Open Access Journal of Gynecology and Obstetrics

ISSN: 2638-5244

Volume 3, Issue 2, 2020, PP: 10-29

An Update on Future Utilization of Extracellular Vesicles in Evaluation Part of These in Maturation of Gametes, Fertilization along with Embryo Implantation for Escalating the Success of Artificial Reproductive Technology : A Systematic Review

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Abstract

After having reviewed the role of Extracellular Vesicles (ECV's) as potential biomarkers along with role in endometriosis and for therapeutic efficacy in diabetes mellitus here we attempted to review the role of ECV's potentially for escalating the success in Artificial reproductive technology(ART). Thus we utilized Pubmed, MEDLINE, Google Scholar, Scopus, Embase, Web of Sciences, Cochrane library search engines wherewe utilized the MeSH terms like Extracellular Vesicles, or micro Vesicles, exososomes, microparticles, prostasomes, Epididymosomes, oviductosomes, uterosomes along with a lot of words in relation to various phases of development varying from ooc ytes, spermatozoa, Fertilization, embryo, implantation, Epididymal fluid, follicular fluid, seminal fluid from 1950's till date in December 2020. We got a total of 2000 articles out of which we selected 148 articles for this review. We utilized both animal along with human studies No meta analysis was done. Different kinds of ECV's were isolated from follicular fluid, seminal fluid, uterine follicular fluid. In cases of males ECV's in seminal fluid correlated with post testicular maturation, along with acquiring sperm motility, as well as decreased oxidative stress. In females follicular fluid possessed miRNA, having probable part in follicular growth, ooc ytes meiosis resuming, steroidogenesis as well as avoiding polyspermy. Besides that ECV's were observed in culture embryos media, pointing that ECV's liberated from embryo as well as uterus might bring about embryo-endometrium communication during implantation. It is still early stages to conclude what are the exact functions of ECV's as well as how they can get utilized as biomarkers of good sperm oocyte/ embryo, utilize addition of miRNA, via ECV's and utilize in specific cases like genetic problems to supplement genetic material. More work is needed with the limitations in human studies.

Keywords: *Extracellular Vesicles, Epididymosomes, oviductosomes, prostasomes, miRNA, exososomes, implantation.*

Introduction

Our attention with regards to intercellular crosstalk has enhanced with greater insight in the complicated nature of it aiding in variety of physiological events, that are control of cell proliferation, differentiation, gametogenesis, embryogenesis as well as generation of embryo. Specifically, the observation of Extracellular Vesicles(ECV's) as innovative mediators of intercellular crosstalk has re-concentrated the research work in this arena. Classically, intercellular communication is made up of 3 modes i) contact-based signalling through membrane bound signalling molecules (receptors) or gap junction,ii) short range paracrine signalling through liberated soluble molecules like cytokines as well as chemokines iii)long range endocrine signalling

through liberated hormones. Iv) With the aid of recent studies presence of ECV's that get liberated by cells in the extracellular surroundings as well as can act as vehicles for the shifting of proteins,lipids as well as RNA's among cells both locally(autocrine along with paracrine) as well as distantly [1,2]. ECV's get liberated by a broad range of cell kinds in both normal as well as pathological situations. ECV's along with their contents might possess crucial part in various angles of biology, that includes reproduction, in the form of candidate biomarkers of health as well as disease as well as probable targets for treatment interventions[3].

Gametogenesis, fertilization implantation as well as early generation of embryo are complicated events that are markedly based on crosstalk among cells as well as organs. Oogenesis represents a multistep event that takes place over a huge time frame (in humans in decades) as well as implicates crosstalk among the generating oocytes as well as cumulus along with granulosa cells which surround it in the follicle. Sperms go via maturation, capacitation molecules along with acrosome reaction, that promote their binding as well as fusion with the liberated oocytes, hence facilitating fertilization to take place. Following, the generating embryos entry into the uterus, apposition along with consequent sticking of the blastocyst to the endometrial luminal epithelium, followed by endometrial invasion, has to take place for a successful implantation[4]. Observation of ECV's in the reproductive biofluids suggests a probable part for them in the intercellular crosstalk essential for as well as following conception. Objective of this review is to illustrate the present proof available regarding the parts of ECV's in conception as well as implantation along with showing the critical gaps in our current insight.

Methods

Thus we utilized Pubmed, MEDLINE, Google Scholar, Scopus, Embase, Web of Sciences, Cochrane library search engines where we utilized the MeSH terms like Extracellular Vesicles; ormicroVesicles; exososomes; microparticles; prostasomes; Epididymosomes; oviductosomes; uterosomes along with a lot of words in relation to various phases of development varying from ooc ytes; spermatozoa; Fertilization; embryo; implantation; Epididymal fluid; follicular fluid; seminal fluidfrom 1950's till date in December 2020.

