



СЕЧЕНОВСКИЙ УНИВЕРСИТЕТ

# **Role of exosomes in pregnancy loss (miscarriage) CD 9, 81, 63**

Assambayeva Ailar

# Plan

- I Extracellular vesicles
- II Types of EVs
- III Function
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- V Oocyte extracellular vesicles
- VI Endometrial EVs
- VII Role of EVs during embryo implantation
- VIII Pregnancy loss
- IX Role of EVs in miscarriage



# Extracellular vesicles:

- Secreted from different types of cells including blood cells, endothelial, trophoblast, cardiac and tumor cells.
- Present in the blood circulation and other biological fluids under normal physiologic conditions
- Level of EVs increase in a wide range of disease states

## Types of Evs:

- **Exosomes** (less than 150 nm in diameter)
- Microvesicles
- Apoptotic bodies

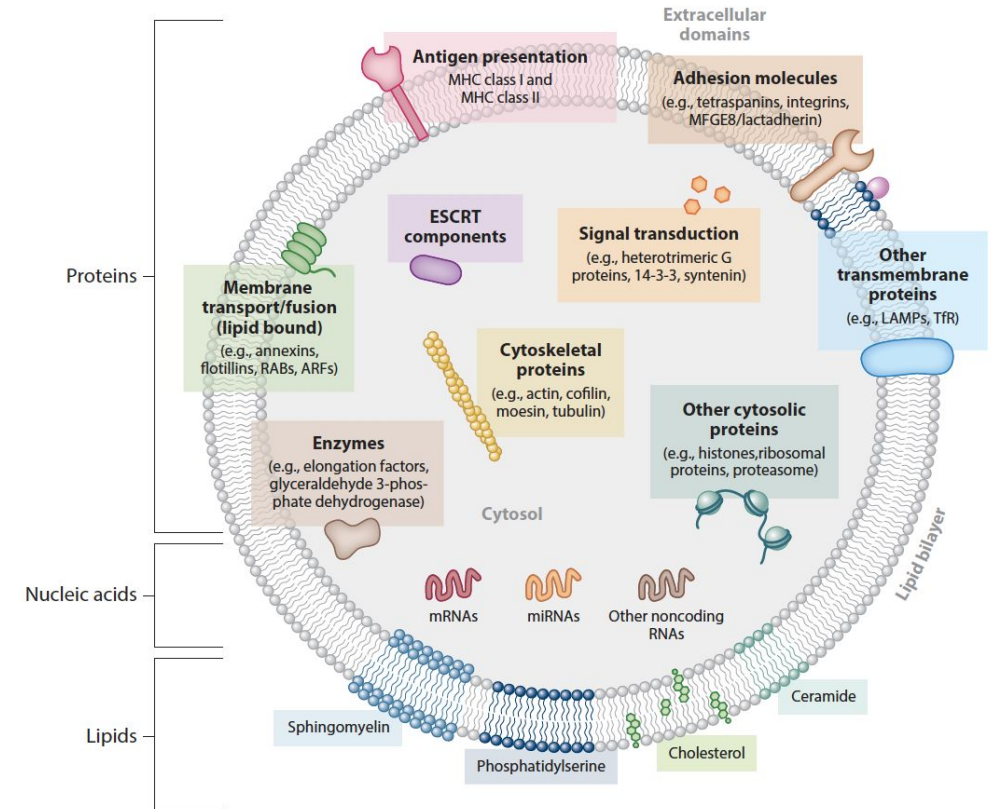


Figure 2

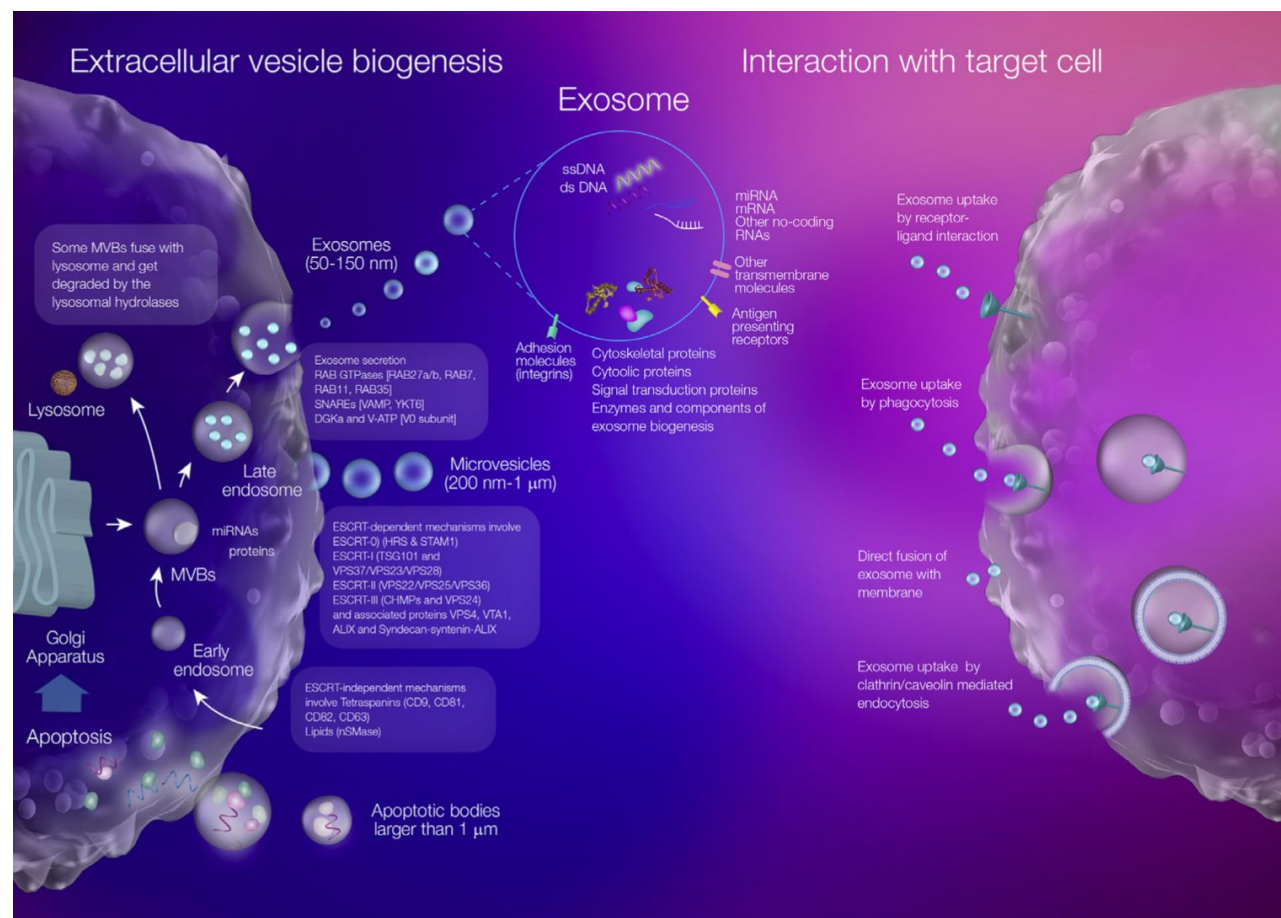
