Instituto de Investigación en Recursos Cinegéticos
CSIC - UCLM - JCCM
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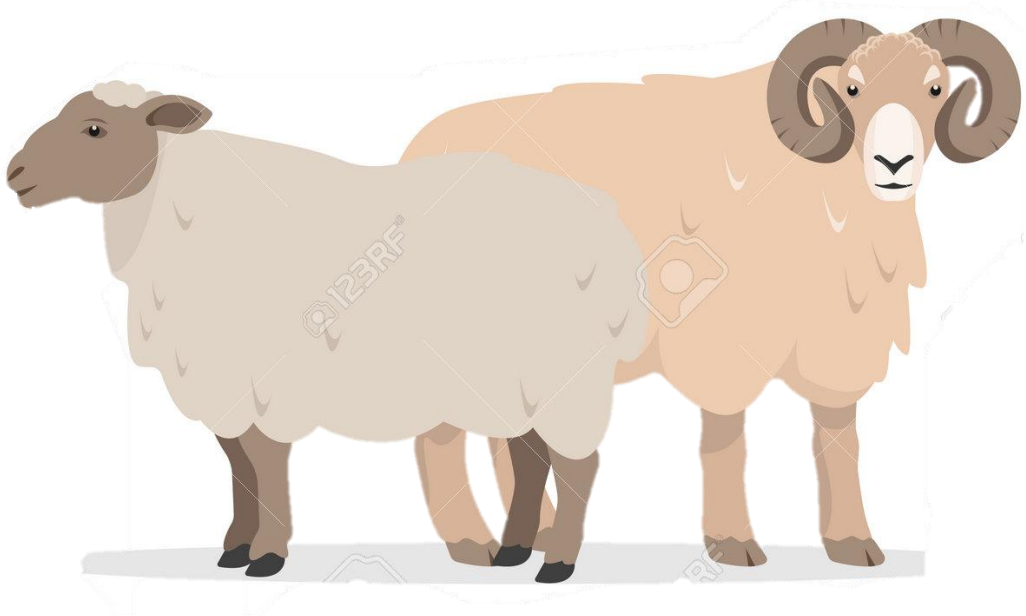
Sanidad y Biotecnología
Health and Biotechnology

UCLM Universidad de Castilla-La Mancha

INTERACTION OF FEMALE REPRODUCTIVE FLUIDS WITH SPERM IN SHEEP



The4th CZU Prague hybrid seminar
Biotechnology in small ruminant reproduction: an international experience - 2025

Faculty of Forestry and Wood Sciences

avantor delivered by VWR

Carmen María Picazo Córdoba, PhD student
University of Castilla-La Mancha, Albacete, Spain
carmenmaria.picazo@uclm.es

1. **BACKGROUND**
2. **EVs ISOLATION**
3. **EVs CHARACTERIZATION**
4. **EVs – SPERM INTERACTION**
5. **CONCLUSIONS**
6. **BIBLIOGRAPHY**

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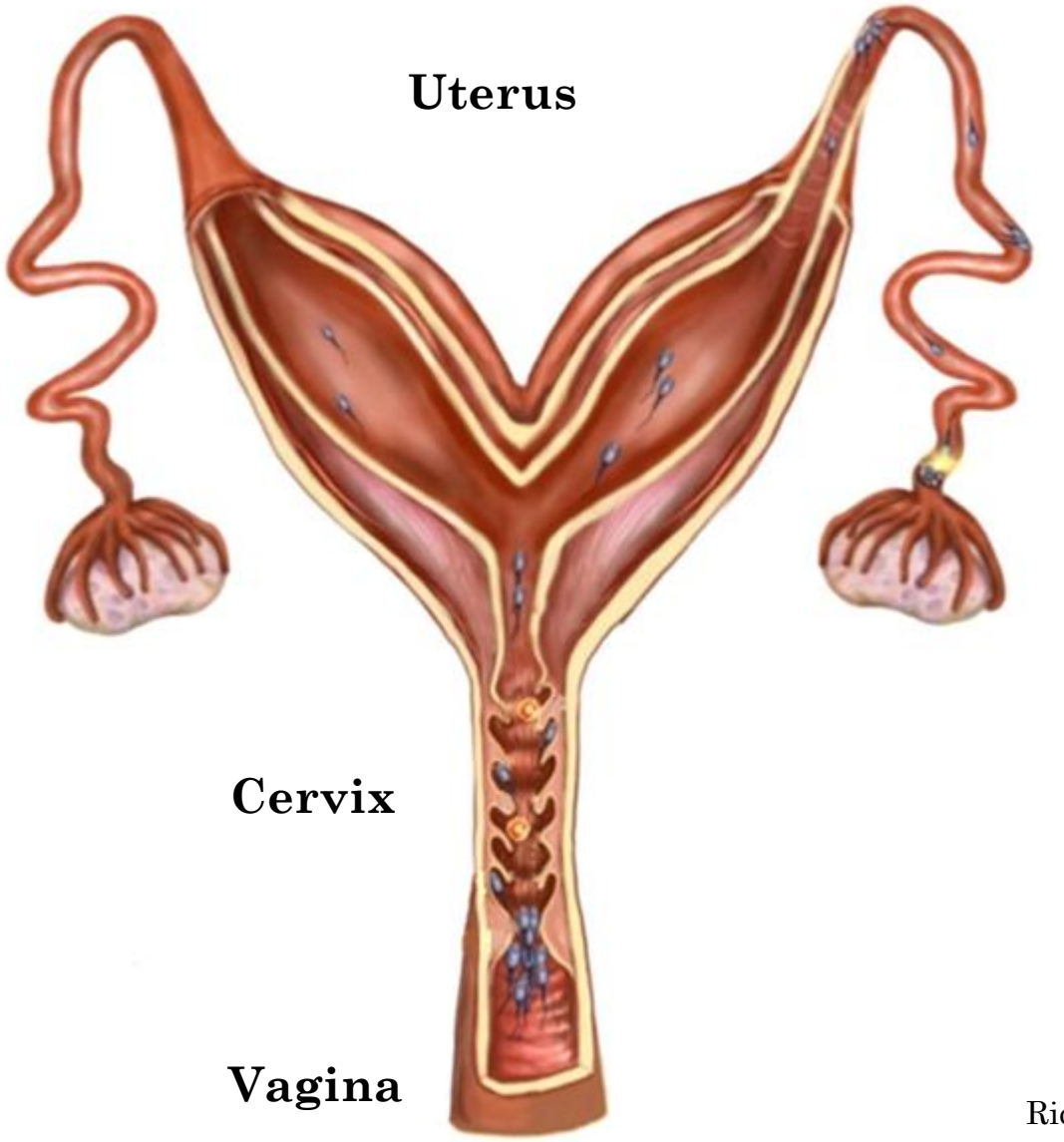
Natural breeding

Uterine depositors

Vaginal depositors



Rickard et al., 2019

THE ESTROUS SHEEP SERUM (ESS) CHALLENGE:

Can we ever do without it?

FIV MEDIUM FOR SMALL RUMINANTS: Synthetic oviductal fluid supplemented with 2% ESS

ESS IS IRREPLACEABLE

Required to induce in vitro capacitation of spermatozoa during *in vitro* fertilization.

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of different media additives on capacitation of frozen–thawed ram spermatozoa as a potential replacement for estrous sheep serum

O. García-Álvarez [a], A. Maroto-Morales [a], P. Jiménez-Rabadán [b], M. Ramón [b], E. del Olmo [a], M. Iniesta-Cuerda [a], L. Anel-López [a], M.R. Fernández-Santos [a], J.J. Garde [a], A.J. Soler [a,*]

Received: 21 March 2022 | Accepted: 14 May 2022

DOI: 10.1111/rda.14161

SHORT COMMUNICATION

Reproduction in Domestic Animals WILEY

Serum supplementation during in vitro fertilization of sheep oocytes influences blastocyst quality through the differential abundance of mRNA transcripts

Irene Sánchez-Ajofrín[1,2] | Patricia Peris-Frau[2,3] | Olga García-Álvarez[2] | María del Rocío Fernández-Santos[2] | Vidal Montoro[2] | José Julián Garde[2] | Ana Josefa Soler[2]

THE ESTROUS SHEEP SERUM (ESS) CHALLENGE:

Can we ever do without it?

FIV MEDIUM FOR SMALL RUMINANTS: Synthetic oviductal fluid supplemented with 2% ESS

ESS IS IRREPLACEABLE	Required to induce *in vitro* capacitation of spermatozoa during in vitro fertilization.

✨*All that glitters is not gold*✨

DRAWBACKS:

-Requires extraction and purification from blood simples (García-Álvarez *et al.*, 2015)

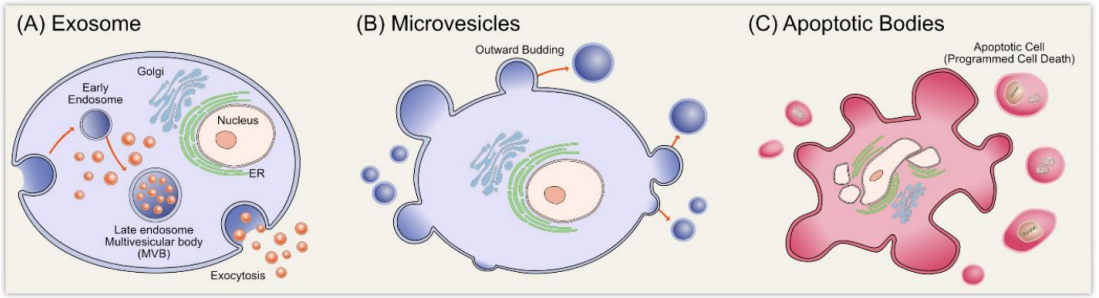
-Its composition can be variable from batch to batch: complicated standarization. (García-Álvarez *et al.*, 2015)