Results

We got a total of 2000 articles out of which we selected 148 articles for this review. We utilized both animal along with human studies No meta analysis was done

Extra Cellular Vesicles(ECV's)

Extra Cellular Vesicles represent membrane bound vesicles, that get liberated by each prokaryotic[5] as well as eukaryotic[6-9]cell kind studied till date. Various terminologies have got utilized for classification of subtypes of ECV's, that have usually been utilized intentionally or without intention for describing overlapping types of ECV's[3]. Despite not getting universally utilized, a usually utilized nomenclature is used by us[3], mainly in view of it not being feasible to find out the particular mode of biogenesis of any particular population of ECV's. Exosomes that are classically 40-100nm in diameter[10] get generated within cells via inward budding of late endosome, also known as multivesicular body(MVB), as well as then get liberated into the Extracellular surroundings by the MVB fusing with the plasma membrane[11,12]. Microvesicles bud straight from the plasma membrane, as well as mostly measure 50-1000nm in diameter. The apoptotic bodies possess a broad range varying from 800-5000nm[10,13-15]. With the knowledge of overlapping size ranges,that ECV formulations remain usually heterogenous to a large extent. Along with that, it becomes tough to differentiate among the various subkinds of ECV's[16]. The word exosomes had been usually utilized traditionally to detail any kind of vesicles observed in the Extracellular biofluid but the word ECV's is preferred [16]. Further ECV's get classified as per the tissue/biofluid from where they got found. Depending on this nomenclature prostatosomes or prostasomes,epididymosomes, oviductosomes as well as uterosomes have got utilized towards pointing to vesicles that have been, identified from seminal fluid, epididymal, oviduct as well as uterine fluids, respectively[17-20]. ECV's have been demonstrated to [possess some proteins,lipids(particularly high amounts of sphingomyelins)DNA as well as a different kind of RNA species that includes miRNAs as well as miRNA fragment[13]. Proteins mostly observed on ECV membranes are tetraspanins, particularly CD63, CD9, CD81,heat shock protein 70(HSP70) as well as

glycophosphatidylinositol-anchoring of proteins. Tissue particular molecular mediators having the properties of the parent cell can get observed both on ECV' as well as within ECV's[17-28]. Molecules existing on the surface of ECV's facilitate crosstalk with rest of cells via sticking of lipids as well as ligands on the surface of the recipient cells, or fusion of the ECV' membrane with the plasma membrane of the recipient cells[13,28]. The participation of extracellular vesicles in many cellular processes, including reproduction,is unquestionable. Although currently, the tetraspanin proteins found in extracellular vesicles are mostly applied as markers, enhancing proof suggests to their role in extracellular vesicle biogenesis,cargo selection, cell targeting, cell uptake under both physiological and pathological conditions.

Recently Jankovicova et. al, reviewed, as well as brought about other insight into the involvement of tetraspanin proteins in extracellular vesicle physiology in mammalian reproduction. They provided knowledge regarding the involvement of extracellular vesicle tetraspanins in these processes in somatic cells. Furthermore, they discussed the future direction as well as towards getting an insight regarding their functions in the tissues and fluids of the mammalian reproductive system in gamete maturation, fertilization, and embryo development;their involvement in mutual cell contact and communication in their complexity. [29] (figure1)