Overall composition of extracellular vesicles (EVs). Schematic representation of the composition (families of proteins, lipids, and nucleic acids) and membrane orientation of EVs. Examples of tetraspanins commonly found in EVs include CD63, CD81, and CD9. Note that each listed component may in fact be present in some subtypes of EVs and not in others. For instance, histones and proteasome and ribosome components are probably secreted in large plasma membrane-derived EVs and/or apoptotic vesicles rather than exosomes. Abbreviations: ARF, ADP ribosylation factor; ESCRT, endosomal sorting complex required for transport; LAMP, lysosome-associated membrane protein; MHC, major histocompatibility complex; MFG8, milk fat globule-epidermal growth factor-factor VIII; RAB, Ras-related proteins in brain; TfR, transferrin receptor.



## Current knowledge on exosome biogenesis and release

Nina Pettersen Hessvik<sup>1,2</sup> · Alicia Llorente<sup>1,2</sup>

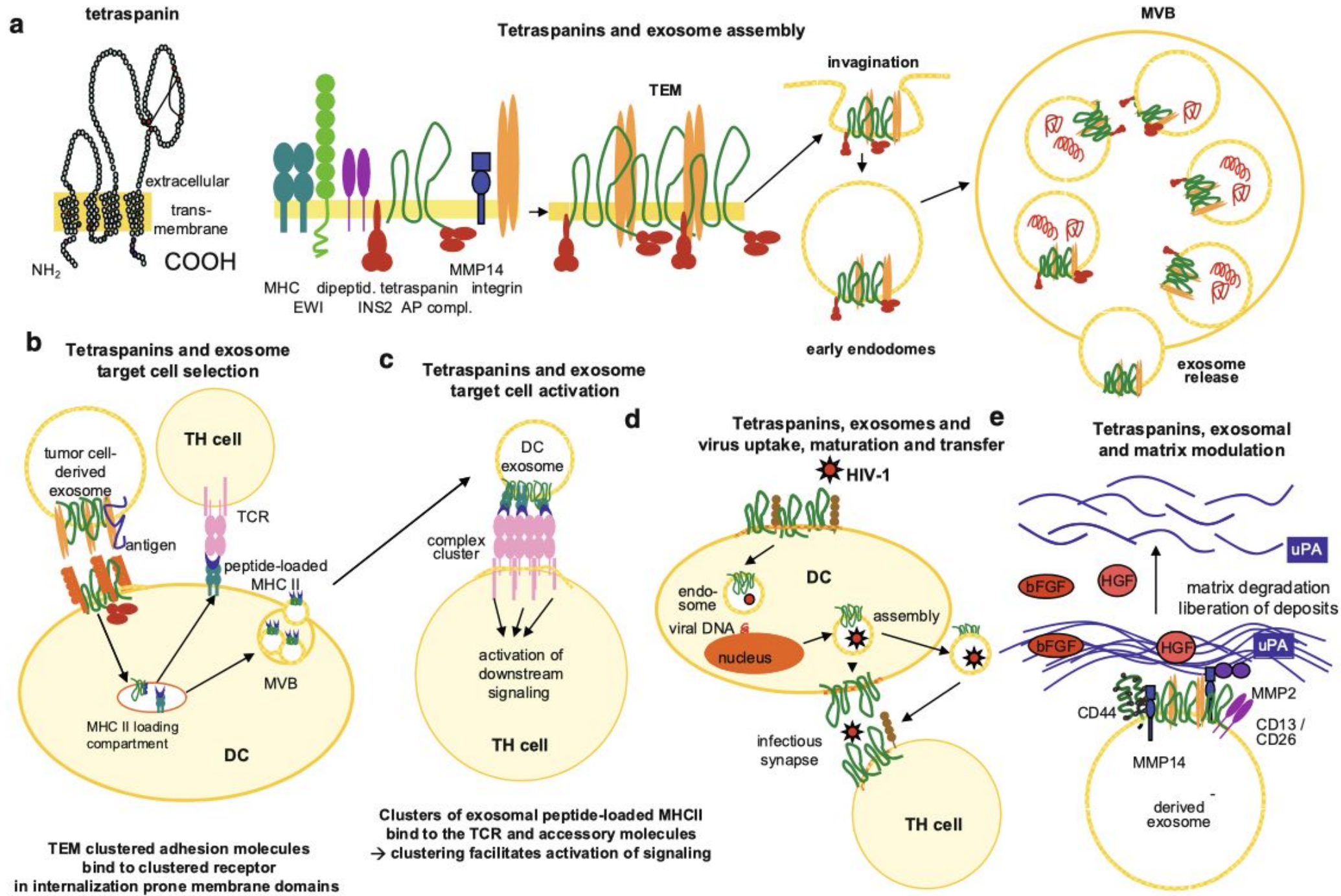
- Contains RNA and proteins, that is secreted into the extracellular space by exocytosis when multivesicular bodies fuse with the plasma membrane
- Consist of lipid bilayer