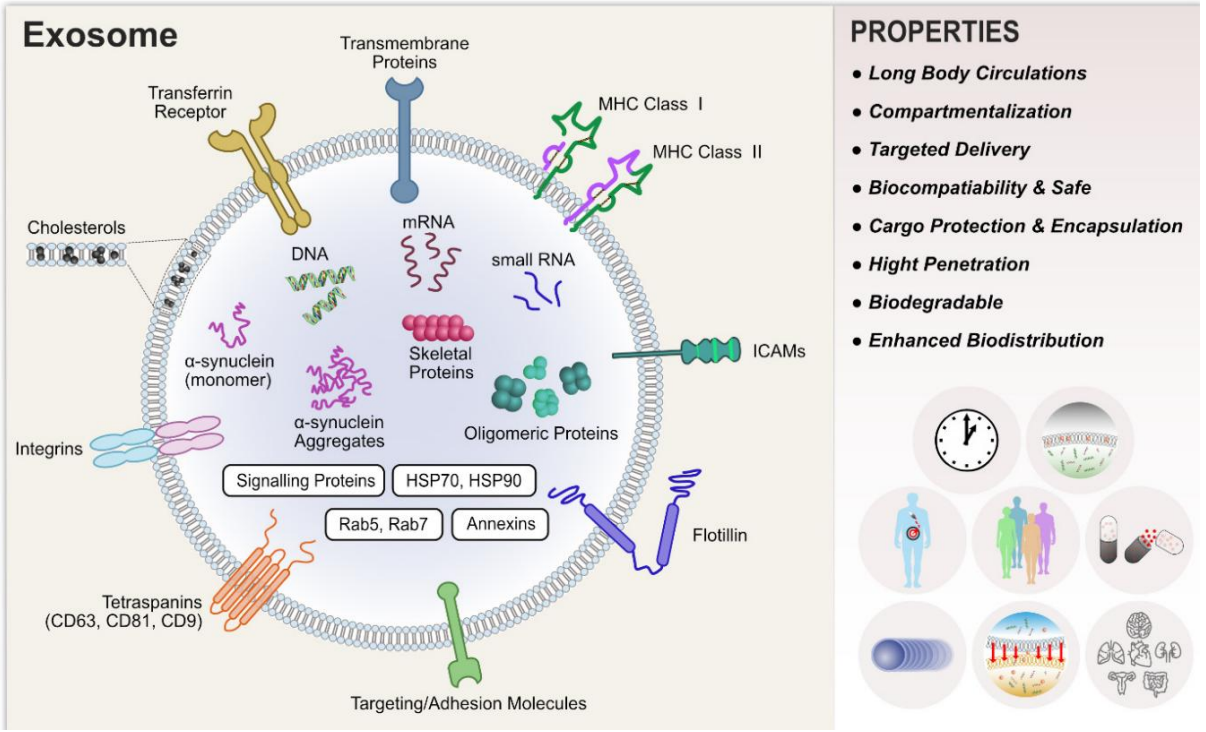
-Alterations in the embryo gene expression and cryotolerance (Rizos *et al.*, 2003)

-Developmental anormalities (Young *et al.*, 2001)

AND ONE MORE ATTEMPT: EXTRACELLULAR VESICLES (EVs) SYSTEM IN IVF

EVs: nanoparticles involved in cellular communication releasing miRNAs, proteins and lipids into target cells modulating cellular response, and are present in biological fluids.

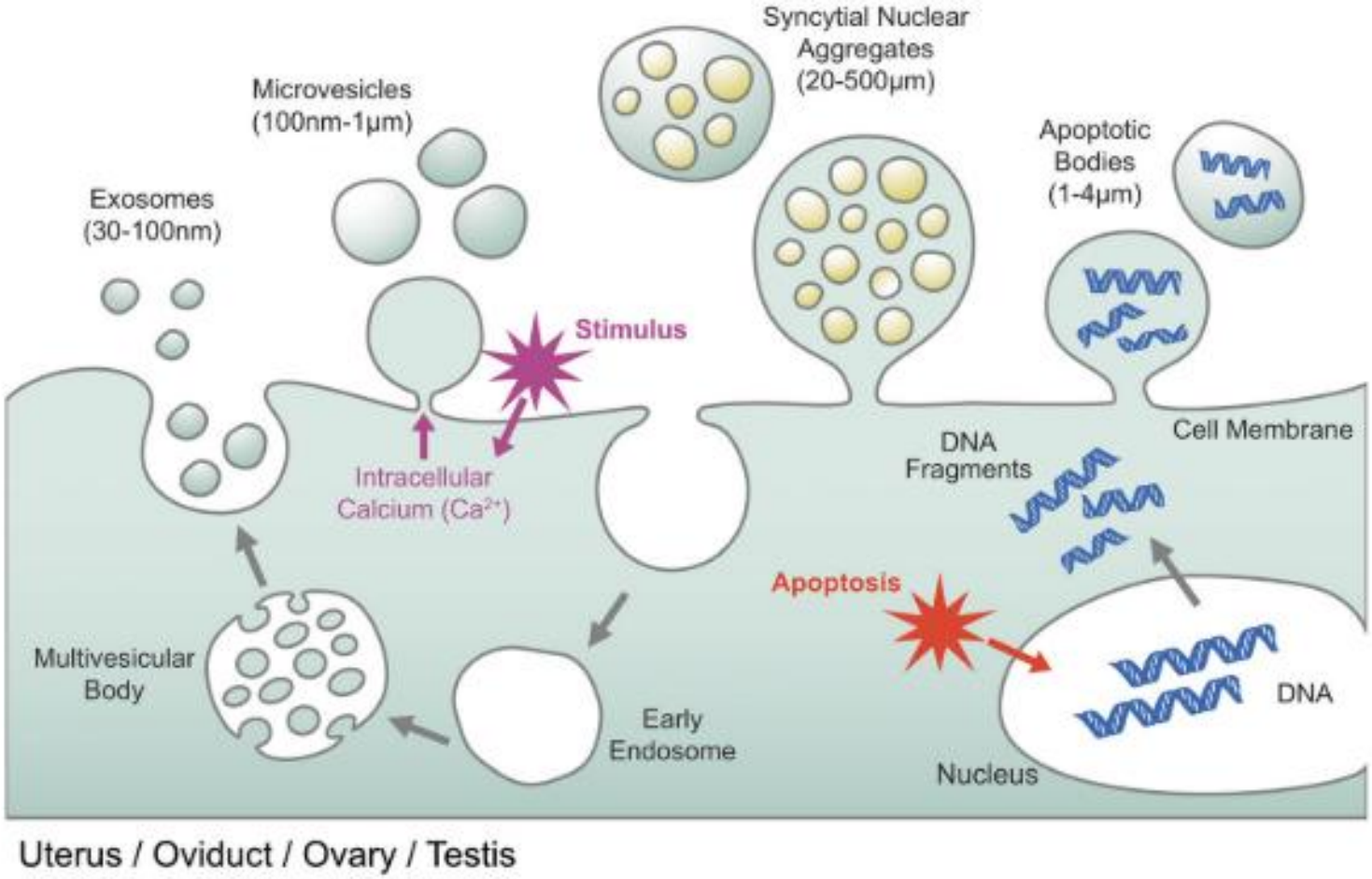




Gurunathan *et al.*, 2019

AND ONE MORE ATTEMPT: EXTRACELLULAR VESICLES (EVs) SYSTEM IN FIV

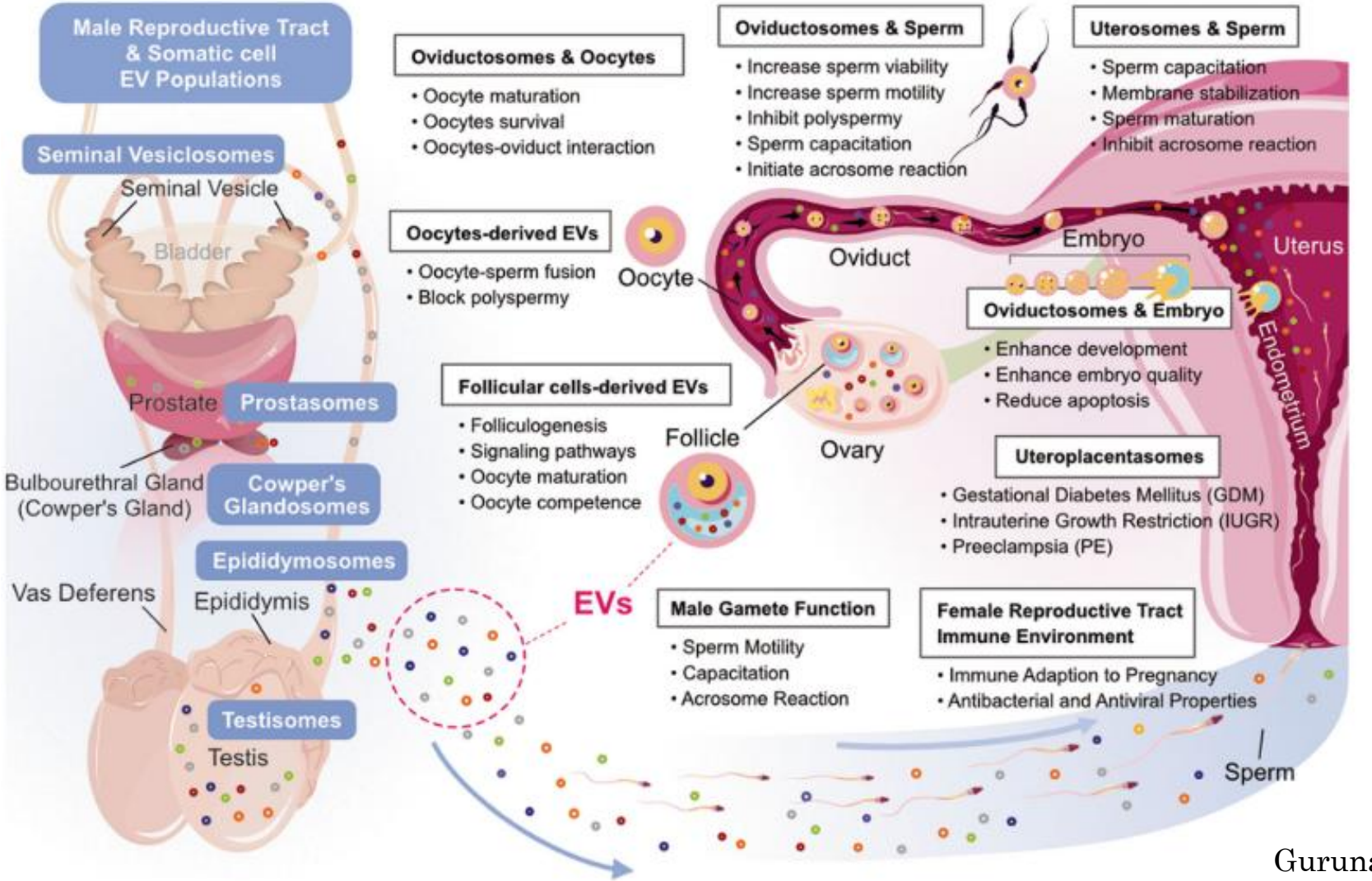
Biogenesis of extracellular vesicles in male and female reproductive systems



Gurunathan *et al.*, 2022

AND ONE MORE ATTEMPT: EXTRACELLULAR VESICLES (EVs) SYSTEM IN FIV

Multifunctional roles of EVs in male and female reproduction organs.



