, Accepted 28th September 2011

DOI: 10.1039/c1mb05358b

Table 1 Metabolites identified through RR-RP-HPLC–MRM-ESI/MS

Metabolite	PubChem ID	Monoisotopic mass	MS/MS	Retention time/min	Standard curves	Linearity: orders of magnitude	Linear correlation coefficient	Ion mode
Lactic acid	612	90.0317	43	2.8	$Y = 56\,251.36X + 140.227$	4	0.997532	–
Glucose-6-phosphate	69 507	260.0297	97	2.6	$Y = 43\,748.99X + 325.442$	4	0.994423	–
α-Ketoglutaric acid	164 533	146.0215	57	3.0	$Y = 44\,133.18X + 479.082$	4	0.996623	–
6-Phosphogluconic acid	91 493	276.0246	79	3.1	$Y = 79\,336.8X + 572.385$	4	0.995031	–
Glutamic acid	611	147.0532	128	2.5	$Y = 2679.885X + 151.267$	5	0.999611	+
ATP	5957	506.9957	410	3.5	$Y = 9315.342X + 678.455$	4	0.995439	+
NAD^+	5893	663.1091	524	3.9	$Y = 3512.349X + 221.776$	4	0.996154	+
NADH	3687	665.1248	649	4.1	$Y = 4399.545X + 135.633$	5	0.998344	+
NADPH	5884	745.4209	729	5.4	$Y = 59\,788.351X + 121.301$	4	0.996572	+

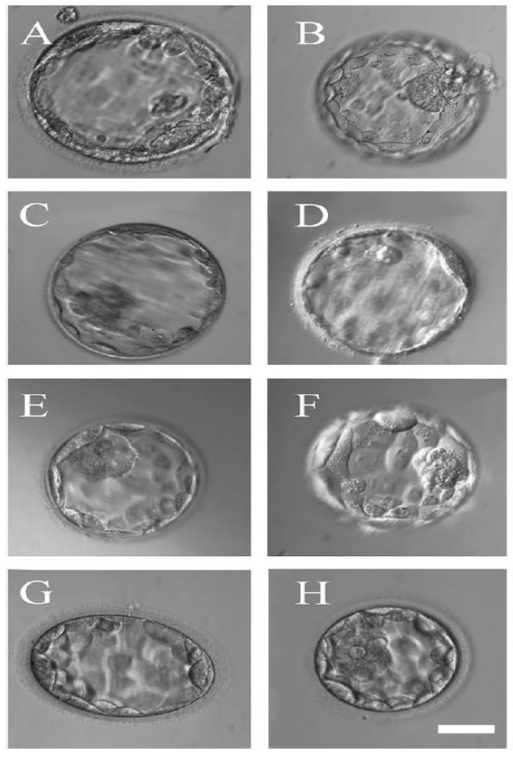


Fig. 1 Microscopic images of human blastocysts, according to classification based on "Grading criteria for human blastocyst".[34]

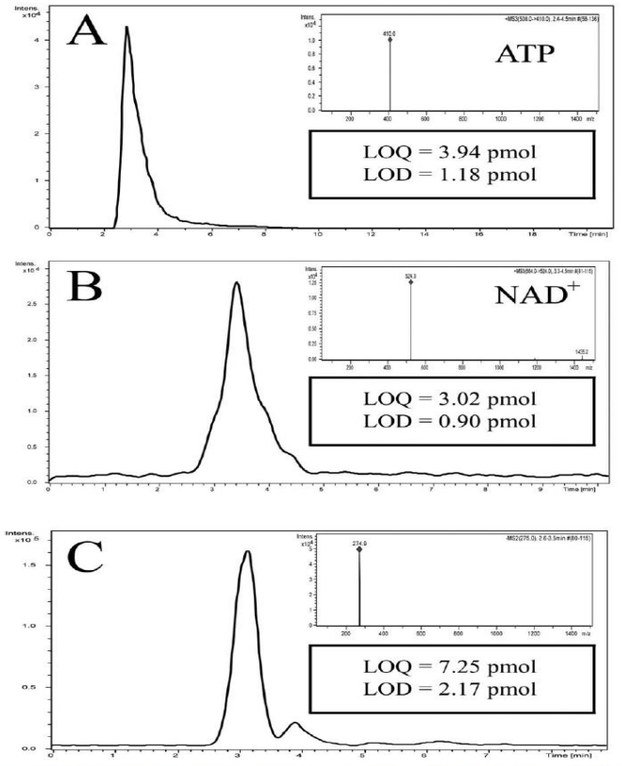


Fig. 2 Three representative chromatograms of metabolites detected in blastocoele fluid through multiple reaction monitoring (MRM) in MS-based targeted analysis: ATP (A), NAD^+ (B) and 6-phosphogluconic acid (6PG) (C). In the right side of each graph the MS and MS/MS spectra are indicated for 6PG and ATP, NAD^+, respectively. Limits of detection (LOD) and quantification (LOQ) are reported as total injected picomoles (injection volume = 20 μl). For each metabolite, LOD was calculated as 3× standard deviation of 20 blank runs, while the LOQ as 10× standard deviation of the blank.

Studi di Genomica

Reproductive BioMedicine Online (2013) xxx, xxx–xxx

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

ARTICLE

Genomic DNA in human blastocoele fluid

S Palini [a,*,1], L Galluzzi [b,1], S De Stefani [a], M Bianchi [b], D Wells [c], M Magnani [b], C Bulletti [a]

[a] IVF Unit, 'Cervesi' Hospital Cattolica, 47841 Cattolica (Rn), Italy; [b] Department of Biomolecular Sciences, University of Urbino 'Carlo Bo', 61029 Urbino (PU), Italy; [c] University of Oxford, Institute of Reproductive Sciences, Oxford Business Park North, Oxford, United Kingdom

* Corresponding author. *E-mail address:* simonepalini@yahoo.it (S Palini). [1] These authors contributed equally to this work.

RBM Online

6th October 2004

Dear Dr Palini

Robert G. Edwards Prize Paper Award for 2013

Genomic DNA in human blastocoele fluid
Palini, S.; Galluzzi, L.; De Stefani, S.; Bianchi, M.; Wells, D.; Magnani, M.; Bulletti, C.
RBM Online (2013) 26, 603–610.

We are very pleased to be able to let you know that the above paper has won the **Robert G. Edwards Prize Paper Award** for the best paper published in the journal in 2013. An announcement to this effect will appear on the journal website later this week.

Many congratulations! It was an extremely competitive field and you should be very pleased that your paper has achieved this accolade.

In due course, Grazina Peoples from Elsevier will be in touch to arrange details of the award of 1000 Euros, and how to access the complimentary SciVerse Expert Ambassador Account.

As your paper has multiple authors, we would be grateful if you could jointly with your co-authors be ready to let Grazina know who the more junior of the main authors is, as that person will be given the one year's access to the entire Elsevier Science Direct database. You will also receive a certificate of recognition on which all authors' names will appear. It is hoped that a mutually convenient location and time can be arranged for a certificate award ceremony.

We look forward to receiving further excellent papers from your laboratory in the future.

Yours sincerely

Jacques Cohen
Carla Coudroplan
Martin Johnson

Senior Editor and Editors,
Reproductive BioMedicine Online
Email: office@rbmonline.com

Future Science OA

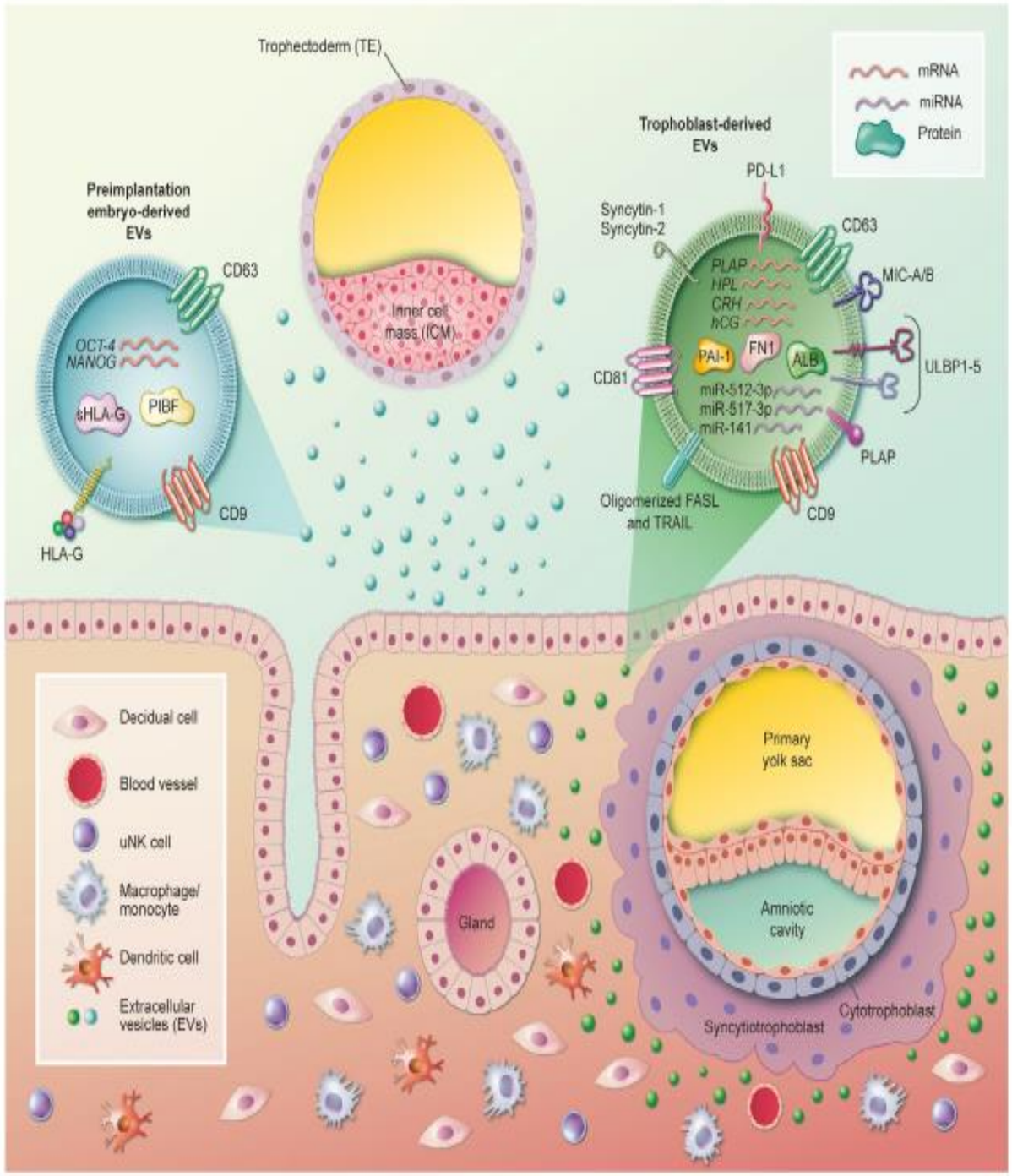
Extracellular embryo genomic DNA and its potential for genotyping applications

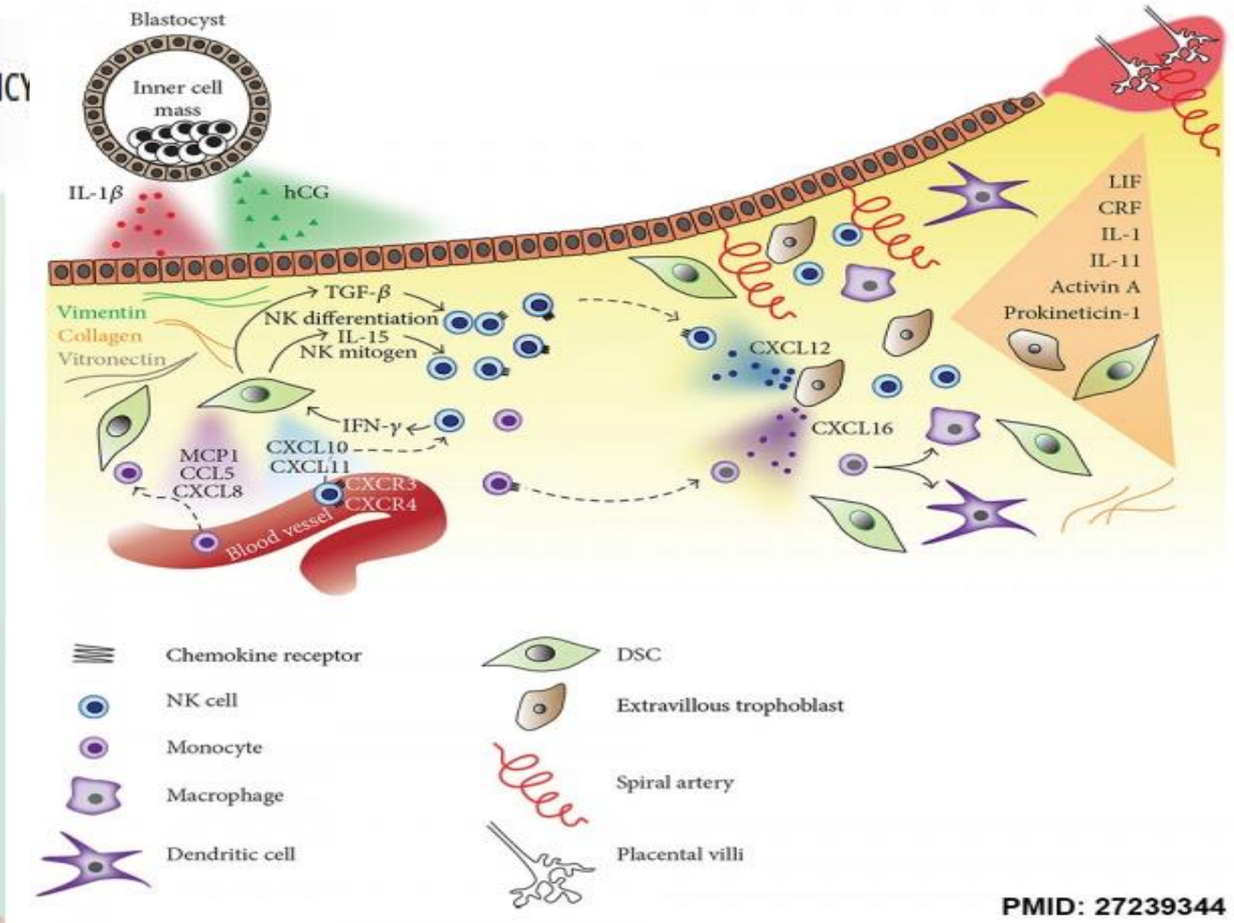
Background: Preimplantation genetic diagnosis (PGD) currently relies on biopsy of one or few embryo cells. Our aim was to evaluate the embryo extracellular matrices (spent medium and blastocoele fluid) as source of DNA for embryo genotyping. **Results/methodology**: We first evaluated the amplifiability and the amount of genomic DNA in spent embryo culture media from day 3 (n = 32) and day 5/6 (n = 54). Secondly, we evaluated the possibility to genotype the *MTHFR* polymorphism C677T from media at day 5/6 (n = 8) and blastocoele fluids (n = 9) by direct sequencing. The C677T polymorphism detection rate was 62.5 and 44.4% in medium and fluid, respectively. **Conclusion**: A noninvasive approach for embryo genotyping was possible, but still with limitations due to low detection rate and possible allele dropout.

Luca Galluzzi*[,‡,1], Simone Palini[‡,2], Silvia De Stefani[2], Francesca Andreoni[1], Mariangela Primiterra[2], Aurora Diotallevi[1], Carlo Bulletti[2] & Mauro Magnani[1]

[1]Department of Biomolecular Sciences, University of Urbino 'Carlo Bo', 61029 Urbino (PU), Italy

[2]IVF Unit, 'Cervesi' Hospital Cattolica, 47841 Cattolica (RN), Italy





PMID: 27239344

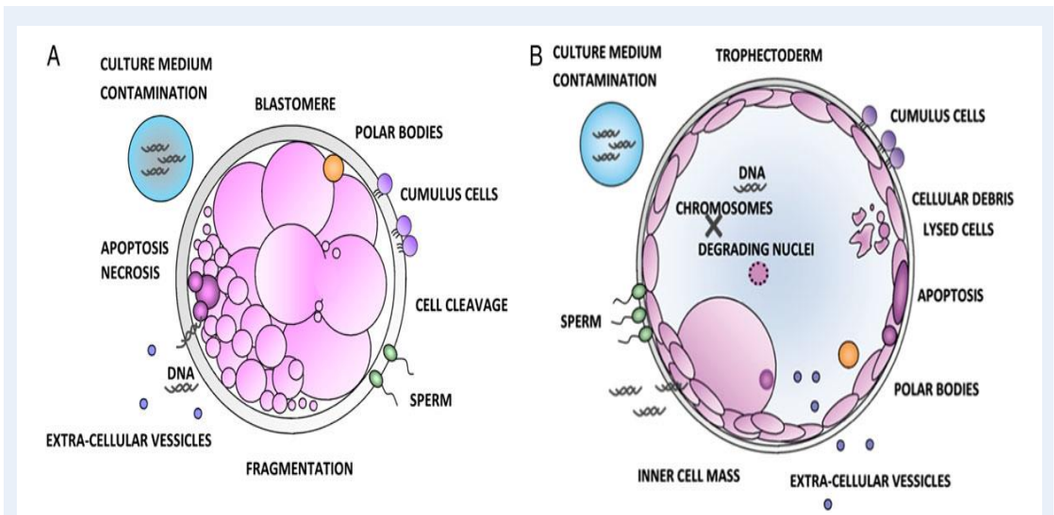
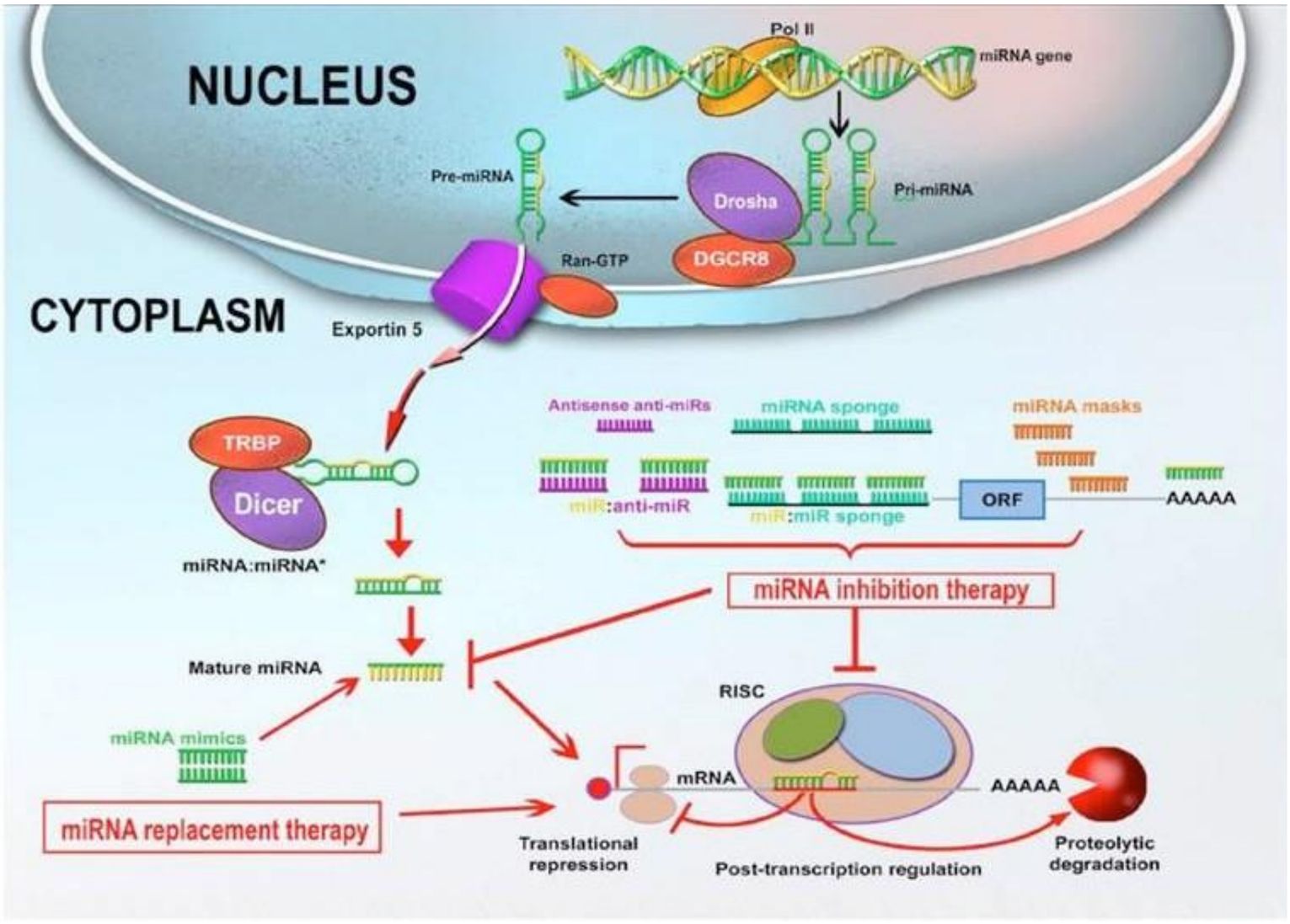


Fig. 1. Cargos of extracellular vesicles released by the preimplantation embryo and by trophoblasts with reported effects on the local maternal immune cells. PIBF = progesterone-induced blocking factor; ALB = albumin; ULBP1-5 = UL-16 binding proteins 1-5; PLAP = placental alkaline phosphatase; PD-L1 = programmed death-ligand 1; MIC-A/B = major histocompatibility complex (MHC) class I-related protein; PAI-1 = plasminogen activator inhibitor-1; hCG = human chorionic gonadotrophin; CRH = corticotrophin-releasing hormone; HPL = human placental lactogen; FN1 = fibronectin-1.