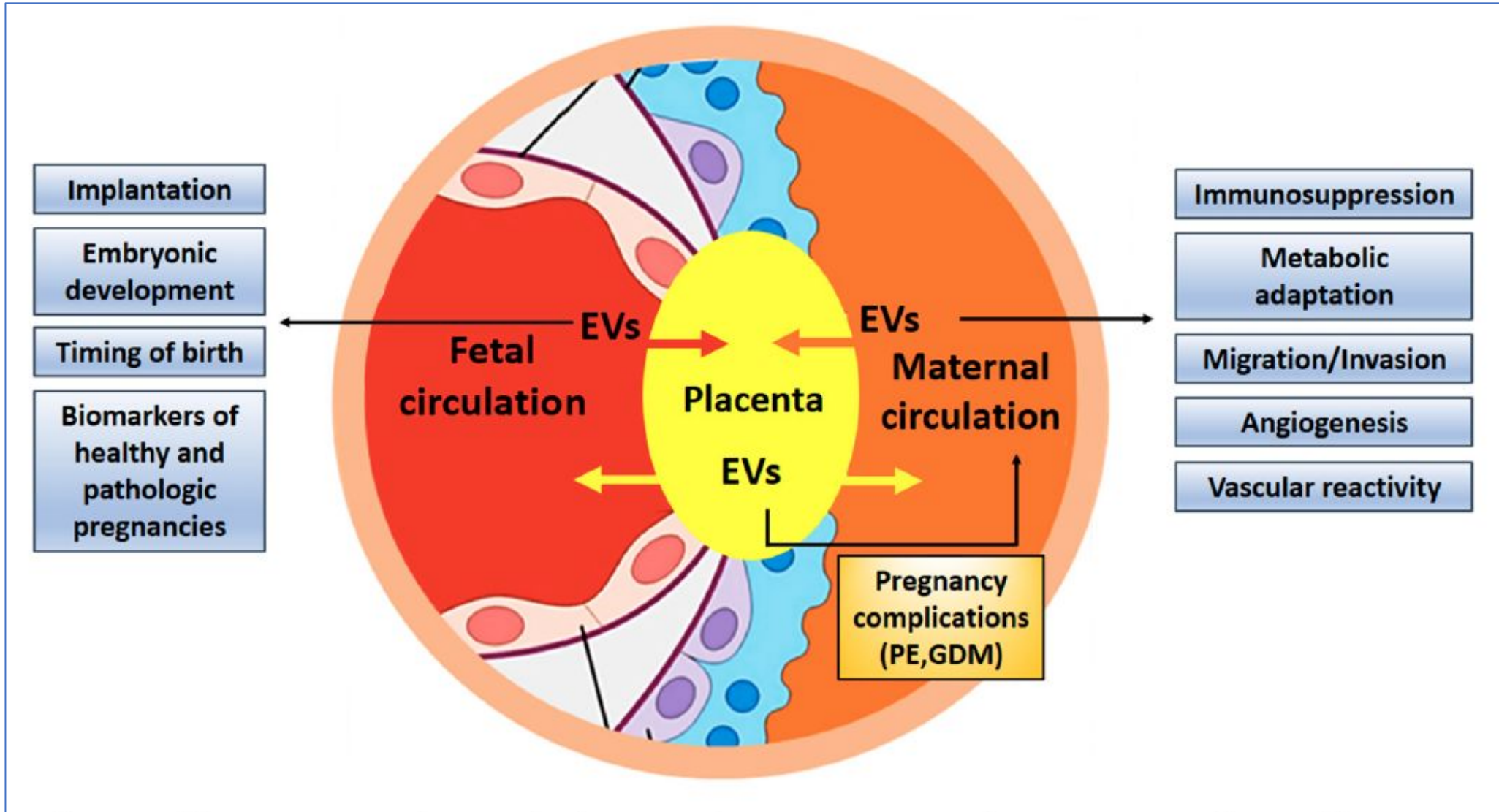


**Exosomes  
can  
regulate  
target cell  
activity via:**

- Alteration in translational activity
- Angiogenesis
- Proliferation
- Metabolism,
- Apoptosis