Gurunathan *et al.*, 2022

EVs IN REPRODUCTION

Importance of EVs of reproductive fluids

OPEN ACCESS Freely available online

PLOS ONE

Extracellular Vesicles in Luminal Fluid of the Ovine Uterus

Gregory Burns[1], Kelsey Brooks[1], Mark Wildung[2], Raphatphorn Navakanitworakul[3], Lane K. Christenson[3], Thomas E. Spencer[1]*

SCIENTIFIC REPORTS

OPEN

Characterization and Small RNA Content of Extracellular Vesicles in Follicular Fluid of Developing Bovine Antral Follicles

Received: 12 November 2015
Accepted: 18 April 2016
Published: 09 May 2016

Raphatphorn Navakanitworakul[1,2,3,*], Wei-Ting Hung[1,*], Sumedha Gunewardena[1,4], John S. Davis[5], Wilaiwan Chotigeat[2,6] & Lane K. Christenson[1]

Animal Reproduction

THEMATIC SECTION: VIII INTERNATIONAL SYMPOSIUM ON ANIMAL BIOLOGY OF REPRODUCTION (ISABR 2020/2021)

The role of the oviduct and extracellular vesicles during early embryo development in bovine

Natália Marins Bastos[1], Juliana Germano Ferst[1], Rodrigo Silva Goulart[2], Juliano Coelho da Silveira[1]*

[1]Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil
[2]Departamento de Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil

1. BACKGROUND
2. **EVs ISOLATION**
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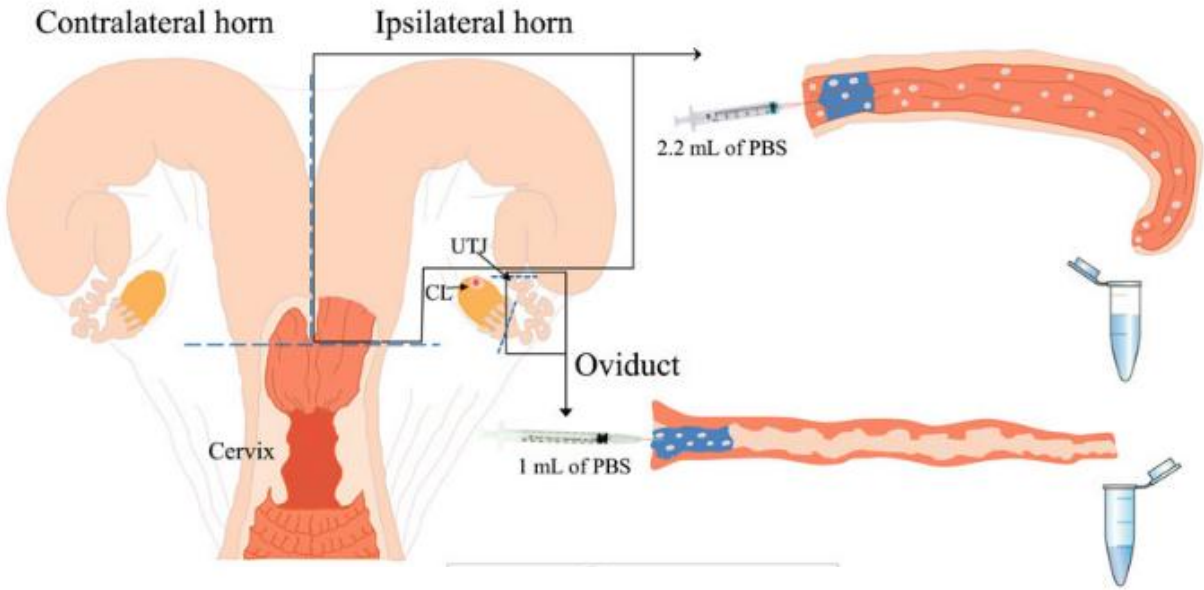
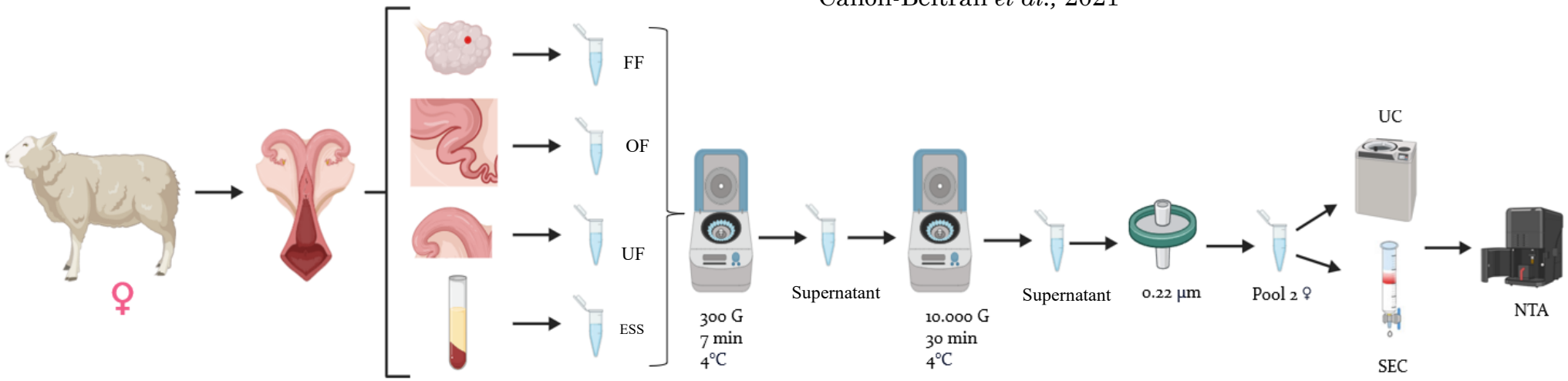
1. FLUID EXTRACTION

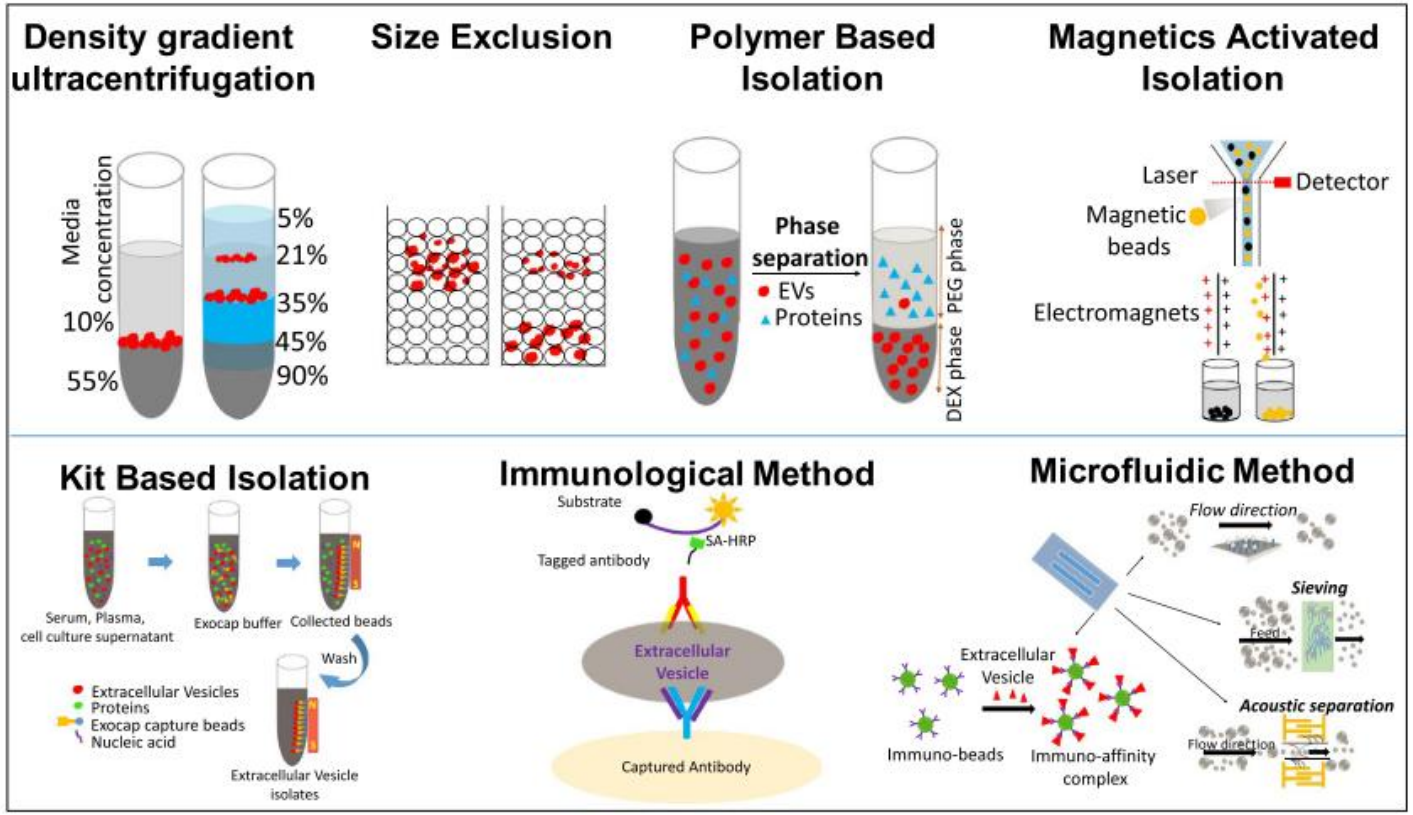
Preovulatory follicle



Cañón-Beltrán *et al.*, 2021



2. EV ISOLATION

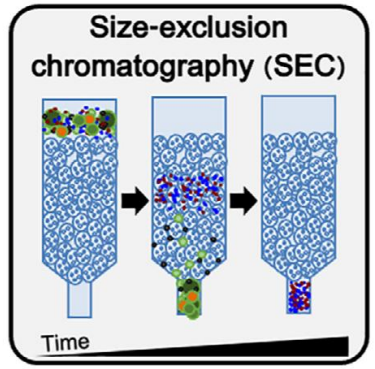


Tiwari et al., 2021

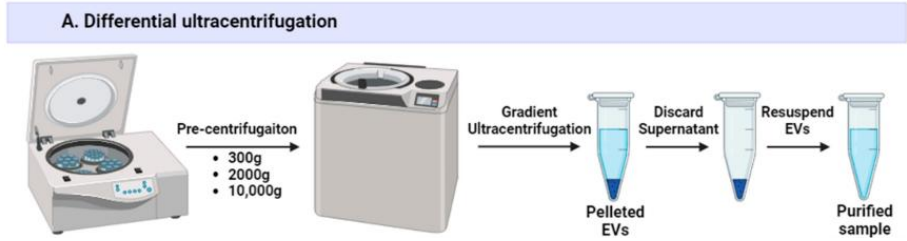
POSITION PAPER

ISEV

Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches



Welsh et al., 2024



Brezgin et al., 2024

2. EV ISOLATION

Table 1. Comparison of different EV isolation approaches.

