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Review Article

The Extracellular Vesicles: Their Role and Future in Reproduction and Embryonic Implantation

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Abstract

Intercellular communication is an integral part of the physiological process in a living organism. Evidence has shown that a significant amount of human protein-coding genes is endowed with the production of membrane and secreted protein types. Some of these proteins possessed post-translational influence that interferes with cellular functions. Recently the concept of cell communication gravitated to the use of membrane-bound particles called Extracellular Vesicles (EVs). EVs are released from the host cell and impact on the recipient cell through its contents (Cargo). The cargo contains various particles such as lipids, proteins, RNA, and glycolipids derived from the host cells. The current widespread research in EVs has brought its mediating role in physiological and pathological processes to limelight. Though the regulatory part of EVs has been widely embraced by researchers, the discrepancy associated with its collection and isolation as well as its analysis has remained a subject of debate in the literature. The facts emanating from the literature suggest that EVs, with its prospective application in Reproductive biology, has prompted extensive research in the last decade. Its knowledge has broadened the scope of understanding the physiology and pathology scenarios involved in reproductive processes. As a potential biomarker, it has become a valuable tool for diagnosis, prognostic, and therapeutic purposes, especially in the context of reproductive processes. However, the challenges of standardizing the isolation, purification, and analyzing EVs have remained a nightmare that need to be surmounted. Emerging evidence has demonstrated its impact in gamete development, fertilization, and embryo implantation. Thus, it could serve as a platform to understand the mechanism of conception and implantation. By extension, define a therapeutic approach for women with recurrent pregnancy loss.

Keywords: Extracellular vesicles, Reproduction, Embryo implantation.

BACKGROUND

Intercellular communication is an integral part of the physiological process in a living organism (Carnino, Lee *et al.,* 2019). The earlier concept was base on various modulating factors, such as endocrine, paracrine, autocrine and direct contact (Simón, Bolumar *et al.*, 2018). At the same time, other mediators include the hormones, growth factors and neurotransmitters (De Jong, Van Balkom *et al.,* 2014, Simón, Bolumar *et al.*, 2018). Evidence has shown that a significant amount of human protein-coding genes is endowed with the production of membrane and secreted protein types (Lamichhane, Sokic *et al.,* 2015). Some of these proteins possessed post-translational influence (Zaborowski, Balaj *et al.,* 2015). Thus, interfere with cellular functions (Zaborowski, Balaj *et al.,* 2015, Simón, Bolumar *et al.,* 2018). In light of this, a large proportion of these molecules play a central role in therapeutic activities in physiological and pathological processes (György, Hung *et al.*, 2015).

Recently, the concept of cell communication gravitated to the use of membrane-bound particles called 'Extracellular Vesicles'(EVs) (Heath, Grant *et al.*, 2018). EVs, are released from the host cell and impact on the recipient cell through its contents (Cargo) (Es-Haghi, Godakumara *et al.,* 2019). The cargo

contains various particles such as lipids, proteins, RNA and glycolipids derived from the host cells (Sáenz-Cuesta 2015, Carnino, Lee *et al.,* 2019). While in transit, the cargo is protected from enzymatic degradation (Tannetta, Dragovic *et al.,* 2014) and gain access to the host by surface interaction driven via specific receptors(Abels and Breakefield 2016). Subsequently, release its content by endocytosis or cell fusion (Zha, Yao *et al.,* 2017).

EVs has been isolated in various body fluids (Carnino, Lee *et al.,* 2019), such as urine (Serrano-Pertierra, Oliveira-Rodríguez *et al.,* 2019), sputum (Ogawa, Miura *et al.,* 2011), semen(Boukouris and Mathivanan 2015), breast milk (Cecilia Lässer, Alikhani *et al.,* 2011) and amniotic fluid (Asea, Jean-Pierre *et al.,* 2008). Until recently, EV was seen as a cellular by-product (György, Szabó *et al.,* 2011), with little or no attention given to it (De Jong, Van Balkom *et al.,* 2014). The current widespread research in EV has brought its mediating role in physiological and pathological processes to the limelight (György, Hung *et al.,* 2015). Though the regulatory part of EVs has been widely embraced by researchers (Reiner, Witwer *et al.,* 2017), the discrepancy associated with its collection and isolation as well as its analysis has remained a subject of debate in the literature (Carnino, Lee *et al.,* 2019).

Historical Perspective

Charles Darwin, 150 years (Margolis and Sadovsky 2019) ago proposed the concept of EV. Through its pangenesis theory, he demonstrated that every cell produces gemmules. Gemmules described as small particles with the inherent ability to mediate cellular interactions (Liu and Chen 2018). Also, the potential value was involved in meterno- fetal relationship culminating in successful pregnancy outcome (Margolis and Sadovsky 2019). However, the concept did not receive the needed attention for lack of evidence (Ouyang, Mouillet *et al.,* 2014). Though the current concept of EVs seems different from Darwin gemmules, the involvement of genetic materials and Materno-fetal crosstalk could be a source of inspiration (T. Nguyen, J. Simpson *et al.,* 2016).

The current widespread research on EV has clouded the literature with lack of consensus on its isolation and analysis (Simón, Bolumar *et al.,* 2018), compounded by its heterogeneity in the Biophysical composition and functions (Srinivasan, Yeri *et al.,* 2019). In light of this, guidelines put in place by the Extracellular RNA communication consortium (ERCCI) and the international society for Extracellular vesicles (ISEV) attempt to unravel the mysteries (Margolis and Sadovsky 2019). However, failure to achieve standard technique has continued to pose a big challenge.

Classification of Extracellular Vesicles

EV's can be classified into three major subgroups based on their genetic and Biophysical characteristics (Simón, Bolumar *et al.,* 2018). These are Micro-vesicles (MVs), Exosomes (EXOs) and Apoptotic bodies (Abs) (György, Szabó *et al.,* 2011, Colombo, Raposo *et al.,* 2014).

Microvesicles

Evolve by direct budding of the plasma membrane of the host cells (Raposo and Stoorvogel 2013), initiated by different cellular activities and associated with varied membrane composition to suit the content of engaged cargo (D'Souza-Schorey and Clancy 2012). The size of MVs ranged between 100 to 1000 nm (Carnino, Lee *et al.,* 2019) with tendency to rise when submerged (Akers, Gonda *et al.,* 2013). Achieving sedimentation at high-speed centrifugation has created the belief that the size may range as high as 2000 nm (Simón, Bolumar *et al.,* 2018). The membrane primarily made up of cholesterol, diacylglycerol and phosphatidylserine. While integrins, selectin and CD40 as the dominant protein markers (S, Mäger *et al.,* 2013).

The exact mechanism for the initiation of budding and determination of the protein markers remain unclear (Taylor and Gercel-Taylor 2013). However, it has been proposed that ARF-6, a guanosine triphosphate binding protein (Tricarico, Clancy *et al.,* 2017) triggers the packaging of the cargo. Also, it facilitates the budding and the release of the MV through the activation of the phospholipase D (Muralidharan-Chari, Clancy *et al.,* 2009). Furthermore, other proteomic studies (Kowal, Arras *et al.,* 2016, Zha, Yao *et al.,* 2017), have suggested a wide range of proteins markers (De Jong, Van Balkom *et al.,* 2014), depending on the technique and the sources the EVs are derived (De Jong, Van Balkom *et al.,* 2014). Thus, creating a state of debate in the literature.

Despite, the controversies associated with its mechanism of actions (Taylor and Gercel-Taylor 2013), MVs central role has been demonstrated in cancer cells invasion (Menck, Scharf *et al.,* 2015), Embryo-maternal crosstalk (Szekeres-Bartho, Šućurović *et al.,* 2018), and autoimmune regulations (Kurian and Modi 2019).

Exosomes

They are a group of Nano-size EVs of the approximately 50-100nm overlapping size of viruses (Raposo and Stoorvogel 2013), produced and released enzymatically by the multi-vesicular bodies (MVB) (György, Szabó *et al.,* 2011, Simón, Bolumar *et al.,* 2018). MVB is inward budding of endosome initiated through the Endosome pathway (Xu, Greening *et al.,* 2016). Enhanced through the Endosomal sorting complex required for transport (ESCRTs) (Simón, Bolumar *et al.,* 2018). The inherent intraluminal vesicles known as the EXOs are released spontaneously or induced with enzymatic fusion to release their

content at the recipient plasma membrane (Colombo, Raposo *et al.*, 2014). Though secretion of EXOs is either ESCRT- dependent or ESCRT- independent pathway depending on the cell or tissue concerned (Mathivanan, Ji *et al.*, 2010). The exact mechanism for its formation and its fate on the endocytic route remains unclear (Akers, Gonda *et al.*, 2013).

The outer membrane is typically made up of phosphatidylserine (György, Hung *et al.*, 2015), while the incorporated markers include CD63, CD81, CD9, TSG 101 and LAMP 1 (Colombo, Raposo *et al.*, 2014). The increasing interest in the research into EVs has shown significant numbers of these proteins markers spectrum across the various EV sub-population (Tannetta, Dragovic *et al.*, 2014) with relative variation in its concentration (Nawaz, Fatima *et al.*, 2016). Therefore, determination of the peculiar marker for the population of interest requires further investigation.

Evidence has shown that EXO is categorized into two sub-type with its unique protein and RNA cargo, respectively (Chen, Xu *et al.*, 2016). The biological relevance of these in both physiological and pathological entities is yet to unravel (Simon, Greening *et al.*, 2018). However, a study in this field has established the pivotal role of EXOs in immune modulation (De Toro, Herschlik *et al.*, 2015), and emerging potential in the reproductive biology, evidence by its association with seminal fluid in the reproductive tract (Burns, Brooks *et al.*, 2014).

Apoptotic Bodies

The Apoptotic bodies are a group of EVs originate by bulging of the plasm membrane of cells destined for cell death (Carnino, Lee *et al.*, 2019). The size of 1-5um characterizes the term 'Apoptotic bodies' designated by Kerr *et al.*, in 1972 (Kerr, Wyllie *et al.*, 1972, Simon, Greening *et al.*, 2018) and diameter close to the size of platelets (Elmore 2007). unlike other EVs, associated with fragmented DNA and organelles of the cells of origin (Carnino, Lee *et al.*, 2019). The membrane contains phosphatidylserine (PS) (Konoshenko, Lekchnov *et al.*, 2018) and known for cytoskeletal variation in a holistic manner (Mathivanan, Ji *et al.*, 2010). Thus, creating accessibility for phagocytosis (Colombo, Raposo *et al.*, 2014). Studies (Cvjetkovic, Lötvall *et al.*, 2014, Gámez-Valero, Monguió-Tortajada *et al.*, 2016), have demonstrated the relationship between annexin V and initiation of PS Fling. Indicating annexin V as a useful marker for Abs (Wlodkowic, Telford *et al.*, 2011). However, its peculiarity as a marker for Abs is yet to be established (Akers, Gonda *et al.*, 2013).

Other features associated with Abs are the oxidatively generated recognition sites, for thrombospondin aid C3b complement (Simon, Greening *et al.*, 2018). Other markers include VDAC1, required for electron transport in the mitochondria (Takizawa, Tsuji *et al.*, 1996, Wlodkowic, Telford *et al.*, 2011), and Calreticulin (Jeppesen, Hvam *et al.*, 2014). Despite the extensive research, the specificity of these markers still lacks consensus in the literature.

The primary function centres on the immuno-suppression and trafficking of genetic materials between cells (Elmore 2007). Thus, a potential for the spread of oncogenes among cancer cells (Bergsmedh, Szeles *et al.*, 2001, Wlodkowic, Telford *et al.*, 2011).

Isolation and Purification of Extracellular Vesicles

Various methods have been developed for the isolation and purification of EVs (Xu, Greening *et al.*, 2016). The herculean task bothers on getting a significant amount of the spectrum homogeneously (Jeppesen, Hvam *et al.*, 2014). As a result, interest seems to have gravitated towards the use of surface markers as a means of defining the EVs sub-types (Sáenz-Cuesta 2015). The approach appears paramount, as single and rapid method tends to be laden with EV sub-types and contaminants (Greening, Xu *et al.*, 2015).

Differential Centrifugation

DC constitute a widely applied method for isolating the EVs from cell culture isolation (Konoshenko, Lekchnov *et al.*, 2018). The approach involves the use of centrifugation of the sample at various speed and duration (Carnino, Lee *et al.*, 2019). In most cases, at 300g for 10min to 100000g for 70min depending on the EV sub-type (Lucchetti, Fattorossi *et al.*, 2019).

The method is cheap and can process a large amount of sample in a cycle (Jeppesen, Hvam *et al.*, 2014). Thus, make it a useful option for isolating EVs in the human subject (Xu, Greening *et al.*, 2016). Despite these perceived advantages, universal acceptance has centred only on the isolation of Exosome (Heath, Grant *et al.*, 2018). The concept of size variability and overlap with other human cells such as platelets has made it difficult to define the standard method of isolation of MVs and ABs in the literature (Cvjetkovic, Lötvall *et al.*, 2014). Furthermore, the act of higher centrifugation to separate the various EV population could predispose to fragmentation of the vesicle (Ford, Graham *et al.*, 1994, Taylor and Shah 2015). Also, the attendant barrage of contaminants could compromise the quality of the harvested EVs (Nawaz, Fatima *et al.*, 2016). However, the combination of ultra-centrifugation with sucrose gradient may circumvent these challenges (Witwer, Buzás *et al.*, 2013).