**Functioning as a  
mechanism for  
intercellular  
communication.**







Review

## Exosomes as Messengers between Mother and Fetus in Pregnancy

Liliana Czernek and Markus Döchler \*

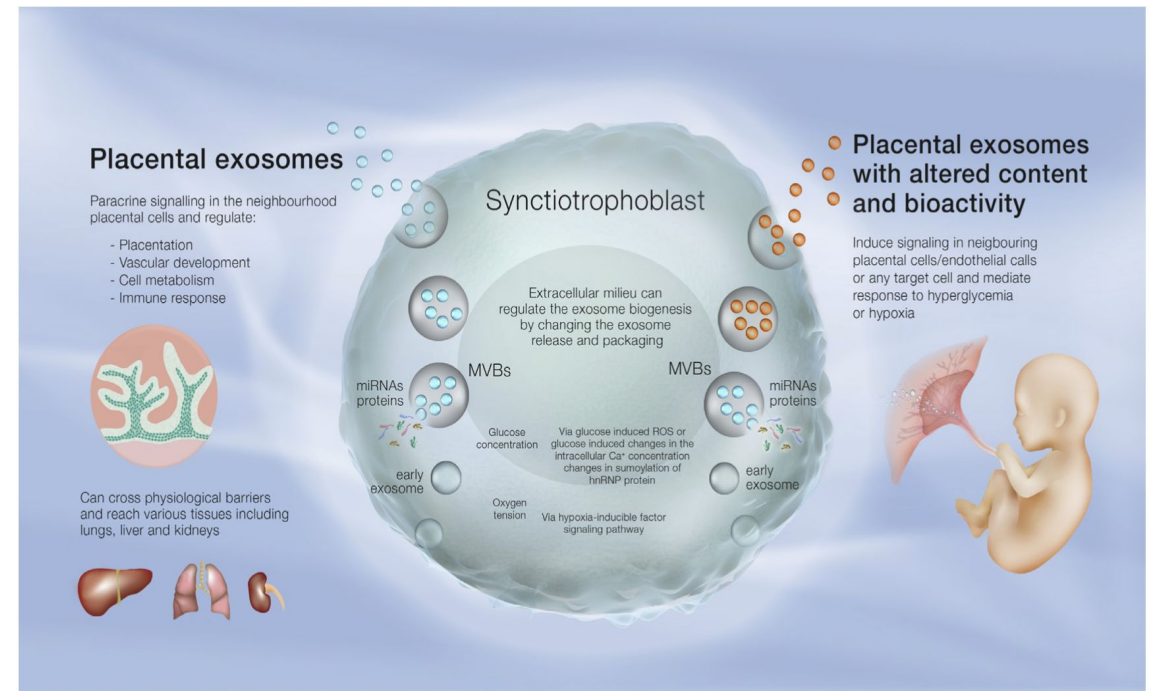
Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112, Sienkiewicza Street, 90-363 Lodz, Poland; lilianaczernek@gmail.com

\* Correspondence: mduchler@cbmm.lodz.pl; Tel.: +48-42-6803-220

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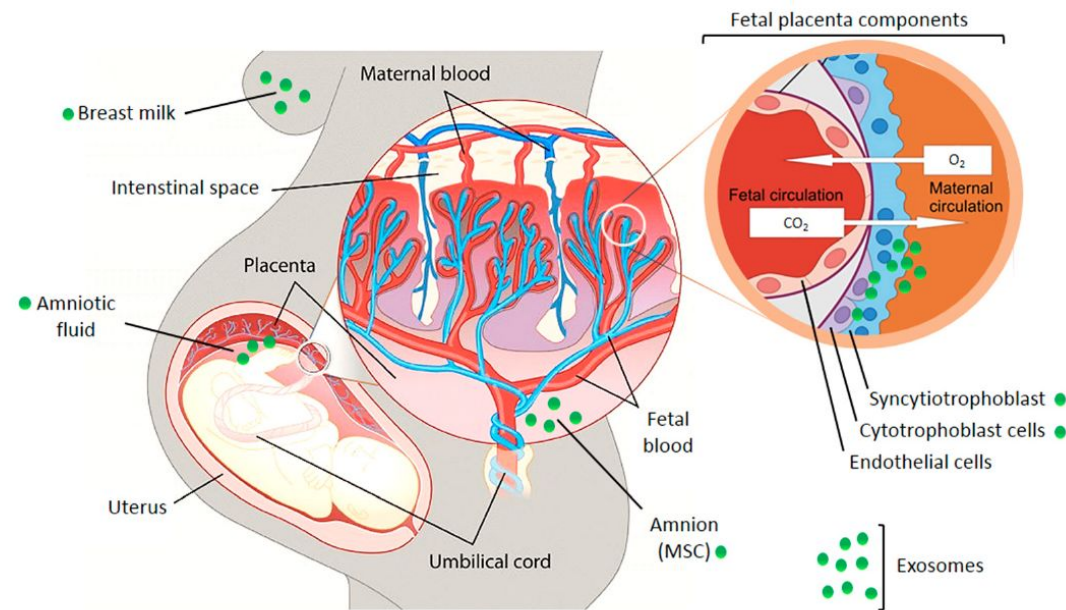
- Placenta secretes exosomes into maternal circulation and play important roles in several different aspects of pregnancy including fetomaternal signaling.
- Size 40-120 nm
- Released via exocytosis into maternal plasma
- Contain many signaling molecules including proteins, mRNA, microRNA, and noncoding RNAs
- Regulate target cell activity, angiogenesis, proliferation, metabolism, and apoptosis