MicroRNAs: regolatori dell'espressione genica



- Sono RNA non codificati
- Agiscono inibendo i mRNA target
- Sono coinvolti nella patogenesi di molte patologie neoplastiche
- Sono possibili bersagli farmacologici
- I miRNA circolanti sono possibili marcatori di patologie
- Rivestono un ruolo importante nelle prime fasi dell'embriogenesi

Published in final edited form as:
Int J Cancer. 2010 March 15; 126(6): 1283–1290. doi:10.1002/ijc.25014.

miRNA control of tumor cell invasion and metastasis

Somesh Baranwal[1] and **Suresh K. Alahari**[1,2,@]

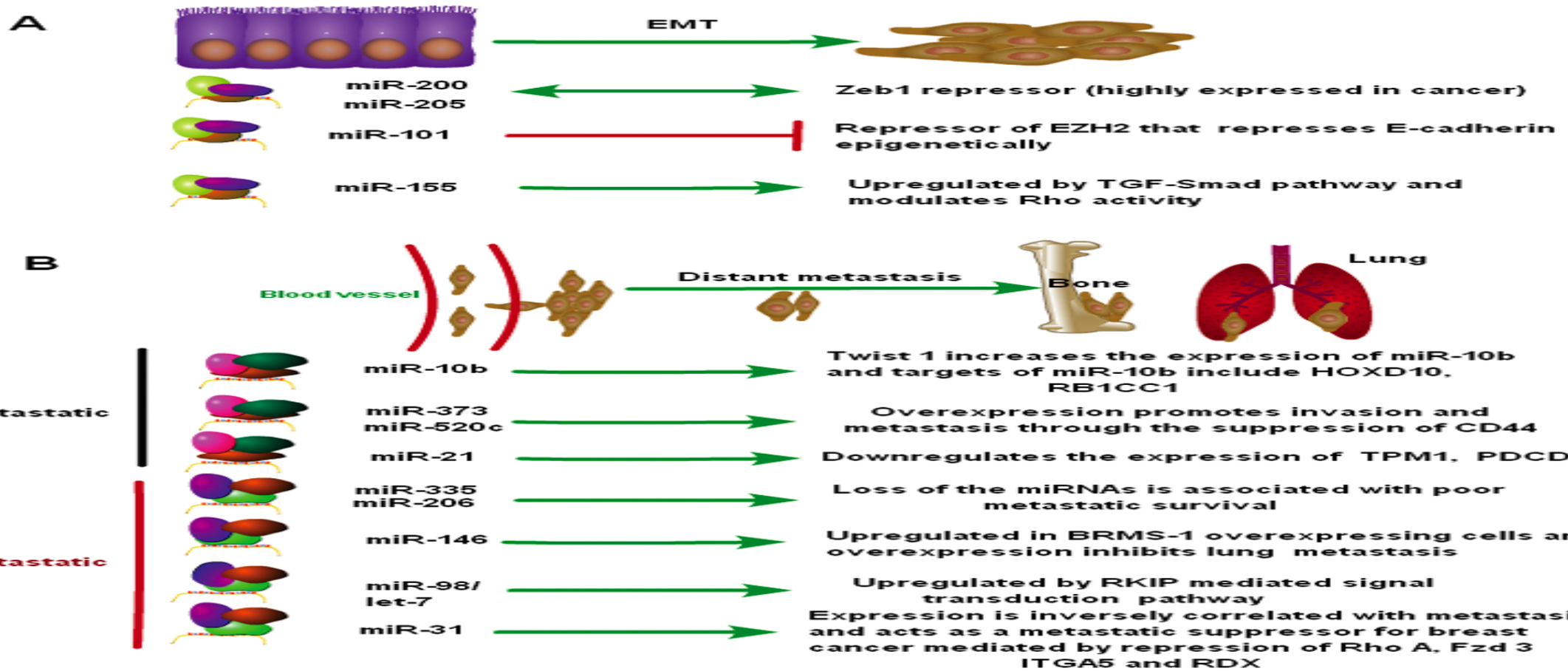


Figure 2. Role of miRNAs in metastasis

(A) EMT regulation by miRNAs: miRNAs such as miR-200 and miR-205, modulate the ZEB family of transcription factors and regulate epithelial-mesenchymal transition. miR-101 modulates E-cadherin expression epigenetically via targeting Zeste Homolog 2 (EZH2) in prostate cancer. miR-155 modulates TGFβ pathway, which plays an important role in cancer metastasis. (B) Suppression of metastasis by miRNAs:. miR-10b is the first miRNA identified to be upregulated in breast cancer that is mediated by Twist1, MMPs, urokinase plasminogen activator(uPA), and various integrins. miR-373 and miR-520c are prometastatic miRNAs that serve as metastasis promoters (partly mediated by modulation of CD44). miR-335, miR-206 and miR-31 are identified as antimetastatic miRNAs that target RhoA, Fzd3, RDX, and integrin α5. miR-146 and miR-98/let-7 are shown to be upregulated in response to metastatic suppressor proteins BRMS-1 and RKIP respectively. miR-10b, miR-373, miR-520c and miR-21 are pro-metastatic miRNAs, while miRNAs such as miR-335, miR-206 and miR-31 are anti-metastatic. Prometastatic miRNAs are depictedpink, dark green and red, while anti-metastatic ones are shown in violet, red and light green.

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 70), pp: 115787-115802

Review

MicroRNAs in cancer metastasis and angiogenesis

Weiyang Lou[1,*], Jingxing Liu[2,*], Yanjia Gao[3,*], Guansheng Zhong[1], Danni Chen[1], Jiaying Shen[1], Chang Bao[1], Liang Xu[4], Jie Pan[1], Junchi Cheng[5], Bisha Ding[1] and Weimin Fan[1,6]

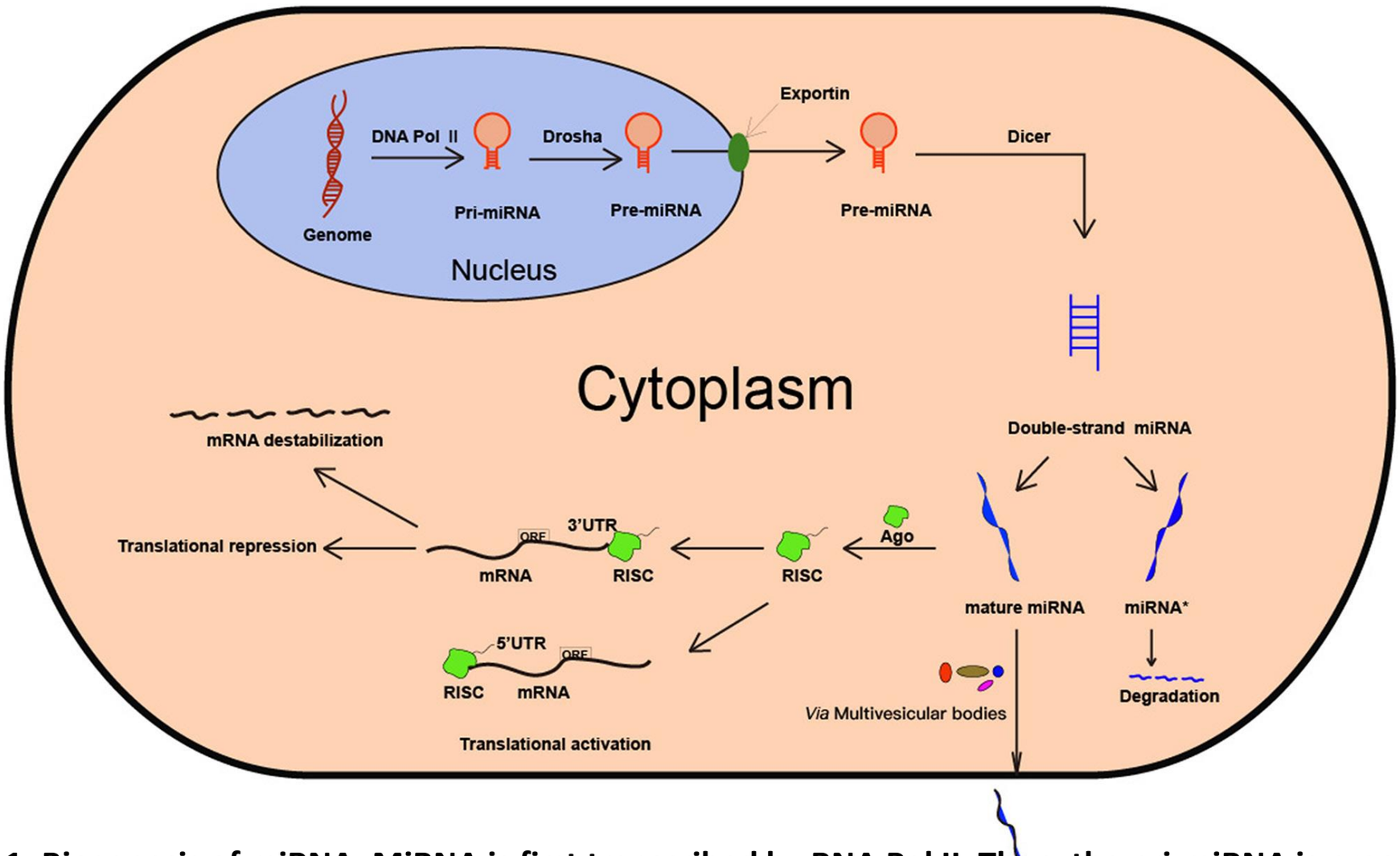


Figure 1: Biogenesis of miRNA. MiRNA is first transcribed by RNA Pol II. Then, the pri-miRNA is processed by the enzyme Drosha and Dicer. The mature miRNA is integrated into RISC, thereby leading to mRNA degradation, translational repression or translational activation.

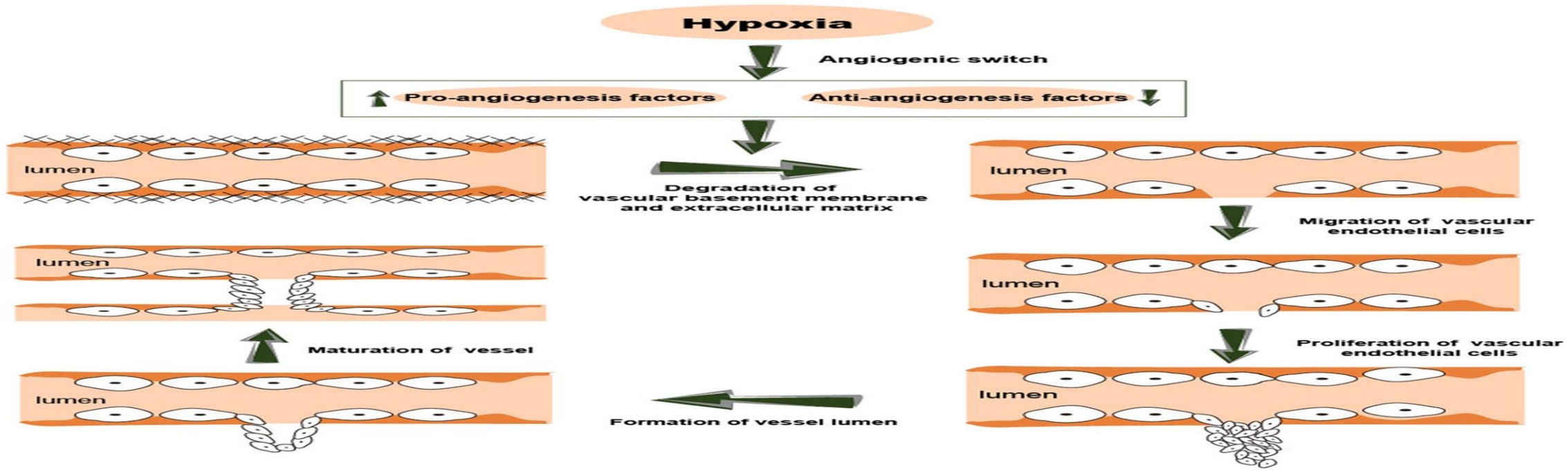


Figure 2: The process of cancer angiogenesis. This process is usually activated in a low oxygen microenvironment. Cancer angiogenesis involves multiple steps, including degradation of vascular basement membrane and extracellular matrix, proliferation and migration of vascular endothelial cells, formation of a new vessel lumen and vessel branches, and maturation of the new vessels.

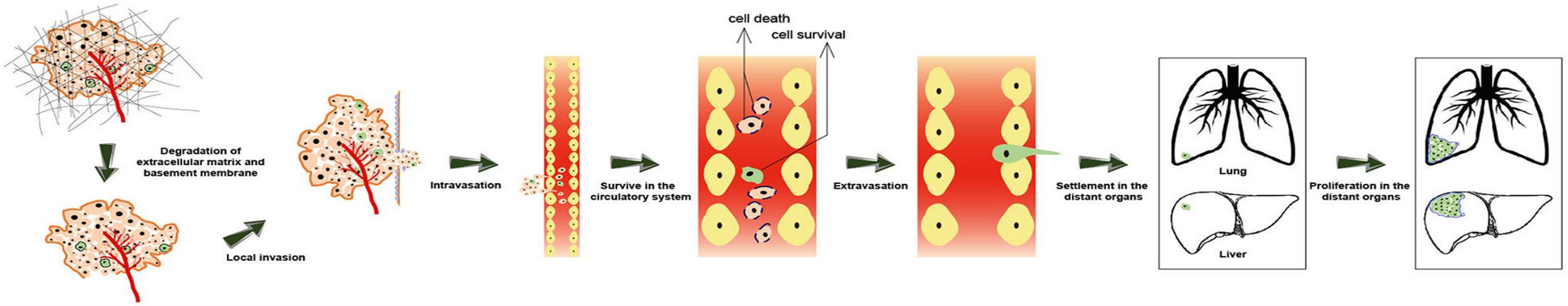


Figure 3: The process of cancer metastasis. A series of sequential steps are involved in cancer metastasis, such as alteration and rearrangement of cytoskeleton, degradation of extracellular matrix, local invasion, intravasation, transfer and survive in the circulatory system, extravasation, settlement and proliferation in a new organ (like lung and liver).

Clinical & Experimental Immunology
The Journal of Translational Immunology

doi:10.1111/cei.13304

Clinical and Experimental Immunology REVIEW ARTICLE

REVIEW SERIES: IMMUNOLOGY OF PREGNANCY
Series Editors: Angelo A. Manfredi and Patrizia Rovere-Querini

Embryonic extracellular vesicles as informers to the immune cells at the maternal–fetal interface

OTHER ARTICLES PUBLISHED IN THIS REVIEW SERIES
The immunology of the fetal-placental unit comes of age. Clinical and Experimental Immunology 2019, 198:11–14.
The role of neutrophil activation in determining the outcome of pregnancy and modulation by hormones and/or cytokines. Clinical and Experimental Immunology 2019, 198: 24–36.
Overview of procalcitonin in pregnancy and in pre-eclampsia. Clinical and Experimental Immunology 2019, 198: 37–46.
Influence of maternal microbiota during pregnancy on infant immunity. Clinical and Experimental Immunology 2019, 198: 47–56.

E. Giacomini,* E. Alleva,† G. Fornelli,† A. Quartucci,† L. Privitera,† V. S. Vanni*† and P. Viganò *
**Reproductive Sciences Laboratory, Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, and †Obstetrics and Gynecology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy.*

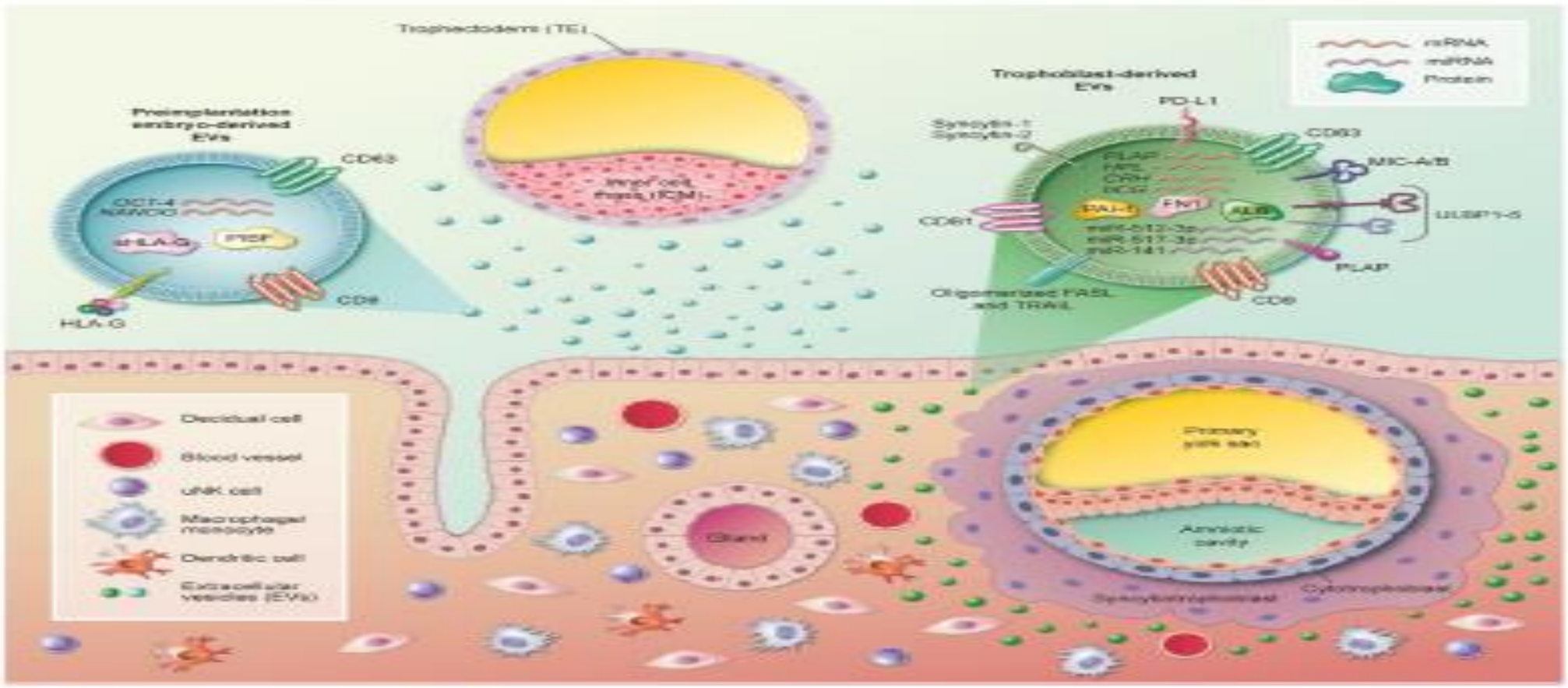


Fig. 1. Cargos of extracellular vesicles released by the preimplantation embryo and by trophoblasts with reported effects on the local maternal immune cells. PIBF = progesterone-induced blocking factor; ALB = albumin; ULBP1-5 = UL-16 binding proteins 1-5; PLAP = placental alkaline phosphatase; PD-L1 = programmed death-ligand 1; MIC-A/B = major histocompatibility complex (MHC) class I-related protein; PAI-1 = plasminogen activator inhibitor-1; hCG = human chorionic gonadotrophin; CRH = corticotrophin-releasing hormone; HPL = human placental lactogen; FN1 = fibronectin-1.