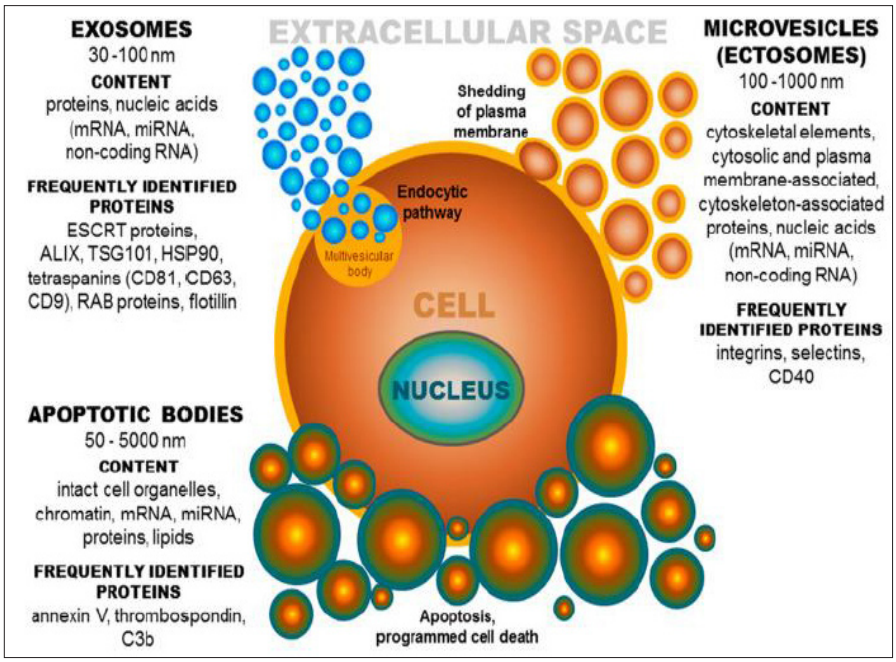

Fig1. *Courtesy ref no-29-Extracellular vesicles: Their origin, size, and cargo. ESCRT-endosomal sorting complex required for transport; ALIX-protein regulating cellular mechanisms, including endocytic membrane trafficking and cell adhesion; TSG101-tumor susceptibility gene 101 protein; HSP-heat shock protein, CD-cluster of differentiation; RAB-proteins included in regulation of endocytosis and secretory processes, C3b-complement component.*

Sperm Maturation as well as Extra Cellular Vesicles

The capacity of the Sperm to be capable of fertilizing an oocyte is smoothly acquired at the time of transition in epididymis, crosstalk with seminal fluid at the time of ejaculation,passage within vagina,contact with epithelium of the oviduct as well as fusion with the oocyte [30]. Epididymis possesses various functions, that are Sperm transportation, maturation as well as storing of gametes[31,32]. There is a division of epididymis into three major segments namely caput, corpus as well as cauda. Every segment generates its own microenvironment,with various proteins getting liberated along with genes getting expressed, as well as get optimized for every stage of the sperm maturation [33,34]. The caput, as well as corpus have the job in sperm maturation,whereas the cauda, works in the form of a sperm reservoir [35,36].

At the time of transition via the epididymis, sperm expels its cytoplasmic droplets as well as their plasma membrane surface proteins go through remodelling (like alteration in phospholipid composition as well as cholesterol:phospholipid ratio, an enhancement in complete negative charges along with the modulation of surface proteins). On ejaculation, sperm gets mixed with the seminal fluid,that is made up of liberations from the prostate, seminal vesicles along with bulbourethral glands[37]. Through these liberations sperms get the capacity to survive as well as shift in the hostile vagina possessing acidic pH, bind with the zona pellucida(ZP), along with merge with oocyte plasma membrane. These alterations in sperm morphology as well as function all occur secondary to crosstalk among the intraluminal fluid along the epididymis, as well as are based on liberation along with uptake of proteins as well as lipids from the surrounding micro environment[30].

Current results show that proteins as well as miRNA in the epididymis fluid correlated with post testicular maturation get transferred to the sperms by ECV's. ECV's got 1st isolated in the human seminal fluid in the form of organelles that got expelled along with liberations from the prostate hence known as prostasomes[17,38-40], that measured 50-500nm as well as possess proteins, lipids as well as nucleic acids. Human prostasomes transport a minimum of 140 proteins, that are prostate specific antigens(PSA), as well as PAP), prostate stem cell antigens, structural proteins, signal transduction proteins, guanosine triphosphate proteins as well as adenosine triphosphate [41, 42, reviewed in 43] (figure2a). These proteins possess antimicrobial, antioxidant as well as immune regulatory actions[44-50]. Prostasomes possess an active part in regulation as well as timing of capacitation as well as acrosomal reaction. Specifically, prostasomes take part in prevention of premature sperm capacitation,along with acrosomal reaction. Actually the prostasome membrane has high content of sphingomyelin, with a greater cholesterol: phospholipid ratio, that aids in its stabilization in acidic vaginal surroundings[51-56]. Moreover in vitro experiments have demonstrated that prostasomes further aid in capacitation as well as acrosomal reaction. Incubation carried out in vivo at a little acidic pH causes merging of human sperms with prostasomes[57] that causes reduced sperm membrane fluidity, that causes the sperm to become more receptive to next fertilization signals[57,58].