# Placental exosome

- Can be detected in maternal plasma from as early as 6 weeks of gestation
- Number increases with advancing gestation
- Increases in number in complicated pregnancies
- Contain members of the chromosome 19 microRNA cluster



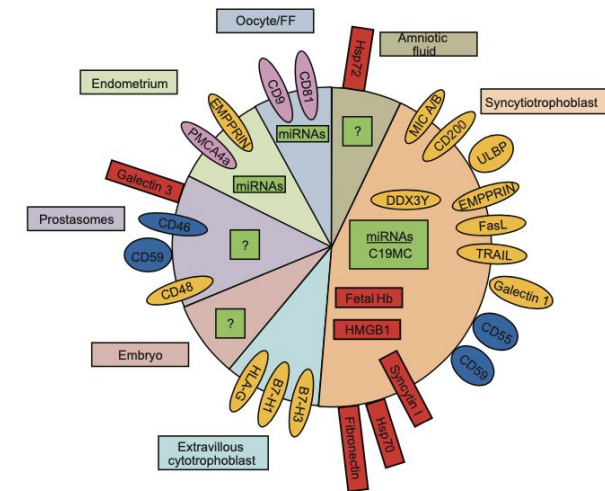
**Figure 2.** Illustration of the fetal placental barrier that separates fetal and maternal circulations in the human placenta. Places of exosome appearance are indicated. MSC—mesenchymal stem cells. The picture was composed using publicly available graphics from The Alcohol Pharmacology Education Partnership at the Duke University Medical Center [13] and from Christiane Albrecht, University of Bern, with her friendly permission [14].

# Oocyte extracellular vesicles

- Tetraspanin CD9 play an important role in gamete fusion
- Oocytes transfer CD9 to the sperm head via “exosome-like” vesicles
- CD9 associated EVs localized in perivitelline space of the oocyte

- Expressed on the surface of the oocyte and surrounding somatic cells
- Play role in a sperm-oocyte membrane fusion

- Expression of CD9 and CD81 are predominantly localized in the Perivitelline space and zona pellucida respectively.
- CD9 is primarily produced by the oocyte
- CD81 is produced in the surrounding cumulus cells