International Journal of *Molecular Sciences*

MDPI

Review

Inside the Endometrial Cell Signaling Subway: Mind the Gap(s)

Sofia Makieva *, Elisa Giacomini, Jessica Ottolina, Ana Maria Sanchez, Enrico Papaleo and Paola Viganò

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Received: 4 July 2018; Accepted: 4 August 2018; Published: 21 August 2018

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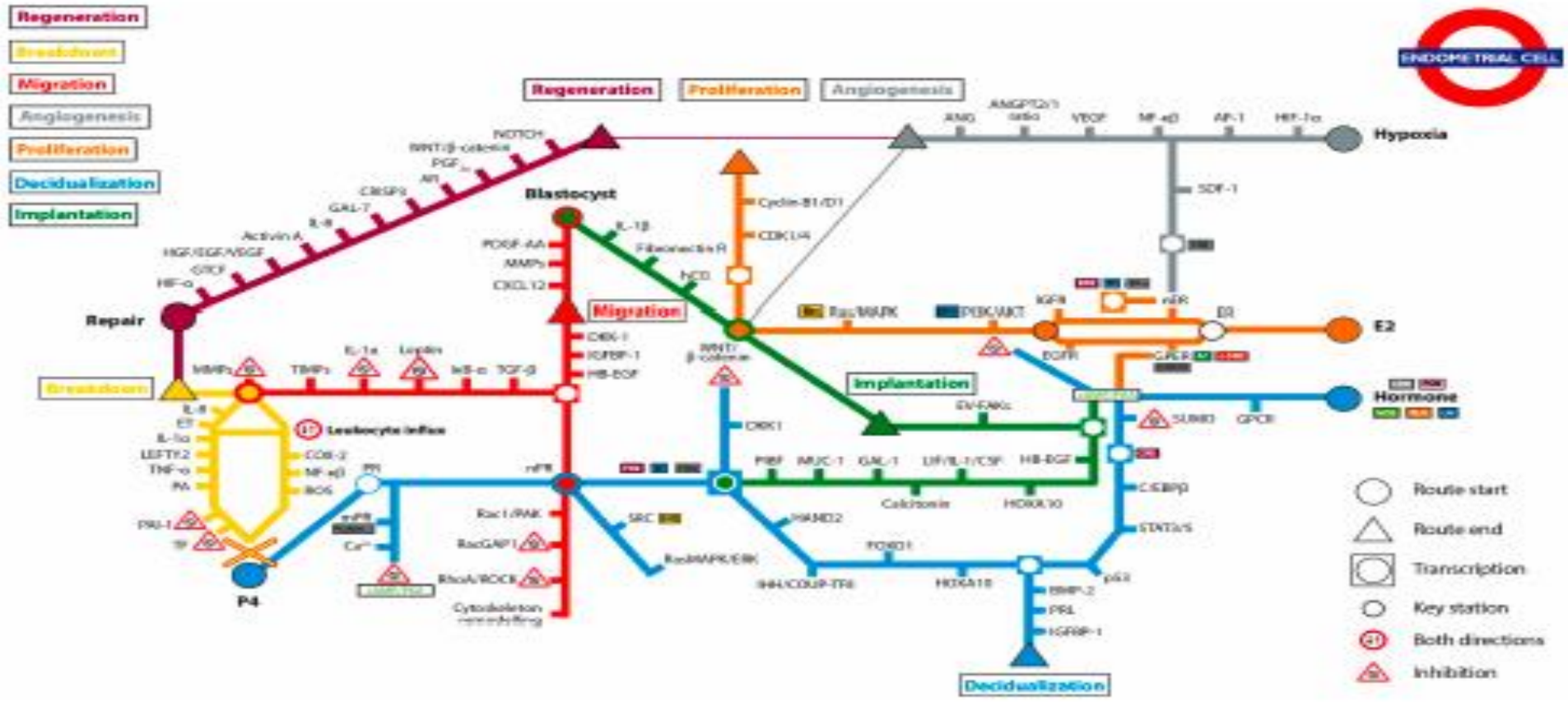


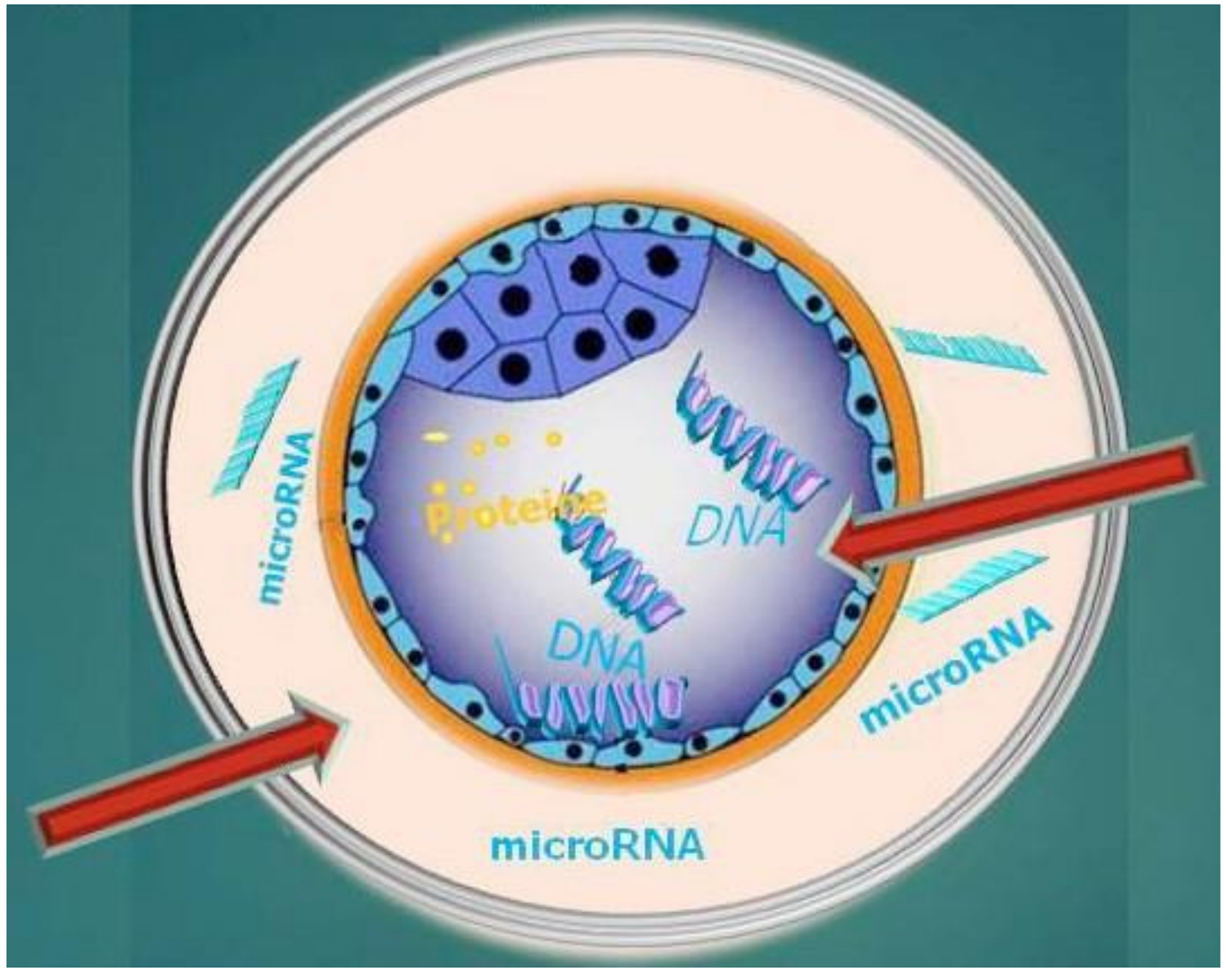
Figure 1. Endometrial cell signaling network illustrated as a subway map showing the seven routes operated by different molecules, narrated in the review. TF in blue boxes denotes transcription factors. All abbreviations are expanded in the main text. The X mark in the red circle indicates progesterone withdrawal.

OBIETTIVO DELLO STUDIO

Identificare e caratterizzare i microRNAs secreti dalla blastocisti nel fluido del blastocele.

MicroRNAs: possibili marcatori molecolari

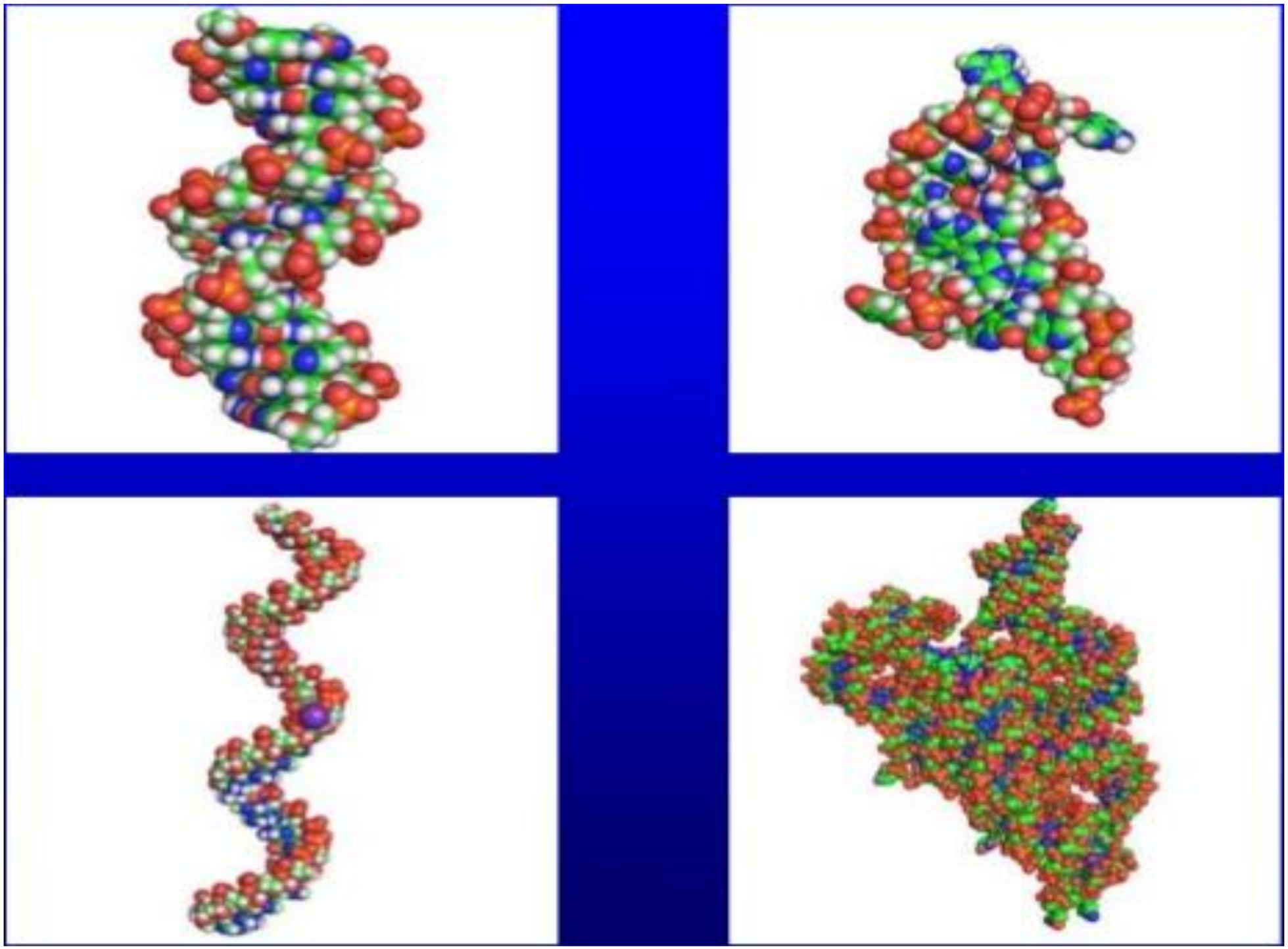
Ottenuti evitando procedure invasive



Terreno di coltura

Blastocele

MiRNA in RAS Mol

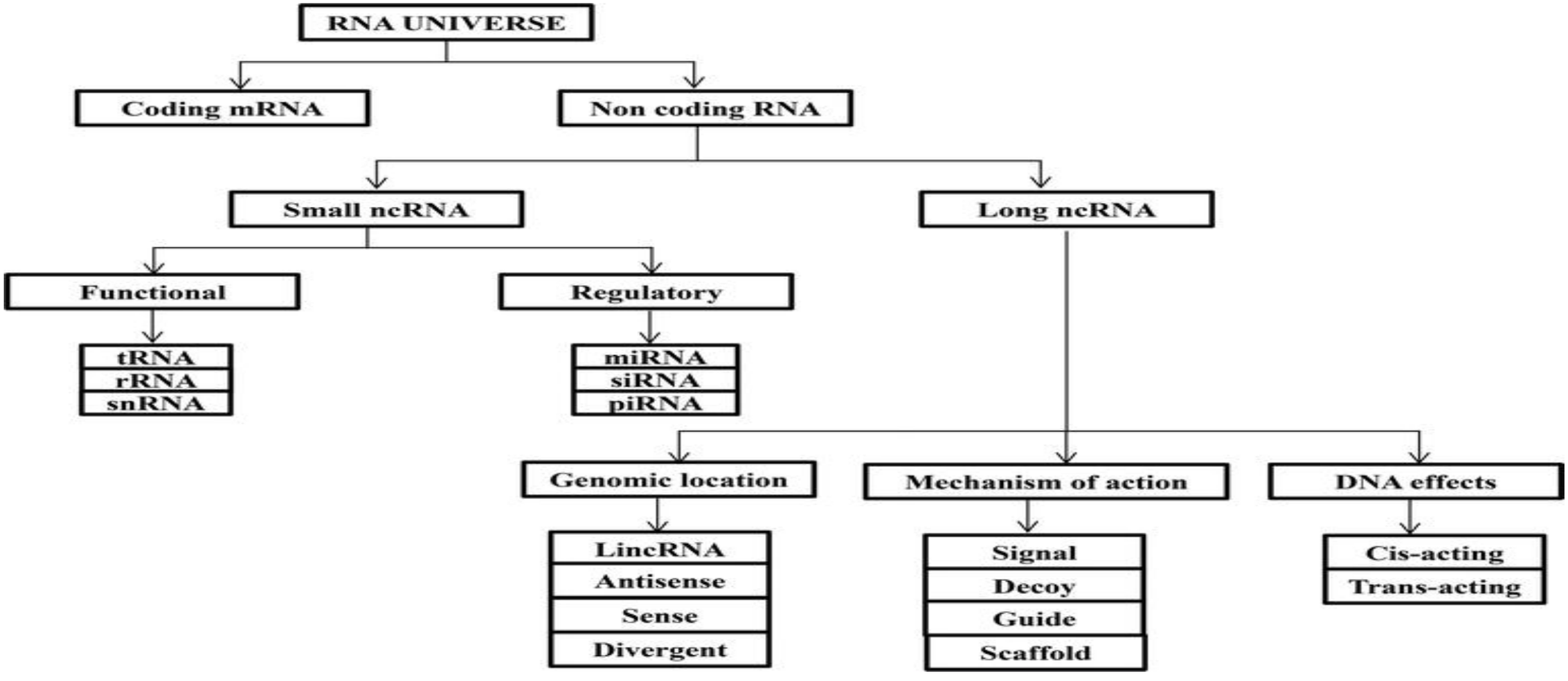


Functional RNAs

Non-coding genes codify for a functional RNA product rather than for a protein.

Family of functional RNAs:

Biological function	RNA family
Involved in protein synthesis	tRNA, rRNA, SRP RNA, tmRNA
Post-trascriptional modification or DNA replication	snRNA, snoRNA, SmY, scaRNA, gRNA, RNAse P, RNAse MRP, Y RNA, telomerase RNA
Regulatory RNAs	aRNA, NAT, crRRNA, long ncRNA, miRNA, piRNA, siRNA, tasiRNA, rasiRNA, 7SK
Parasitic RNA	Retrotransposon, Viroid, satellite RNA



Tecniche biologiche molecolari utilizzate

Real Time PCR

TaqMan Low Density Array

- Analisi di 384 microRNAs
- Tecnologie consolidate
- Conoscenza vantaggi e limiti

Nanostring

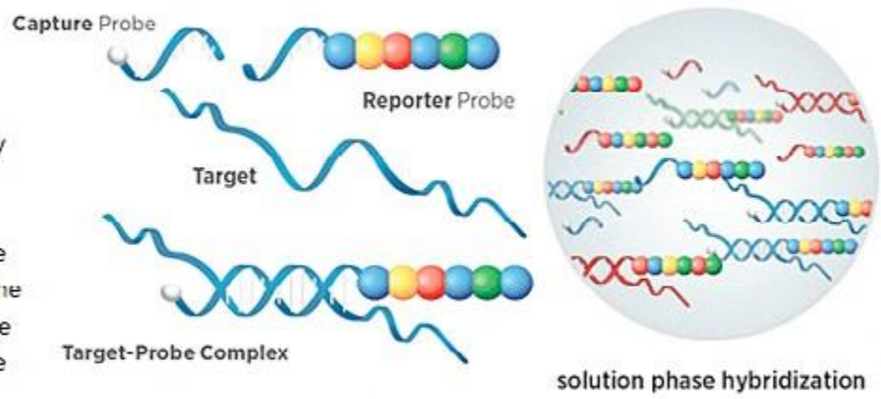
- Analisi di 800 microRNAs
- Tecnologia recente
- No amplificazione
- Sensibile e specifica
- Ridotta quantita' campione ci ha indotto all'utilizo del TLDA

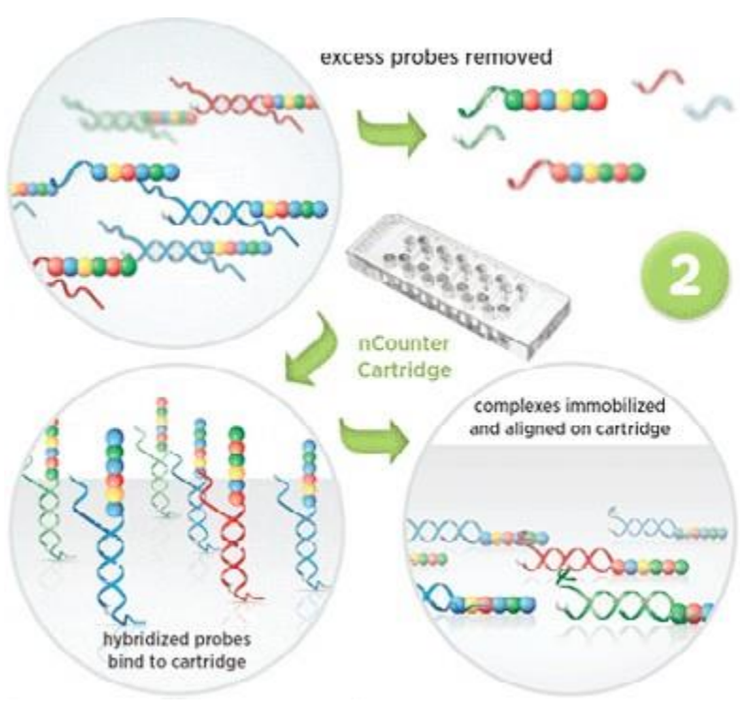
Nanostring, tre fasi principali:

1

Hybridization

NanoString's Technology employs two ~50 base probes per mRNA that hybridize in solution. The Reporter Probe carries the signal; the Capture Probe allows the complex to be immobilized for data collection.





2

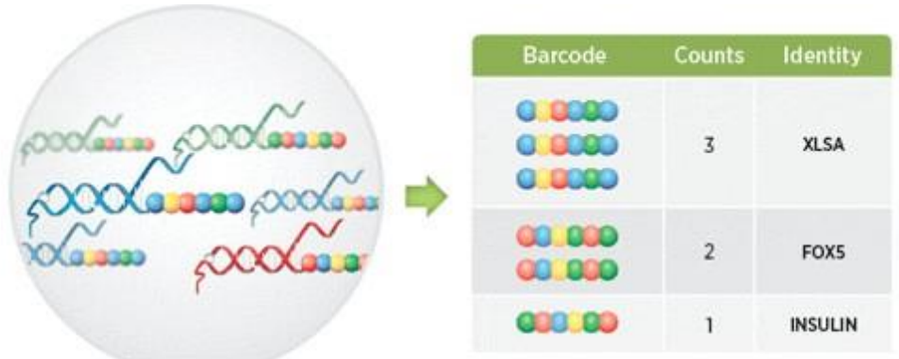
Purify and Immobilize

After hybridization, the excess probes are removed and the probe/target complexes aligned and immobilized in the nCounter Cartridge.