Method	Principle	Scalability	Yield (Recovery)	EV Damage	Purity	Equipment Requirement	Cost	Additional Pre/Post-Steps	Time	Ref.
Differential ultracentrifugation (dUC)	Serial UC steps	+	+ (5–30%)	↑↑↑	++	+++	+	No	↑↑↑	[22,24–29]
Density gradient ultracentrifugation (DGU)	Separation of EVs by density using gradient medium	+	+ (5–30%)	↑	++++	+++	++	Yes (media removal)	↑↑↑	[12,22,31]
Ultrafiltration (UF)	Filtration through semi-permeable membranes	+++	+++ (30–80%)	↑↑	+++	++	++	No	↑/↑↑	[12,22,32–36]
Asymmetric depth filtration (DF)	Filtration through porous medium	++	++ (40–60%)	↑	+++	++	++	No	↑↑	[37]
Tangential flow filtration (TFF)	Cross-flow filtration through membranes	++++	+++ (up to 90%)	↑	+++	++	++	No	↑↑	[12,18,38–41]
Precipitation approaches	EV sedimentation using polymers	+++	++++ (up to 90–95%)	↑↑/↑↑↑	+	+	++	Yes (polymer removal)	↑↑↑	[12,18,48–50]
Affinity-based isolation	EV capture via specific interactions with EV markers	+/++	++ (50–70%)	↑/↑↑	++++	++	+++	No	↑↑	[35,52–55,57]
Size exclusion chromatography (SEC)	Separation by size through a bead-filled column	+++ (combined with UF/TFF)	++ (40–75%)	↑	+++	++	+	Yes (pre-Concentration)	↑↑	[43–47]
Multimodal flowthrough chromatography (MFC)	Combination of size-exclusion and bind–elute chromatography	+++ (combined with pre-concentration)	++/+++ (up to 80%)	↑	++++	++	++	Yes (pre-Concentration)	↑↑	[59]
Anion-exchange chromatography (AIEX)	Binding of EVs to positively charged column	+++	+++ (40–90%)	↑↑	++	++	++	Yes (buffer exchange)	↑↑↑	[43,61–63]

The symbols "+" or "↑" represent the lowest quality, while "++++" or "↑↑↑↑" indicate the highest quality.

Brezgin et al., 2024

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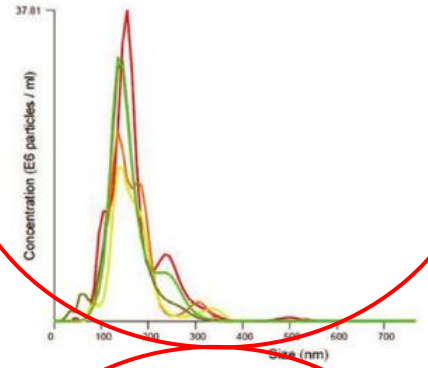
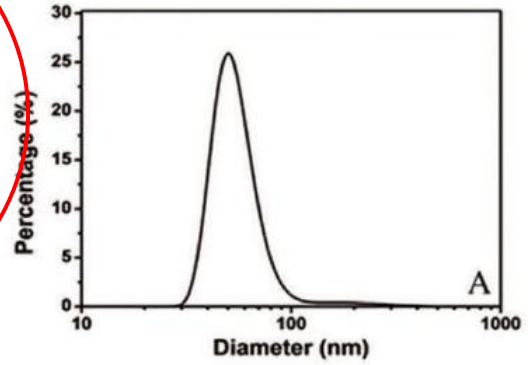
EVs CHARACTERIZATION

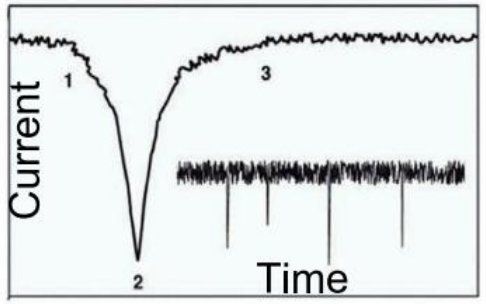
Exosome tracking



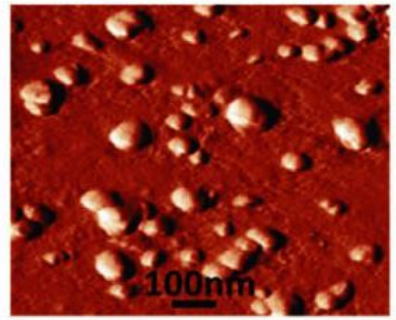
Dynamic scattering



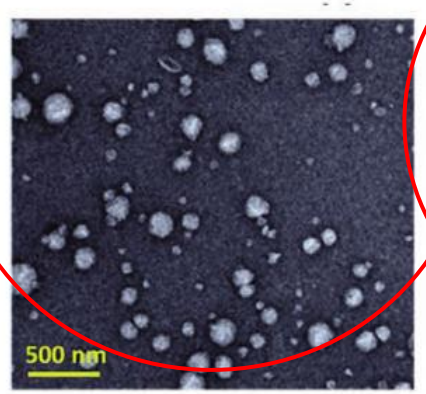
Resistive pulse analysis



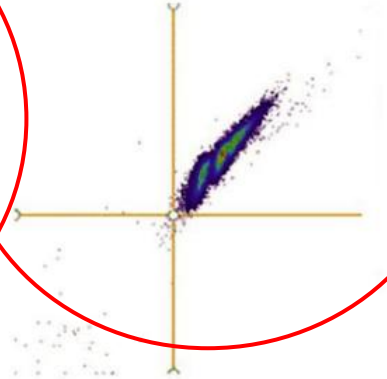
Atomic force microscopy



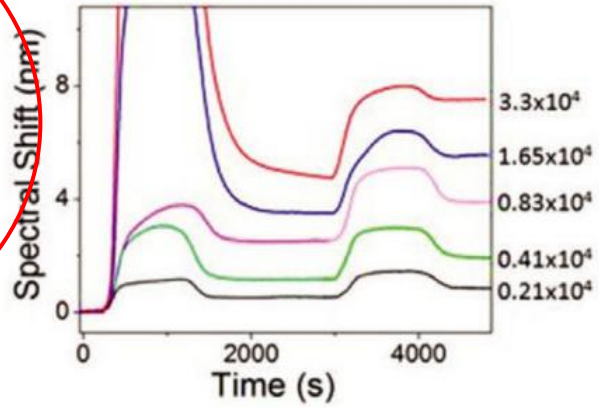
Electron microscopy



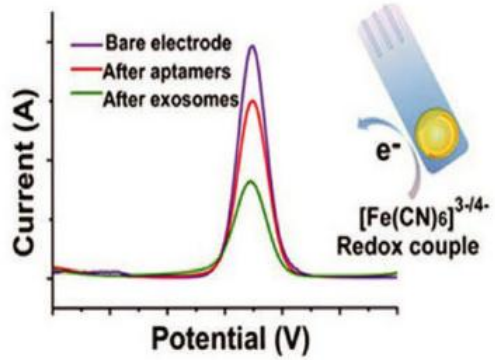
Flow cytometry



Surface plasmon resonance



Electrochemical method



Tiwari et al., 2021

EVs CHARACTERIZATION

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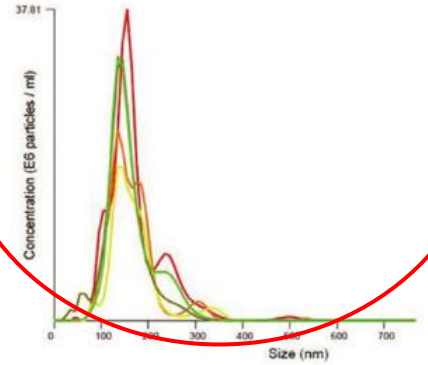

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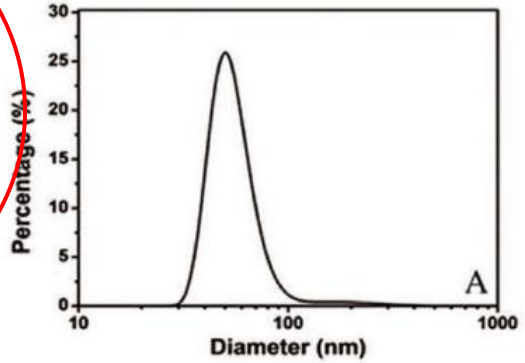

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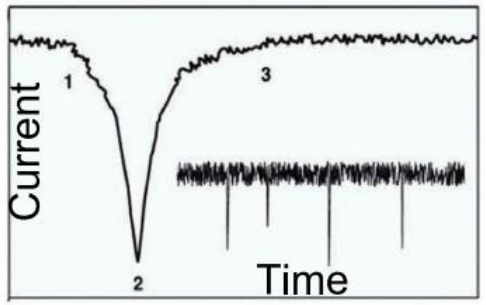

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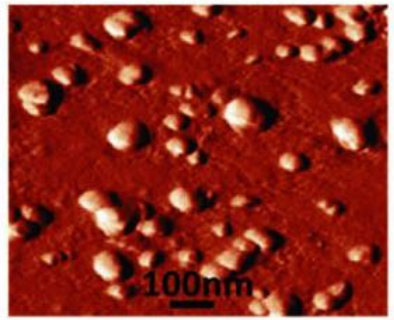

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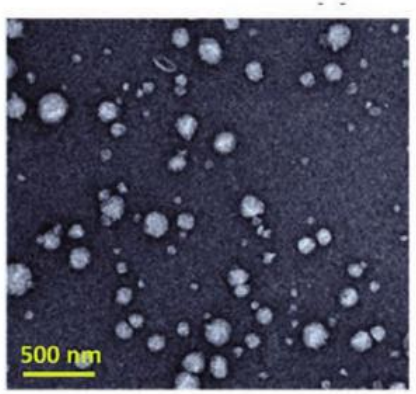

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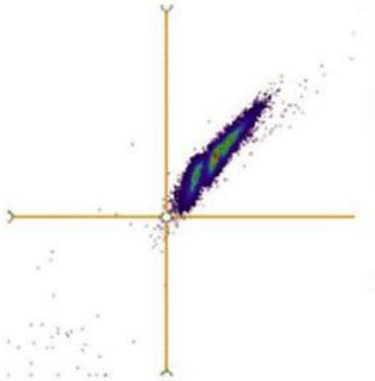

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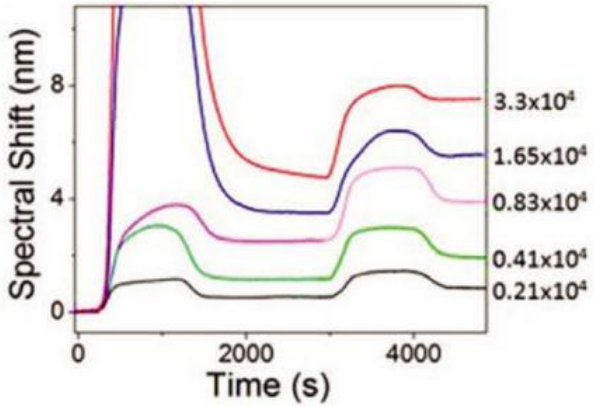