Epididymosomes represent ECV's that get liberated from epididymal epithelial cells through apocrine liberation. [33,35,36,59]. Epididymosomes identification has been done from hamster, rat, ram, mouse as well as a bovine species along with from humans [36,59-63]. These epididymosomes possess adhesion molecules like tetraspanins, integrins as well as milk fat globulin-epidermal growth factor8 protein (MFGE8)[13,64,65] (figure 2b). Bovine epididymosomes possess various proteins which take part in the acquiring of sperm motility, fertilization capacity as well as protection from reactive oxygen species (ROS) [66-69]. These are aldo-keto reductase family 1, member1 (aldose reductase)(AKR1B1), phatidylinositolethanolamine binding protein (PEBP1), macrophage migration inhibitory factor(MIF), enzymes belonging to polyol pathway,HES/CD52,type5 glutathione peroxidise(GPX 5), ubiquitin, sperm adhesion molecule1(SPAM 1/PH-20 and P25b(in humans called dicarbonyl /L-xylulose reductase(DCXR) or P34H)(figure2b). These proteins are implicated in sperm maturation as well as fertilization, with MIF as well as enzymes belonging to polyol pathway facilitate sperm motility[66-68,70], GPX 5 avoids premature acrosomal reaction[63], as well as along with ubiquitin, gives protection to sperms from oxidative stress. AKR1B1 as well as PEBP1, observed in caudal epididymosomes aid in sustaining epididymal sperm in a quiet phase at transition time[71]. SPAM 1/PH-20 is a hyaluronidase that escalates sperm penetrating via the cumulus cell layer surrounding the oocyte as well as is implicated in sperm-ZP binding [72-74].

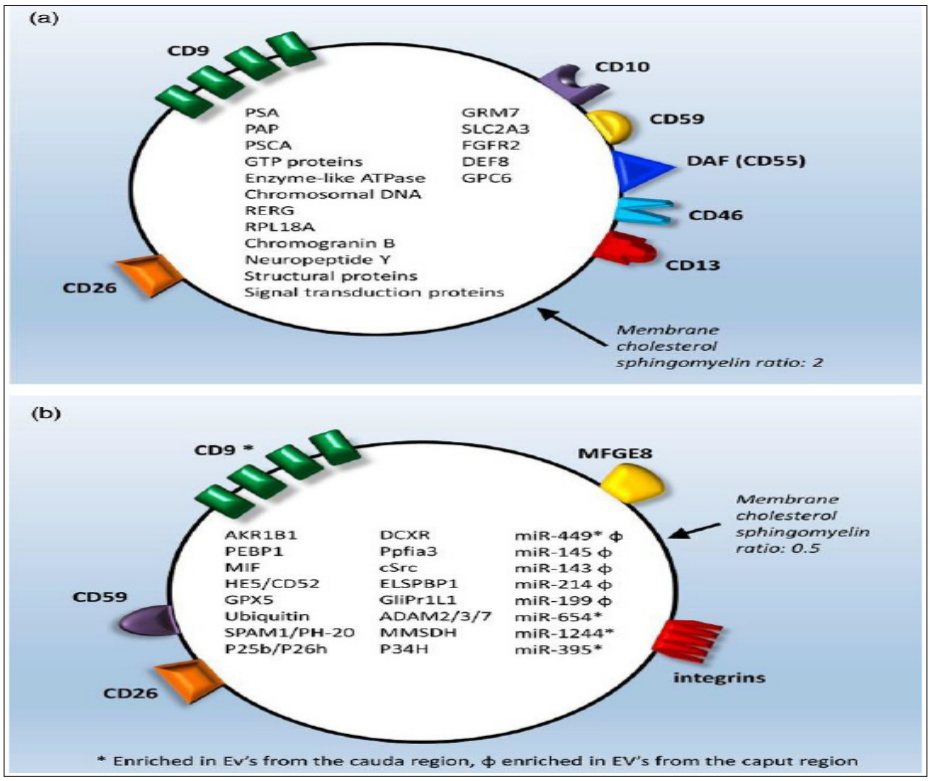

Fig2a. *Prostasome structure and content.* **Fig2b.** *Epididymosome structure and content.*

In the bovine system, epididymosomes from various epididymis areas carry separate contents [33,71]. Certain miRNA, like miR-449 are rich in epididymosomes from both caput as well as cauda areas, whereas rest are differentially expressed in between these areas, with miR-145 as well as miR-143, miR-214, along with miR-199,being present in greater numbers in epididymosomes from caput, whereas miR-654, miR-1224, along with miR-395 get expressed mainly in epididymosomes from cauda areas[33,71].

ECV's the seminal fluid have been evaluated less, though they take origin from various cell kinds in the male genital tract [77,78] as well as merge with the sperm membrane for shifting molecules which aid in sperm survival in the vaginal acidic surroundings[54,79].

Crosstalk among sperms as well as ECV's are pH based [57,79,80]. This helps with the knowledge of alkaline pH of prostatic liberations,that avoid early ECV's as well as sperm fusion, along with the acidic pH of the vagina,that works as a stimulus for merger.