3

Count

Sample Cartridges are placed in the Digital Analyzer for data collection. Color codes on the surface of the cartridge are counted and tabulated for each target molecule.



Risultati

Nanostring

Sono stati identificati nel fluido del blastocele

36 miRNAs su 800 ricercati

Nanostring Results

Detector	count
hsa-miR-125b-5p	1308,5
hsa-let-7a-5p	649,5
hsa-miR-15b-5p	492,5
hsa-let-7b-5p	358,5
hsa-miR-93-5p	312,5
hsa-miR-191-5p	**204,5**
hsa-miR-23a-3p	192,5
hsa-miR-100-5p	161,5
hsa-miR-125a-5p	160,5
hsa-miR-200c-3p	156,5
hsa-miR-302b-3p	**148,5**
hsa-let-7i-5p	144,5
hsa-miR-181a-5p	142,5
hsa-miR-25-3p	**123,5**
hsa-miR-20a-5p+hsa-miR-20b-5p	**119,5**
hsa-let-7e-5p	101,5
hsa-miR-4454+hsa-miR-7975	100,5
hsa-miR-16-5p	**99,5**
hsa-let-7c-5p	97,5
hsa-miR-1246	92,5
hsa-miR-302a-3p	**91,5**
hsa-miR-1260a	90,5
hsa-let-7d-5p	87,5
hsa-miR-302d-3p	80,5
hsa-let-7g-5p	75,5
hsa-miR-4443	70,5
hsa-miR-342-3p	69,5
hsa-miR-146a-5p	65,5
hsa-miR-92a-3p	**58,5**
hsa-miR-107	57,5
hsa-miR-99b-5p	54,5
hsa-miR-873-3p	53,5
hsa-miR-106a-5p+hsa-miR-17-5p	51,5

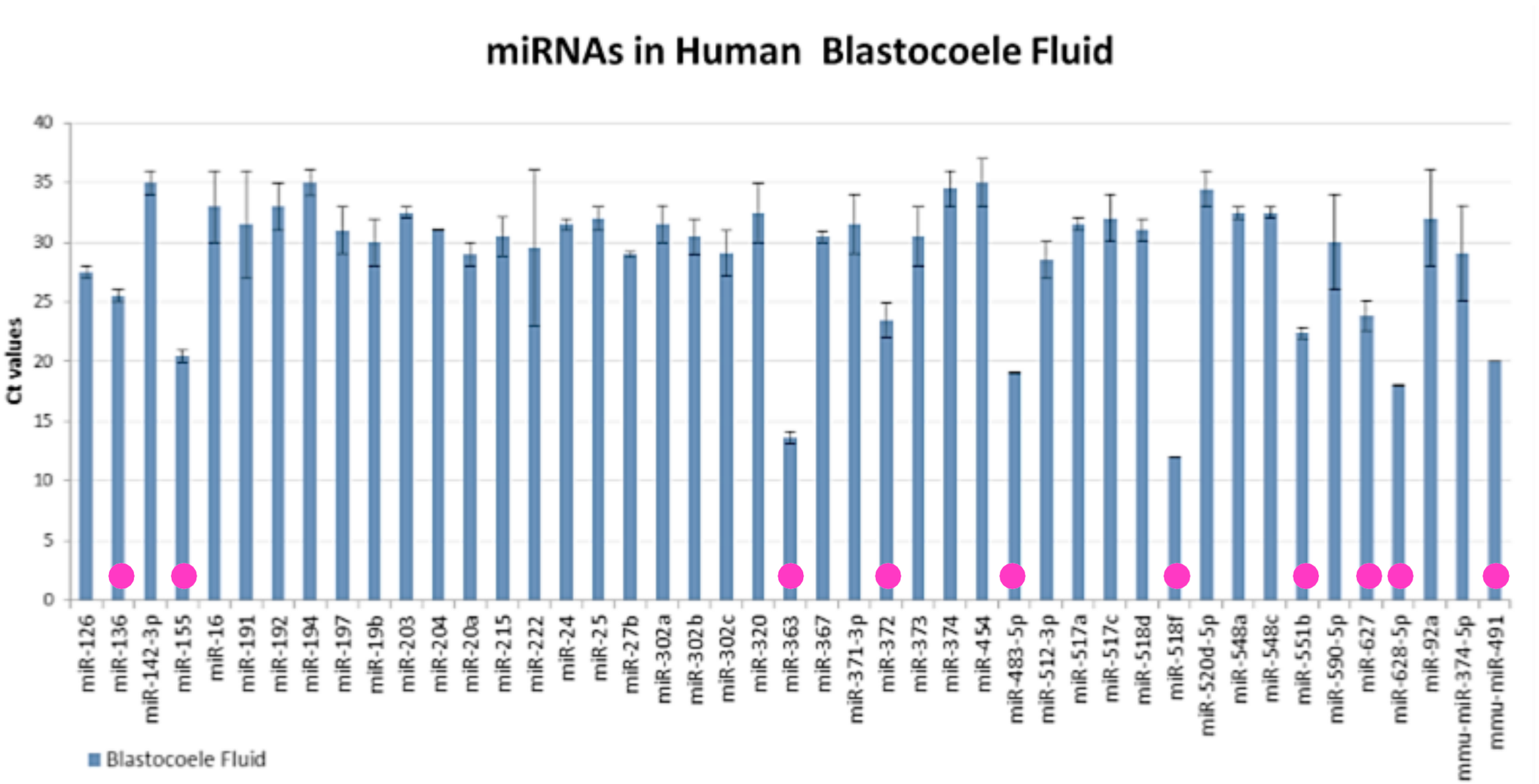
Risultati

Real Time PCR

TaqMan Low Density Array

Sono stati identificati nel fluido del blastocele

80 miRNAs su 384 ricercati



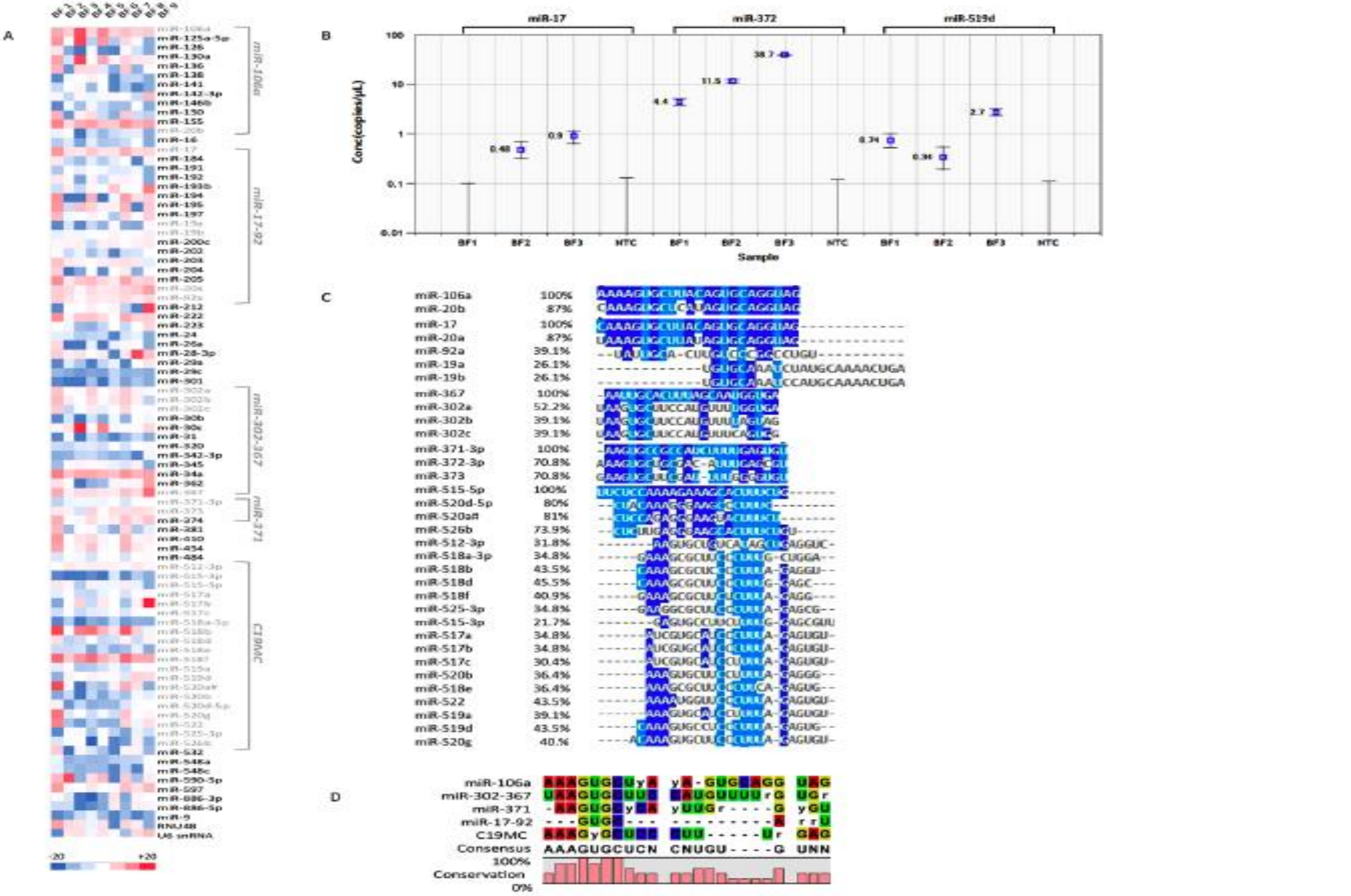


Figure 2. miRNA expression in human Blastocoel Fluid (BF). (**A**) Heat map representation of normalized expression data (−ΔCT values) for 89 miRNAs from nine BF samples. The red and blue colors represent miRNA expression levels. (**B**) Quantification of BF miRNAs using droplet digital PCR (ddPCR). Measurements for miR-17, miR-372 and miR-519d are shown as miRNA copies/µl ddPCR mix. All NTC controls do not show positive droplets. (**C**) Alignment of human BF miRNAs. Blue shading indicates nucleotides of miRNA sequences with identical positions in the alignment. Light blue shading indicates nucleotides conserved in the major groups of BF miRNAs. (**D**) Consensus clustering and frequency of nucleotide conservation for groups of BF-miRNAs. Most of the mature miRNAs conserve the seed sequence AAGUGC.

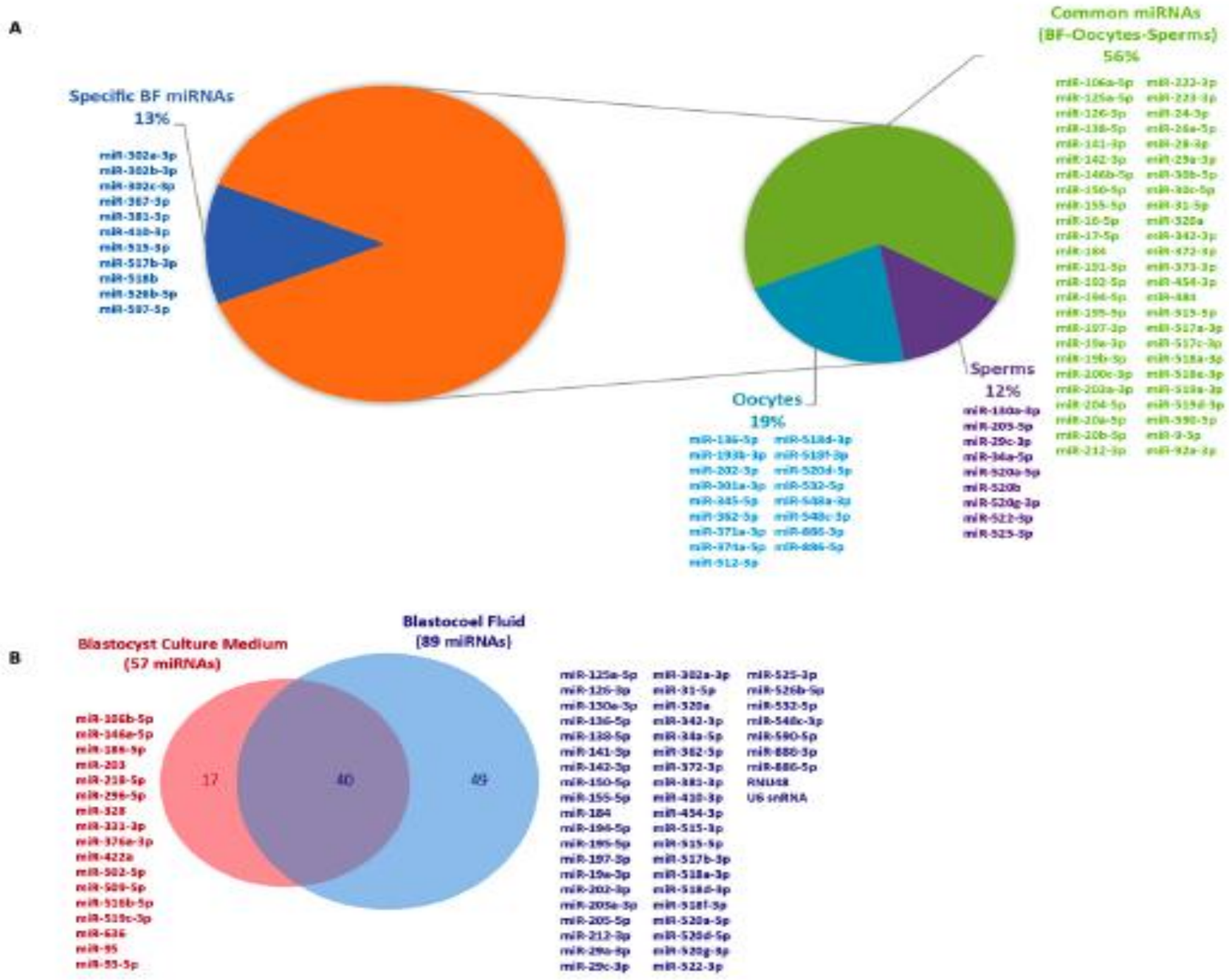
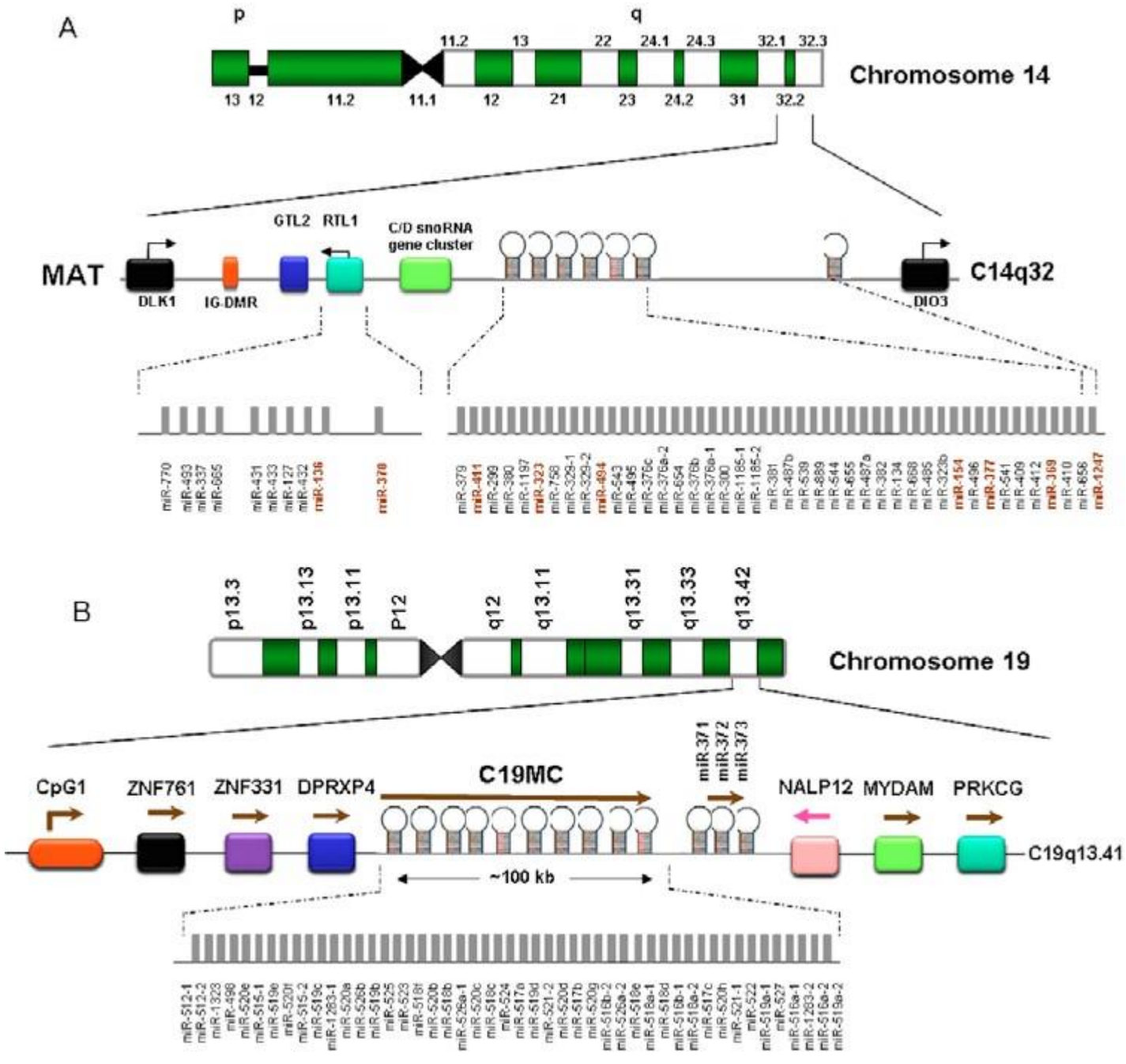


Figure 3. Comparison of BF miRNA expression profile in human oocyte, sperm and blastocyst culture medium. (**A**) Pie charts representing miRNAs from BF identified in human oocytes and sperms. Most of the detected miRNAs (87%) were shared among human gametes. Their distribution is indicated in the small Pie chart. (**B**) Venn diagram comparing BF miRNA expression with culture medium miRNAs.

Cluster



MicroRNAs identificati appartenenti ai Cluster:

C14MC

Sviluppo embrionale, regolazione trascrizione e metabolismo RNA

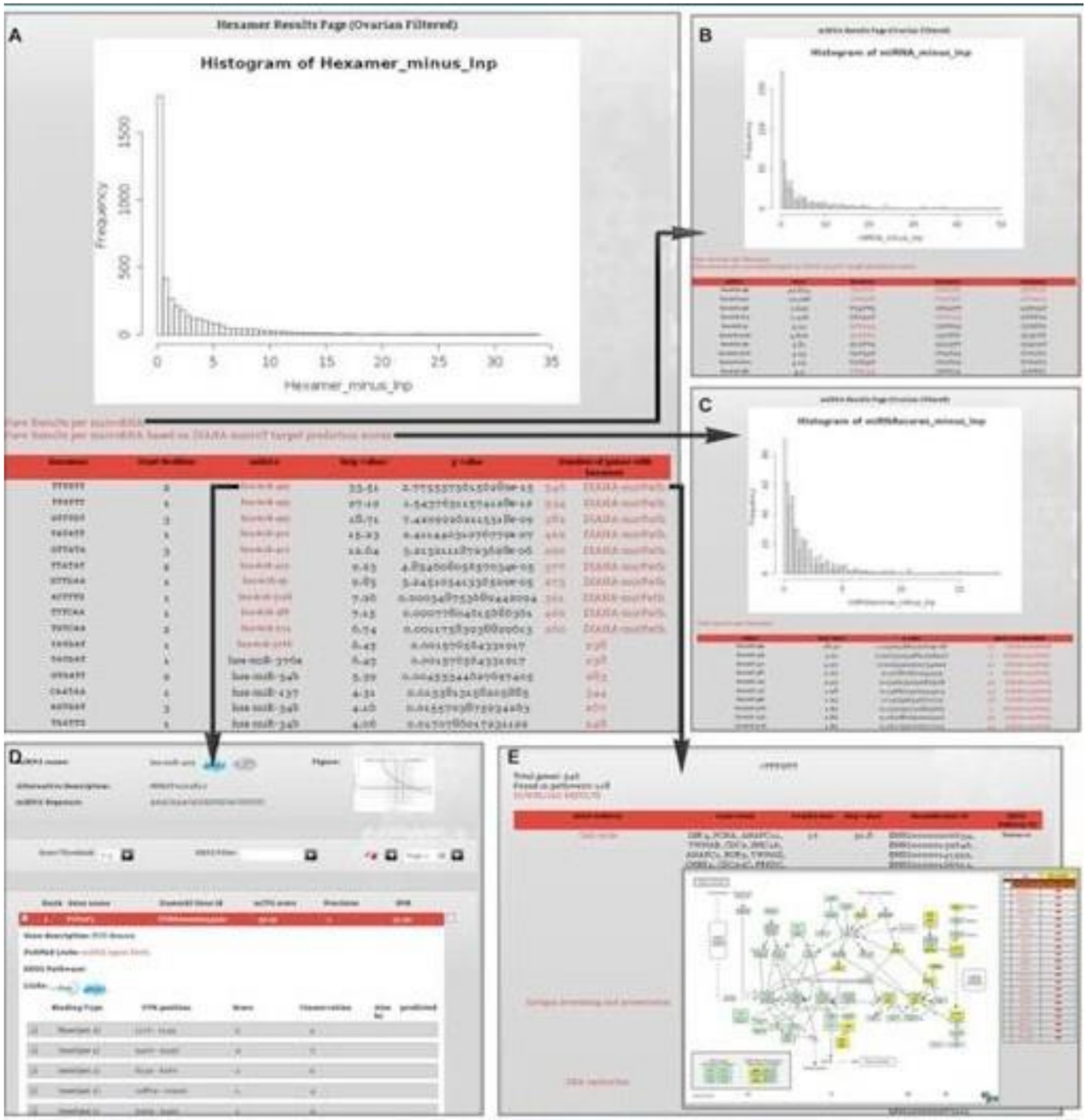
mir-371-3 cluster

Ciclo cellulare, proliferazione, apoptosi

C19MC

Il più grande cluster identificato nella **placenta e specifico per I primati,** è stato correlato all'evoluzione dei primati.
Importante nelle prime fasi di sviluppo embrionale.