Density Gradient Centrifugation

The method primarily aims to improve on the quality of EVs harvested from the Differential Centrifugation (Taylor and Shah 2015). It involves the preparation of the EV pellets in the appropriate buffer solution such as phosphate-buffered saline (PBS)

(Colombo, Raposo *et al.,* 2014) and introduced into a well-developed sucrose gradient cushion (Greening, Xu *et al.,* 2015). The subsequent ultra-centrifugation results in the sedimentation of the EVs at various level of the cushion influenced by their buoyant density (Johansson, Admyre *et al.,* 2008). Alternatively, the use of non-ionic iodixanol (Ford, Graham *et al.,* 1994, Witwer, Buzás *et al.,* 2013), has a better safety profile in biological samples and excellent clinical application (Wang, Yu *et al.,* 2020).

Though it has the potential to separate EVs from a wide range of contaminants, it is associated with complexity and time consumption (Lamichhane, Sokic *et al.,* 2015). Makes its optimal efficiency largely depends on the size of centrifugation and the sample volume (Simons and Raposo 2009, Carnino, Lee *et al.,* 2019).

Size – Exclusion Chromatography

The method complements other protocols to enhance optimal isolation of EVs in a size-dependent manner (György, Szabó *et al.,* 2011). It involves a column of porous beads whose pores can only admit particles less than the size of EV of interest (Böing, van der Pol *et al.,* 2014). The mechanism of action entails the absorption of the smaller size particles (Onódi, Pelyhe *et al.,* 2018). In the process, guarantee the EVs Biophysical integrity (Benedikter, Bouwman *et al.,* 2017), and prevention of aggregation (Wei, Zhao *et al.,* 2020). However, the retention of contaminants remains a challenge (Gámez-Valero, Monguió-Tortajada *et al.,* 2016). Especially those particles of the same size and absorbent with EVs of interest (Monguió-Tortajada, Gálvez-Montón *et al.,* 2019). Also, the column size determines the sample volume (Mol, Goumans *et al.,* 2017). Thus, detrimental in case of large sample volume (Crowell, Wall *et al.,* 2013).

The technicalities required are complicated, and the size overlap among EVs sub-types still pose a nightmare, necessitating another protocol to unravel (Jeppesen, Hvam *et al.,* 2014).

Commercial Kits for Polymer Precipitation

Several commercial kits have been developed in a bid to ease and rapidly isolated EVs from culture solution (Konoshenko, Lekchnov *et al.,* 2018). Though different in composition and trade names, they are necessarily polyethylene glycerol (PEG) based (Momen-Heravi, Balaj *et al.,* 2013). PEG is a super-hydrophilic polymer (Zhang, Yeo *et al.,* 2019) and enhances the sedimentation of EVs mixed with culture media at low centrifugation (Jeon, Kang *et al.,* 2020). The harvested pellets are further washed in sterile PBS and analyzed (Lane, Korbie *et al.,* 2017). Studies have shown that Exoquik, when compared to other methods such as ultra-centrifugation, provide optima yields of Exosomal proteins and RNA (Brownlee, Lynn *et al.,* 2014). Thus, renders the methods a valuable tool for the isolation of quality Exosome RNA (Simón, Bolumar *et al.,* 2018). However, it is saddled with some drawbacks. Ranging from being expensive, accumulation of contaminants and inability to discriminate between some of the EVs sub-type (Peterson, Otoc *et al.,* 2015). Renders the methods deficient in the face of developing potential miRNA related biomarkers for EVs subtypes, especially those outside the range of Exosomes (Taylor and Shah 2015).

To further exploit the potential benefits of precipitation of EVs with chemicals, the ionic affinity of some organic solvents such as sodium acetals and protamine was developed (Konoshenko, Lekchnov *et al.,* 2018). EVs endowed with the negatively charged phosphatidylserine (Brownlee, Lynn *et al.,* 2014) and the introduction of the positively charged organic solvent interferes with EVs hydration tendency(Purushothaman 2019). Thus, resulting in sedimentation of the EVs following centrifugation (Carnino, Lee *et al.,* 2019). The harvested pellets are further purified through gel filtration to free it of the solvents and other impurities (Deregibus, Figliolini *et al.,* 2016). The retention of contaminants and other non-EV proteins renders the approach non-suitable for EVs subtypes (Brown and Yin 2017). Hence, it becomes paramount to design other methods that could isolate EVs sub-population using its specific Biomarkers (Jella, Nasti *et al.,* 2018).

Immuno-Precipitation

The method explores the affinity of antibody immersed magnetic beads to the EVs surface proteins (Heath, Grant *et al.,* 2018). It involves the passage of the cultured solutions through the bead and selectively isolates EVs with specific surface proteins CD63, CD9 and CD8 (Popovic, Mazzega *et al.,* 2018). Though the method has the advantage of enhancing the quality of EVs (Pocsfalvi, Stanly *et al.,* 2016), it has a discriminatory effect on the EVs with the requisite surface protein (Kowal, Arras *et al.,* 2016). However, the lack of consensus on the specific EVS sub-type surface markers, constitute its drawbacks (Padda, Deng *et al.,* 2019). Also, the high selectively and the sub-optimal separation from the anti-body may predispose to poor yield (Yoshioka, Konishi *et al.,* 2013).

Micro Fluidic Devices

Recently, developed technology whose mechanism of action based on immunoprecipitation and size determined separation through the application of porous device (Chen, Skog *et al.,* 2010). The protocol also aids direct RNA recovering from lysed EVs (Santana, Antonyak *et al.,* 2014). While the introduction of the Scanning Electron Microscope (SEM), conferred added advantage (Li, Kaslan *et al.,* 2017). It involves the injection of the cultured solution into a device followed by rinsing with PBS (Zeringer, Barta *et al.,* 2015) and the outcome of the harvest mostly influenced by the size and specific surface

protein of the EVs (Gholizadeh, Shehata Draz *et al.*, 2017). Recent evidence has shown its prominent role in the purification of Exosome when combined with Acoustic pressure for size selection (Ludwig, Whiteside *et al.*, 2019).

Considering the uncertainty associated with isolation and purification of EVs, the modification of these technologies may unravel some of the mysteries. Thus, meet the much-desired clinical diagnostic and therapeutic purposes.

Characterization of Extracellular Vesicles

Extracted EVs are often a mixture of EVs sub-population and contaminants of varying degree (Willis, Kourembanas *et al.*, 2017). The concept of characterization enhances the isolation of EVs with its peculiar features and source of origin (Simón, Bolumar *et al.*, 2018). Thus, renders its clinical application more robust (Zabeo, Cvjetkovic *et al.*, 2017). Besides, research on EVs requires optimal analysis of data to deduce findings (Yamashita, Takahashi *et al.*, 2018). Achieving these sets goals requires adequate characterization of the various EVs sub-population in line with the unique composition and biomarkers (Libregts, Arkesteijn *et al.*, 2018). Evidence has shown that EVs are associated with variations in the proteins and RNA composition (Arraud, Linares *et al.*, 2014) as well as the stability of the microparticles integrity at different conditions (Zhang, Li *et al.*, 2015). Also, cellular surface antigen has been used to categories EVs into cells of origin (Szatanek, Baj-Krzyworzeka *et al.*, 2017). Thus, a prognostic tool for the affected cells and tissues (Arraud, Linares *et al.*, 2014). The analytic findings of these characteristics' features confer the potential application of these EVs in physiological and pathological processes (Joyce, Kerin *et al.*, 2016).

Microscopy: Morphology and Size Analysis

The use of electron microscopy has remained a central approach in the demonstration of EVs morphology relatives to their size (Colombo, Moita *et al.*, 2013). The data obtained from the method primarily depends on the protocols adopted in the preparation of EVs (Li, Corbett *et al.*, 2019) for example, Transmission Electron Microscopy (TEM) has been a veritable tool for delineating the shape of EVs in the mixed of contaminants of similar size (Cizmar and Yuana 2017). However, its associated preparatory protocol of fixation and contracting renders the approach unsuitable (Momen-Heravi, Balaj *et al.*, 2013) as most of the data reported were noted to be laden with artefacts (Johansson, Admyre *et al.*, 2008). This assertion corroborated by the use of cryo-electron microscopy on frozen EVs in their natural state (Brisson, Tan *et al.*, 2017). The application of this approach refuted some of the earlier data related to the shape of EXOs (Willis, Kourembanas *et al.*, 2017). While, the size distribution and quantification analysis involve the use of Atomic Force Microscopy (AFM) (Yuana, Oosterkamp *et al.*, 2010). The approach has the advantage of analyzing the EVs in solution (Chiang and Chen 2019). Thus guarantee, the EVs natural state. Ashcroft *et al.*, demonstrated that its combination with microfluidic could serve as a valuable tool for optimal purification and characterization of EVs (Ashcroft, de Sonneville *et al.*, 2012).

Nanoparticles Tracking Analysis (NTA)

Evaluates EVs size distribution and concentration with the application of light scattering laser beams based on Brownian Motion of particles in an aqueous medium (Soo, Song *et al.*, 2012). The microscope picks the subsequent rays, and the means velocity used to determine the size of the particles(Jamaludin, Thurston *et al.*, 2019). The drawbacks bother on the inability of NTA to discriminate against other particles of similar motion with EVs (Lerner, Avissar *et al.*, 2017). Also, not suitable for large EVs such as Abs (Szatanek, Baj-Krzyworzeka *et al.*, 2017). Recently, concern for the standard approach with refraction index has proposed the use of silica nanosphere (Simon, Greening *et al.*, 2018) in the future.

Dynamic Light Scattering (DLC)

Similar to NTA in principles and technique, in the determination of EVs size distribution and Zeta potential (Im, Shao *et al.*, 2015). Its optimal performances are primarily based on the homogeneity of the EVs Solution (Kwizera, O'Connor *et al.*, 2018). However, the disparity in the size of the particles in a given solution may render the data obtained incongruous (Szatanek, Baj-Krzyworzeka *et al.*, 2017).

Flow Cytometry

Measure and characterized EVs with its size distribution and concentration (Libregts, Arkesteijn *et al.*, 2018). Its analytic power limits the light variant for small size EVs (Padda, Deng *et al.*, 2019). However, the advanced option, Nano-scale flow cytometry, is endowed with antibiotics (Libregts, Arkesteijn *et al.*, 2018). Thus, EVs of interest are selectively separated from the mixture in solution (Arraud, Linares *et al.*, 2014). The application of specific Fluorescence aid in the identification of EVs cells of origin (Arraud, Gounou *et al.*, 2016) and approaches a valuable tool that would harmonize EVs surface markers towards discernment on the types of cells involved (Arraud, Linares *et al.*, 2014)

Molecular Marker Characterization

Characterization of EVs through sedimentation of the specific surface marker constitutes an optimal approach for purifying the EVs of interest (Yoshioka, Konishi *et al.*, 2013). Foremost in this regards, is the use of ELISA (Lane, Korbie *et al.*, 2017). The micronuclear Magnetic Resonance Spectrometry (UNMR) endowed with magnetic Nanoparticles, especially in the context of labelled EVs with specific

molecular surface antibodies (Sunkara, Woo *et al.,* 2016), confers better sensitivity when compared to the ELISA (Ludwig, Whiteside *et al.,* 2019). Another similar approach with some degree of better sensitivity is the use of Nano-plasmonic EXO assay (Im, Shao *et al.,* 2015). The principle-based on transmission surface resonance, and it involves the affinity of Exospecific proteins to specifically developed antibodies lining Nanopores (Greening, Xu *et al.,* 2015).

Recent studies have demonstrated the concept of proteomic analysis aim at determining the markers that could play a characteristic feature of the EVs sub-population (Sunkara, Woo *et al.,* 2016, Xu, Greening *et al.,* 2016). As a result, several markers identified among the EXO and MV (Kowal, Arras *et al.,* 2016). However, the application of these proteins as discriminatory markers relative to the cell type still requires further research to unravel (Makler and Asghar 2020).

Extracellular Vesicles Cargo

The cargo content widely influenced by its biogenesis, cell of origin and clinical application (Tricarico, Clancy *et al.,* 2017). Constituent significant characteristic features of EVs (Peterson, Otoc *et al.,* 2015). Several studies have attempted to demonstrate and characterize these contents, discriminatory with EVs sub-population (Akers, Gonda *et al.,* 2013, Jamaludin, Thurston *et al.,* 2019). The consensus described EVs cargo with varieties of proteins, lipids, nucleic acids and some other cell modulatory particles (Pocsfalvi, Stanly *et al.,* 2016). However, emphasize seems to focus on the proteins, RNA and miRNA contents due to their accessibility of reliable approach for the isolation, purification and analysis (Witwer, Buzás *et al.,* 2013).