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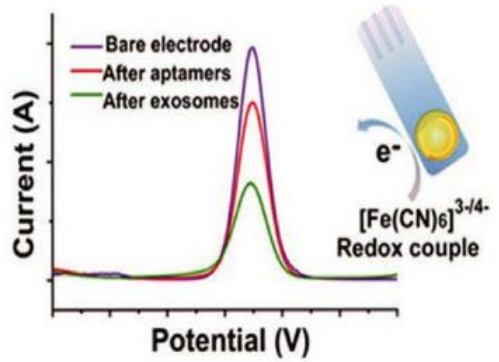
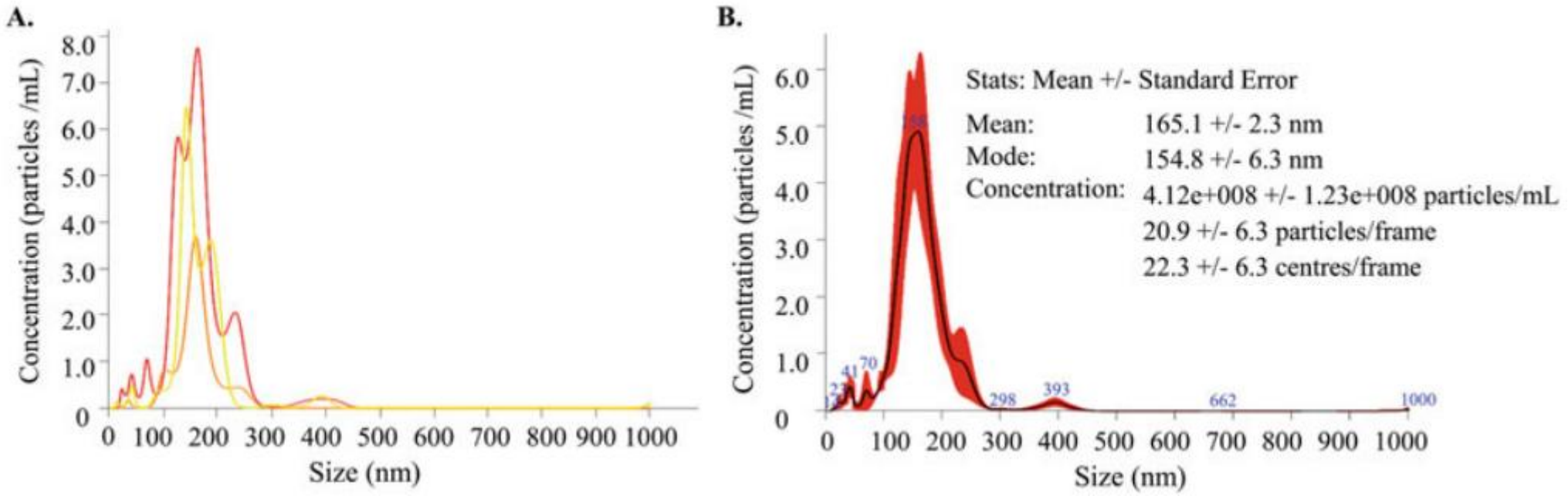


Tiwari et al., 2021

Nanoparticle tracking analysys (NTA)



Cañón-Beltrán *et al.*, 2021

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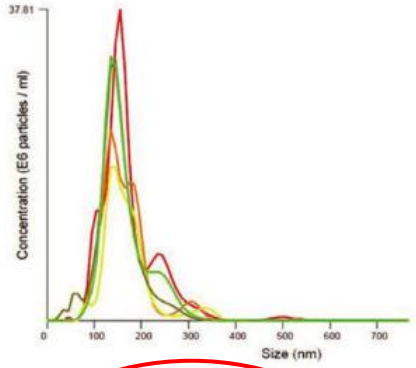

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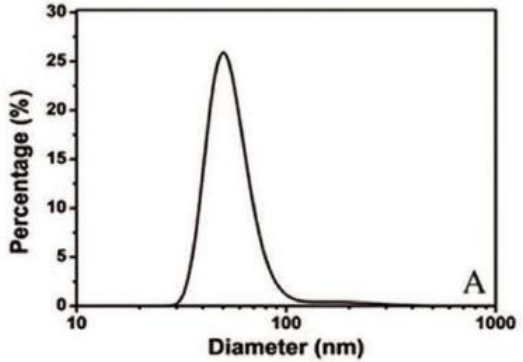

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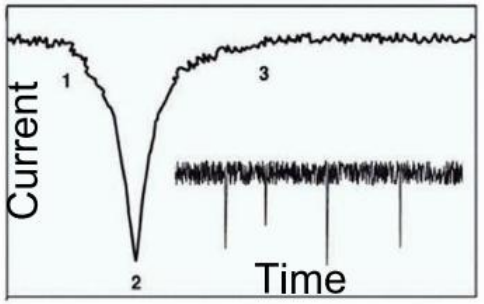

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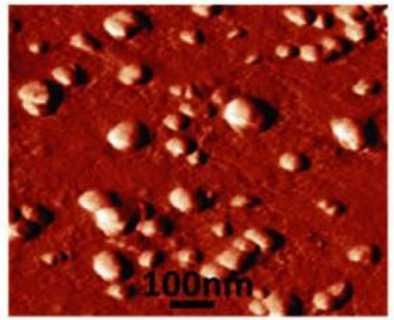

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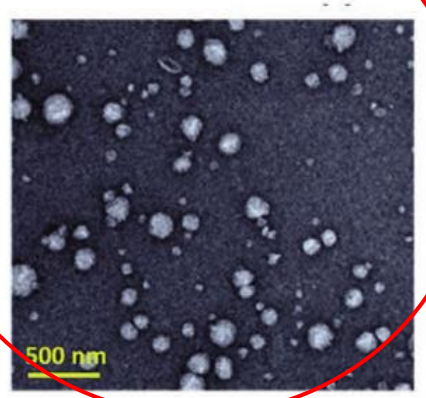

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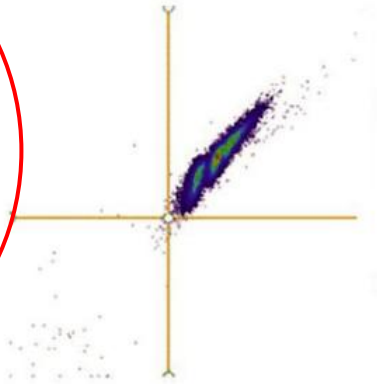

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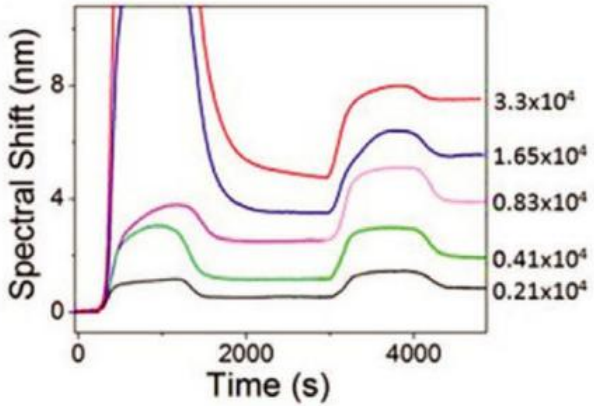

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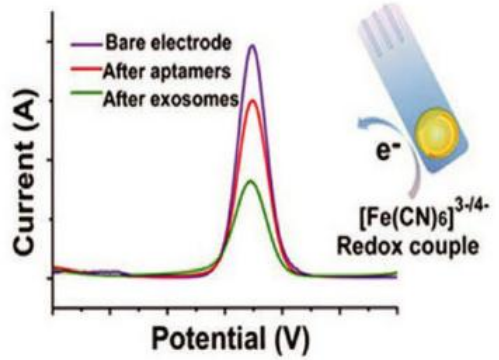
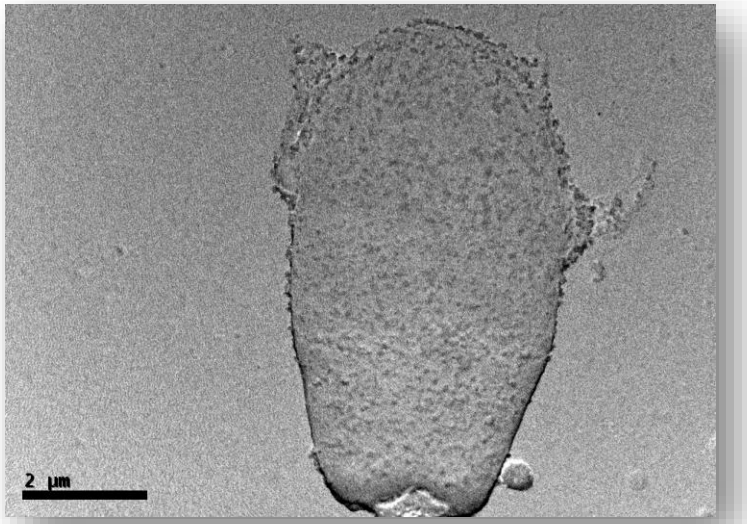
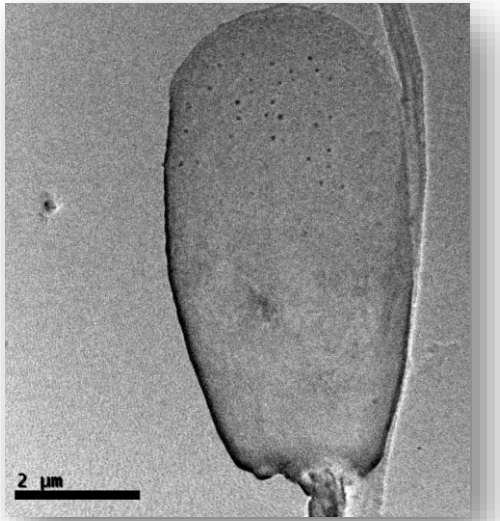