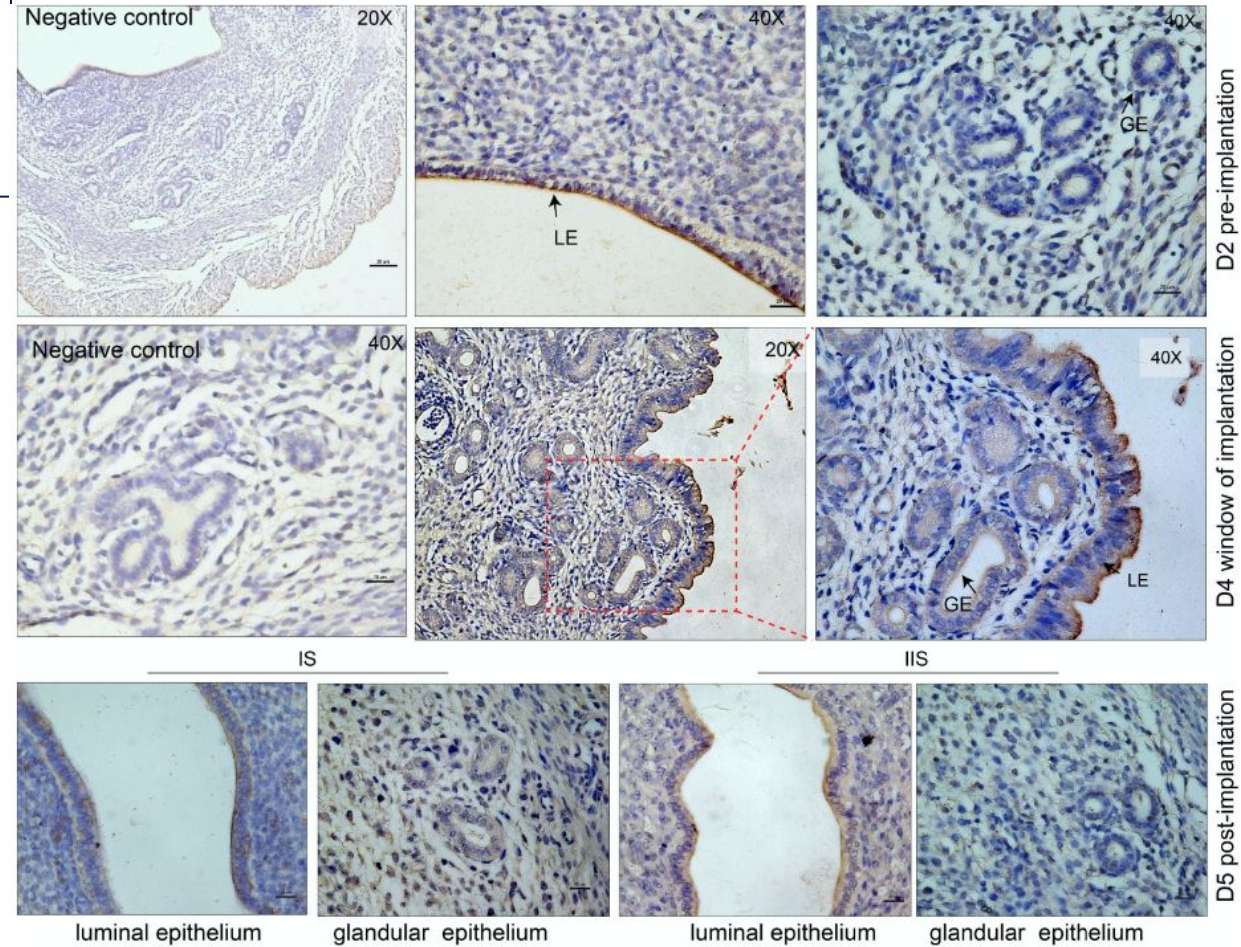


**Figure 3** Schematic diagram summarizing various functional moieties carried by reproductive EVs. Proinflammatory mediators (red fill) include HSP70, HSP72, HMGB1, fibronectin, fetal haemoglobin and Syncytin 1. Reproductive EVs have also been shown to contain immunoregulatory molecules (orange fill), with the potential to suppress NK and T cell responses, such as MIC A/B, EMPPRIN, CD200, minor histocompatibility antigen DDX3Y and galectin 1 and complement regulatory proteins (blue fill) CD46, CD55 and CD59. Finally, follicular fluid, endometrial and STB EVs contain miRNAs (green fill) with the potential to further modulate recipient cell responses. HMGB1, high mobility group box 1; MIC A/B, MHC class I chain-related proteins A and B; miRNA, micro-interfering RNA; MV, microvesicle; NK, natural killer; STB, syncytiotrophoblast.

Article  
**MicroRNAs in Small Extracellular Vesicles Indicate Successful Embryo Implantation during Early Pregnancy**

Qiang Tan <sup>1,†</sup>, Shuang Shi <sup>1,†</sup>, Jingjie Liang <sup>1</sup>, Xiaowei Zhang <sup>2</sup>, Dingren Cao <sup>1</sup> and Zhengguang Wang <sup>1,\*</sup>

Extracellular Vesicles are present and secreted by the endometrium



**Figure 2.** Immunohistochemical staining of CD63 in mouse glandular and luminal epithelium during pre-implantation (D2), window of implantation (WOI) (D4), post-implantation (D5) stage, and in the negative control (no primary antibody). LE: luminal epithelium. GE: glandular epithelium. IS: implantation site. IIS: inter implantation site.

## **Circulating Exosomal miRNA Profile During Term and Preterm Birth Pregnancies: A Longitudinal Study**

Ramkumar Menon,<sup>1\*</sup> Chirantan Debnath,<sup>2\*</sup> Andrew Lai,<sup>3</sup> Dominic Guanzon,<sup>3</sup> Shinjini Bhatnagar,<sup>2</sup> Pallavi K. Kshetrapal,<sup>2</sup> Samantha Sheller-Miller,<sup>1</sup> and Carlos Salomon,<sup>3,4</sup> The Garbhini Study Team

<sup>1</sup>Division of Maternal-Fetal Medicine and Perinatal Research, Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, Texas 77555; <sup>2</sup>Translational Health Science and Technology Institute, Faridabad, Haryana 121001, India; <sup>3</sup>Exosome Biology Laboratory, Centre for Clinical Diagnostics, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, University of Queensland, Brisbane, Queensland 4029, Australia; and <sup>4</sup>Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy, University of Concepción, Concepción, Chile

**ORCID numbers:** [0000-0001-9213-6105](https://orcid.org/0000-0001-9213-6105) (R. Menon); [0000-0002-3991-1747](https://orcid.org/0000-0002-3991-1747) (S. Sheller-Miller).



Exosomal miRNAs in pre-term birth play role in cell-to-cell signaling and interaction, cellular growth and proliferation, and cellular development.

The data suggest that the miRNA content of circulating exosomes in maternal blood might represent a biomolecular “fingerprint” of the progression of pregnancy.