Protein Content

The protein content of EVs has received extensive research (Abels and Breakefield 2016). However, its composition tends to widely varied with isolation and purification techniques adopted for the EVs sub-population (Lucchetti, Fattorossi *et al.,* 2019). Despite the short-coming, EVs are endowed with certain specific proteins determined by their biogenesis and Endosomal pathway (Monguió-Tortajada, Gálvez-Montón *et al.,* 2019). They include the tetraspanins such as CD63, CD81 and CD9, while the MHC1 and MHC and EGFR are the antigenic and transduction proteins, respectively (Abels and Breakefield 2016). Others proteins such as Golgi and other cellular organelles are often scanty in the EVs cargo (Pocsfalvi, Stanly *et al.,* 2016). Genarally, the protein composition of the EVs cargo are influenced by the strength of the stimuli (Onódi, Pelyhe *et al.,* 2018). Necessitating standard technique for isolation and purification (Li, Corbett *et al.,* 2019).

Lipid Content

The lipid content, as in protein, has been widely researched (Abels and Breakefield 2016). The consensus revealed comparable composition with increased lipid/protein ratio to the originating cell (Willis, Kourembanas *et al.,* 2017, Zabeo, Cvjetkovic *et al.,* 2017). Though, some studies have demonstrated some discriminatory tendency across the EVs sub-population (Höög and Lötvall 2015, Peterson, Otoc *et al.,* 2015). EVs are generally endowed with sphingomyelin, cholesterol, phosphatidylserine and ceremide (Szatanek, Baj-Krzyworzeka *et al.,* 2017). Unlike the originating cell, it has a remarkable decrease in phosphatidylcholine and diacyl-glycerol (Tricarico, Clancy *et al.,* 2017). Despite the relative similarity of the lipid composition of MVs to its originating cells, elevated PS tends to be characteristics features in EVs cargo (Abels and Breakefield 2016).

Nucleic Acid Content

EVs are composed of varying amounts of genetic particles (Witwer, Buzás *et al.,* 2013). Predominantly, endowed with an array of small RNAs (Ludwig, Whiteside *et al.,* 2019). Though, Valadi *et al.,* demonstrated that mRNA and miRNA were central in the physiological process of EVs (Valadi, Ekström *et al.,* 2007). Other studies have shown the presence of other RNAs such as mRNA, miRNA, rRNA and other non-coding RNAs (Simons and Raposo 2009, Abels and Breakefield 2016). The RNAs packaged in fragments of the varied length of nucleotides with some degree of guaranteed membrane protection in the extracellular space (György, Szabó *et al.,* 2011).

One striking feature of EVs cargo is the RNA profile in a manner different from the cell of origin, often described as a sorting mechanism (Akers, Gonda *et al.,* 2013). The bid to unravel the phenomenon had geared effort towards the search for the principle behind the discriminatory approach (Raposo and Stoorvogel 2013). However, the concept seems based on the biogenesis and physiological activities of the cell of origin (S, Mäger *et al.,* 2013). In light of this, recent studies have shown the modification of miRNA profile in EVs influenced by the activation of the originating cell to conform with its clinical application (Taylor and Gercel-Taylor 2013, Abels and Breakefield 2016). Ng *et al.,* corroborated the assertion with in-vitro endometrial culture (Ng, Rome *et al.,* 2013). Suggesting the central role of the differential of EXOs miRNA during embryo and maternal crosstalk (Tannetta, Dragovic *et al.,* 2014). The target was genes for implantation, and miR-30d EXOs were mainly involved (Simón, Bolumar *et al.,* 2018). Thus, giving credence to its clinical implication in Embryo Implantation and Pregnancy outcome (Es-Haghi, Godakumara *et al.,* 2019, Kurian and Modi 2019).

Analyzing the RNA from EVs can be cumbersome (Yoshioka, Konishi *et al.,* 2013), often influenced by the approach adopted by its isolation and purification (Witwer, Buzás *et al.,* 2013). Though, the challenge initially tackled with the introduction of RNase (Zaborowski, Balaj *et al.,* 2015). The formation of a protein complex with miRNA renders the approach sub-optimal (Li, Kaslan *et al.,* 2017). While the use of proteinase K attempt to circumvent the drawbacks (Witwer, Buzás *et al.,* 2013). However, the associated Lysis of EVs renders the method unprofitable (Simons and Raposo 2009).

The limited data on DNA and EVs have shown that DNA was more of single-stranded and mainly involved in oncogenic cells with MV predominantly involved (Peterson, Otoc *et al.,* 2015). Similarly, mitochondrial DNA involved in EV is pathognomonic of the pathological process in the recipient cell(De Toro, Herschlik *et al.,* 2015).

Uptake of Extracellular Vesicles

Uptake of the EVs involves recognition of the recipient cell as the target cells mediated by Ligand / Receptors Molecules (Zhang, Li *et al.,* 2015). Subsequently, bind and internalized by fusing to the recipient cell membrane or endocytosis (Lerner, Avissar *et al.,* 2017). Endocytosis seems more favoured mediated through clathrin, caveolin, lipid- raft, micropinocytosis and phagocytosis (Abels and Breakefield 2016). While, adhesive property constitute an integral part of the EVs recognition, (Menck, Scharf *et al.,* 2015) mediated by adhesion proteins such as the integrin (Lässer, Seyed Alikhani *et al.,* 2011). Some of which demonstrated in lungs and liver metastasis (Simon, Greening *et al.,* 2018). Suggesting the prognostic value of the integrin profile in EV subpopulation. Studies have shown the regulatory role of Exo tetraspanin on the EVs integrin (Bergsmedh, Szeles *et al.,* 2001), interfering with its adhesive potential (Cecilia Lässer, Alikhani *et al.,* 2011, Ludwig, Whiteside *et al.,* 2019). In addition to other blocking agents, could be of therapeutic value in such EXOs related pathological condition. Also, recent studies have shown the impact of lipid contents of the cargo on the efficiency of the delivered EXOs (Makler and Asghar 2020, Wang, Yu *et al.,* 2020). Trigger by proteoglycans, lecithin and heparainsuphate proteoglycans associated with the recipient cell membrane (Menck, Scharf *et al.,* 2015). Blocking with heparin could compromise EV uptake in culture (Abels and Breakefield 2016).

The fusion of the EV with the recipient cell is another method of uptake often associated with tumour cells (Yoshioka, Konishi *et al.,* 2013). The process requires low PH to guarantee optimal interaction (Parolini, Federici *et al.,* 2009). As the stability of the lipid contents of the EXO membrane is PH dependent (Brownlee, Lynn *et al.,* 2014). The adopted approach mostly influenced by the prevailing circumstance between cells concerned (Abels and Breakefield 2016). Thus, determined the physiological and pathological impact of the EVs (Willis, Kourembanas *et al.,* 2017). EVs internalization through the various Endocytic processes has been widely reported (Christianson, Svensson *et al.,* 2013, Abels and Breakefield 2016). The various mechanisms with which it occurs with respect to the EVs sub-population needs further clarifications(Christianson and Belting 2014).

The effort at determining the EVs uptake in culture has triggered several studies using EVs labelled with fluorescent (Arraud, Gounou *et al.,* 2016). The consensus on the characteristics of the EVs in both in-vitro and in- vivo models are still subject to debate (Greening, Xu *et al.,* 2015). Thus, the need for further research into the standardization of the isolation techniques for the EVs subpopulation. Also, the biogenesis, cargo and impact on the recipient cells (Witwer, Buzás *et al.,* 2013). Good knowledge of these could render it a new vista in the clinical entity.

Extracellular Vesicles in Reproductive Physiology

Reproductive physiology involved a complex process highly predicated on intercellular communication at various designated stages (Tannetta, Dragovic *et al.,* 2014). The central role of intercellular communication has engendered focus on the place of EVs in the male and female reproductive system (Machtinger, Laurent *et al.,* 2015). Recent studies have demonstrated the presence of EVs in reproductive processes, serving as a platform in the transfer of protein and RNAs between cells (Burns, Brooks *et al.,* 2014, Trigg, Eamens *et al.,* 2019). Studies have also demonstrated EVs in Epididymal fluid (A Trigg, L Eamens *et al.,* 2019), Prostatic fluid (Padda, Deng *et al.,* 2019), Seminal fluid (Höög and Lötvall 2015), Follicular fluid (Franz, Böing *et al.,* 2016), Endometrial / Uterine fluid (Ng, Rome *et al.,* 2013), Amniotic fluid (Hell, Wisgrill *et al.,* 2017) and Breast milk (Cecilia Lässer, Alikhani *et al.,* 2011). Besides, the regulatory potentials of EVs in the reproductive processes have been demonstrated in gametogenesis, fertilization, endometrial-embryo crosstalk culminating in implantation (Machtinger, Laurent *et al.,* 2015). Successful pregnancy outcome following effective implantation has linked to placenta production of EVs aim at modulating maternal immune response for innate or adaptive response to the foetus (Kurian and Modi 2019).

Furthermore, the inflammatory and pro-coagulatory role of EVs has been demonstrated in amniotic fluid (Hell, Wisgrill *et al.,* 2017). At the same time, its role in bone formation, immune modulation of gene expression has been shown in breast milk (Cecilia Lässer, Alikhani *et al.,* 2011). Therefore, it has become evident that EVs and its related cargo seems paramount in Reproductive processes (Tannetta,

Dragovic *et al.,* 2014). By extension, it could be a suitable biomarker in the physiological and pathological entities as well as a potential prognostic tool in the future (Machtinger, Laurent *et al.,* 2015).

Extracellular Vesicles in the Male Reproductive Tract

Spermatozoa are produced from the seminiferous tubules as immature cells (Baskaran, Panner Selvam *et al.,* 2020). Following its release, they transverse through the caput to the caudal segment of the epididymis (Robert Sullivan and Fabrice Saez 2013). While in transit, the spermatozoa undergo a series of morphological and physiological changes culminating in mature form endowed with motility and fertilization potentials (Machtinger, Laurent *et al.,* 2015). The epididymis is made up of three regions, caput, corpus and caudal (Sostaric, Aalberts *et al.,* 2008). Each characterized with distinct protein and genetic phenotype aim at sperm development (Höög and Lötvall 2015). Sperm maturation takes place in the caput and corpus, while the caudal region is mainly for sperm reserve (Sullivan and Saez 2013).

Ejaculated semen is a combination of the spermatozoa and seminal fluid. Produced from seminal vesicles, prostate and bulbourethral glands (Höög and Lötvall 2015). The seminal fluid guarantees the survival of the sperm through the vaginal by altering the PH (Tannetta, Dragovic *et al.,* 2014) and subsequently capacitated through the cervix, the uterus and fallopian tube to reach the oocyte for fertilization (Simón, Bolumar *et al.,* 2018). The mechanism involved in these processes may be predicated on intercellular communication, between the spermatozoa and the intraluminal fluid (Burns, Brooks *et al.,* 2014). Evidence has demonstrated the association of the epididymal fluid derived EVs with its cargo protein and miRNA (epididymosomes) in post-testicular mature sperm (Sullivan and Saez 2013). Suggesting the central role of intraluminal secretion in optimizing the sperm for fertilization.

Epididymosomes

These are collections of epididymal associated EVs secreted via apocrine with varied morphological and physiological disposition; in line with the anatomical regions of the epididymis (Sostaric, Aalberts *et al.,* 2008). The EVs demonstrated in animal and human are endowed with more sphingomyelin compared to other phospholipids (A Trigg, L Eamens *et al.,* 2019). In contrast, the amount of the cholesterol contents tends to vary with the anatomical region of the epididymis (Belleannée 2015). Unlike, the spermatozoa, it has a higher proportion of saturated acid (Robert Sullivan 2016). Suggesting a converse morphological disposition between the epididymosome and the spermatozoa (Baskaran, Panner Selvam *et al.,* 2020). Thus, its impact on sperm motility and fertilization potential (Sostaric, Aalberts *et al.,* 2008).

Evidence has demonstrated the presence of two sets of Epididymosomes (Frenette, Girouard *et al.,* 2010). The CD9-positive are associated with live spermatozoa. While the Epididymal sperm binding protein 1 (ELSPBP1) discriminately bind to dead spermatozoa (Frenette, Girouard *et al.,* 2010, Robert Sullivan 2016). The CD9-positive are nanoparticles, predominantly in the caudal of the epididymis and with the aid of CD 26, they release P25b, GliPriLi and MIF proteins for sperm maturation (Caballero, Frenette *et al.,* 2013). Therefore, epididymosomes associated with ELSPBP1 could serve as a valuable biomarker for dead spermatozoa (D'Amours, Frenette *et al.,* 2012, Sullivan 2015). However, the introduction of Biliverdin Reductase A (BLVRA) to the ELSPBP renders its protective qualities (Trigg, Eamens *et al.,* 2019). The protective phenomenon prevents the exposure of the developing spermatozoa to oxidative stress, orchestrated by reactive oxygen species associated with dead and immature spermatozoa (Robert Sullivan and Fabrice Saez 2013).