Tiwari et al., 2021

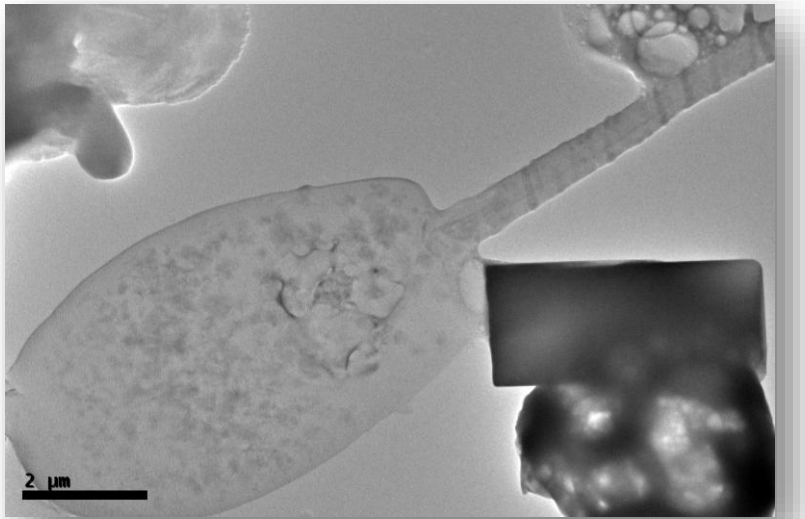
Transmission electron mycroscopy (TEM)



UF view by TEM



OF view by TEM

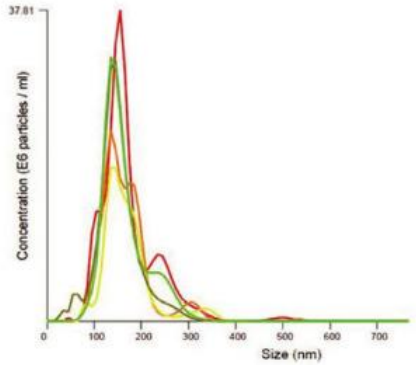


FF view by TEM

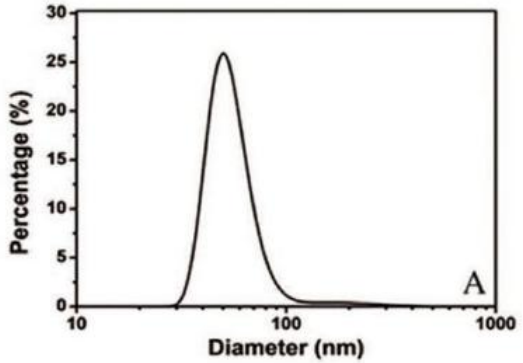
(Images provided by the author)

EVs CHARACTERIZATION

Exosome tracking



Dynamic scattering



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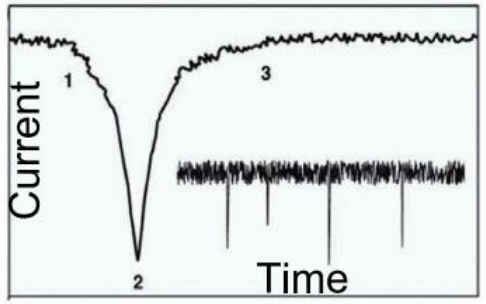
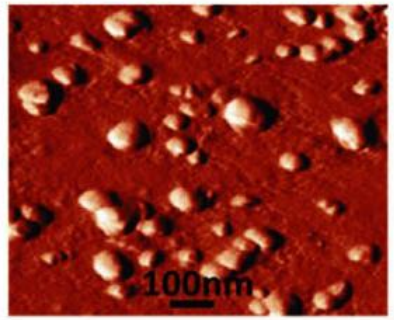
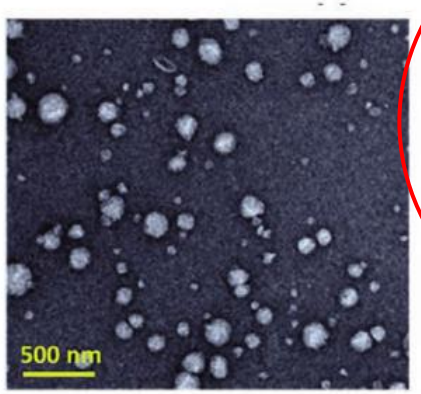