# The correlation between unexplained infertility and exosomes

Yıldız Uyar<sup>1</sup>, Melike Özgül<sup>1</sup>, Sevtap Gökalp<sup>1</sup>, Gülay Ok<sup>1</sup>, Ayça Tan<sup>1</sup>, Hafize Seda Vatansever<sup>1</sup>

Manisa Celal Bayar University Medical Faculty, Manisa, Turkey

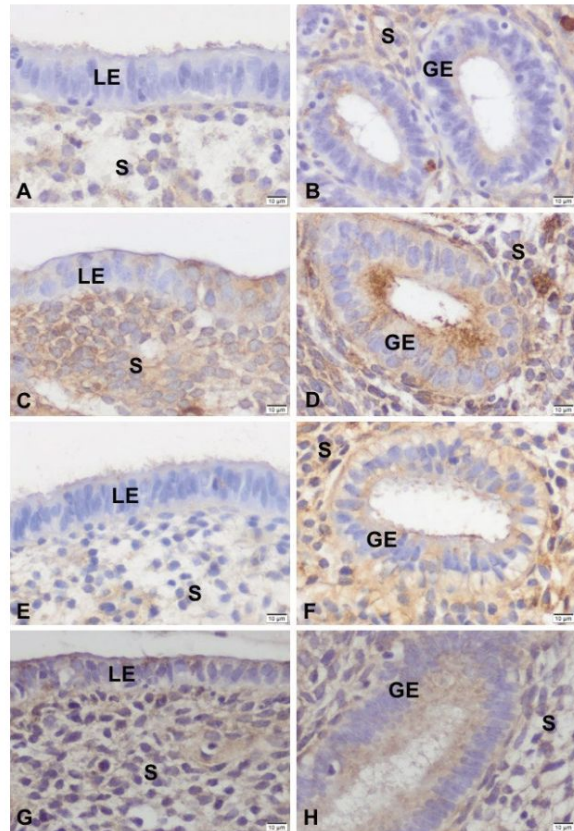


Figure 2. Immunohistochemical distribution of CD63. Scale Bars: 10 µm

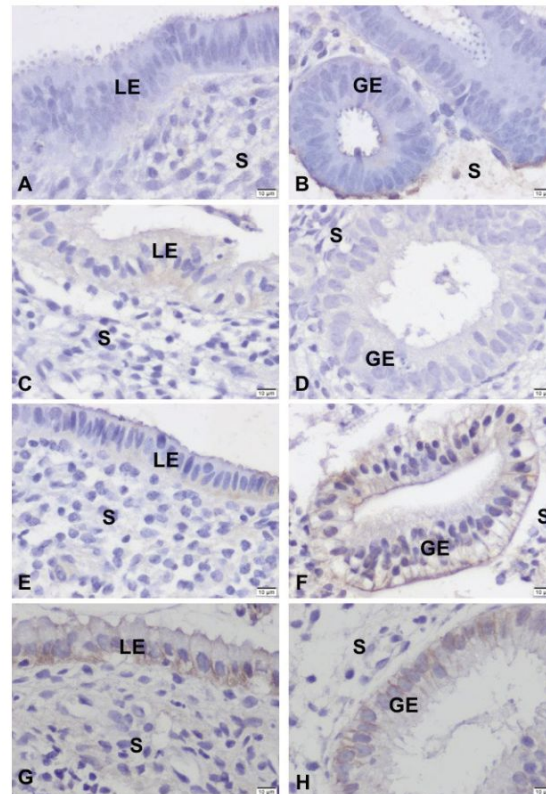


Figure 4. Immunohistochemical distribution of CD9. Scale Bars: 10 µm

Table 1. p values of statistical comparisons of CD63 and CD9 HSCORE results

CD63	
Compared Groups	p Value
Fertile proliferation vs infertile proliferation (luminal epithelium)	p = 0.009
Fertile proliferation vs infertile proliferation (glandular epithelium)	p = 0.008
Fertile secretion vs infertile secretion (luminal epithelium)	p = 0.034
Fertile secretion vs infertile secretion (glandular epithelium)	p = 0.037
CD9	
Compared Groups	p Value
Fertile proliferation vs infertile proliferation (luminal epithelium)	p = 0.025
Fertile proliferation vs infertile proliferation (glandular epithelium)	p = 0.005
Fertile secretion vs infertile secretion (luminal epithelium)	p = 0.037
Fertile secretion vs infertile secretion (glandular epithelium)	p = 0.037

# Pregnancy loss



## Pregnancy loss (miscarriage): Risk factors, etiology, clinical manifestations, and diagnostic evaluation

**Authors:** Sarah Prager, MD, MAS, Elizabeth Micks, MD, MPH, Vanessa K Dalton, MD, MPH

**Section Editors:** Robert L Barbieri, MD, Courtney A Schreiber, MD, MPH

**Deputy Editor:** Kristen Eckler, MD, FACOG

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Literature review current through: **Nov 2020**. | This topic last updated: **Jun 29, 2020**.

Pregnancy loss, also referred to as miscarriage or spontaneous abortion, is generally defined as a nonviable intrauterine pregnancy up to 20 weeks gestation.