Specific adhesion molecules have been shown in epididymosomes (Machtinger, Laurent *et al.,* 2015). These include tetraspanins, integrins and milk fat globule-epidermal growth factor 8 (MFGE8) (Sullivan and Saez 2013, Machtinger, Laurent *et al.,* 2015). Notable among these, is the Sperm Adhesion Molecule 1 (SPAM 1), hyaluronidase, an indicator for sperm maturity required for cumulus attachment and subsequent fertilization of the oocyte (A Trigg, L Eamens *et al.,* 2019). Other relevant proteins associated with epididymosomes are ADAM 7 for sperm motility (Oh, Han *et al.,* 2009); membrane $ATPase_4$, ca^{2+} pump and Glutathione peroxidase requires to prevent lipid peroxidation associated with oxidative stress (Cho 2012).

In addition to the protein, Epididymosomes involves in the release of miRNA with distinct profile influence by the prevailing circumstance at the different region of the Epididymis (A Trigg, L Eamens *et al.,* 2019). The associated sorting mechanism gives credence to the assertion of Epididymosome impact on gene expression modulation at the various regions of the epididymis (Robert Sullivan and Fabrice Saez 2013). Emerging evidence has demonstrated the release of transfer RNA fragments (tRFs) from Epididymosome to post-testicular spermatozoa in mice (Simon, Greening *et al.,* 2018). Though the modulatory gene impact of tRNA was earlier demonstrated in pathological scenarios involving viral infections (Sharma, Sun *et al.,* 2018). The abundant tRFs in spermatozoa, in a maturity-dependent manner, has been shown to have a profound impact on an epigenomic modification and subsequent trait to offspring (Reilly, McLaughlin *et al.,* 2016). Though the mechanism remains unclear, it could be a valuable biomarker for

sperm selection in the clinical entity (Nixon, De Iuliis *et al.*, 2019).

Prostasomes

These are EVs of 50-500nm first demonstrated from the seminal fluid as an organelle following its release from the prostate epithelial cells (Robert Sullivan and Fabrice Saez 2013, Machtinger, Laurent *et al.*, 2015). The exocytotic or diacytotic events culminating in the release of prostasomes mostly influenced by Mg^{2+} and ca^{2+} dependent ATPase phenomenon (Ronquist 2015). Prostasomes is rich in protein, lipid and nucleic acid (Baskaran, Panner Selvam *et al.*, 2020) and vectorize wide range of proteins such as prostate-specific protein like PSA and PAP and signal transduction protein (Sostaric, Aalberts *et al.*, 2008). These qualities confer immune-regulatory, antioxidant and anti-microbial properties on the prostasomes (Robert Sullivan and Fabrice Saez 2013).

The membrane predominantly consists of cholesterol and sphingomyelin with remarkably high cholesterol/phospholipid ratio (Ronquist 2015). A recent report has demonstrated the attribute of these features in sustaining its physiological state in a vaginal acidic milieu as well as its regulatory functions in spermatozoa capacitation and acrosome reaction (Taylor and Gercel-Taylor 2013). Emerging evidence has demonstrated that the release of protasome tends to reduce the fluidity of the sperm membrane in PH dependent manner (Machtinger, Laurent *et al.*, 2015). Thus, prevent premature capacitation and acrosome reaction (Sullivan and Saez 2013). In the process, enhance acquisition of optimal signals for fertilization (Ronquist and Nilsson 2004).

On the other hand, protasome has been shown to facilitate fertilization through the release of the progesterone receptor (Burden, Holmes *et al.*, 2006, Robert Sullivan and Fabrice Saez 2013). Thus, trigger acrosome reaction by cumulus cells in a progesterone-dependent phenomenon prior to sperm contact with the zona pellucida (Gadella 2012). Suggesting a two-prong approach of the protasome in the process of fertilization.

The role of Ca^{2+} in spermatozoa motility has been widely documented (Park, Kim *et al.*, 2011). However, recent Hamster study has demonstrated the relationship of the protasome with the influx of Ca^{2+} in spermatozoa (Achikanu, Pendekanti *et al.*, 2018). Though data on the mechanism seems sketchy, emerging evidence is related to progesterone trigger in PH dependent manner (Ronquist 2015). Other related proteins associated with Ca^{2+} stability include $PMCA_4$ and Nitric Oxide synthesis (NOs) (Sullivan and Saez 2013). The dynamics of these proteins aim at preventing oxidative stress that could compromise the motility and morphology of the spermatozoa (Sostaric, Aalberts *et al.*, 2008).

Fertilization occurs when spermatozoa penetrate the oocyte in the fallopian tube (Gadella 2012). As a result, the spermatozoa must transverse the gateway pose by the cervix to the uterine cavity and then the fallopian tube (Machtinger, Laurent *et al.*, 2015). A recent report has shown cervical associated EVs in Sialidase that climax in the ovulatory phase of the woman cycle (A Trigg, L Eamens *et al.*, 2019). Thus, modulate the cervical mucus and enhance the spermatozoa transition to the uterine cavity (Machtinger, Laurent *et al.*, 2015).

The presence of nucleic acid as a constituent of its cargo has been reported and most of which being RNAs with varied regulatory activities (Wang, Lv *et al.*, 2014). However, the paucity of data on their physiological impact in the male reproductive system makes further research imperative (Simon, Greening *et al.*, 2018).

Extracellular Vesicles in the Female Reproductive Tract

Fertilization involves the interaction of the mature spermatozoa and well-developed oocyte at the appropriate site in the female reproductive tract (Tannetta, Dragovic *et al.*, 2014). The oocyte undergoes stepwise development pattern with the initial arrest of the meiosis at prophase 1 of the primordial follicle (Machtinger, Laurent *et al.*, 2016). The acquisition of secondary sexual characteristics, culminating in the female maturity and the development of Hypothalamus-Pituitary-Ovarian axis, trigger the process of the menstrual cycle (Andronico, Battaglia *et al.*, 2019). The process initiates cyclical recruitment of oocyte cohort for follicular development with the simultaneous resumption of meiotic division orchestrated by luteinizing hormone (Sun, Ma *et al.*, 2019). Subsequently, extruding of the first polar body to attain metaphase II at ovulation (Machtinger, Laurent *et al.*, 2016). The ovulated oocyte, with the aid of cumulus cells, navigates through the fallopian tube to reach the ampulla for interaction with the sperm (Andronico, Battaglia *et al.*, 2019).

Following fertilization, the Nascent embryo transverse the tube to reach the uterine cavity within 4-6 days (Aplin 2000). In comparison, the process of implantation occurs within a time frame of approximately 6-10 days often described as Window of Implantation (Singh, Nardo *et al.*, 2010, Carlomagno, Minini *et al.*, 2018). Evidence has shown that the successful outcome of these processes involved significant impact of EVs (Ng, Rome *et al.*, 2013). Suggesting the mediating role of EVs in the intercellular communication between the female reproductive tract and the gametes in transit (Machtinger, Laurent *et al.*, 2015).

Extracellular Vesicles in Ovarian Follicle and Oocyte Development

The development of oocyte occurs within the follicular fluid, produced in the form of exudate from surrounding granulosa and theca cells (Franz, Böing *et al.,* 2016). These components involve in a coordinated communication modulated by various factors domicile within the follicular fluid (Andronico, Battaglia *et al.,* 2019). Studies have shown the impact of communication in the optimal follicular growth as well as oocyte and pre-implantation embryo development (Machtinger, Laurent *et al.,* 2015, Andronico, Battaglia *et al.,* 2019).

The recent demonstration of EVs in follicular fluid and granulosa cells gravitated interest in its place in follicular growth (Adam, Elfeky *et al.,* 2017, Pavani, Alminana *et al.,* 2017). Suggesting its valuable communicating tool within the ovary. The impact on follicular growth and oocyte maturation are mediated by transforming growth factor-beta (TGF_B), Wingless Signaling Pathway (WNT) and mitogen-activated protein kinase (MAPK) (Machtinger, Laurent *et al.,* 2015).

Available data from Bovine study demonstrated contrast miRNA profile relative to follicles of the mature and immature oocyte (Sohel, Hoelker *et al.,* 2013). Though, some studies adduce it to Interswitch in genetic programme synchronous with follicular development (Di Pietro 2016, Andronico, Battaglia *et al.,* 2019). Whether the release exosome is related to the growing process of the oocyte is yet to unravel, considering the dynamics involved in oocyte maturation (Machtinger, Laurent *et al.,* 2015). In light of this, EVs extracted from cultured granulosa cells could be used to enhance the quality of the immature oocyte. Thus, improve IVF outcome in future. Similarly, emerging evidence has demonstrated discrepancy in the follicular fluid EV miRNA profile among the young and older female (da Silveira, de Andrade *et al.,* 2015). Suggesting its prognostic application in the context of ovarian ageing and possible therapeutic intervention to counter age impact on the quality of oocyte from the older female (da Silveira, Veeramachaneni *et al.,* 2012).

Extracellular Vesicles in Fallopian Tubes and Fertilization

Fertilization takes place when the capacitated spermatozoa fuse with the oocyte; haven attained acrosomal reaction (Machtinger, Laurent *et al.,* 2015). The process typically occurs in the ampullary region of the fallopian tube (Andronico, Battaglia *et al.,* 2019). It is characterized by cumulus expansion and the release of protease and hyaluronidase to aid sperm access the zona pellucida. And subsequently in the perivitelline space (Adam, Elfeky *et al.,* 2017). The activity highly regulated by the presence of intracellular Ca^{2+} modulated by $PMCA_4$ pump (Franz, Böing *et al.,* 2016). Emerging evidence has demonstrated the presence of abundant $PMCA_4$a, an active component of $PMCA_4$ in CD9 positive EVs in fallopian intraluminal fluid (Burns, Brooks *et al.,* 2014). Suggesting the regulatory potential of EVs during sperm capacitation and acrosome reaction in the process of fertilization (Simon, Greening *et al.,* 2018). Similarly, Mouse study further strengthen the central role of CD9 positive EVs as a point of attachment of the sperm and the oocyte at the perivitelline space (Barraud-Lange, Chalas Boissonnas *et al.,* 2012, Machtinger, Laurent *et al.,* 2016) corroborating the regulatory role of EVs in the fusion of the gametes (Jankovičová, Simon *et al.,* 2015). However, study with Mice and Hamster failed to justified this assertion (Machtinger, Laurent *et al.,* 2015).

Further research on the mechanism of sperm oocyte fusion leads to the concept of Izumo on the surface of the sperm and Juno on the oocyte (Gupta 2014). These proteins facilitate sperm oocyte fusion necessary for effective fertilization (Bianchi, Doe *et al.,* 2014). Interestingly, the highly expressed Juno on the oocyte shed off following fusion with Izumo in a process aim to prevent polyspermy (Bianchi and Wright 2014). The resulted early-stage embryo development occurs in the fallopian tube, and the impact of EVs protein on the quality of the early-stage embryo has been demonstrated in a Bovine in-vitro study (Lopera-Vasquez, Hamdi *et al.,* 2017). However, the discrepancy in the cargo protein content relative to the in-vivo model with HSPA8 and OVGP and only HSPA8 invitro require further study (Lopera-Vásquez, Hamdi *et al.,* 2016).

Extracellular Vesicles in the Uterus

The uterine cavity is lined by endometrial epithelial cells whose glandular component secretes a uterine fluid (Saadeldin, Oh *et al.,* 2015). The secreted fluid mostly influenced by the regulatory hormones base on the menstrual phase (Ng, Rome *et al.,* 2013). The uterine fluid characterized by sets of proteins and other modulatory factors serves as a background for maternal and embryo interactions (Kurian and Modi 2019). These interactions result in embryonic and placenta modulation, culminating in epigenetic impact on the foetus (Bidarimath, Khalaj *et al.,* 2017).

Studies have demonstrated the presence of EVs in the uterine fluid in both in-vivo and invitro models irrespective of the phase of the menstrual cycle (Burns, Brooks *et al.,* 2014) (Szekeres-Bartho, Šućurović *et al.,* 2018). These EVs carry specific miRNA with sorting mechanism aim to modulate the relevant genes required for embryo implantation (Kurian and Modi 2019). In contrast, the protein content and functions widely varied with the phase of the menstrual cycle influenced by the prevailing steroid hormones (Bidarimath, Khalaj *et al.,* 2017). Its protein profile conforms to the sorting mechanism relative to

the parent cells (Tannetta, Dragovic *et al.*, 2014). While it promotes endometrial proliferation in the estrogenic setting and facilitates coordination of extra-cellular matrix post-ovulatory under the influence of progesterone for embryo implantation (Kurian and Modi 2019).

Furthermore, in-vivo and in-vitro studies have demonstrated the impact of Endometrial EXOs on the adhesive potential of the trophoblastic cells through the uptake of HTR-8 mediated by Focal Adhesion Kinase (FAK) (Greening, Nguyen *et al.*, 2016). Thus, indicating that optimal Endometrial Receptivity may require well-coordinated endometrial EXOs and Trophoblast cells. Also, recent Bovine study has shown the discriminatory release of EVs proteins relative to pre-implantation and post-implantation (Kurian and Modi 2019). EVs proteins in the pre-implantation stage target genes for apoptosis while that of post-implantation are involved in cell adhesion in a synchronised manner with Endometrial cycle (Imakawa, Bai *et al.*, 2018). Indicating the paracrine approach of the Endometrial EVs in modulating Receptivity and implantation (Kusama, Nakamura *et al.*, 2018).