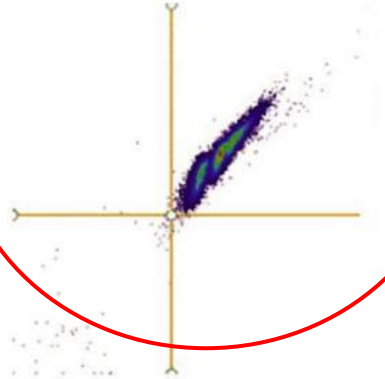
Atomic force microscopy



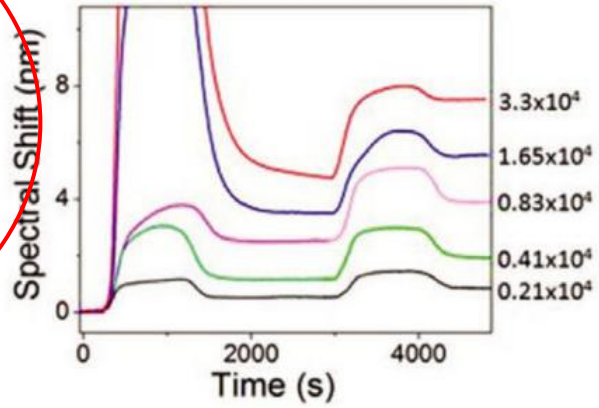
Electron microscopy



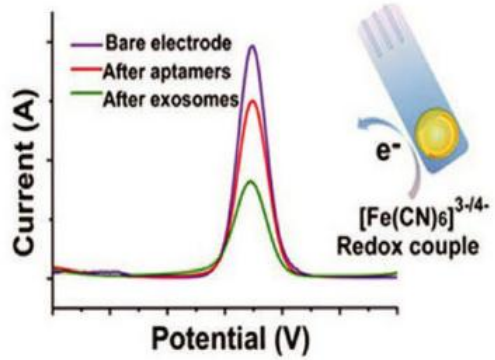
Flow cytometry



Surface plasmon resonance

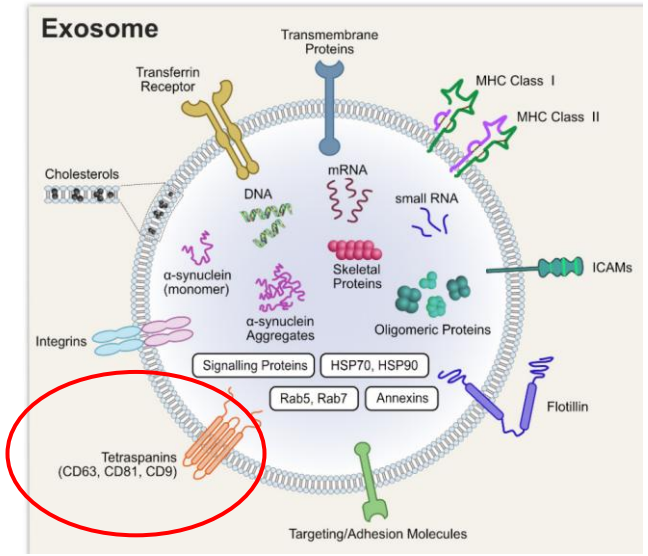


Electrochemical method



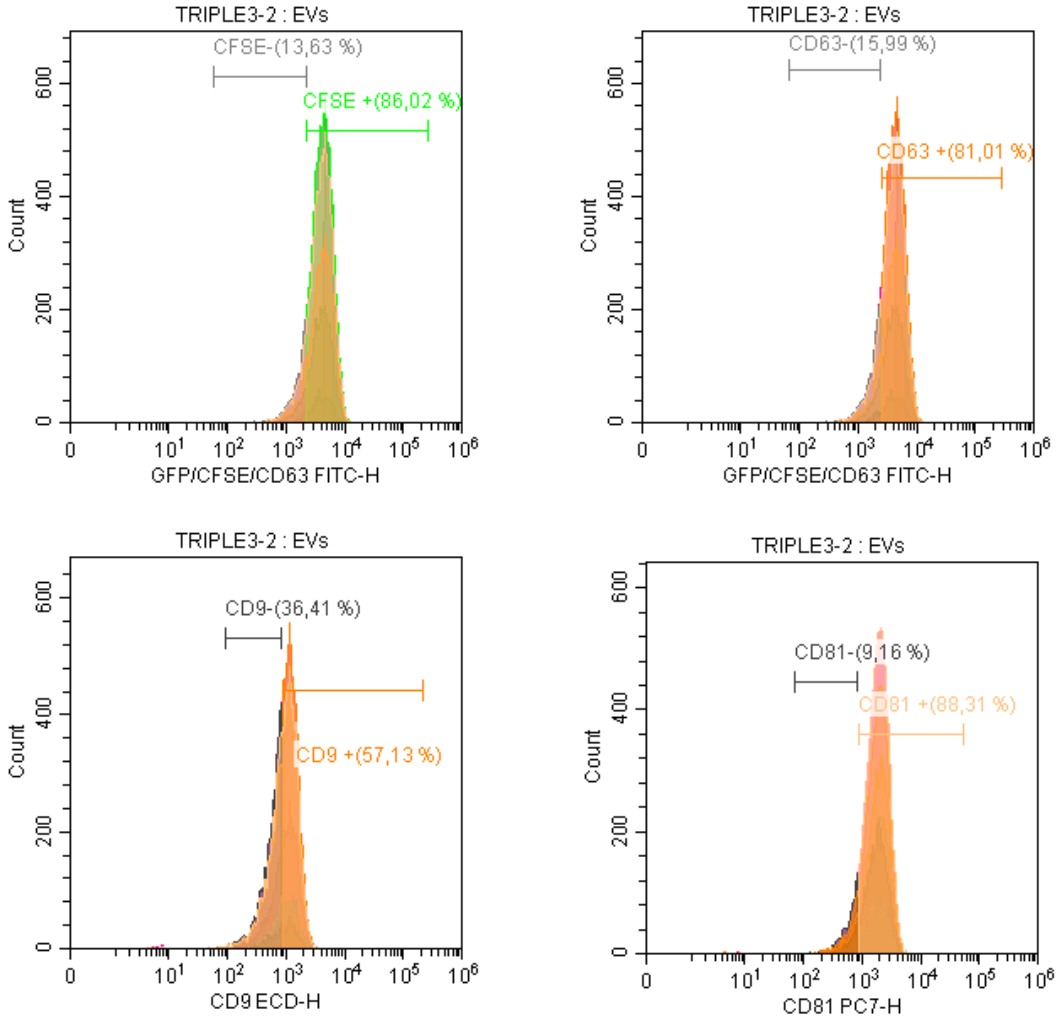
Tiwari et al., 2021

TETRASPANIN CHARACTERIZATION BY FLOW CYTOMETRY



Detection of CD9, CD63 and CD81 tetraspanins

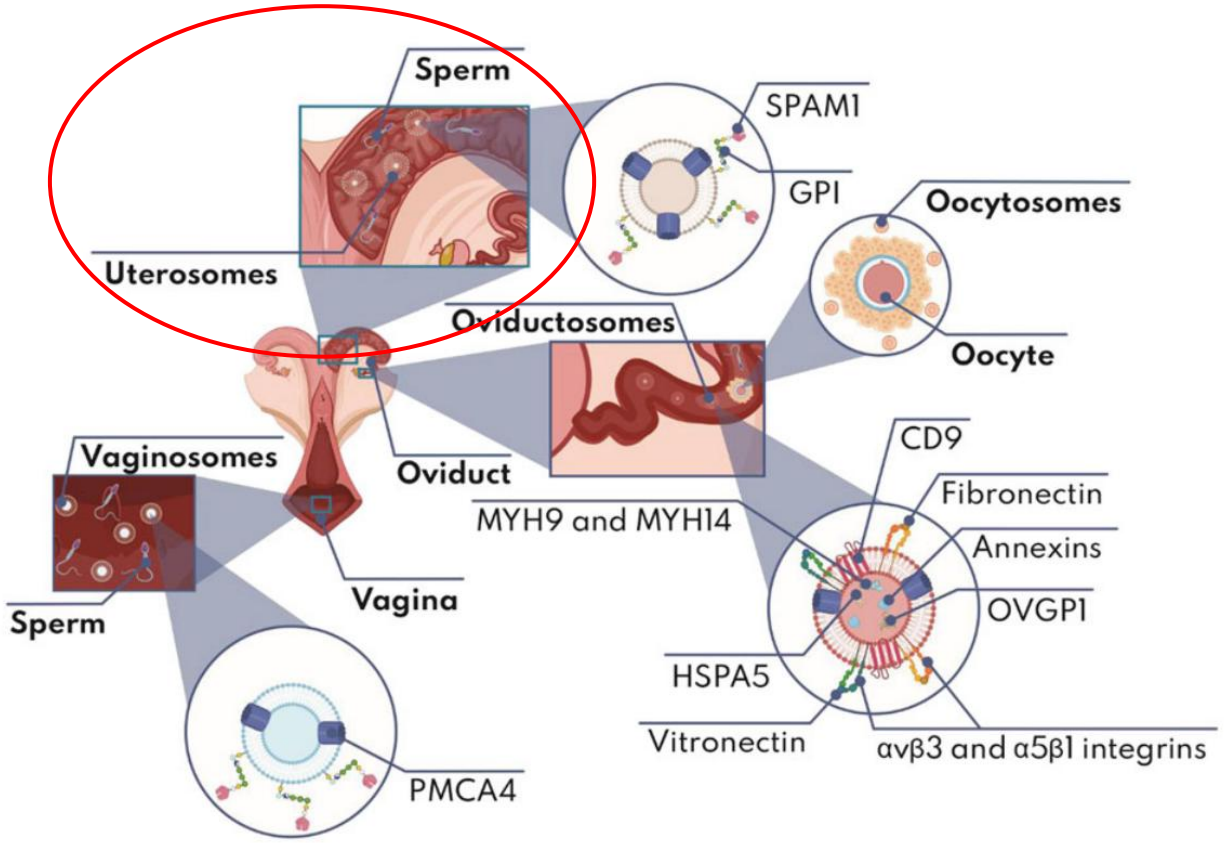
Gurunathan *et al.*, 2019



Tetraspanin characterization by flow cytometry.

Image prepared by the author

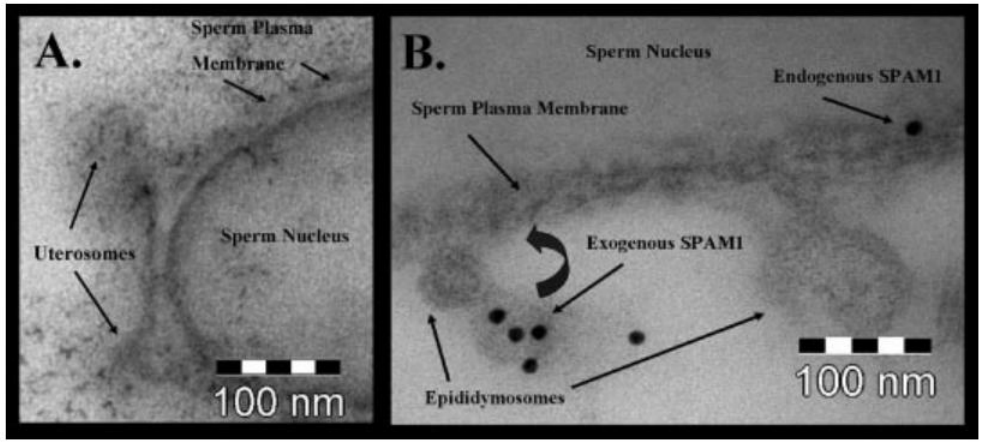
1. BACKGROUND
2. EVs ISOLATION
3. EVs CHARACTERIZATION
4. **EVs – SPERM INTERACTION**
5. CONCLUSIONS
6. BIBLIOGRAPHY



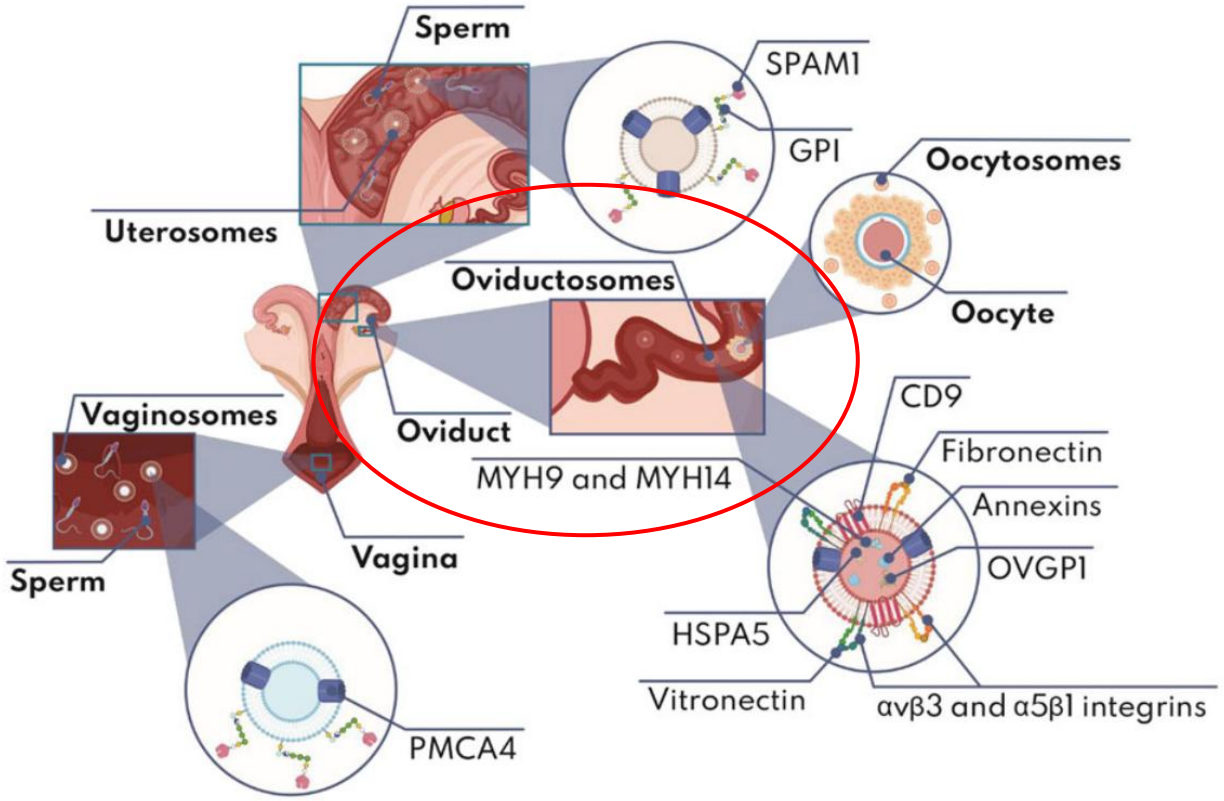
Uterine fluid: uterosomes

-Capacitation: spz membrane remodeling due to SPAM 1 anchoring to GPI binding to mid piece and spz head producing cholesterol release.

-Endometriosomes incubation increase sperm capacitation and acrosomal reaction owing to ptyr increase.



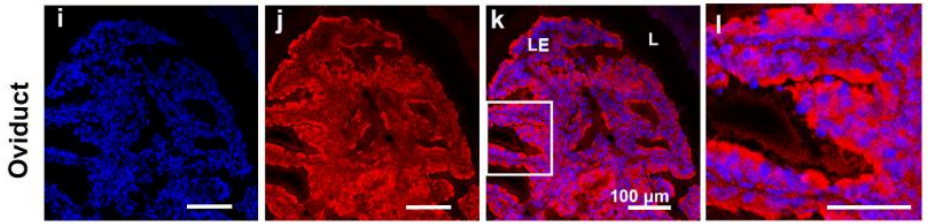
Griffiths et al., 2008



Oviductal fluid: oviductosomes

-PMCA4: calcium pump, is a multifunctional protein involved in viability, motility, capacitation, acrosomal reaction and regulation of sperm calcium homeostasis