Analisi bioinformatiche



Il software **DIANA miRPath v3.0** è stato utilizzato per lo studio:

- mRNA target
- Gene Ontology dei geni bersaglio
- Analisi dei Pathways

Permette l'utilizzo contemporaneo di GO e KEGG

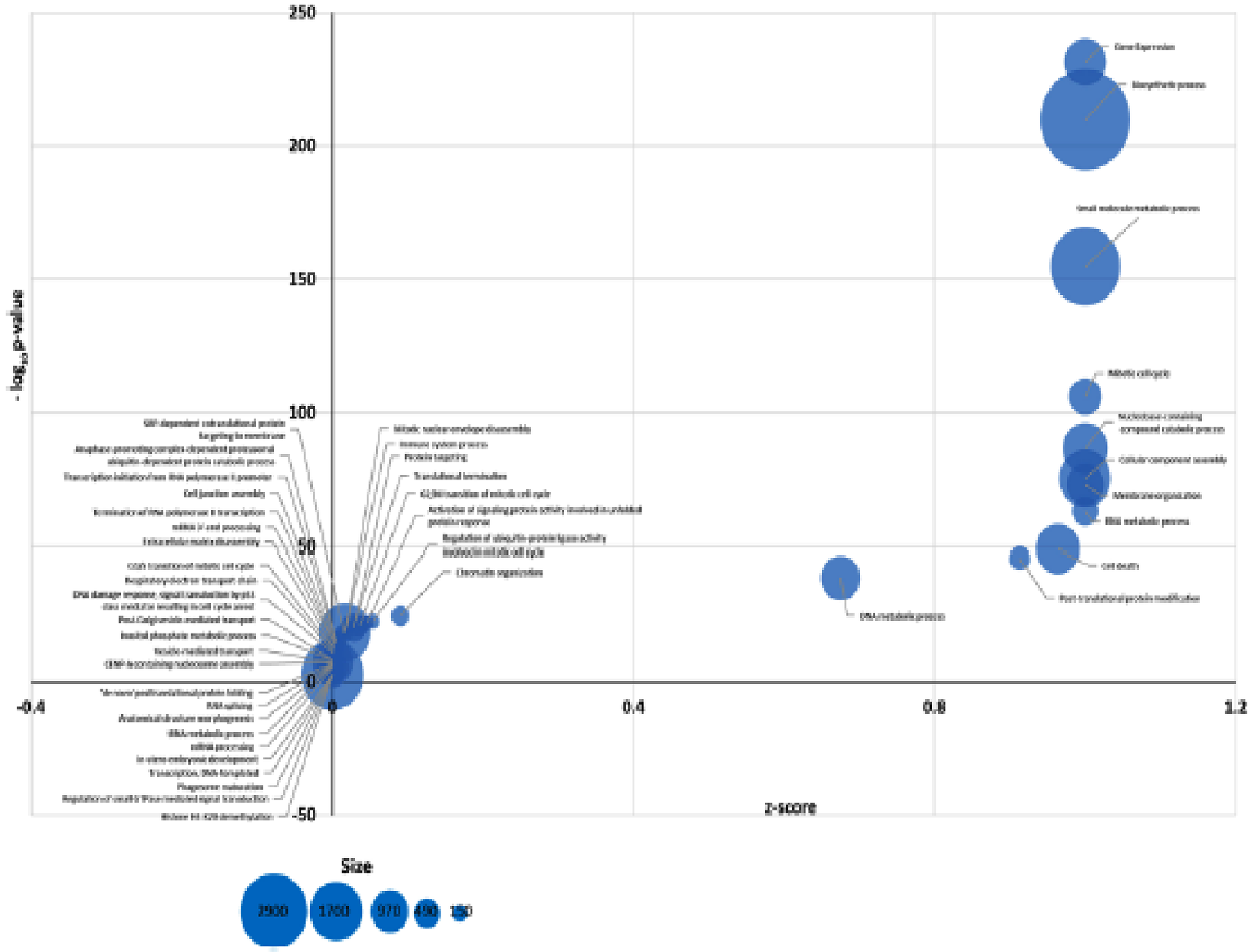


Figure 4. Significant GOs, biological processes, for miRNAs identified in human BFs. Bubble chart representing the most enriched functional categories for miRNA targets in BF. The y-axis represents the -$\log_{10}$ (P-value) and the x-axis the corresponding z-scores. The radius of the bubble is proportional to the size of the mRNA functional category (in terms of number of genes).

Analisi bioinformatica

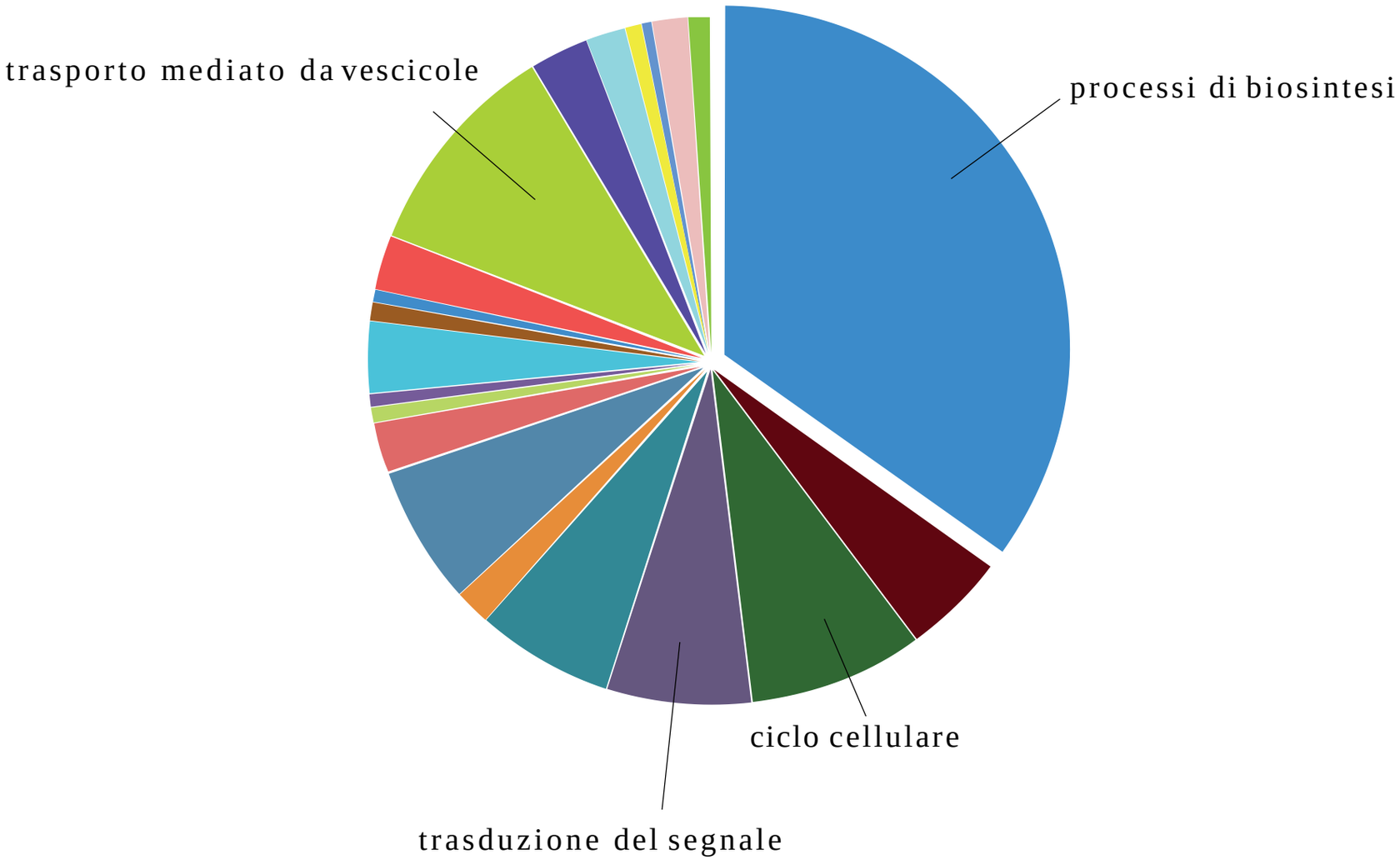
DIANA miRPath v3.0

Gene Ontology

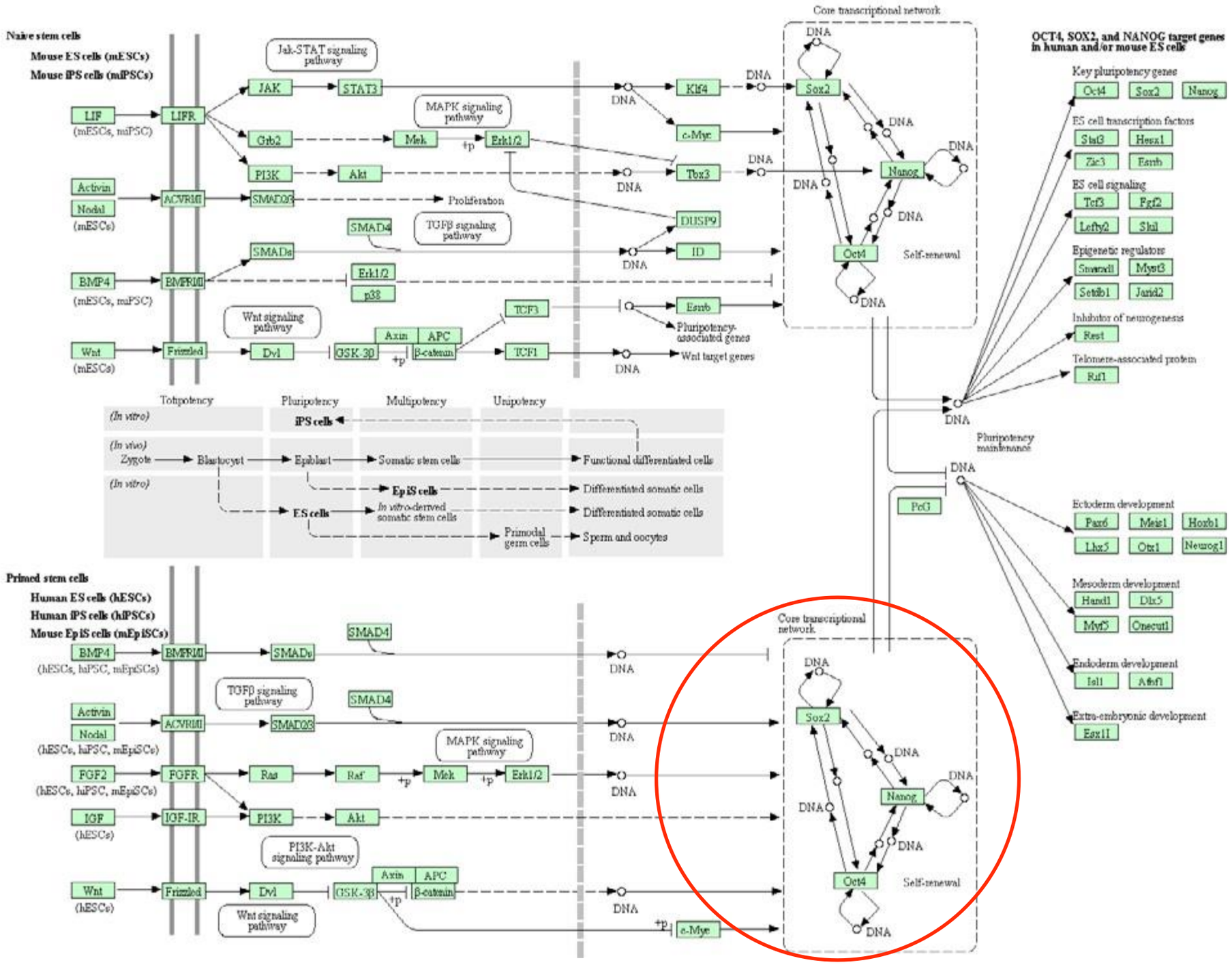
Biological Process Terms

→

Classificazione funzionale dei geni



Signaling Pathways regulating pluripotency of stem cells



Signaling Pathways regulating pluripotency of stem cells

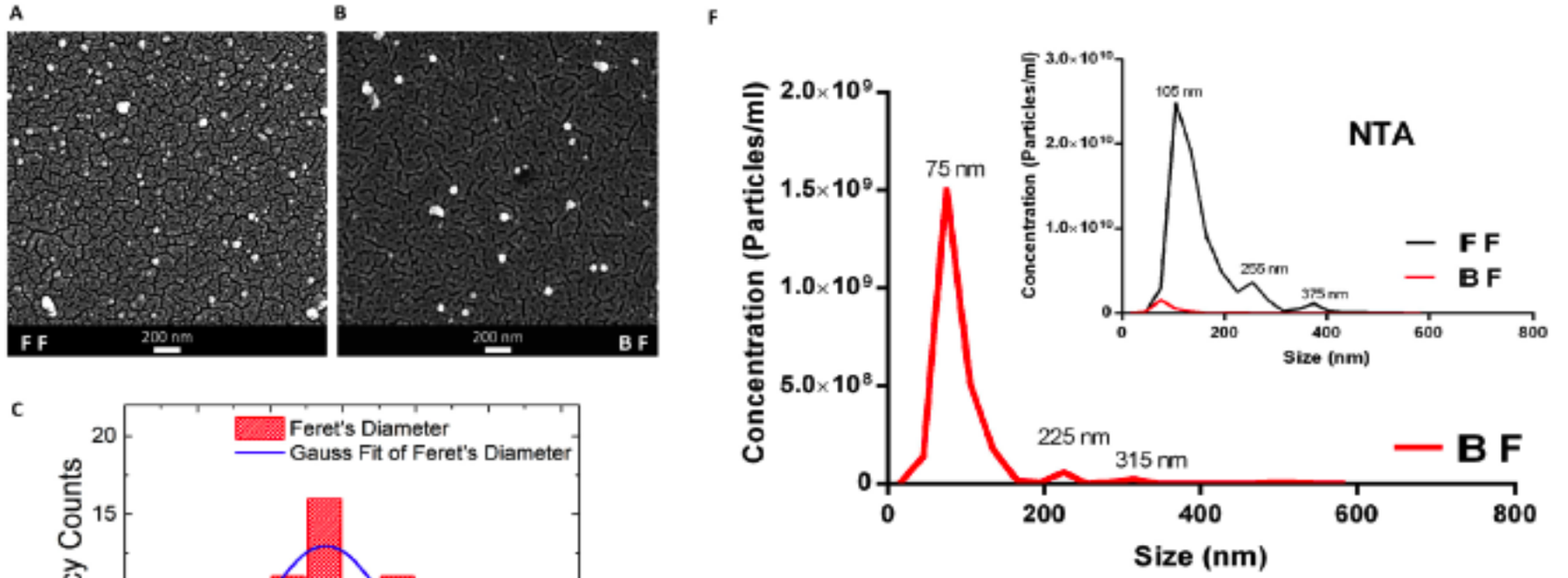
Sox2

Nanog

Oct4

Sono coinvolti nella regolazione:

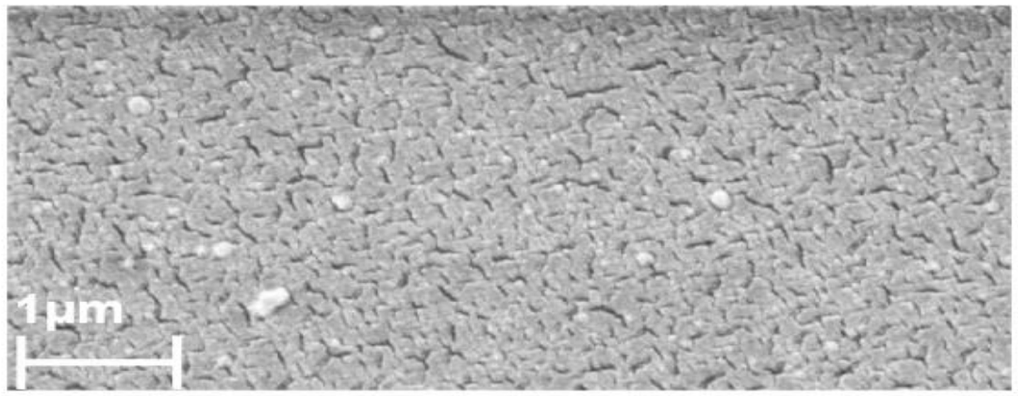
- **Pluripotenza delle cellule staminali embrionali**
- **Proliferazione cellulare nell'embriogenesi**
- **Migrazione delle cellule primordiali germinali**



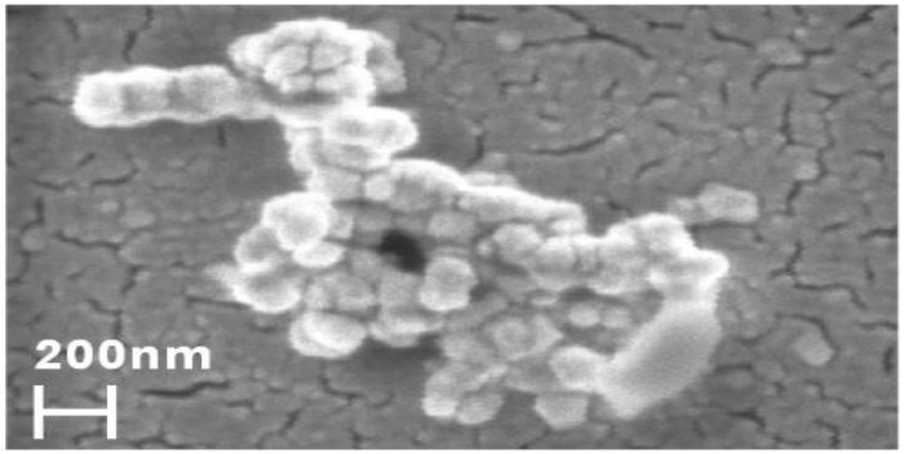
NANOSIGHT	BF	FF
Mean	94.0 ± 8.2 nm	151.0 ± 2.7 nm
Mode	78.4 ± 1.7 nm	115.9 ± 2.9 nm
SD	39.9 ± 13.5 nm	64.6 ± 2.3 nm
D10	32.1 ± 0.8 nm	65.2 ± 0.6 nm
D50	53.1 ± 3.3 nm	101.7 ± 2.8 nm
D90	121.5 ± 25.4 nm	210.1 ± 7.9 nm
Nanoparticles/ml	2.46e+009 ± 2.73e+008	7.06e+010 ± 2.05e+009

Figure 6. Morphological and Molecular Characterization of Exosomes from human Blastocoel Fluid. (**A**,**B**) Scanning Electron Micrographs of extracellular vesicles isolated from Follicular Fluid (FF) and Blastocoel Fluid (BF) respectively. (**C**) Diameter distribution of exosomes from BFs. Gauss fit of the Feret's diameter histogram measured on SEM microscopies show an average BF diameter of 75 ± 3 nm and a full width at half maximum (FWHM) of 38 ± 8 nm. (**D**) Transmission Electron Microscopy images of exosomes from BFs. (**E**) Transmission Electron Microscopy images of exosomes from BFs marked with CD81. (**F**) Nanoparticle Tracking Analysis (NTA) of BF extracellular vesicles. Extracellular vesicles from Follicular Fluid were used as reference control (inset). Diameters and concentration of vesicles are indicated in the table. (**G**) ELISA assay with the tetraspanin CD63 antibody of BF exosomes. Amount (µg) of CD63 protein and EV concentration (number of particles/100 µl) evaluated in BFs. Follicular Fluid (FF) samples were used as reference control. Results are expressed as mean ± SEM.