Extracellular Vesicles in Embryonic Development and Trophectoderms

The better outcome of Embryos culture in a group compare to a single culture, has been attributed to the autocrine and paracrine phenomenon; exhibited by the embryo in their micro-environment (Saadeldin, Oh *et al.*, 2015). Emerging evidence from Porcine study has shown that the interaction predicated on a wide range of vesicles of varied sizes depending on the stages of the embryo development (Kurian and Modi 2019). The vesicles cargo enriched with mRNA and specific proteins such as SOX2, KLF4 and OCT4 with significant number exhibiting CD9 exosome surface marker (Saadeldin, Kim *et al.*, 2014). However, no consensus on the exact impact of EVs on embryonic development, especially in human (Tannetta, Dragovic *et al.*, 2014, Machtinger, Laurent *et al.*, 2015).

The role of EVs from trophectoderm in the Materno-embryo crosstalk has been widely reported (Adam, Elfeky *et al.*, 2017, Es-Haghi, Godakumara *et al.*, 2019). Invitro Porcine studies have demonstrated the impact of trophectoderm EVs on the Endometrial angiogenesis in the course of implantation (Saadeldin, Kim *et al.*, 2014, Su, Liu *et al.*, 2014, Bidarimath, Khalaj *et al.*, 2017). The validity of the data in human, is still subject to debate in the literature (Machtinger, Laurent *et al.*, 2015). Also, the invasive tendency, of the trophoblast cells and its link with the released MVs from the inner cell mass to trophectoderm has been demonstrated in Mouse (Simón, Bolumar *et al.*, 2018). The mechanism involves Laminin and Fibronectin initiated cargo attachment to the integrin on the trophoblast (Qu, Qing *et al.*, 2017). Subsequently, trigger series of adhesive processes required for trophoblast transition (Bidarimath, Khalaj *et al.*, 2017). Furthermore, the introduction of the EVs into the Blastocoele has shown to facilitate optimal implantation process (Niakan and Eggan 2013). Suggesting the central role of EVs in the Embryo implantation. Though, the phenomenon cannot be extrapolated for human (Desrochers, Bordeleau *et al.*, 2016), considering the contrast orientation of the human Blastocyst relative to the site of implantation (Bidarimath, Khalaj *et al.*, 2017). However, a recent study with human trophectoderm associated EXOs demonstrated the modulatory role of the vesicles on the spiral artery invasion by vascular smooth muscles cells (Salomon, Yee *et al.*, 2014). A process required for optimal placenta development and successful pregnancy outcome (Adam, Elfeky *et al.*, 2017).

Extracellular Vesicles in Endometrial – Embryo Crosstalk

Optimal implantation is anchored on a robust, coordinated interaction between the maternal endometrium and the nascent embryo (Machtinger, Laurent *et al.*, 2015). Endometrial release of EVs with distinct cargo has been reported (Ng, Rome *et al.*, 2013). Suggesting EVs as the background with which the cross-talk is established (Saadeldin, Oh *et al.*, 2015). Evidence has shown that the EVs are endowed with miRNA profile in sorting mechanism aim at modulating genes programmed for implantation (Kurian and Modi 2019). Also, studies have demonstrated the presence of CD9 and CD63 exosome markers in EVs isolated from Luminal and glandular endometrial epithelial cells (Machtinger, Laurent *et al.*, 2016). Asserting to EVs as the platform for Endometrial communication with the embryo for implantation.

The Trophoblastic internalization of the EVs triggers its adhesion potential, mediated by the active FAK (Adam, Elfeky *et al.*, 2017). Thus, enhance successful implantation (Andronico, Battaglia *et al.*, 2019). In-vitro Mouse studies have demonstrated miR-30d in EVs associated with receptive phase of the Endometrial cycle and its impact on embryo adhesion when cultured with Embryo (Simon, Greening *et al.*, 2018, Balaguer, Moreno *et al.*, 2019). Supporting the maternal endometrial EVs transcriptomic effect on the nascent embryo (Liu, Niu *et al.*, 2016).

Similarly, the role of Trophoblast EVs in the Endometrial-Embryo Crosstalk has been reported (Saadeldin, Oh *et al.*, 2015, Es-Haghi, Godakumara *et al.*, 2019). Also, isolation of EVs from trophoblast has been demonstrated in an in-vitro model with trophoblastic cell line and placenta culture (Kurian and Modi 2019). While the in-vivo model has revealed its presence in the maternal blood (Adam, Elfeky *et al.*, 2017). The volume as well as its physiological impact influenced mainly by the gestational age and serum

level of oxygen and glucose respectively (Mitchell, Peiris *et al.*, 2015, Kurian and Modi 2019)

The cargo is characterized with miRNA and varieties of specific protein markers such as NKGD2 Ligand TRIAL, FasL as well as syncytin and TGF-B indicating its immune suppressive/ tolerance tendency (Saadeldin, Kim *et al.*, 2014). Thus, constitute qualities required to facilitate optimal growth of the embryonic allograft on the maternal endometrium (Mincheva-Nilsson and Baranov 2014, Tannetta, Dragovic *et al.*, 2014). Good knowledge of this mechanism could be of Diagnostic and therapeutic values in clinical settings like recurrent pregnancy loss often orchestrated by implantation failure.

Clinical Correlations and Future application

The concept of EVs as a platform for the exchange of genetic and specific protein between cells has become a new vista in reproductive biology. Given the propensity for its stability in biological fluids and host-recipient specificity, it has become a proposed veritable tool in the clinical entity, especially in the areas of diagnosis, prognosis and therapeutic purposes. The role of EVs in reproductive biology has been widely reported (Simón, Bolumar *et al.*, 2018). Thus, creating a background to better understand the physiological and pathological conditions such as implantation failure associated with this entity. As a result, the effort is beginning to focus on its potential application to improve reproductive success in the future (Machtinger, Laurent *et al.*, 2015). Evidence has shown its rapid cellular uptake when compared to other biomolecular carriers (Hood 2016, Lu and Huang 2020). Indicating the advantage of EVs in clinical applications. Therefore, Engineers EVs for tissue-specific delivery could be a source of therapeutic intervention in the context of gamete, embryo and implantation culminating in successful Assisted Reproduction Technology (ART) outcome (Machtinger, Laurent *et al.*, 2015).

CONCLUSION

The facts emanating from the literature suggest that EVs, with its prospective application in Reproductive biology, has prompted extensive research in the last decade. Its knowledge has broadened the scope of understanding the physiological and pathological scenarios involved in reproductive processes. As a potential biomarker, it has become a valuable tool for diagnosis prognostic and therapeutic purposes, especially in the context of reproductive processes. However, the challenges of standardizing the isolation, purification and analyzing EVs have remained a nightmare that needs to be surmounted.

In most conditions, the physiological roles of the EVs are poorly understood, but emerging evidence has demonstrated its impact in gamete development, fertilization and embryo implantation. Thus, it could serve as a platform to understand the mechanism of conception and implantation. By extension, define a therapeutic approach for women with recurrent pregnancy loss.

REFERENCES

- Abels, E. R. and X. O. Breakefield (2016). "Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake." Cell Mol Neurobiol 36(3): 301-312.
- Achikanu, C., V. Pendekanti, R. Teague and S. Publicover (2018). "Effects of pH manipulation, CatSper stimulation and Ca2+-store mobilization on [Ca2+]i and behaviour of human sperm." Hum Reprod 33(10): 1802-1811.
- Adam, S., O. Elfeky, V. Kinhal, S. Dutta, A. Lai, N. Jayabalan, Z. Nuzhat, C. Palma, G. E. Rice and C. Salomon (2017). "Fetal-maternal communication via extracellular vesicles–implications for complications of pregnancies." Placenta 54: 83-88.
- Adam, S., O. Elfeky, V. Kinhal, S. Dutta, A. Lai, N. Jayabalan, Z. Nuzhat, C. Palma, G. E. Rice and C. Salomon (2017). "Review: Fetal-maternal communication via extracellular vesicles – Implications for complications of pregnancies." Placenta 54: 83-88.
- Akers, J. C., D. Gonda, R. Kim, B. S. Carter and C. C. Chen (2013). "Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies." J Neurooncol 113(1): 1-11.
- Andronico, F., R. Battaglia, M. Ragusa, D. Barbagallo, M. Purrello and C. Di Pietro (2019). "Extracellular Vesicles in Human Oogenesis and Implantation." International Journal of Molecular Sciences 20(9): 2162.
- Andronico, F., R. Battaglia, M. Ragusa, D. Barbagallo, M. Purrello and C. Di Pietro (2019). "Extracellular Vesicles in Human Oogenesis and Implantation." Int J Mol Sci 20(9).
- Aplin, J. D. (2000). "The cell biological basis of human implantation." Baillieres Best Pract Res Clin Obstet Gynaecol 14(5): 757-764.
- Arraud, N., C. Gounou, D. Turpin and A. R. Brisson (2016). "Fluorescence triggering: A general strategy for enumerating and phenotyping extracellular vesicles by flow cytometry." Cytometry A 89(2): 184-195.
- Arraud, N., R. Linares, S. Tan, C. Gounou, J. M. Pasquet, S. Mornet and A. R. Brisson (2014). "Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration." J Thromb Haemost 12(5): 614-627.
- Asea, A., C. Jean-Pierre, P. Kaur, P. Rao, I. M. Linhares, D. Skupski and S. S. Witkin (2008). "Heat shock protein-containing exosomes in mid-

trimester amniotic fluids." J Reprod Immunol 79(1): 12-17.
- Ashcroft, B. A., J. de Sonneville, Y. Yuana, S. Osanto, R. Bertina, M. E. Kuil and T. H. Oosterkamp (2012). "Determination of the size distribution of blood microparticles directly in plasma using atomic force microscopy and microfluidics." Biomed Microdevices 14(4): 641-649.
- A Trigg, N., A. L Eamens and Brett Nixon (2019). "The contribution of epididymosomes to the sperm small RNA profile." 157: R209-R223.
- Balaguer, N., I. Moreno, M. Herrero, M. Gonzáléz-Monfort, F. Vilella and C. Simón (2019). "MicroRNA-30d deficiency during preconception affects endometrial receptivity by decreasing implantation rates and impairing fetal growth." Am J Obstet Gynecol 221(1): 46.e41-46.e16.
- Barraud-Lange, V., C. Chalas Boissonnas, C. Serres, J. Auer, A. Schmitt, B. Lefèvre, J. P. Wolf and A. Ziyyat (2012). "Membrane transfer from oocyte to sperm occurs in two CD9-independent ways that do not supply the fertilising ability of Cd9-deleted oocytes." Reproduction 144(1): 53-66.
- Baskaran, S., M. K. Panner Selvam and A. Agarwal (2020). "Exosomes of male reproduction." Adv Clin Chem 95: 149-163.
- Belleannée, C. (2015). "Extracellular microRNAs from the epididymis as potential mediators of cell-to-cell communication." Asian J Androl 17(5): 730-736.
- Benedikter, B. J., F. G. Bouwman, T. Vajen, A. C. A. Heinzmann, G. Grauls, E. C. Mariman, E. F. M. Wouters, P. H. Savelkoul, C. Lopez-Iglesias, R. R. Koenen, G. G. U. Rohde and F. R. M. Stassen (2017). "Ultrafiltration combined with size exclusion chromatography efficiently isolates extracellular vesicles from cell culture media for compositional and functional studies." Sci Rep 7(1): 15297.
- Bergsmedh, A., A. Szeles, M. Henriksson, A. Bratt, M. J. Folkman, A. L. Spetz and L. Holmgren (2001). "Horizontal transfer of oncogenes by uptake of apoptotic bodies." Proc Natl Acad Sci U S A 98(11): 6407-6411.
- Bianchi, E., B. Doe, D. Goulding and G. J. Wright (2014). "Juno is the egg Izumo receptor and is essential for mammalian fertilization." Nature 508(7497): 483-487.
- Bianchi, E. and G. J. Wright (2014). "Izumo meets Juno: preventing polyspermy in fertilization." Cell Cycle 13(13): 2019-2020.
- Bidarimath, M., K. Khalaj, R. T. Kridli, F. W. Kan, M. Koti and C. Tayade (2017). "Extracellular vesicle mediated intercellular communication at the porcine maternal-fetal interface: A new paradigm for conceptus-endometrial cross-talk." Sci Rep 7: 40476.
- Bidarimath, M., K. Khalaj, R. T. Kridli, F. W. K. Kan, M. Koti and C. Tayade (2017). "Extracellular vesicle mediated intercellular communication at the porcine maternal-fetal interface: A new paradigm for conceptus-endometrial cross-talk." Scientific Reports 7(1): 40476.
- Boukouris, S. and S. Mathivanan (2015). "Exosomes in bodily fluids are a highly stable resource of disease biomarkers." PROTEOMICS - Clinical Applications 9(3-4): 358-367.
- Brisson, A. R., S. Tan, R. Linares, C. Gounou and N. Arraud (2017). "Extracellular vesicles from activated platelets: a semiquantitative cryo-electron microscopy and immuno-gold labeling study." Platelets 28(3): 263-271.
- Brown, P. N. and H. Yin (2017). "Polymer-Based Purification of Extracellular Vesicles." Methods Mol Biol 1660: 91-103.
- Brownlee, Z., K. D. Lynn, P. E. Thorpe and A. J. Schroit (2014). "A novel "salting-out" procedure for the isolation of tumor-derived exosomes." J Immunol Methods 407: 120-126.
- Burden, H. P., C. H. Holmes, R. Persad and K. Whittington (2006). "Prostasomes--their effects on human male reproduction and fertility." Hum Reprod Update 12(3): 283-292.
- Burns, G., K. Brooks, M. Wildung, R. Navakanitworakul, L. K. Christenson and T. E. Spencer (2014). "Extracellular Vesicles in Luminal Fluid of the Ovine Uterus." 9(3): e90913.
- Burns, G., K. Brooks, M. Wildung, R. Navakanitworakul, L. K. Christenson and T. E. Spencer (2014). "Extracellular Vesicles in Luminal Fluid of the Ovine Uterus." PLoS ONE 9(3): e90913.
- Böing, A. N., E. van der Pol, A. E. Grootemaat, F. A. Coumans, A. Sturk and R. Nieuwland (2014). "Single-step isolation of extracellular vesicles by size-exclusion chromatography." J Extracell Vesicles 3.
- Caballero, J. N., G. Frenette, C. Belleannée and R. Sullivan (2013). "CD9-positive microvesicles mediate the transfer of molecules to Bovine Spermatozoa during epididymal maturation." PLoS One 8(6): e65364.
- Carlomagno, G., M. Minini, M. Tilotta and V. Unfer (2018). "From Implantation to Birth: Insight into Molecular Melatonin Functions." Int J Mol Sci 19(9).
- Carnino, J. M., H. Lee and Y. Jin (2019). "Isolation and characterization of extracellular vesicles from Broncho-alveolar lavage fluid: a review and comparison of different methods." Respiratory research 20(1): 240.
- Cecilia Lässer, Vesta S. Alikhani, Karin Ekström, Maria Eldh, Patricia T. Paredes, Apostolos Bossios, Margareta Sjöstrand, Susanne Gabrielsson, Jan Lötvall and Hadi Valadi (2011). "Human saliva, plasma and breast milk exosomes contain