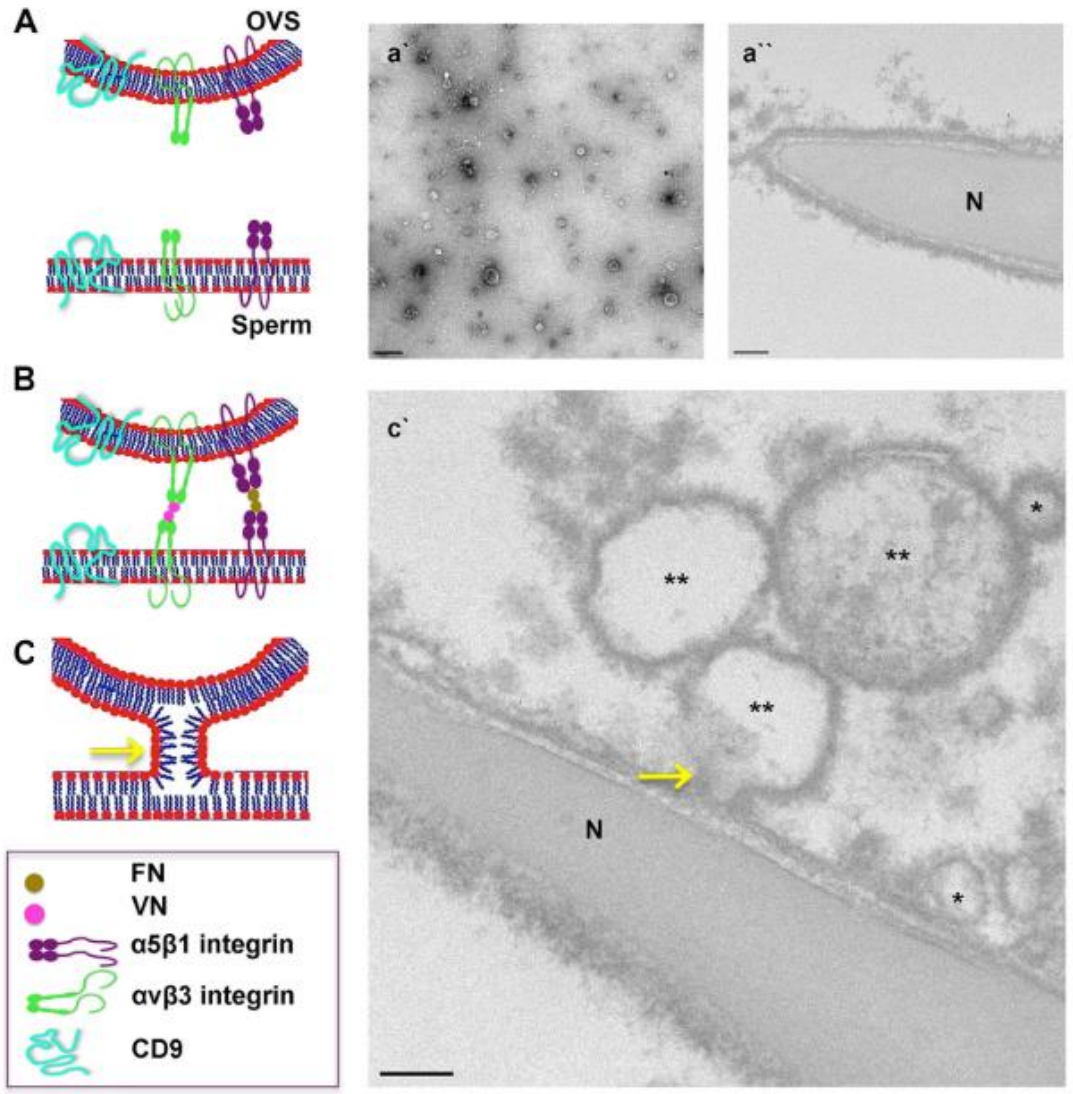
This allows the spermatozoon to free itself from the isthmus sperm reservoir



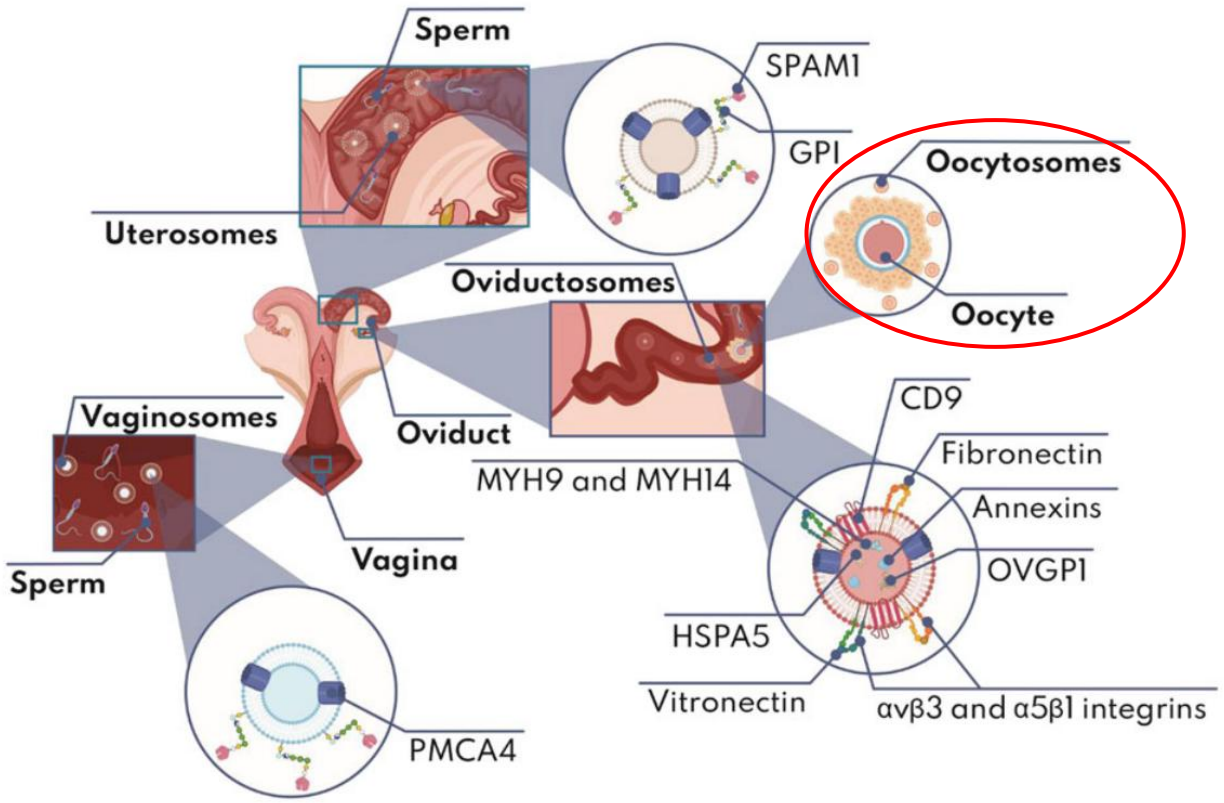
Expression of PMCA4a in mouse oviductal tissues following superovulation.

Al-Dossary *et al.*, 2013

Sperm-Microvesicle (Oviductosome) Fusion Revealed byNanoscopy



Al-Dossary *et al.*, 2015



Follicular fluid: folliculosomes

Estrus period: FSH, LH

↓

MAPK/ERK gene expression

↓

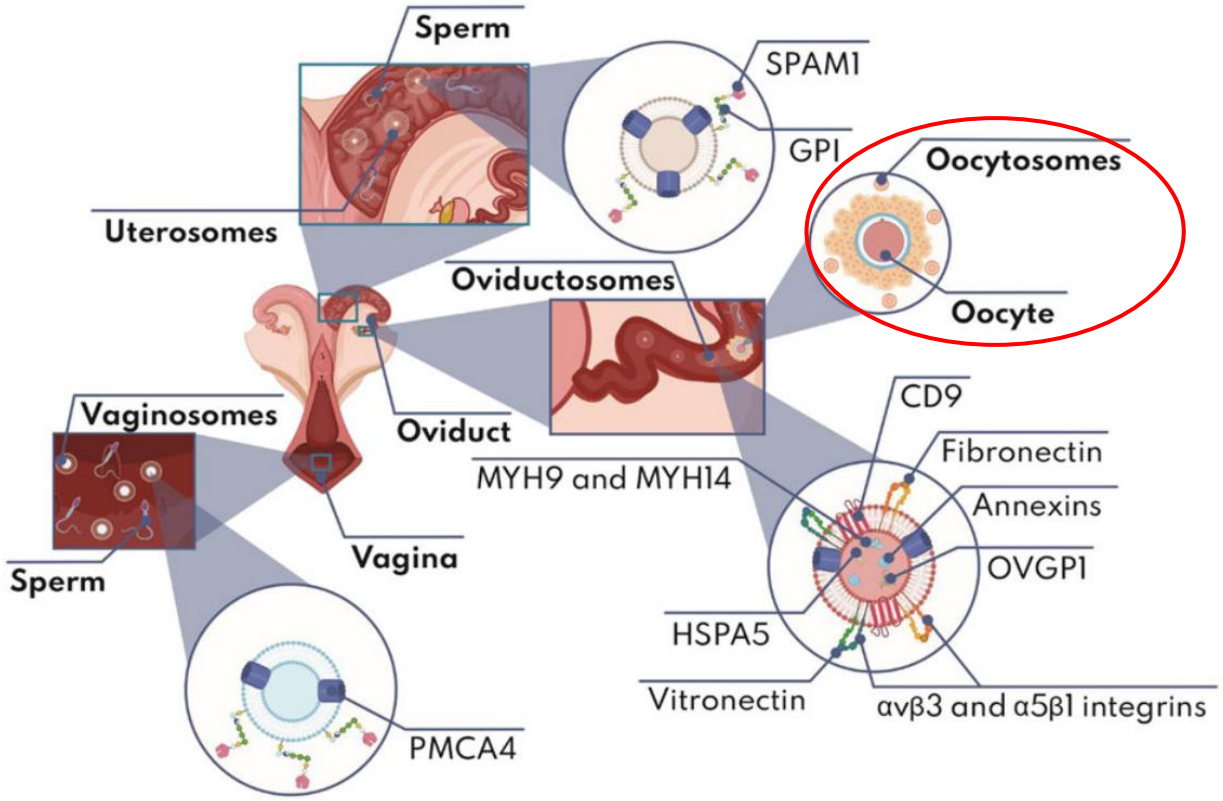
-Granulosa cells proliferation

-Cumulus cells expansion

↓

-Follicular growth

-Oocyte maduration



Follicular fluid: folliculosomes

What about sperm???

biology MDPI

Article

Bovine Follicular Fluid Derived Extracellular Vesicles Modulate the Viability, Capacitation and Acrosome Reaction of Bull Spermatozoa

Mohammad Mehedi Hasan [1,2], Qurat Ul Ain Reshi [1,2], Freddy Lättekivi [2], Janeli Viil [3], Kasun Godakumara [1,2], Keerthie Dissanayake [1,2], Aneta Andronowska [4], Ülle Jaakma [1] and Alireza Fazeli [1,2,5,*]

JBRA Assisted Reproduction 2016;**20**(1):38-40
doi: 10.5935/1518-0557.20160009

Review Article

Human follicular fluid and effects on reproduction

Laís Basuino[1], Carolina F. Silveira[1]

Folliculosomes and sperm: an untapped researching area

1. BACKGROUND
2. EVs ISOLATION
3. EVs CHARACTERIZATION
4. EVs – SPERM INTERACTION
5. **CONCLUSIONS**
6. BIBLIOGRAPHY

CONCLUSIONS

TAKE HOME MESSAGES

1. Further research should be done on the components of gamete maintenance media to establish a clear composition, determining compounds that can compensate for the disadvantages of ESS use.

2. The use of EVs could be an option, since although there is still no established isolation method, they are physiologically present in reproductive fluids.

3. It is necessary to establish an EVs isolation method that allows high yield and high purity to be obtained.

4. A new generation of flow cytometers with detection limits suitable for EVs samples allows their accurate characterization.

1. BACKGROUND
2. EV ISOLATION
3. EV CHARACTERIZATION
4. SPERM PHYSIOLOGY
5. CONCLUSIONS
6. **BIBLIOGRAPHY**

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Thanks you for your attention!