From these animal as well as human studies good proof is there for the way ECV's aid in sperm maturation. Still the available proof is quite minimal for the part of different kinds of ECV's. Further Xu et al. , reviewed that ECVs regulate multiple physiological processes. Seminal plasma possesses various ECVs that may deliver functional molecules such as small RNAs (sRNAs) to the sperm. However, the RNA profiles in the boar seminal plasma extracellular vesicles (SP-ECVs) and its function have not been characterized. The aim of this study was to characterize the functions and sRNA profiles in the boarSP-EVs using deep sequencing technology. Briefly, boar SP-ECVs were isolated bydifferential ultracentrifugation and confirmed with a transmission electron microscope(TEM), nanoparticle tracking analysis (NTA), and Western blot. The isolated boar SP-EVscontained numerous and diverse sRNA families, including microRNAs (miRNAs, 9. 45%of the total reads), PIWI-interacting RNAs (piRNAs, 15. 25%of the total reads), messengerRNA fragments (mRNA, 25. 30% of the total reads), and tRNA-derived small RNAs(tsRNA, 0. 01% of the total reads). A total of 288 known miRNAs, 37 novel miRNA, and 19, 749 piRNAs were identified in boar SP-EVs. The identified ssc-miR-21-5p may confernegative effects on sperm fertility based on a dual-luciferase reporter experiment. This study therefore yields an efficacious way for identification of SP-EVs along with exploring the properties of these RNA profile. [81]

Extra Cellular Vesicles, Crosstalk in the Ovarian Follicle along with Oocyte Maturation

The mature ovarian follicle consists of an oocyte, the somatic cells (cumulus,mural granulosa cells as well as theca cells) along with follicular fluid(FF) (Figure3). This FF gets obtained from components of circulating plasma which crosses the blood-follicular barrier through theca capillaries, along with from granulosa as well as theca cells constituents that are hormones, proteins, amino acids, as well as antiapoptotic factors[82,83]. Crosstalk that is in both directions among gametes as well as granulosa cells in the follicle takes place either directly by a network comprising of gap junctions or via paracrine, autocrine as well as endocrine signalling factors in the FF[84,85]. These crosstalks are key for normal follicular growth, proliferation as well as differentiation of granulosa cells, oocyte maturation, manipulation of transcriptional activity, fertilization as well as preimplantation embryonic generation [85,86-92]. ECV's put an extra layer of transmission as well as regulation, since they possess miRNA which are anticipated to target crucial elements in the pathway linked to follicular growth as well as oocyte maturation in mammals like the wingless signalling pathway (WNT), transforming growth factor beta (TGFβ), Mitogen activated protein kinase (MAPK), neurotrophin, epidermal growth factor receptor (Erb B) pathways as well as ubiquitin modulated pathways[93-95]. WNT molecules represent glyco proteins implicated in follicular growth, luteogenesis along with steroidogenesis. TGFβ superfamily members like inhibin, activin, Bone morphogenetic protein (BMP)15 as well as Growth differentiation factor9 (GDF9) get expressed in the oocytes from right from initial stages as well as are implicated in follicular growth as well as oocyte maturation[95-97]. The MAPK pathway induces granulosa cell proliferation along with cumulus expansion. Moreover along with the Erb B) pathways, this MAPK pathway facilitates meiosis getting resumed in the oocyte[1,98z]. The ubiquitin modulated pathway manipulates oocyte meiotic maturation [99], as well as early mitotic division in embryos[100], whereas the pathway signalling utilizing neurotrophin controls oogenesis as well as follicle generation[101].