RNA: uptake by macrophages." Journal of Translational Medicine 9(1): 9.

- Chen, C., J. Skog, C. H. Hsu, R. T. Lessard, L. Balaj, T. Wurdinger, B. S. Carter, X. O. Breakefield, M. Toner and D. Irimia (2010). "Microfluidic isolation and transcriptome analysis of serum microvesicles." Lab Chip 10(4): 505-511.
- Chen, M., R. Xu, H. Ji, D. W. Greening, A. Rai, K. Izumikawa, H. Ishikawa, N. Takahashi and R. J. Simpson (2016). "Transcriptome and long noncoding RNA sequencing of three extracellular vesicle subtypes released from the human colon cancer LIM1863 cell line." Sci Rep 6: 38397.
- Chiang, C. Y. and C. Chen (2019). "Toward characterizing extracellular vesicles at a single-particle level." J Biomed Sci 26(1): 9.
- Cho, C. (2012). "Testicular and epididymal ADAMs: expression and function during fertilization." Nat Rev Urol 9(10): 550-560.
- Christianson, H. C. and M. Belting (2014). "Heparan sulfate proteoglycan as a cell-surface endocytosis receptor." Matrix Biol 35: 51-55.
- Christianson, H. C., K. J. Svensson, T. H. van Kuppevelt, J. P. Li and M. Belting (2013). "Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity." Proc Natl Acad Sci U S A 110(43): 17380-17385.
- Cizmar, P. and Y. Yuana (2017). "Detection and Characterization of Extracellular Vesicles by Transmission and Cryo-Transmission Electron Microscopy." Methods Mol Biol 1660: 221-232.
- Colombo, M., C. Moita, G. van Niel, J. Kowal, J. Vigneron, P. Benaroch, N. Manel, L. F. Moita, C. Théry and G. Raposo (2013). "Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles." J Cell Sci 126(Pt 24): 5553-5565.
- Colombo, M., G. Raposo and C. Théry (2014). "Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles." Annu Rev Cell Dev Biol 30: 255-289.
- Crowell, A. M., M. J. Wall and A. A. Doucette (2013). "Maximizing recovery of water-soluble proteins through acetone precipitation." Anal Chim Acta 796: 48-54.
- Cvjetkovic, A., J. Lötvall and C. Lässer (2014). "The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles." J Extracell Vesicles 3.
- D'Amours, O., G. Frenette, L. J. Bordeleau, N. Allard, P. Leclerc, P. Blondin and R. Sullivan (2012). "Epididymosomes transfer epididymal sperm binding protein 1 (ELSPBP1) to dead spermatozoa during epididymal transit in bovine." Biol Reprod 87(4): 94.
- D'Souza-Schorey, C. and J. W. Clancy (2012). "Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers." Genes & Development 26(12): 1287-1299.
- da Silveira, J. C., G. M. de Andrade, M. F. Nogueira, F. V. Meirelles and F. Perecin (2015). "Involvement of miRNAs and Cell-Secreted Vesicles in Mammalian Ovarian Antral Follicle Development." Reprod Sci 22(12): 1474-1483.
- da Silveira, J. C., D. N. Veeramachaneni, Q. A. Winger, E. M. Carnevale and G. J. Bouma (2012). "Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle." Biol Reprod 86(3): 71.
- De Jong, O. G., B. W. Van Balkom, R. M. Schiffelers, C. V. Bouten and M. C. Verhaar (2014). "Extracellular vesicles: potential roles in regenerative medicine." Frontiers in immunology 5: 608.
- De Toro, J., L. Herschlik, C. Waldner and C. Mongini (2015). "Emerging roles of exosomes in normal and pathological conditions: new insights for diagnosis and therapeutic applications." Front Immunol 6: 203.
- Deregibus, M. C., F. Figliolini, S. D'Antico, P. M. Manzini, C. Pasquino, M. De Lena, C. Tetta, M. F. Brizzi and G. Camussi (2016). "Charge-based precipitation of extracellular vesicles." Int J Mol Med 38(5): 1359-1366.
- Desrochers, L. M., F. Bordeleau, C. A. Reinhart-King, R. A. Cerione and M. A. Antonyak (2016). "Microvesicles provide a mechanism for intercellular communication by embryonic stem cells during embryo implantation." Nat Commun 7: 11958.
- Di Pietro, C. (2016). "Exosome-mediated communication in the ovarian follicle." J Assist Reprod Genet 33(3): 303-311.
- Elmore, S. (2007). "Apoptosis: a review of programmed cell death." Toxicol Pathol 35(4): 495-516.
- Es-Haghi, M., K. Godakumara, A. Häling, F. Lättekivi, A. Lavrits, J. Viil, A. Andronowska, T. Nafee, V. James, Ü. Jaakma, A. Salumets and A. Fazeli (2019). "Specific trophoblast transcripts transferred by extracellular vesicles affect gene expression in endometrial epithelial cells and may have a role in embryo-maternal crosstalk." Cell Commun Signal 17(1): 146.
- Ford, T., J. Graham and D. Rickwood (1994). "Iodixanol: a nonionic iso-osmotic centrifugation medium for the formation of self-generated gradients." Anal Biochem 220(2): 360-366.
- Franz, C., A. N. Böing, M. Montag, T. Strowitzki, U. R. Markert, S. Mastenbroek, R. Nieuwland and B. Toth (2016). "Extracellular vesicles in human follicular fluid do not promote coagulation." Reproductive BioMedicine Online 33(5): 652-655.

- Frenette, G., J. Girouard, O. D'Amours, N. Allard, L. Tessier and R. Sullivan (2010). "Characterization of two distinct populations of epididymosomes collected in the intraluminal compartment of the bovine cauda epididymis." Biol Reprod 83(3): 473-480.
- Gadella, B. M. (2012). "Dynamic regulation of sperm interactions with the zona pellucida prior to and after fertilisation." Reprod Fertil Dev 25(1): 26-37.
- Gholizadeh, S., M. Shehata Draz, M. Zarghooni, A. Sanati-Nezhad, S. Ghavami, H. Shafiee and M. Akbari (2017). "Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: Current status and future directions." Biosens Bioelectron 91: 588-605.
- Greening, D. W., H. P. Nguyen, K. Elgass, R. J. Simpson and L. A. Salamonsen (2016). "Human Endometrial Exosomes Contain Hormone-Specific Cargo Modulating Trophoblast Adhesive Capacity: Insights into Endometrial-Embryo Interactions." Biol Reprod 94(2): 38.
- Greening, D. W., R. Xu, H. Ji, B. J. Tauro and R. J. Simpson (2015). "A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods." Methods Mol Biol 1295: 179-209.
- Gupta, S. K. (2014). "Unraveling the intricacies of mammalian fertilization." Asian J Androl 16(6): 801-802.
- György, B., M. E. Hung, X. O. Breakefield and J. N. Leonard (2015). "Therapeutic applications of extracellular vesicles: clinical promise and open questions." Annual review of pharmacology and toxicology 55: 439-464.
- György, B., M. E. Hung, X. O. Breakefield and J. N. Leonard (2015). "Therapeutic Applications of Extracellular Vesicles: Clinical Promise and Open Questions." Annual Review of Pharmacology and Toxicology 55(1): 439-464.
- György, B., T. G. Szabó, M. Pásztói, Z. Pál, P. Misják, B. Aradi, V. László, É. Pállinger, E. Pap, Á. Kittel, G. Nagy, A. Falus and E. I. Buzás (2011). "Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles." Cellular and Molecular Life Sciences 68(16): 2667-2688.
- Gámez-Valero, A., M. Monguió-Tortajada, L. Carreras-Planella, M. Franquesa, K. Beyer and F. E. Borràs (2016). "Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents." Sci Rep 6: 33641.
- Heath, N., L. Grant, T. M. De Oliveira, R. Rowlinson, X. Osteikoetxea, N. Dekker and R. Overman (2018). "Rapid isolation and enrichment of extracellular vesicle preparations using anion exchange chromatography." Scientific Reports 8(1).
- Hell, L., L. Wisgrill, C. Ay, A. Spittler, M. Schwameis, B. Jilma, I. Pabinger, P. Altevogt and J. Thaler (2017). "Procoagulant extracellular vesicles in amniotic fluid." Transl Res 184: 12-20.e11.
- Hood, J. L. (2016). "Post isolation modification of exosomes for nanomedicine applications." Nanomedicine (Lond) 11(13): 1745-1756.
- Höög, J. L. and J. Lötvall (2015). "Diversity of extracellular vesicles in human ejaculates revealed by cryo-electron microscopy." J Extracell Vesicles 4: 28680.
- Im, H., H. Shao, R. Weissleder, C. M. Castro and H. Lee (2015). "Nano-plasmonic exosome diagnostics." Expert Rev Mol Diagn 15(6): 725-733.
- Imakawa, K., R. Bai and K. Kusama (2018). "Integration of molecules to construct the processes of conceptus implantation to the maternal endometrium." J Anim Sci 96(7): 3009-3021.
- Jamaludin, N. A., L. M. Thurston, K. J. Witek, A. Meikle, S. Basatvat, S. Elliott, S. Hunt, A. Andronowska and A. Fazeli (2019). "Efficient isolation, biophysical characterisation and molecular composition of extracellular vesicles secreted by primary and immortalised cells of reproductive origin." Theriogenology 135: 121-137.
- Jankovičová, J., M. Simon, J. Antalíková, P. Cupperová and K. Michalková (2015). "Role of tetraspanin CD9 molecule in fertilization of mammals." Physiol Res 64(3): 279-293.
- Jella, K. K., T. H. Nasti, Z. Li, S. R. Malla, Z. S. Buchwald and M. K. Khan (2018). "Exosomes, Their Biogenesis and Role in Inter-Cellular Communication, Tumor Microenvironment and Cancer Immunotherapy." Vaccines (Basel) 6(4).
- Jeon, H., S. K. Kang and M. S. Lee (2020). "Effects of different separation methods on the physical and functional properties of extracellular vesicles." PLoS One 15(7): e0235793.
- Jeppesen, D. K., M. L. Hvam, B. Primdahl-Bengtson, A. T. Boysen, B. Whitehead, L. Dyrskjøt, T. F. Orntoft, K. A. Howard and M. S. Ostenfeld (2014). "Comparative analysis of discrete exosome fractions obtained by differential centrifugation." J Extracell Vesicles 3: 25011.
- Johansson, S. M., C. Admyre, A. Scheynius and S. Gabrielsson (2008). "Different types of in vitro generated human monocyte-derived dendritic cells release exosomes with distinct phenotypes." Immunology 123(4): 491-499.
- Joyce, D. P., M. J. Kerin and R. M. Dwyer (2016). "Exosome-encapsulated microRNAs as circulating biomarkers for breast cancer." Int J Cancer 139(7): 1443-1448.