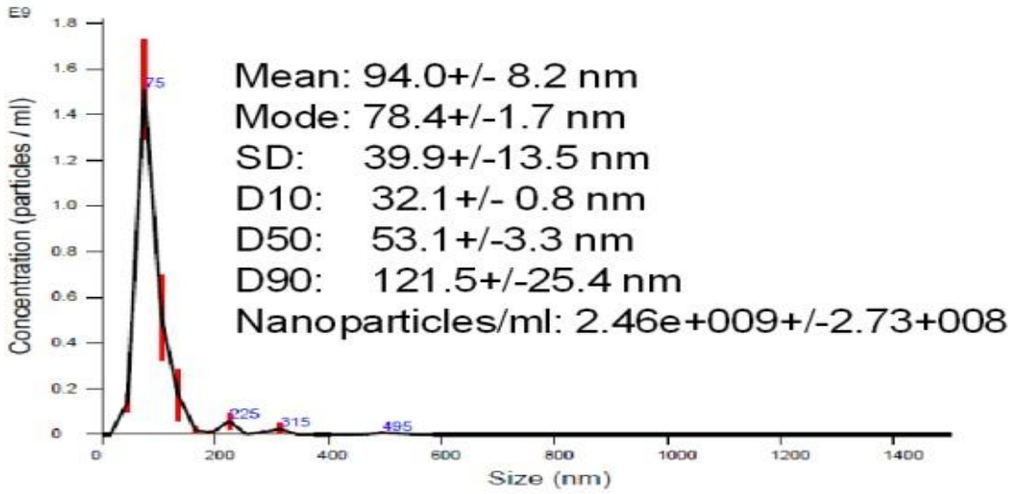
A


1µm

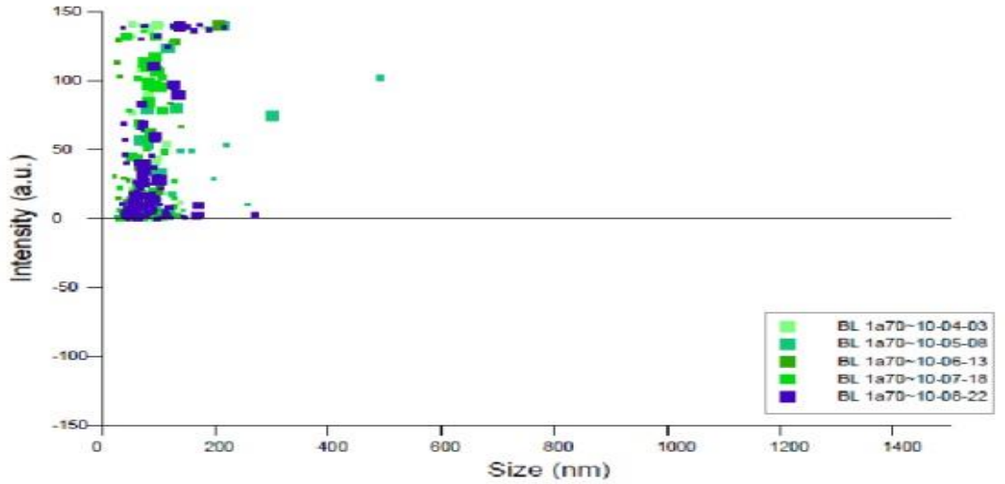
B


200nm

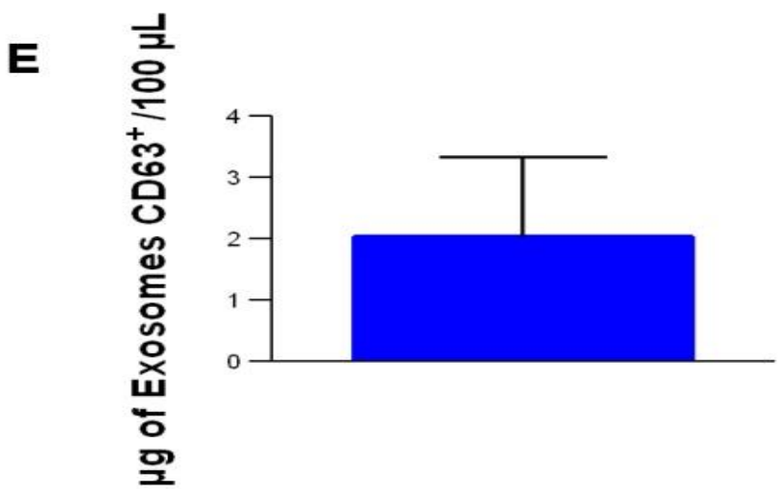
C


E9
Concentration (particles / ml)
Size (nm)
Mean: 94.0+/- 8.2 nm
Mode: 78.4+/-1.7 nm
SD: 39.9+/-13.5 nm
D10: 32.1+/- 0.8 nm
D50: 53.1+/-3.3 nm
D90: 121.5+/-25.4 nm
Nanoparticles/ml: 2.46e+009+/-2.73+008

D


Intensity (a.u.)
Size (nm)
BL 1a70~10-04-03
BL 1a70~10-05-08
BL 1a70~10-06-13
BL 1a70~10-07-18
BL 1a70~10-08-22

E


µg of Exosomes CD63+ /100 µL

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Identification of extracellular vesicles and characterization of miRNA expression profiles in human blastocoel fluid

Received: 16 August 2018
Accepted: 19 November 2018
Published online: 14 January 2019

R. Battaglia[1], S. Palini[1,2], M. E. Vento[1], A. La Ferlita[1,2], M. J. Lo Faro[1,2], E. Caroppo[3], P. Borzì[4], L. Falzone[1], D. Barbagallo[1], M. Ragusa[1,7], M. Scalia[1], G. D'Amato[3], P. Scollo[4], P. Musumeci[5], M. Purrello[1], E. Gravotta[6] & C. Di Pietro[1]

CONCLUSIONI

I) I MiRNA trovati nel fluido del blastocele potrebbero costituire uno strumento per lo studio della biologia delle cellule staminali embrionali non invasivo.

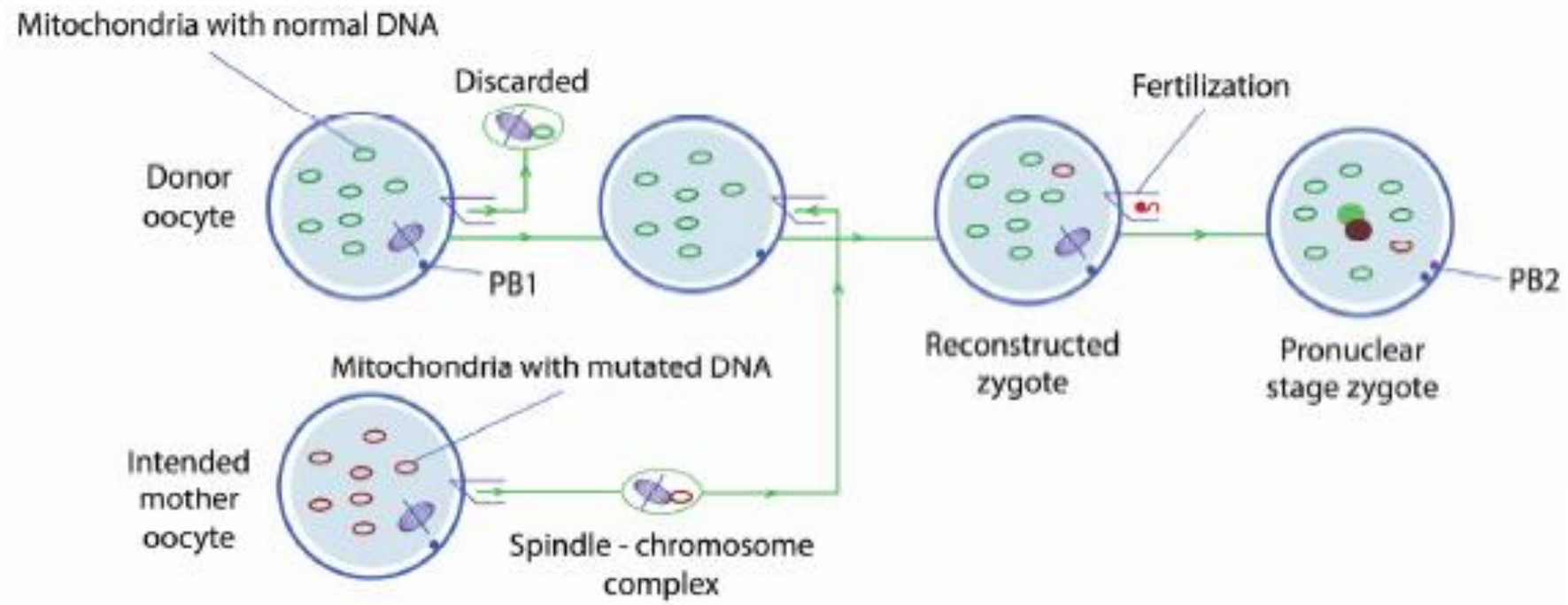
II) Essendo prodotti della blastocisti, il loro profilo di espressione potrebbe essere correlato alla qualita' embrionaria

III) Quindi potrebbero essere utilizzati come biomarcatori non invasivi di qualita' embrionaria e per il cross talk tra embrione ed endometrio

IV) Dati preliminari evidenziano correlazioni dirette tra MiRNA e gravidanza prescindendo dalla stimolazione, dal terreno e correlando positivamente con i criteri mofologici!!

Sept 2016: First three parent baby (Leigh syndrome)

Maternal spindle transfer: removal of the nucleus from one of the mother's oocytes and inserted it into a donor oocyte that had had its own nucleus removed. The resulting oocyte – with nuclear DNA from the mother and mitochondrial DNA from a donor – was then fertilised with the father's sperm.



Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in Human Assisted Reproduction

Dori C. Woods, PhD[1] Jonathan L. Tilly, PhD[1,2]

[1] Laboratory of Aging and Infertility Research (LAIR), Department of Biology, Northeastern University, Boston, Massachusetts
[2] Office of the Dean, College of Science, Northeastern University, Boston, Massachusetts

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Semin Reprod Med 2015;33:410–421

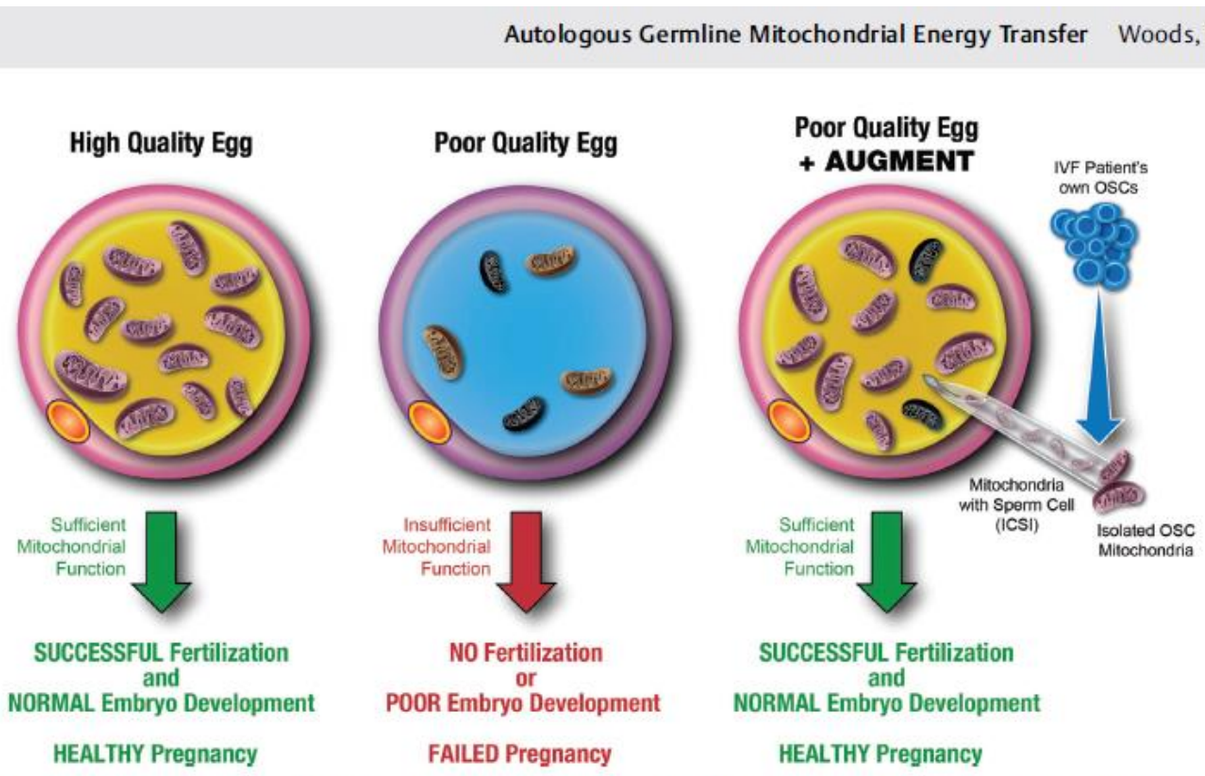

Fig. 1 Conceptual depiction of AUGMENT in human assisted reproduction. In high-quality eggs, mitochondria are sufficient in number, function, and quality to provide the energy required for successful fertilization and normal preimplantation embryonic development, resulting in a healthy pregnancy (*left* panel). With age, the functional integrity of the mitochondrial pool in women's eggs decreases, leading to poor egg quality that results in reduced rates of fertilization, compromised embryonic development, and ultimately failed pregnancy (*center* panel). By providing a source of germline mitochondria from an IVF patient's own OSCs, AUGMENT introduces a proprietary amount of autologous egg precursor cell-derived mitochondria into the egg along with the sperm at the time of ICSI. This bolus of pristine mitochondria provides an otherwise compromised egg with sufficient energetic potential for successful fertilization and subsequent embryonic development, restoring the natural potential to achieve a healthy pregnancy (*right* panel). AUGMENT, autologous germline mitochondrial energy transfer; ICSI, intracytoplasmic sperm injection; OSCs, oogonial stem cells.

cells

MDPI

Review

Implications and Current Limitations of Oogenesis from Female Germline or Oogonial Stem Cells in Adult Mammalian Ovaries

Jessica J. Martin, Dori C. Woods and Jonathan L. Tilly *

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Received: 29 November 2018; Accepted: 16 January 2019; Published: 28 January 2019

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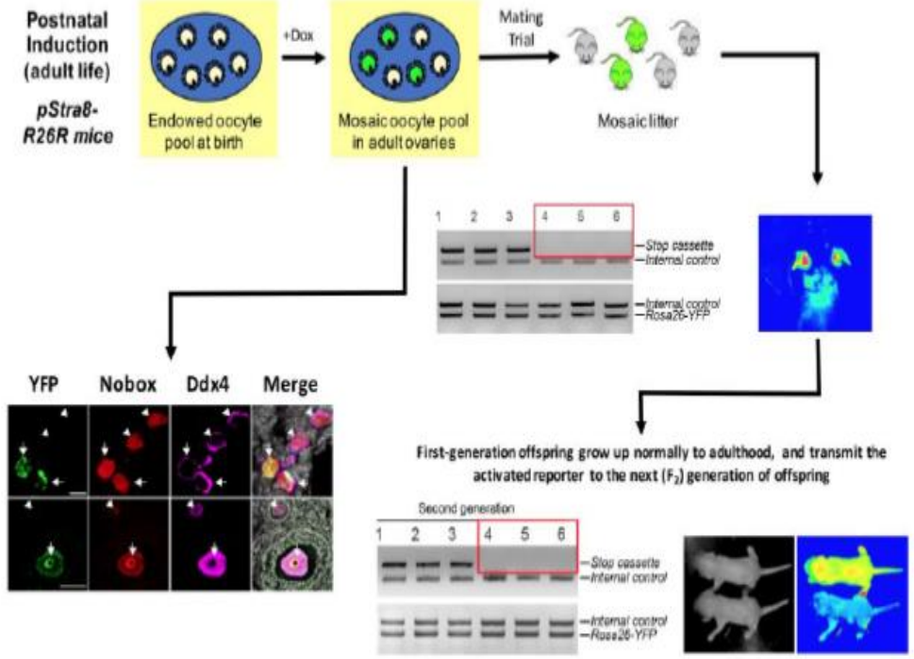


Figure 1. Oocytes formed during adult life in mice contribute directly to offspring production. Schematic representation of an inducible genetic lineage-tracing model to 'mark' new oocytes formed during the doxycycline (dox) induction phase, which specifically activates a triple-transgenic Cre-*loxP*–based reporter system tied to *stimulated by retinoic acid gene 8* (*Stra8*) expression in pre-meiotic germ cells (viz. OSCs) committing to meiosis followed by de novo oogenesis. Portions of this figure were adapted with permission from Wang et al. [84].

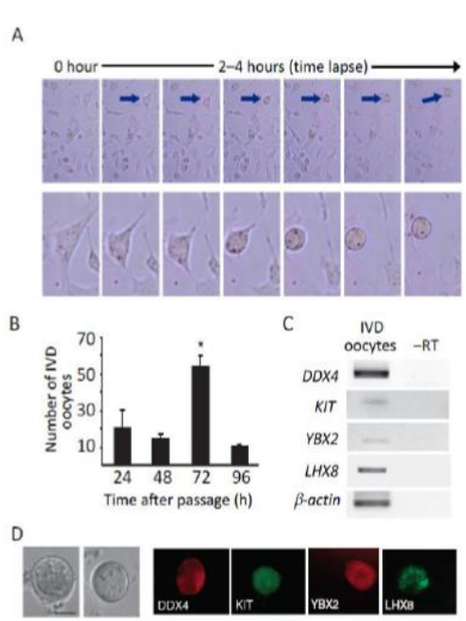


Figure 2. In vitro oogenesis from human OSCs. (**A**) Time lapse images of human OSCs in culture, with a typical OSC (blue arrow) followed as it undergoes progressive differentiation into an IVD oocyte (oocyte-like cell). (**B**) Numbers of IVD oocytes formed in human OSC cultures over time post-passage. (**C**) Expression analysis of germ cell (*DDX4*) and oocyte (*KIT* oncogene or *KIT*; *Y-box protein 2* or *YBX2*, also referred to as *MSY2* or *CONTRIN*; *LIM homeobox protein 8* or *LHX8*) marker genes, as well as of *β-actin* expression as a loading control, in IVD oocytes collected from human OSC cultures (–RT, PCR analysis performed on the RNA template without reverse transcription, as a control to rule out genomic DNA amplification). (**D**) Representative images of human IVD oocytes by light microscopy (two left panels; scale bar, 50-µm), and by immunofluorescence microscopy for the presence of DDX4, KIT, YBX2 and LHX8 proteins. Portions of this figure were adapted with permission from White et al. [73].