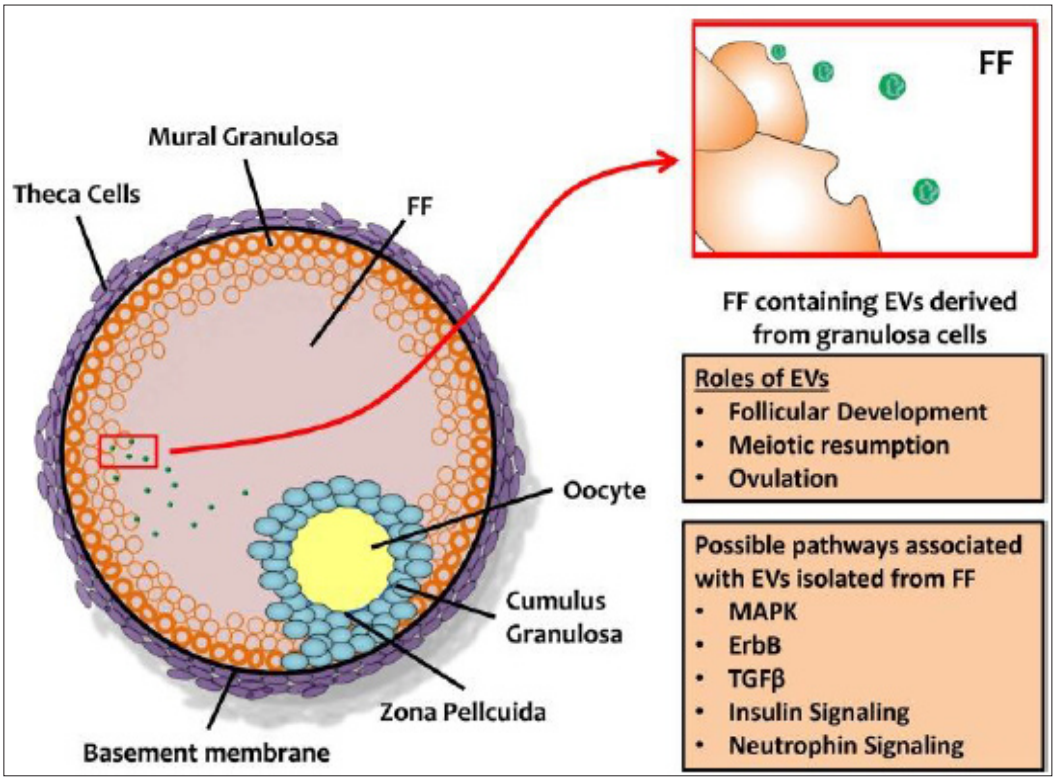

Fig3. *Extracellular vesicles and the ovarian follicle. EV: extracellular vesicles; FF: follicular fluid.*

Equine, bovine along with human studies have shown the existence of ECV's in FF[93-95]. In the ovarian follicle, ECV's get utilized in shifting RNAs, micro RNAs(miRNA) in addition to proteins to the recipient cells[93,95]. Certain miRNAs are only observed in ECV's in FF, whereas rest can be observed in the non –vesicular fraction of FF as well. Significantly, equine as well as bovine ECV's observed in in FF possess miRNA anticipated to target transcripts of proteins correlated with focal adhesion as well as control of actin skeleton [93,95]. In the bovine system pathway of significance correlated with exosomal miRNAs are the ubiquitin modulated pathway, MAPK, insulin as well as neurotrophin signalling [93]. In the equine miRNAs correlated with the MAPK pathway get observed in larger ECV's, like microvesicles instead of exosomes[[95]. In human beings,maximum of ECV's miRNAs are anticipated to work in the form of targets which control the WNT, MAPK, Erb B as well as TGFβ signalling pathway, all of which work across separate compartments in the ovarian follicle as well as assist to follicle generation, meiosis getting resumed as well as ovulation. Generally, granulosa as well as /or cumulus cells can pick up miRNAs from the ECV's in FF,in vivo as well as in vitro[93].

Results correlating ECV's with follicular maturation as well as oocyte competence are at present correlative alongwithdescriptive.Acurrentbovinestudycompared the profiles of miRNAs that were encapsulated in ECV's from follicles possessing mature oocyte with those of follicles possessing immature oocytes. Those follicles that possessed immature oocytes, the ECV's in FF that surrounded these possessed a greater amount of upregulated miRNAs pointing towards the existence of a greater transcriptional activity at the time of oocyte growth[93]. It is not sure if these greater amount of upregulated miRNAs found is secondary to either an enhancement in the liberation of exosomes –possessed RNAs at the time of oocyte growth or from an enhancement of miRNAs amounts. The anticipated targets for these upregulated miRNAs are ubiquitin, neurotrophin, MAPK as well as a insulin signalling pathways that are correlated with ovarian follicle growth, oocyte meiotic maturation [102], as well as early mitotic division in embryos [93]. In equine FF, miRNAs from the exosomes can control gene expression of the TGFβ superfamly in granulosa cells at the time of oocyte maturation[95]. In various species FF, exosomes possess miRNAs like miR-30b, let-7, miR-181a, miR-375, miR-503, as well as miR-513a-p,that might assist in follicle growth as well as oocyte maturation[95,103-106]. A human study contrasted the exosomal profile of plasma as well as pooled FF,from 15women less than 35yrs who went through in vitro fertilization(IVF)secondary to male factor infertility [94]. Santonocito etal. , isolated 37 miRNAs that were upregulated in FF as compared to plasma, 32 of which were transported by exosomes. Certain miRNAs that were identified from FF exosomes were further observed in cumulus as well as granulosa cells. Pathway evaluation illustrated that these miRNAs targeted factors, like WNT, MAPK, Erb B as well as TGFβ which aid in follicular generation as well as meiosis getting resumed. This study nevertheless,evaluated pooled FF,that is anticipated to possess follicles from various maturation stages as well as thus could not yield knowledge regarding the part of ECV's in FF across the various steps of oocyte maturation. Detailing the dynamic shift of ECV's miRNAs in FF, right through maturation of the follicle needs future studies evaluating ECV's from follicles at every stage of maturation. These studies need invasive sampling procedures and cannot be easily conducted in the general population. Essential knowledge could be derived from animal experiments or in case of humans, by recruitment of patients going through IVF as well as obtaining FF along with paired data on oocyte maturation from oocyte pick ups.