- Kerr, J. F., A. H. Wyllie and A. R. Currie (1972). "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics." Br J Cancer 26(4): 239-257.
- Konoshenko, M. Y., E. A. Lekchnov, A. V. Vlassov and P. P. Laktionov (2018). "Isolation of Extracellular Vesicles: General Methodologies and Latest Trends." Biomed Res Int 2018: 8545347.
- Konoshenko, M. Y., E. A. Lekchnov, A. V. Vlassov and P. P. Laktionov (2018). "Isolation of extracellular vesicles: general methodologies and latest trends." BioMed research international 2018.
- Kowal, J., G. Arras, M. Colombo, M. Jouve, J. P. Morath, B. Primdal-Bengtson, F. Dingli, D. Loew, M. Tkach and C. Théry (2016). "Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes." Proc Natl Acad Sci U S A 113(8): E968-977.
- Kurian, N. K. and D. Modi (2019). "Extracellular vesicle mediated embryo-endometrial cross talk during implantation and in pregnancy." J Assist Reprod Genet 36(2): 189-198.
- Kusama, K., K. Nakamura, R. Bai, K. Nagaoka, T. Sakurai and K. Imakawa (2018). "Intrauterine exosomes are required for bovine conceptus implantation." Biochem Biophys Res Commun 495(1): 1370-1375.
- Kwizera, E. A., R. O'Connor, V. Vinduska, M. Williams, E. R. Butch, S. E. Snyder, X. Chen and X. Huang (2018). "Molecular Detection and Analysis of Exosomes Using Surface-Enhanced Raman Scattering Gold Nanorods and a Miniaturized Device." Theranostics 8(10): 2722-2738.
- Lamichhane, T. N., S. Sokic, J. S. Schardt, R. S. Raiker, J. W. Lin and S. M. Jay (2015). "Emerging Roles for Extracellular Vesicles in Tissue Engineering and Regenerative Medicine." Tissue Engineering Part B: Reviews 21(1): 45-54.
- Lane, R. E., D. Korbie, M. Trau and M. M. Hill (2017). "Purification Protocols for Extracellular Vesicles." Methods Mol Biol 1660: 111-130.
- Lerner, N., S. Avissar and E. Beit-Yannai (2017). "Extracellular vesicles mediate signaling between the aqueous humor producing and draining cells in the ocular system." PLoS One 12(2): e0171153.
- Li, P., M. Kaslan, S. H. Lee, J. Yao and Z. Gao (2017). "Progress in Exosome Isolation Techniques." Theranostics 7(3): 789-804.
- Li, X., A. L. Corbett, E. Taatizadeh, N. Tasnim, J. P. Little, C. Garnis, M. Daugaard, E. Guns, M. Hoorfar and I. T. S. Li (2019). "Challenges and opportunities in exosome research-Perspectives from biology, engineering, and cancer therapy." APL Bioeng 3(1): 011503.
- Libregts, S., G. J. A. Arkesteijn, A. Németh, E. N. M. Nolte-'t Hoen and M. H. M. Wauben (2018). "Flow cytometric analysis of extracellular vesicle subsets in plasma: impact of swarm by particles of non-interest." J Thromb Haemost 16(7): 1423-1436.
- Liu, W., Z. Niu, Q. Li, R. T. Pang, P. C. Chiu and W. S. Yeung (2016). "MicroRNA and Embryo Implantation." Am J Reprod Immunol 75(3): 263-271.
- Liu, Y. and Q. Chen (2018). "150 years of Darwin's theory of intercellular flow of hereditary information." Nature Reviews Molecular Cell Biology 19(12): 749-750.
- Lopera-Vasquez, R., M. Hamdi, V. Maillo, A. Gutierrez-Adan, P. Bermejo-Alvarez, M. Ramírez, M. Yáñez-Mó and D. Rizos (2017). "Effect of bovine oviductal extracellular vesicles on embryo development and quality in vitro." Reproduction 153(4): 461-470.
- Lopera-Vásquez, R., M. Hamdi, B. Fernandez-Fuertes, V. Maillo, P. Beltrán-Breña, A. Calle, A. Redruello, S. López-Martín, A. Gutierrez-Adán, M. Yañez-Mó, M. Ramirez and D. Rizos (2016). "Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality." PLoS One 11(2): e0148083.
- Lu, M. and Y. Huang (2020). "Bioinspired exosome-like therapeutics and delivery nanoplatforms." Biomaterials 242: 119925.
- Lucchetti, D., A. Fattorossi and A. Sgambato (2019). "Extracellular Vesicles in Oncology: Progress and Pitfalls in the Methods of Isolation and Analysis." Biotechnol J 14(1): e1700716.
- Ludwig, N., T. L. Whiteside and T. E. Reichert (2019). "Challenges in Exosome Isolation and Analysis in Health and Disease." Int J Mol Sci 20(19).
- Lässer, C., V. Seyed Alikhani, K. Ekström, M. Eldh, P. Torregrosa Paredes, A. Bossios, M. Sjöstrand, S. Gabrielsson, J. Lötvall and H. Valadi (2011). "Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages." Journal of Translational Medicine 9(1): 9.
- Machtinger, R., L. C. Laurent and A. A. Baccarelli (2015). "Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation." Human Reproduction Update: dmv055.
- Machtinger, R., L. C. Laurent and A. A. Baccarelli (2015). "Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation." Human Reproduction Update 22(2): 182-193.
- Machtinger, R., L. C. Laurent and A. A. Baccarelli (2016). "Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation." Hum Reprod Update 22(2): 182-193.
- Makler, A. and W. Asghar (2020). "Exosomal biomarkers for cancer diagnosis and patient monitoring." Expert Rev Mol Diagn 20(4): 387-400.

- Margolis, L. and Y. Sadovsky (2019). "The biology of extracellular vesicles: The known unknowns." PLOS Biology 17(7): e3000363.
- Mathivanan, S., H. Ji and R. J. Simpson (2010). "Exosomes: extracellular organelles important in intercellular communication." J Proteomics 73(10): 1907-1920.
- Menck, K., C. Scharf, A. Bleckmann, L. Dyck, U. Rost, D. Wenzel, V. M. Dhople, L. Siam, T. Pukrop, C. Binder and F. Klemm (2015). "Tumor-derived microvesicles mediate human breast cancer invasion through differentially glycosylated EMMPRIN." J Mol Cell Biol 7(2): 143-153.
- Mincheva-Nilsson, L. and V. Baranov (2014). "Placenta-derived exosomes and syncytiotrophoblast microparticles and their role in human reproduction: immune modulation for pregnancy success." Am J Reprod Immunol 72(5): 440-457.
- Mitchell, M. D., H. N. Peiris, M. Kobayashi, Y. Q. Koh, G. Duncombe, S. E. Illanes, G. E. Rice and C. Salomon (2015). "Placental exosomes in normal and complicated pregnancy." Am J Obstet Gynecol 213(4 Suppl): S173-181.
- Mol, E. A., M. J. Goumans, P. A. Doevendans, J. P. G. Sluijter and P. Vader (2017). "Higher functionality of extracellular vesicles isolated using size-exclusion chromatography compared to ultracentrifugation." Nanomedicine 13(6): 2061-2065.
- Momen-Heravi, F., L. Balaj, S. Alian, P. Y. Mantel, A. E. Halleck, A. J. Trachtenberg, C. E. Soria, S. Oquin, C. M. Bonebreak, E. Saracoglu, J. Skog and W. P. Kuo (2013). "Current methods for the isolation of extracellular vesicles." Biol Chem 394(10): 1253-1262.
- Monguió-Tortajada, M., C. Gálvez-Montón, A. Bayes-Genis, S. Roura and F. E. Borràs (2019). "Extracellular vesicle isolation methods: rising impact of size-exclusion chromatography." Cell Mol Life Sci 76(12): 2369-2382.
- Muralidharan-Chari, V., J. Clancy, C. Plou, M. Romao, P. Chavrier, G. Raposo and C. D'Souza-Schorey (2009). "ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles." Curr Biol 19(22): 1875-1885.
- Nawaz, M., F. Fatima, I. Nazarenko, K. Ekström, I. Murtaza, M. Anees, A. Sultan, L. Neder, G. Camussi and H. Valadi (2016). "Extracellular vesicles in ovarian cancer: applications to tumor biology, immunotherapy and biomarker discovery." Expert review of proteomics 13(4): 395-409.
- Nawaz, M., F. Fatima, K. C. Vallabhaneni, P. Penfornis, H. Valadi, K. Ekström, S. Kholia, J. D. Whitt, J. D. Fernandes and R. Pochampally (2016). "Extracellular vesicles: evolving factors in stem cell biology." Stem cells international 2016.
- Ng, Y. H., S. Rome, A. Jalabert, A. Forterre, H. Singh, C. L. Hincks and L. A. Salamonsen (2013). "Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for embryo-endometrial cross talk at implantation." PLoS One 8(3): e58502.
- Niakan, K. K. and K. Eggan (2013). "Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse." Dev Biol 375(1): 54-64.
- Nixon, B., G. N. De Iuliis, M. D. Dun, W. Zhou, N. A. Trigg and A. L. Eamens (2019). "Profiling of epididymal small non-protein-coding RNAs." Andrology 7(5): 669-680.
- Ogawa, Y., Y. Miura, A. Harazono, M. Kanai-Azuma, Y. Akimoto, H. Kawakami, T. Yamaguchi, T. Toda, T. Endo, M. Tsubuki and R. Yanoshita (2011). "Proteomic Analysis of Two Types of Exosomes in Human Whole Saliva." Biological & Pharmaceutical Bulletin 34(1): 13-23.
- Oh, J. S., C. Han and C. Cho (2009). "ADAM7 is associated with epididymosomes and integrated into sperm plasma membrane." Mol Cells 28(5): 441-446.
- Onódi, Z., C. Pelyhe, C. Terézia Nagy, G. B. Brenner, L. Almási, Á. Kittel, M. Manček-Keber, P. Ferdinandy, E. I. Buzás and Z. Giricz (2018). "Isolation of High-Purity Extracellular Vesicles by the Combination of Iodixanol Density Gradient Ultracentrifugation and Bind-Elute Chromatography From Blood Plasma." Front Physiol 9: 1479.
- Ouyang, Y., J. F. Mouillet, C. B. Coyne and Y. Sadovsky (2014). "Review: placenta-specific microRNAs in exosomes - good things come in nano-packages." Placenta 35 Suppl: S69-73.
- Padda, R. S., F. K. Deng, S. I. Brett, C. N. Biggs, P. N. Durfee, C. J. Brinker, K. C. Williams and H. S. Leong (2019). "Nanoscale flow cytometry to distinguish subpopulations of prostate extracellular vesicles in patient plasma." Prostate 79(6): 592-603.
- Park, K. H., B. J. Kim, J. Kang, T. S. Nam, J. M. Lim, H. T. Kim, J. K. Park, Y. G. Kim, S. W. Chae and U. H. Kim (2011). "Ca2+ signaling tools acquired from prostasomes are required for progesterone-induced sperm motility." Sci Signal 4(173): ra31.
- Parolini, I., C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. De Milito, C. Coscia, E. Iessi, M. Logozzi, A. Molinari, M. Colone, M. Tatti, M. Sargiacomo and S. Fais (2009). "Microenvironmental pH is a key factor for exosome traffic in tumor cells." J Biol Chem 284(49): 34211-34222.
- Pavani, K. C., C. Alminana, E. Wydooghe, M. Catteeuw, M. A. Ramírez, P. Mermillod, D. Rizos and A. Van Soom (2017). "Emerging role of extracellular vesicles in communication of