Female Fertility Preservation through Stem Cell-based Ovarian Tissue Reconstitution In Vitro and Ovarian Regeneration In Vivo

Taichi Akahori[1,2], Dori C Woods[1] and Jonathan L Tilly[1]

[1]Laboratory for Aging and Infertility Research, Department of Biology, Northeastern University, Boston, MA, USA. [2]On leave from the Department of Obstetrics and Gynecology, Saitama Medical Center, Saitama Medical University, Saitama, Japan

Clinical Medicine Insights: Reproductive Health
Volume 13: 1–10

DOI: 10.1177/1179558119848007
SAGE

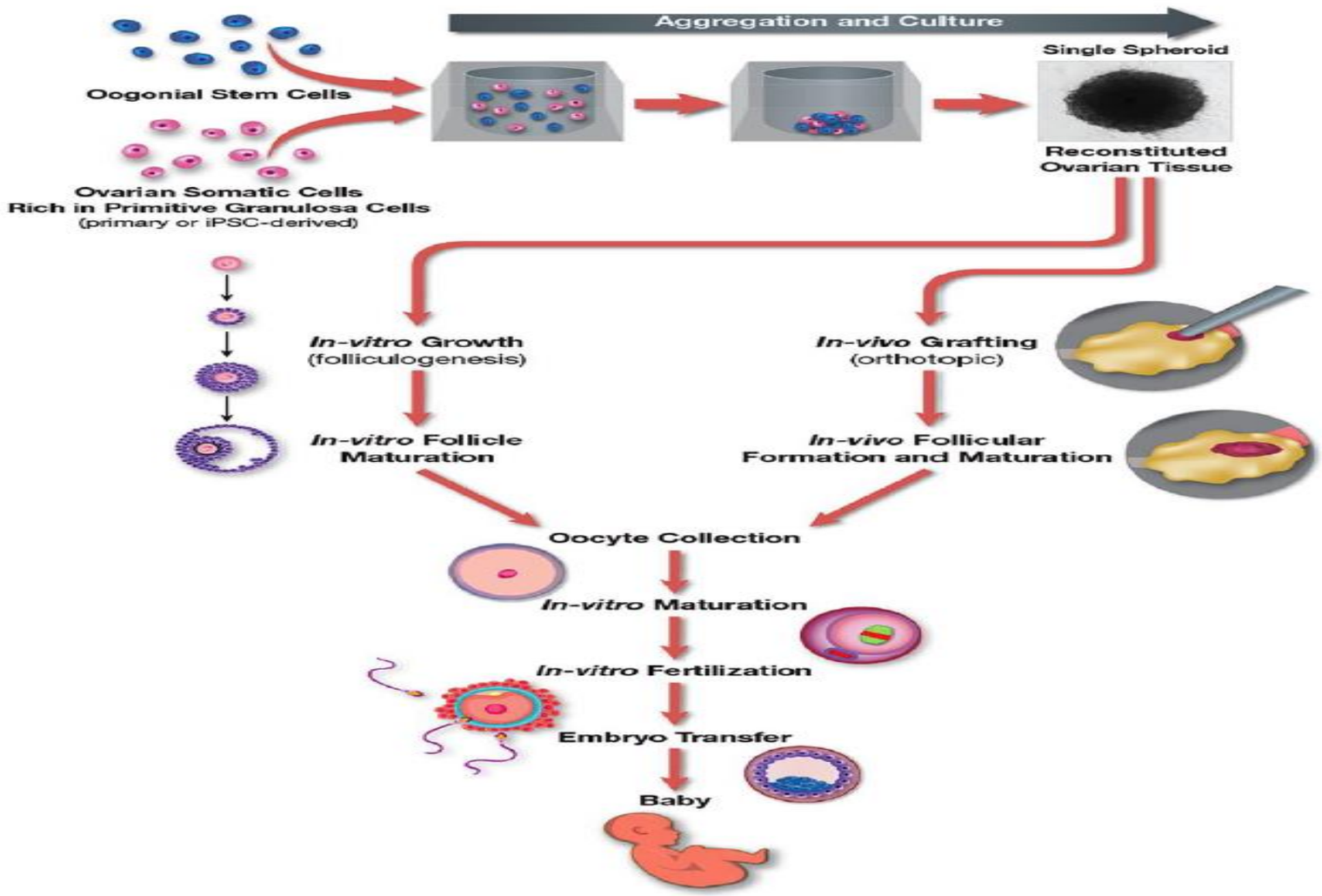
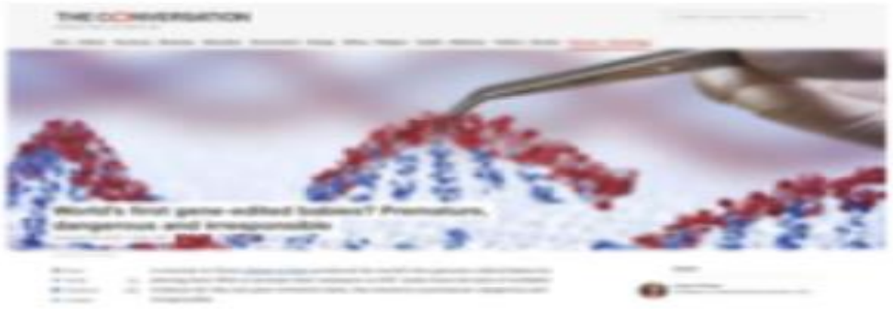


Figure 2. Working model for ex-vivo reconstitution of autologous human ovarian tissue. Aggregation of OSCs with primitive granulosa cells, specified from iPSCs or isolated from ovarian tissue during OSC purification, enables de-novo oogenesis and folliculogenesis in the reconstituted tissue in vitro. The tissue containing new follicles is then used for orthotopic grafting to the ovaries for in-vivo growth to produce maturing follicles for oocyte aspiration or for in-vitro follicle culture to generate oocytes. Oocytes obtained from either approach are subjected to in-vitro maturation and in-vitro fertilization to generate blastocysts for embryo transfer and establishment of successful pregnancies.


THE CONVERSATION
World's first gene-edited babies? Premature, dangerous and irresponsible


China orders inquiry into 'world's first gene-edited babies'


World Update


nature
Genome-edited baby claim provokes international outcry

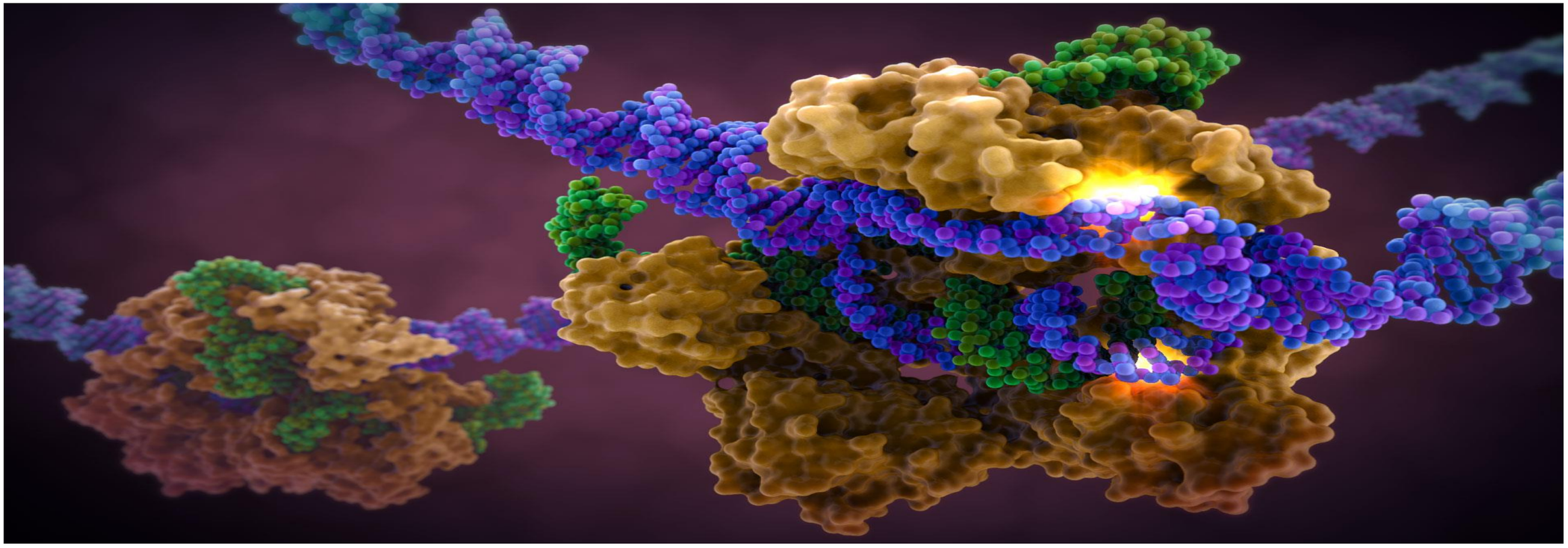

China orders probe into scientist claims of first gene-edited babies

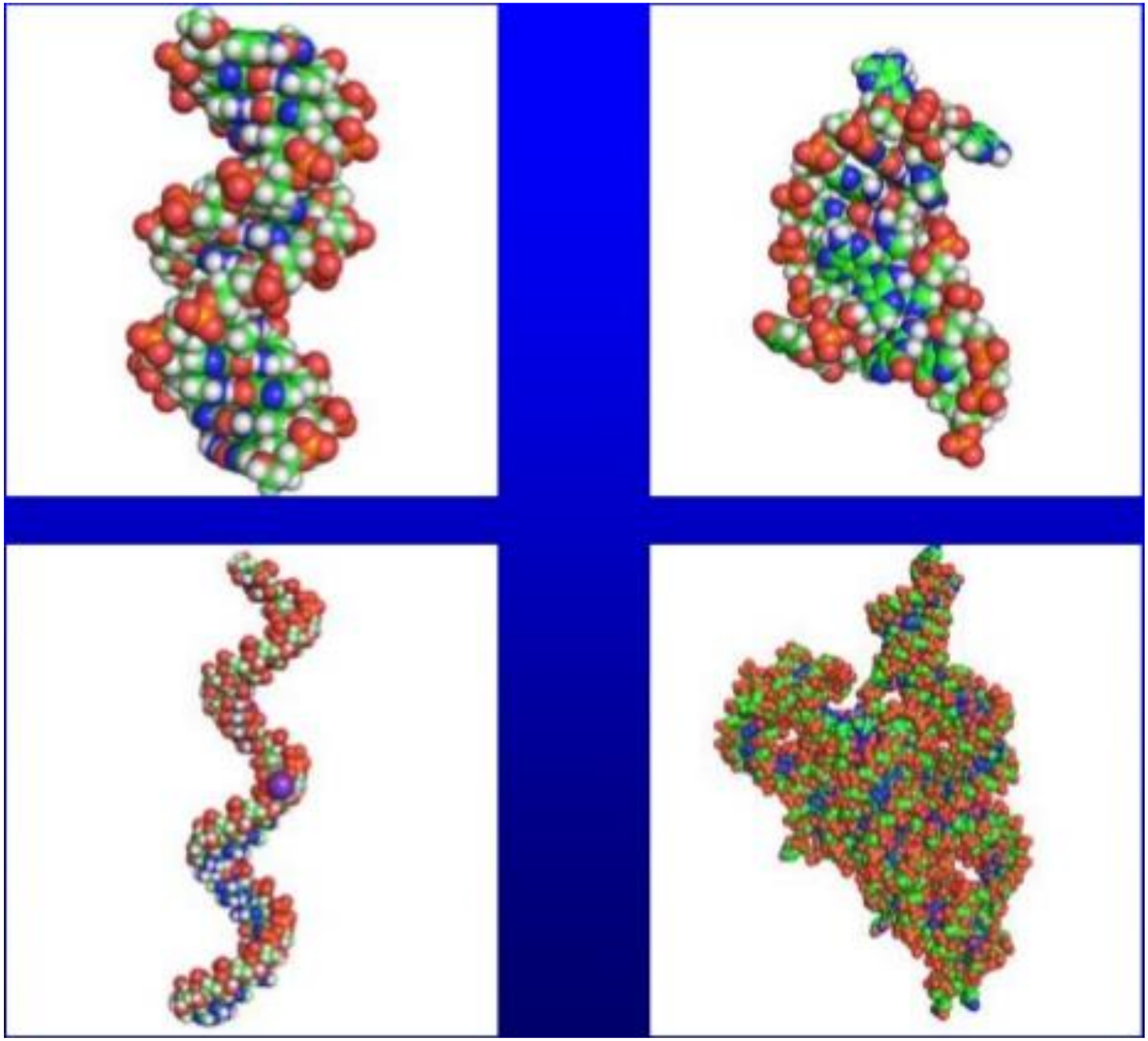

Chinese scientist claims to have created world's first genetically-edited babies


Chinese scientist claims to have created genetically-edited babies

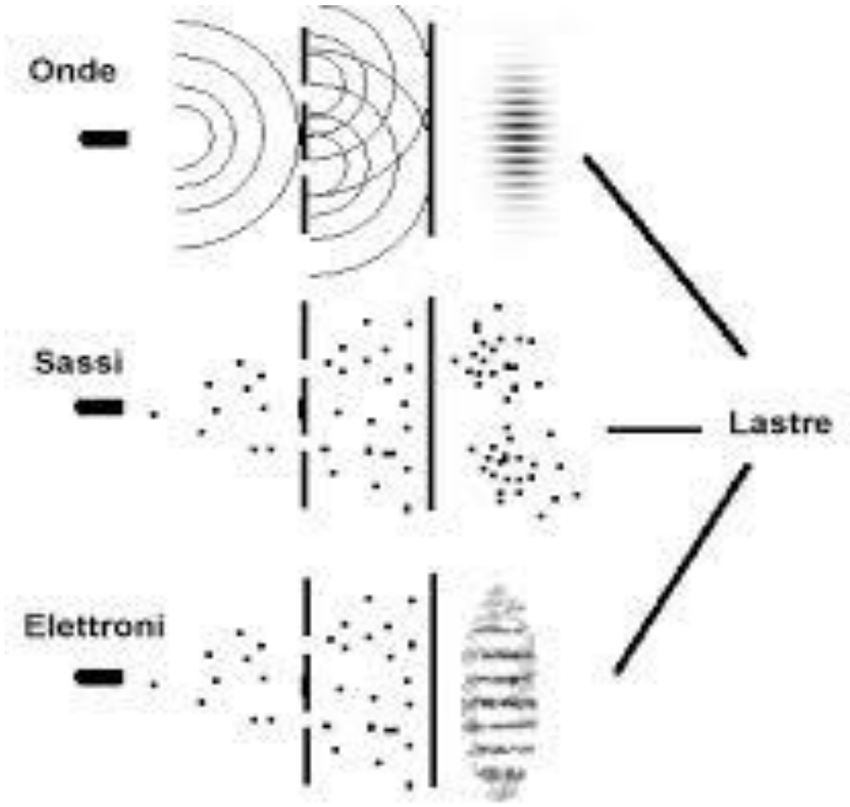

Controversy as researcher claims to have created world's first 'gene-edited' babies


First genetically edited BABIES created by 'dangerous and irresponsible' scientist





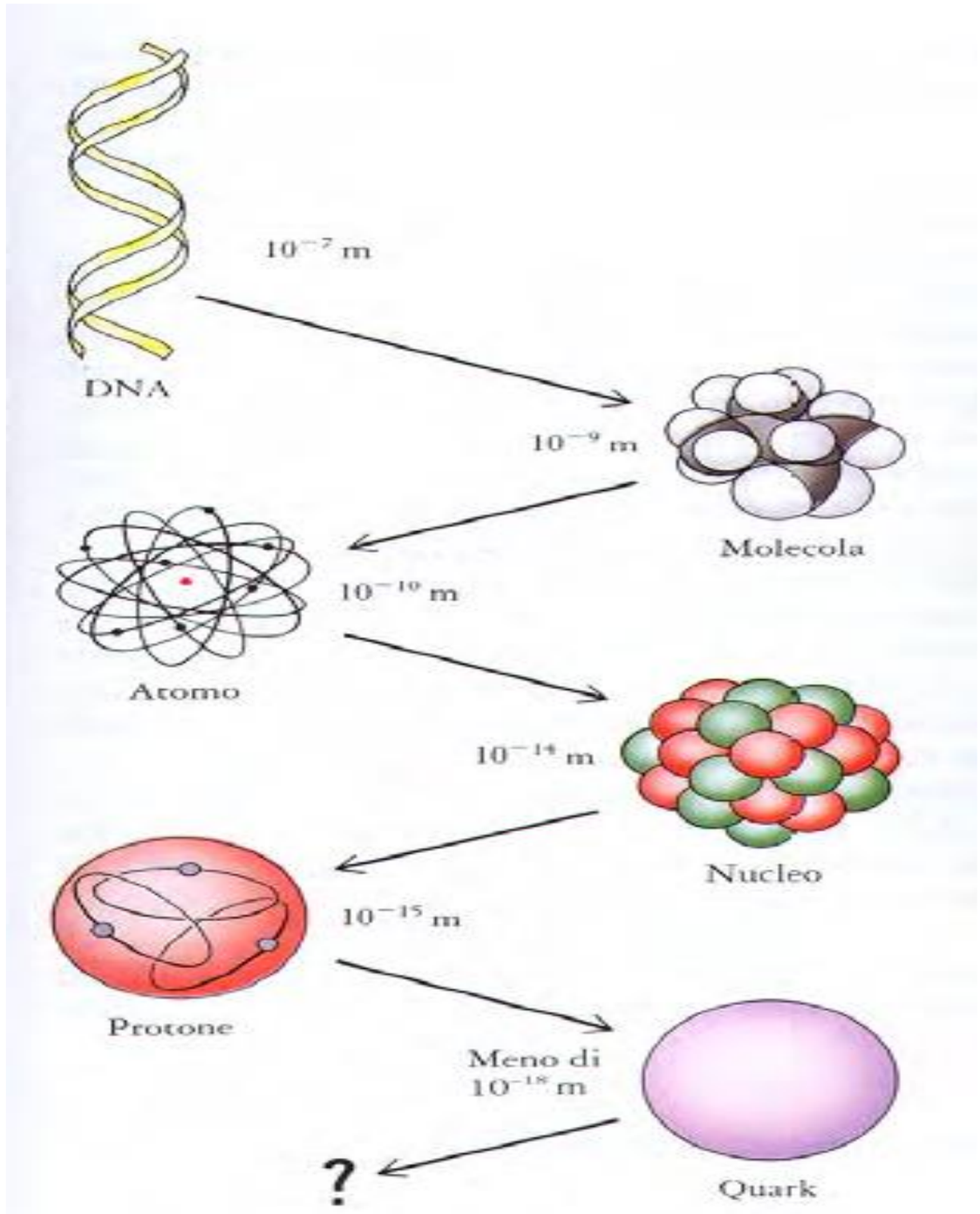
Esperimento più bello della fisica: la doppia fenditura



Relazione fra energia e frequenza

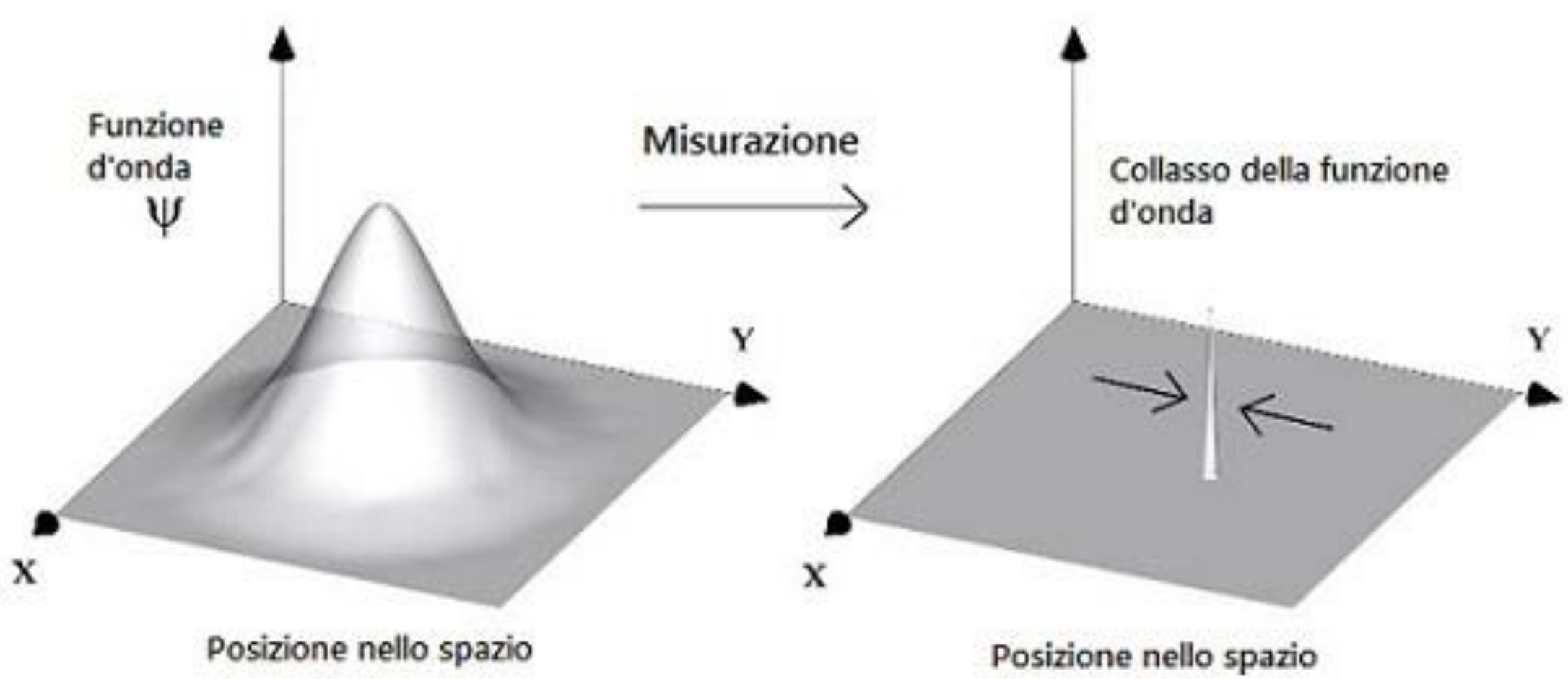
$$E = h\nu$$

h = costante di Plank 6.62 x 10^{-34} J s



Le «bambole russe» della materia, dal DNA ai quark; l'ordine di grandezza cambia a ogni livello.