The major cause of SA is fetal chromosomal abnormalities, contributing to about 50-60% of the cases

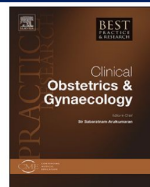


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2

### The biological basis and prevention of preterm birth

Gian Carlo Di Renzo\*, Valentina Tosto, Irene Giardina

*Department of Obstetrics and Gynecology, Centre for Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy*



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### Spontaneous miscarriage in first trimester pregnancy is associated with altered urinary metabolite profile

Chee Wai Ku<sup>a,\*,1</sup>, Zhen Wei Tan<sup>b,\*,1</sup>, Mark Kit Lim<sup>c</sup>, Zhi Yang Tam<sup>d</sup>, Chih-Hsien Lin<sup>d</sup>, Sean Pin Ng<sup>d</sup>, John Carson Allen<sup>e</sup>, Sze Min Lek<sup>a</sup>, Thiam Chye Tan<sup>a</sup>, Nguan Soon Tan<sup>a,b,c,f,\*\*</sup>

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PLOS ONE

## Cell-Free Fetal DNA and Cell-Free Total DNA Levels in Spontaneous Abortion with Fetal Chromosomal Aneuploidy

Ji Hyae Lim<sup>1</sup>, Min Hyoung Kim<sup>2</sup>, You Jung Han<sup>2</sup>, Da Eun Lee<sup>1</sup>, So Yeon Park<sup>1</sup>, Jung Yeol Han<sup>2</sup>, Moon Young Kim<sup>2</sup>, Hyun Mee Ryu<sup>1,2\*</sup>

<sup>1</sup>Laboratory of Medical Genetics, Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Seoul, Korea, <sup>2</sup>Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, KwanDong University College of Medicine, Seoul, Korea

RESEARCH ARTICLE

## New evidence that a large proportion of human blood plasma cell-free DNA is localized in exosomes

M. Rohan Fernando<sup>1,2\*</sup>, Chao Jiang<sup>1</sup>, Gary D. Krzyzanowski<sup>1,2</sup>, Wayne L. Ryan<sup>2</sup>

<sup>1</sup> Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE, United States of America, <sup>2</sup> Department of Research and Development, CFGenome®, Omaha, NE, United States of America

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Ji Hyae Lim<sup>1</sup>, Min Hyoung Kim<sup>2</sup>, You Jung Han<sup>2</sup>, Da Eun Lee<sup>1</sup>, So Yeon Park<sup>1</sup>, Jung Yeol Han<sup>2</sup>, Moon Young Kim<sup>2</sup>, Hyun Mee Ryu<sup>1,2\*</sup>

<sup>1</sup>Laboratory of Medical Genetics, Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Seoul, Korea, <sup>2</sup>Department of Obstetrics and

- Fetal cfDNA is produced
- by the apoptosis of placental cells of the trophoblast.
- Produced during normal pregnancies; however, the process of apoptosis is likely increased during complicated pregnancies, due to increased oxidative stress and inflammatory response

Noninvasive prenatal testing for chromosomal abnormalities can be performed using only a blood sample collected from the mother, which brings about new possibilities for prenatal diagnostics. Such a new technique is beneficial also when the increased concentrations of cell-free fetal DNA are detected in the circulation of mothers with various pregnancy complications





# Take home message:

**TABLE 1** Biogenesis, characteristics and functions of different sub-populations of EVs

Types of EV	Mechanism of biogenesis	Size	Proteins present <sup>a</sup>	Cargo	Method of isolation <sup>b</sup>	Reported functions
Exosomes	Correspond to the ILVs formed inside the late endosomes. Released by fusion of MVBs with the plasma membrane. Proteins belonging to the ESCRT and ESCRT-independent mechanisms (lipids and tetraspanins), Rab GTPases and SNARE are involved in the biogenesis [12]	Present a diameter of 50–150 nm, similar to the diameter of ILVs on electron microscopy [13]	Tetraspanins (CD81, CD63, CD9), ESCRT components, Alix, TSG101, syntenins, flotillins, integrins, disintegrin and metalloproteinase domain-containing protein 10/ADAM10/ADA10, HSC70/HSPA8/HSPC7 [13–15]	Proteins, miRNAs, mRNAs, lncRNAs, small fragments of DNA Devoid of nuclear, mitochondrial, endoplasmic reticulum and golgi complex proteins [13, 14]	Differential centrifugation and ultracentrifugation at 100,000 g for 90 m, flotation in density gradient (sucrose or iodixanol), ultrafiltration, size exclusion chromatography, immunoisolation using antibodies and commercial kits (using precipitation) [16, 17]	Cell to cell communication by transfer of molecular cargo to recipient cells [18, 19]  Immunoregulation [20, 21]  Tumor progression and metastasis [22, 23]

Soumyalekshmi Nair<sup>a</sup>, Carlos Salomon<sup>a,b,c</sup>

<sup>a</sup>Exosome Biology Laboratory, Centre for Clinical Diagnostics, UQ centre for Clinical Research, Royal Brisbane and Women's Hospital, The University of Queensland, St Lucia, QLD, Australia,

<sup>b</sup>Department of Clinical Biochemistry and Immunology, University of Concepción, Concepción,

Chile, <sup>c</sup>Department of Obstetrics and Gynecology, Ochsner Baptist Hospital, New Orleans, LA, United States

# Thank you

## Any questions?



Ailar Assambayeva  
[Ailar.lauren@gmail.com](mailto:Ailar.lauren@gmail.com)  
89857812427