preimplantation embryos in vitro." Reproduction, Fertility and Development 29(1): 66.
- Peterson, M. F., N. Otoc, J. K. Sethi, A. Gupta and T. J. Antes (2015). "Integrated systems for exosome investigation." Methods 87: 31-45.
- Pocsfalvi, G., C. Stanly, I. Fiume and K. Vékey (2016). "Chromatography and its hyphenation to mass spectrometry for extracellular vesicle analysis." J Chromatogr A 1439: 26-41.
- Popovic, M., E. Mazzega, B. Toffoletto and A. de Marco (2018). "Isolation of anti-extra-cellular vesicle single-domain antibodies by direct panning on vesicle-enriched fractions." Microb Cell Fact 17(1): 6.
- Purushothaman, A. (2019). "Exosomes from Cell Culture-Conditioned Medium: Isolation by Ultracentrifugation and Characterization." Methods Mol Biol 1952: 233-244.
- Qu, P., S. Qing, R. Liu, H. Qin, W. Wang, F. Qiao, H. Ge, J. Liu, Y. Zhang, W. Cui and Y. Wang (2017). "Effects of embryo-derived exosomes on the development of bovine cloned embryos." PLoS One 12(3): e0174535.
- Raposo, G. and W. Stoorvogel (2013). "Extracellular vesicles: Exosomes, microvesicles, and friends." The Journal of Cell Biology 200(4): 373-383.
- Reilly, J. N., E. A. McLaughlin, S. J. Stanger, A. L. Anderson, K. Hutcheon, K. Church, B. P. Mihalas, S. Tyagi, J. E. Holt, A. L. Eamens and B. Nixon (2016). "Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome." Sci Rep 6: 31794.
- Reiner, A. T., K. W. Witwer, B. W. M. Van Balkom, J. De Beer, C. Brodie, R. L. Corteling, S. Gabrielsson, M. Gimona, A. G. Ibrahim, D. De Kleijn, C. P. Lai, J. Lötvall, H. A. Del Portillo, I. G. Reischl, M. Riazifar, C. Salomon, H. Tahara, W. S. Toh, M. H. M. Wauben, V. K. Yang, Y. Yang, R. W. Y. Yeo, H. Yin, B. Giebel, E. Rohde and S. K. Lim (2017). "Concise Review: Developing Best-Practice Models for the Therapeutic Use of Extracellular Vesicles." STEM CELLS Translational Medicine 6(8): 1730-1739.
- Robert Sullivan (2016). "Epididymosomes Role of extracellular microvesicles in sperm maturation." 8: 106-114.
- Robert Sullivan and Fabrice Saez (2013). "Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology." 146: R21-R35.
- Ronquist, G. (2015). "Prostasomes: Their Characterisation: Implications for Human Reproduction: Prostasomes and Human Reproduction." Adv Exp Med Biol 868: 191-209.
- Ronquist, G. and B. O. Nilsson (2004). "The Janus-faced nature of prostasomes: their pluripotency favours the normal reproductive process and malignant prostate growth." Prostate Cancer Prostatic Dis 7(1): 21-31.
- S, E. L. A., I. Mäger, X. O. Breakefield and M. J. Wood (2013). "Extracellular vesicles: biology and emerging therapeutic opportunities." Nat Rev Drug Discov 12(5): 347-357.
- Saadeldin, I. M., S. J. Kim, Y. B. Choi and B. C. Lee (2014). "Improvement of cloned embryos development by co-culturing with parthenotes: a possible role of exosomes/microvesicles for embryos paracrine communication." Cell Reprogram 16(3): 223-234.
- Saadeldin, I. M., H. J. Oh and B. C. Lee (2015). "Embryonic-maternal cross-talk via exosomes: potential implications." Stem cells and cloning : advances and applications 8: 103-107.
- Salomon, C., S. W. Yee, M. D. Mitchell and G. E. Rice (2014). "The possible role of extravillous trophoblast-derived exosomes on the uterine spiral arterial remodeling under both normal and pathological conditions." Biomed Res Int 2014: 693157.
- Santana, S. M., M. A. Antonyak, R. A. Cerione and B. J. Kirby (2014). "Microfluidic isolation of cancer-cell-derived microvesicles from hetergeneous extracellular shed vesicle populations." Biomed Microdevices 16(6): 869-877.
- Serrano-Pertierra, E., M. Oliveira-Rodríguez, M. Rivas, P. Oliva, J. Villafani, A. Navarro, M. Blanco-López and E. Cernuda-Morollón (2019). "Characterization of Plasma-Derived Extracellular Vesicles Isolated by Different Methods: A Comparison Study." Bioengineering 6(1): 8.
- Sharma, U., F. Sun, C. C. Conine, B. Reichholf, S. Kukreja, V. A. Herzog, S. L. Ameres and O. J. Rando (2018). "Small RNAs Are Trafficked from the Epididymis to Developing Mammalian Sperm." Dev Cell 46(4): 481-494.e486.
- Simon, C., D. W. Greening, D. Bolumar, N. Balaguer, L. A. Salamonsen and F. Vilella (2018). "Extracellular Vesicles in Human Reproduction in Health and Disease." Endocrine Reviews 39(3): 292-332.
- Simons, M. and G. Raposo (2009). "Exosomes--vesicular carriers for intercellular communication." Curr Opin Cell Biol 21(4): 575-581.
- Simón, C., D. Bolumar, N. Balaguer, L. Salamonsen and F. Vilella (2018). "Extracellular Vesicles in Human Reproduction in Health and Disease." Endocrine Reviews 39.
- Singh, H., L. Nardo, S. J. Kimber and J. D. Aplin (2010). "Early stages of implantation as revealed by an in vitro model." Reproduction 139(5): 905-914.
- Sohel, M. M., M. Hoelker, S. S. Noferesti, D. Salilew-Wondim, E. Tholen, C. Looft, F. Rings, M. J. Uddin, T. E. Spencer, K. Schellander and D.

Tesfaye (2013). "Exosomal and Non-Exosomal Transport of Extra-Cellular microRNAs in Follicular Fluid: Implications for Bovine Oocyte Developmental Competence." PLoS One 8(11): e78505.

- Soo, C. Y., Y. Song, Y. Zheng, E. C. Campbell, A. C. Riches, F. Gunn-Moore and S. J. Powis (2012). "Nanoparticle tracking analysis monitors microvesicle and exosome secretion from immune cells." Immunology 136(2): 192-197.
- Sostaric, E., M. Aalberts, B. M. Gadella and T. A. Stout (2008). "The roles of the epididymis and prostasomes in the attainment of fertilizing capacity by stallion sperm." Anim Reprod Sci 107(3-4): 237-248.
- Srinivasan, S., A. Yeri, P. S. Cheah, A. Chung, K. Danielson, P. De Hoff, J. Filant, C. D. Laurent, L. D. Laurent, R. Magee, C. Moeller, V. L. Murthy, P. Nejad, A. Paul, I. Rigoutsos, R. Rodosthenous, R. V. Shah, B. Simonson, C. To, D. Wong, I. K. Yan, X. Zhang, L. Balaj, X. O. Breakefield, G. Daaboul, R. Gandhi, J. Lapidus, E. Londin, T. Patel, R. L. Raffai, A. K. Sood, R. P. Alexander, S. Das and L. C. Laurent (2019). "Small RNA Sequencing across Diverse Biofluids Identifies Optimal Methods for exRNA Isolation." Cell 177(2): 446-462.e416.
- Su, L., R. Liu, W. Cheng, M. Zhu, X. Li, S. Zhao and M. Yu (2014). "Expression patterns of microRNAs in porcine endometrium and their potential roles in embryo implantation and placentation." PloS one 9(2): e87867.
- Sullivan, R. (2015). "Epididymosomes: a heterogeneous population of microvesicles with multiple functions in sperm maturation and storage." Asian J Androl 17(5): 726-729.
- Sullivan, R. and F. Saez (2013). "Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology." REPRODUCTION 146(1): R21-R35.
- Sullivan, R. and F. Saez (2013). "Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology." Reproduction 146(1): R21-35.
- Sun, X., X. Ma, X. Yang and X. Zhang (2019). "Exosomes and Female Infertility." Curr Drug Metab 20(10): 773-780.
- Sunkara, V., H. K. Woo and Y. K. Cho (2016). "Emerging techniques in the isolation and characterization of extracellular vesicles and their roles in cancer diagnostics and prognostics." Analyst 141(2): 371-381.
- Szatanek, R., M. Baj-Krzyworzeka, J. Zimoch, M. Lekka, M. Siedlar and J. Baran (2017). "The Methods of Choice for Extracellular Vesicles (EVs) Characterization." Int J Mol Sci 18(6).
- Szekeres-Bartho, J., S. Šućurović and B. Mulac-Jeričević (2018). "The role of extracellular vesicles and PIBF in embryo-maternal immune-interactions." Frontiers in immunology 9: 2890.
- Sáenz-Cuesta, M. (2015). "Methods for extracellular vesicles isolation in a hospital setting." Frontiers in Immunology 6.
- T. Nguyen, H. P., R. J. Simpson, L. A. Salamonsen and D. W. Greening (2016). "Extracellular Vesicles in the Intrauterine Environment: Challenges and Potential Functions." 95: 109-109.
- Takizawa, F., S. Tsuji and S. Nagasawa (1996). "Enhancement of macrophage phagocytosis upon iC3b deposition on apoptotic cells." FEBS Lett 397(2-3): 269-272.
- Tannetta, D., R. Dragovic, Z. Alyahyaei and J. Southcombe (2014). "Extracellular vesicles and reproduction-promotion of successful pregnancy." Cellular & molecular immunology 11(6): 548-563.
- Tannetta, D., R. Dragovic, Z. Alyahyaei and J. Southcombe (2014). "Extracellular vesicles and reproduction–promotion of successful pregnancy." Cellular & Molecular Immunology 11(6): 548-563.
- Taylor, D. D. and C. Gercel-Taylor (2013). "The origin, function, and diagnostic potential of RNA within extracellular vesicles present in human biological fluids." Frontiers in genetics 4: 142.
- Taylor, D. D. and C. Gercel-Taylor (2013). "The origin, function, and diagnostic potential of RNA within extracellular vesicles present in human biological fluids." Frontiers in Genetics 4.
- Taylor, D. D. and S. Shah (2015). "Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes." Methods 87: 3-10.
- Tricarico, C., J. Clancy and C. D'Souza-Schorey (2017). "Biology and biogenesis of shed microvesicles." Small GTPases 8(4): 220-232.
- Trigg, N. A., A. L. Eamens and B. Nixon (2019). "The contribution of epididymosomes to the sperm small RNA profile." Reproduction 157(6): R209-R223.
- Valadi, H., K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee and J. O. Lötvall (2007). "Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells." Nature cell biology 9(6): 654-659.
- Wang, L., J. Lv, C. Guo, H. Li and C. Xiong (2014). "Recovery of cell-free mRNA and microRNA from human semen based on their physical nature." Biotechnol Appl Biochem 61(3): 342-348.
- Wang, M., F. Yu, P. Li and K. Wang (2020). "Emerging Function and Clinical Significance of Exosomal circRNAs in Cancer." Mol Ther Nucleic Acids 21: 367-383.
- Wei, R., L. Zhao, G. Kong, X. Liu, S. Zhu, S. Zhang and L. Min (2020). "Combination of Size-Exclusion Chromatography and Ultracentrifugation Improves the Proteomic Profiling of Plasma-Derived Small Extracellular Vesicles." Biol Proced Online 22: 12.
- Willis, G. R., S. Kourembanas and S. A. Mitsialis (2017). "Toward Exosome-Based Therapeutics:

Isolation, Heterogeneity, and Fit-for-Purpose Potency." Front Cardiovasc Med 4: 63.

- Witwer, K. W., E. I. Buzás, L. T. Bemis, A. Bora, C. Lässer, J. Lötvall, E. N. Nolte-'t Hoen, M. G. Piper, S. Sivaraman, J. Skog, C. Théry, M. H. Wauben and F. Hochberg (2013). "Standardization of sample collection, isolation and analysis methods in extracellular vesicle research." J Extracell Vesicles 2.
- Wlodkowic, D., W. Telford, J. Skommer and Z. Darzynkiewicz (2011). "Apoptosis and beyond: cytometry in studies of programmed cell death." Methods Cell Biol 103: 55-98.
- Xu, R., D. W. Greening, H.-J. Zhu, N. Takahashi and R. J. Simpson (2016). "Extracellular vesicle isolation and characterization: toward clinical application." Journal of Clinical Investigation 126(4): 1152-1162.
- Xu, R., D. W. Greening, H. J. Zhu, N. Takahashi and R. J. Simpson (2016). "Extracellular vesicle isolation and characterization: toward clinical application." J Clin Invest 126(4): 1152-1162.
- Yamashita, T., Y. Takahashi and Y. Takakura (2018). "Possibility of Exosome-Based Therapeutics and Challenges in Production of Exosomes Eligible for Therapeutic Application." Biol Pharm Bull 41(6): 835-842.
- Yoshioka, Y., Y. Konishi, N. Kosaka, T. Katsuda, T. Kato and T. Ochiya (2013). "Comparative marker analysis of extracellular vesicles in different human cancer types." J Extracell Vesicles 2.
- Yuana, Y., T. H. Oosterkamp, S. Bahatyrova, B. Ashcroft, P. Garcia Rodriguez, R. M. Bertina and S. Osanto (2010). "Atomic force microscopy: a novel approach to the detection of nanosized blood microparticles." J Thromb Haemost 8(2): 315-323.
- Zabeo, D., A. Cvjetkovic, C. Lässer, M. Schorb, J. Lötvall and J. L. Höög (2017). "Exosomes purified from a single cell type have diverse morphology." J Extracell Vesicles 6(1): 1329476.
- Zaborowski, M. P., L. Balaj, X. O. Breakefield and C. P. Lai (2015). "Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study." BioScience 65(8): 783-797.
- Zeringer, E., T. Barta, M. Li and A. V. Vlassov (2015). "Strategies for isolation of exosomes." Cold Spring Harb Protoc 2015(4): 319-323.
- Zha, Q. B., Y. F. Yao, Z. J. Ren, X. J. Li and J. H. Tang (2017). "Extracellular vesicles: An overview of biogenesis, function, and role in breast cancer." Tumor Biology 39(2): 1010428317691182.
- Zhang, J., S. Li, L. Li, M. Li, C. Guo, J. Yao and S. Mi (2015). "Exosome and exosomal microRNA: trafficking, sorting, and function." Genomics Proteomics Bioinformatics 13(1): 17-24.
- Zhang, P., J. C. Yeo and C. T. Lim (2019). "Advances in Technologies for Purification and Enrichment of Extracellular Vesicles." SLAS Technol 24(5): 477-488.