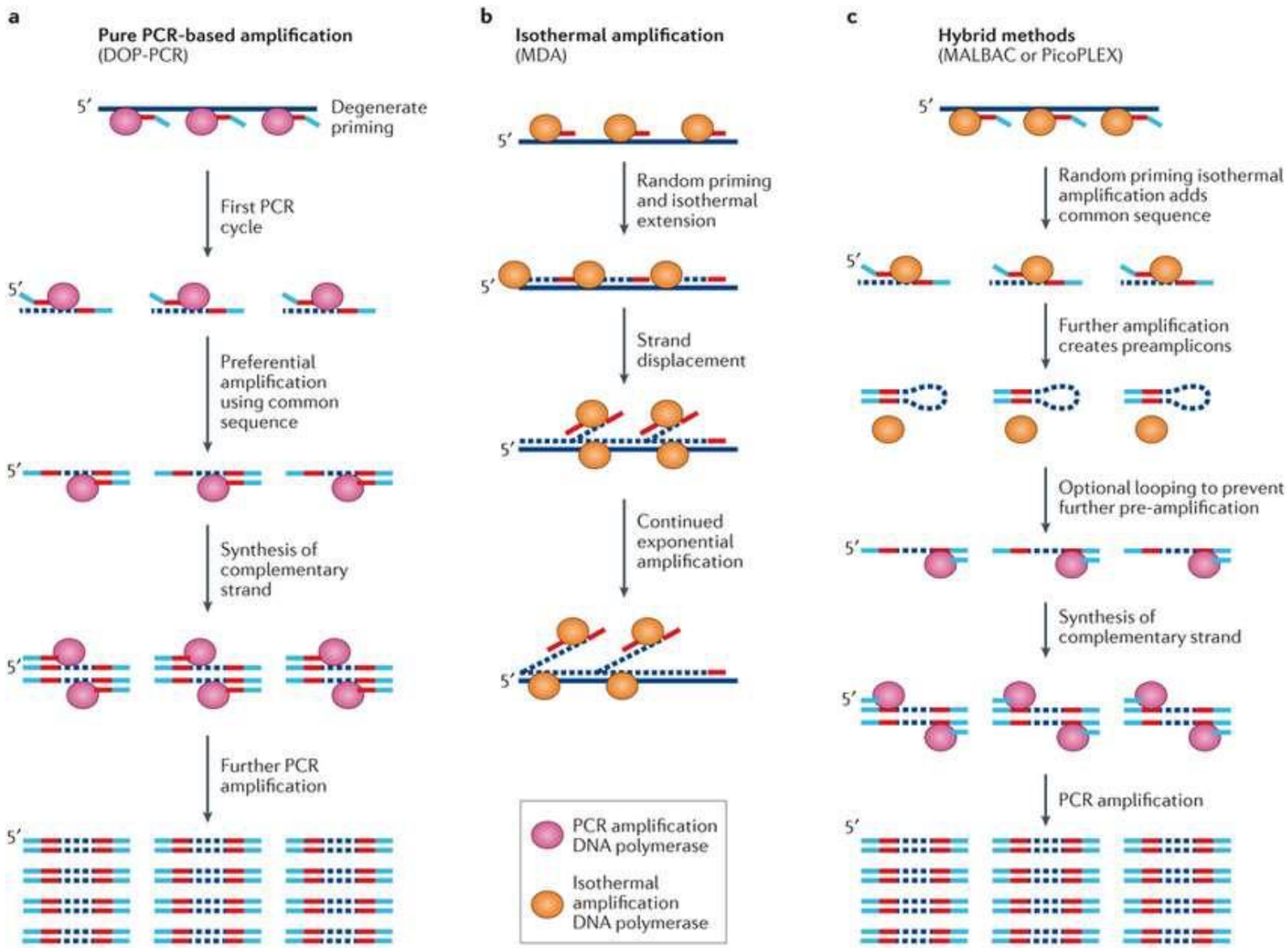
Collasso dell'onda; Schrodinger



Culture conditions and Amplification methods

There are different WGA methods:

- The **DNA integrity** (large fragments vs fragmented) will impact the results of WGA
- WGA enzymes are very **sensitive to buffer** systems, resulting in suboptimal amplification

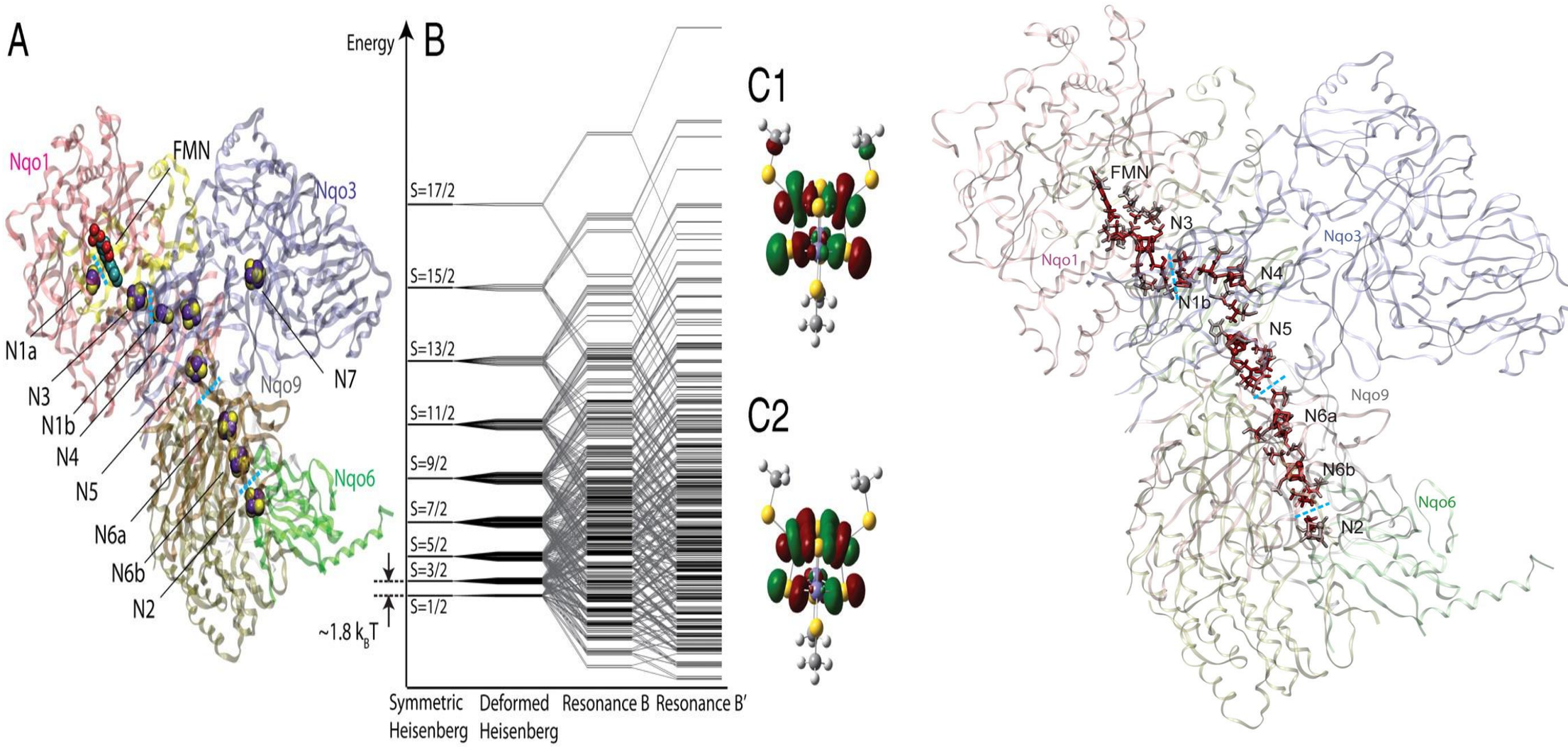


Equazione di Schrodinger

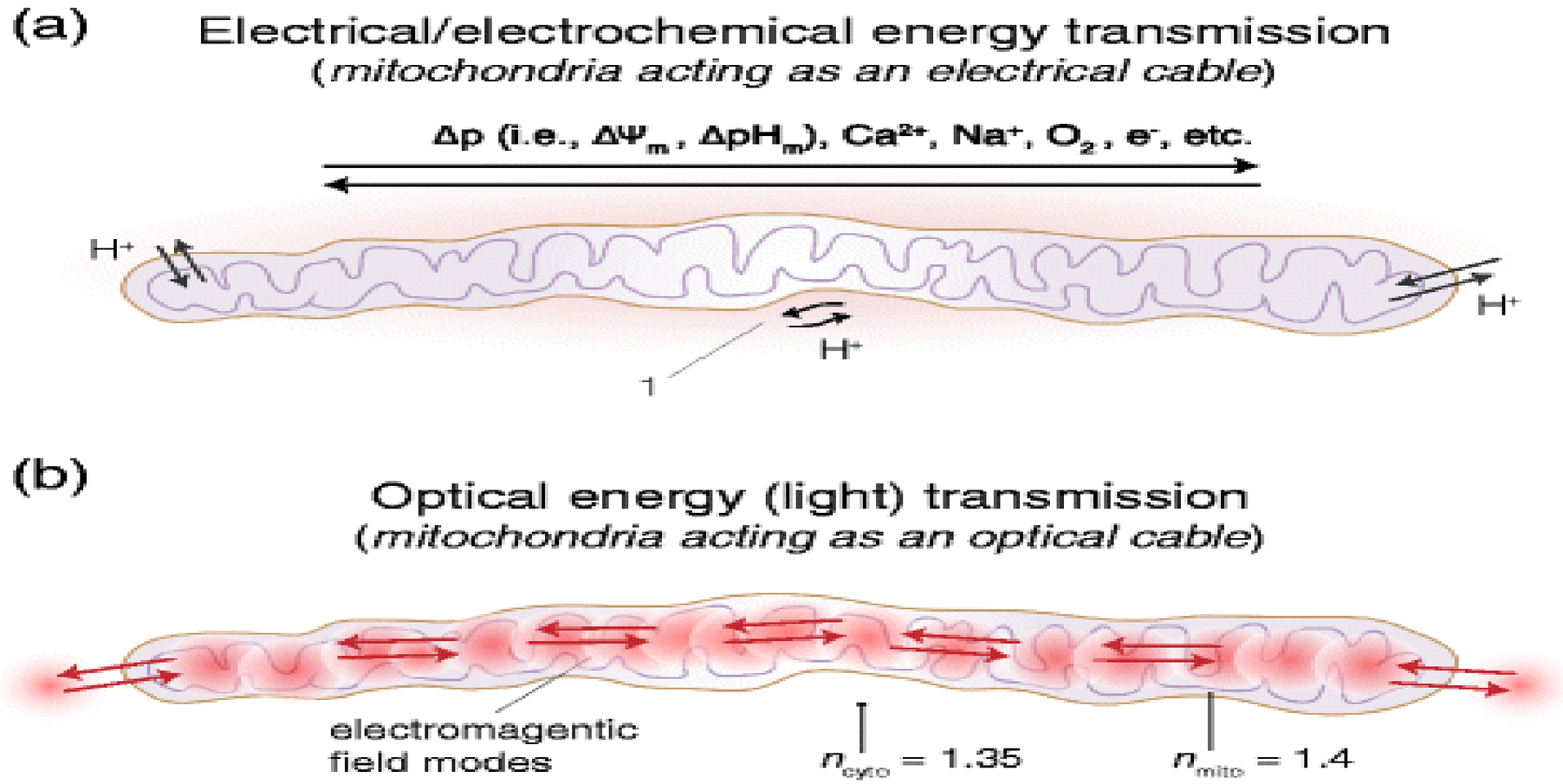
$$i\hbar\frac{\partial}{\partial t}\Psi = H\Psi$$

La realtà e’ in funzione del nostro punto di vista,
siamo noi ad influenzare l’esito di ciò che vogliamo vedere;

(A) Crystal structure of the hydrophilic domain of the respiratory complex I from T. thermophilus (4).



Tomoyuki Hayashi, and Alexei A. Stuchebrukhov PNAS 2010;107:19157-19162



Tomoyuki Hayashi, and Alexei A. Stuchebrukhov PNAS 2010;107:19157-19162

ANNUAL REVIEWS

Annual Review of Physical Chemistry

Why Are DNA and Protein Electron Transfer So Different?

David N. Beratan[1,2]

[1]Department of Chemistry and Department of Physics, Duke University, Durham, North Carolina 27708, USA; email: david.beratan@duke.edu

[2]Department of Biochemistry, Duke University, Durham, North Carolina 27710, USA

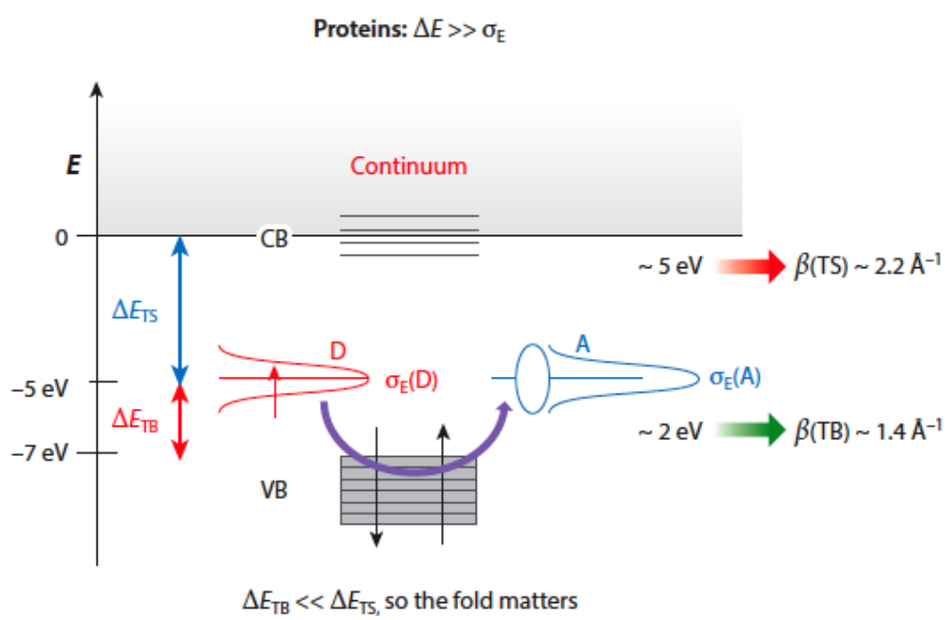


Figure 1

In proteins, the DA energy-level broadening is <10% of the tunneling barrier height. The TB tunneling barrier (ΔE_{TB}) height is about half the height of the TS tunneling barrier (ΔE_{TS}), so the folded protein bonding orbitals (VB) dominate the electron tunneling mediation from D to A (6). Abbreviations: A, acceptor; CB, protein anti-bonding orbitals; D, donor; TB, through-bond; TS, through-space; VB, valence band.

Quindi come la teoria Geo-Elio centrica non hanno cambiato nulla nel quotidiano, questi punti di vista alternativi, scientifici
e reali, dovrebbero, con tempi molto più brevi, visti i coevi mezzi divulgativi a disposizione , prepararci a quello che avvenne 150 anni dopo la scoperta di Giordano Bruno; L'illumnismo!!!
In biologia, grazie all'evolversi delle nanotecnologie, stiamo capendo che la fisica Newtoniana, deterministica intendo,
non funziona sempre bene ed andiamo sempre più a trovare spiegazioni a tali fenomeni applicando le regole che sottendono la meccanica quantistica.

Grazie per l’attenzione

Dott. Palini Simone
Specialista in Genetica,Senior Clinical Embryologist
U.O. Fisiopatologia della Riproduzione Osp. Cervesi, Cattolica (RN)
AUSL Romagna
Membro Direttivo SIRU
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International Journal of
Molecular Sciences

MDPI

Review

Extracellular Vesicles in Human Oogenesis and Implantation

Francesca Andronico [1], Rosalia Battaglia [1,*], Marco Ragusa [1,2], Davide Barbagallo [1], Michele Purrello [1] and Cinzia Di Pietro [1,*]

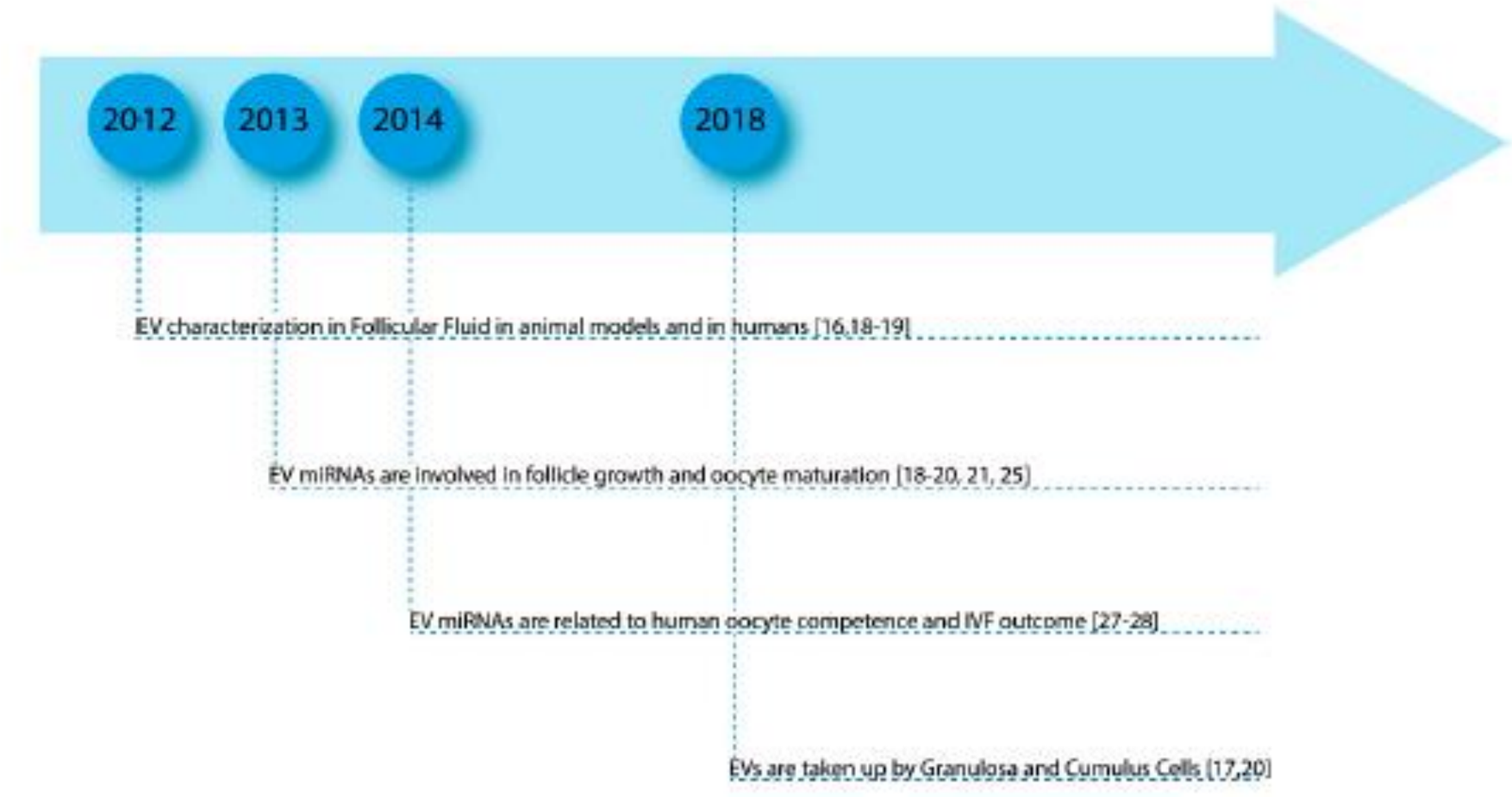

Figure 2. Milestones of the main significant papers about the involvement of EVs in follicle growth and their potential role in fertility disorders.