The catalogue of the macro molecules possessed in FF as well as ECV's in FF is continuously escalating, Nevertheless direct proof of ECV's crosstalk within ovarian follicle is still absent. Present studies possess a lot of drawbacks. In view of the relatively greater cost of FF harvesting as well as evaluation,to limit laboratory costs,in vivo studies of ECV's in FF is classically possess very small sample sizes as well as /or have pooled together FF samples from multiple follicles. Thus there is not sufficient statistical power for arriving at conclusive outcomes. , Moreover the in vivo studies carried on till date have been usually observational kinds, have developed descriptive information, as well as do not yield experimental proof on the functions of ECV's in FF. In vivo functional

studies would be significant to demonstrate functions of ECV's in FF. The ECV's community have utilized in vitro studies for evaluations of the transfer of ECV's from source into recipient cells. Studies akin to this are required immediately regarding ECV's in FF. Like subsequent evaluations might harvest ECV's from granulosa cells cultured in vitro as well as transfer them to an immature oocyte to find out if ECV's can stimulate maturation as well as fertilization. Observations from in vivo descriptive studies can directly tell the experimental results. Various reports have documented or are actively finding out the molecular markers that are encapsulated in ECV's in FF in vivo. The growing prowess for engineering ECV's having predecided constituents could be manipulated to enhance the fertilization rates as well as quality of embryos with the basic aim of enhancement of the fertilization rates as well as IVF success rates.

Other studies evaluated the miRNAs amounts in ECV's in FF, with regard to female age. In equines, exosomal miRNAs expression profiles differed with animal age along with are correlated with variations in fertility. 2 studies documented high amounts of miR-181A, miR-375, as well as miR-513a-3p exosomes from old vis a vis younger mares. All these three miRNAs target TGFβ,can repress the TGFβ pathway as well as result in dysfunctional oocyte maturation in older women[95,97]. A human study also found various miRNAs profiles of ECV's removed from FF of 3 younger (<31yrs old) as compared to three older ones (>38 yr old)[107]. Particularly,four miRNAs had differential expression in FF of younger vis a vis older women namely miR-21-5p was only expressed in the FF of younger women, miR-190b as well as miR-99b-3b were existing just in older women, as well as miR-134 was markedly escalated in ECV's from older women. These associations documented among miRNAs in ECV's in FF along with female age raise new queries like do miRNAs shifted across the FF, aid or even just track with oocyte aging ?If these ECV's in FF were observed to implicate oocyte viability,is it feasible that we utilize ECV's miRNAs in FF from younger women to generate new treatment modalities as well as ameliorate the actions of age on fertility?Although future queries they can be easily examined in vitro. If the miRNAs which vary among younger vis a vis older women also possessed a part in fertilization as well as embryo quality,adding ECV's from younger women to the FF of older women, or better enriching with miRNAs believed to be of relevance or rest of biomolecules which might be implicated in sustaining oocyte viability in younger women, might potentially aid in yielding higher fertilization along with good quality embryos in older cases. **Extracellular vesicles (EVs) contain multiple factors that regulate cell and tissue function. However, understanding of their influence on gametes, including communication with the oocyte, remains limited. In the present study, Almeida Monteiro Melo Ferraz et. al characterized the proteome of domestic cat (*Felis catus*)** follicular**fluid EVs (ffEV). To determine the influence of follicular fluid EVs on gamete cryosurvival and the ability**to undergo ***in vitro*** **maturation, cat oocytes were vitrified using the Cryotop method in thepresenceor absence of ffEV. Vitrified oocytes were thawed with or without ffEVs, assessed for survival by authors, *in vitro*cultured for 26 hours and then evaluated for viability and meiotic status. Cat ffEVs had an average sizeof 129. 3 ± 61. 7 nm (mean** ± SD) and characteristic doughnut shaped circular vesicles in transmission **electron microscopy. Proteomic analyses of the ffEVs identified a total of 674 protein groups out of 1,974 proteins, which were classified as being involved in regulation of oxidative phosphorylation, extracellular matrix formation, oocyte meiosis, cholesterol metabolism, glycolysis/gluconeogenesis,and MAPK, PI3K-AKT, HIPPO and calcium signaling pathways. Furthermore, several chaperone proteinsassociated with the responses to osmotic and thermal stresses were also identified. There were nodifferences in the oocyte survival among fresh and vitrified oocyte; however, the addition of ffEVs tovitrification and/or thawing media enhanced the ability of frozen-thawed oocytes to resume meiosis. In summary, this study is the first to characterize protein content of cat ffEVs and their potential roles insustaining meiotic competence of cryopreserved oocytes. [108**].

Fertilization as well as Extra Cellular Vesicles

Fertilization involves a number of consequent processes,that are (cumulus cell expansion that promotes sperm passage via them, after which

sperm binds to ZP[109]. The crosstalk of capacitated sperm with ZP stimulates acrosomal reaction,that aids in biochemical alterations in the sperm head membrane along with liberation of proteases as well as hyaluronidase from the acrosome, aiding the sperm to recall the ZP as well as to enter the perivitelline space(PVS)[110-112]. Recently PMCA4a, that represents a protein which contributes in sustaining Ca^{2+} homeostasis,was identified from the CD9 positive ECV's in the oviduct (oviductosomes) as well as uterus(uterosomes). In vitro the PMCA4a acquisition by sperms following incubation with the exosomes identified from the luminal fluid. These observations point that ECV's might avoid premature capacitation of the sperm[19].

Following acrosomal reaction the inner acrosomal membrane sticks to the region of the oocyte which are covered with microvilli, as well as the sperm merges with the membrane of the oocyte[114]. These microvilli show CD9[115]. Merging of the sperm as well as oocyte results in depolarization of the oolemma along with stimulates the liberation of cortical granules which are located beneath the oocyte surface,blocking the penetration of any other sperm. The molecular basis by which fusion occurs is not clear. Utilizing an in vitro porcine model, ECV's have the capacity of merging with the sperm a as well as stimulation of acrosomal reaction[116,117]have demonstrated in a mouse model that ECV's get shifted from the oocyte to sperm in the PVS prior to direct crosstalk among the oocyte as well as sperm. The existence of ECV's in PVS was corroborated further by utilizing electron microscopy[118].

CD9 positive ECV's have been observed on plasma membrane of the oocyte, particularly on the oocyte microvilli at the sperm attachment area. CD9 further is present on the surface of the fertilizing sperm at the time point of membrane binding as well as merger is needed in sperm- oocyte. CD9 negative oocytes possess changed microvilli that cant merge with the sperm[115]. In a mouse study, merger among the sperm as well as CD9 negative oocytes got rescued following coincubation with wild type oocytes. Thus the authors concluding that fusion among sperm as well as oocytes gets modulated by exosome like vesicles possessing CD9,that get liberated from the oocyte into the PVS as well as then shifted to the sperm[119]. Nevertheless, 2 other studies in hamster as well as mice did not observe any enhanced capacity of CD9 negative oocytes to merge with sperms on getting inseminated in the existence of wild type oocytes or medium that possessed CD9- correlated ECV's[117,120].

Other tetraspanin CD81,that is basically generated by cumulus cells as well as present majorly in the inner area of ZP, as well as might also take part in fertilization, basically in fusion correlated processes before membrane merger as well as particularly in acrosomal reaction[121]. , CD81, as well as CD9 seem to be shifted to the sperm through ECV's on the sperm penetration of the PVS[119,121]. CD81 might aid in the shifting of CD9 from oocyte to the sperm membrane prior to sperm- oocyte merger[122]. A new protein known as Izumo was isolated on the surface of sperms that had gone through acrosomal reaction in 2005[122,123]. In sperms where Izumo protein was absent lack the capacity to fuse with the oocyte. Atthe time of sperm- oocyte merger, Izumo I binds to a folate receptor on the oocyte known as Juno[124]. Astonishingly, following fertilization, Juno that is markedly expressed on oocyte prior to fertilization,gets shed from the membrane of the oocyte along with getting redistributed in PVS since ECV's are probably obtained from the microvillus rich oolemma. ECV's might bind as well as neutralize consequent acrosome –reacted sperms,adding a probable mode for avoiding polyspermy [124,125]. Studies conducted in vitro have demonstrated that oocytes lacking juno do not have the capacity to get fertilized [124]. These observations give proof that the Izumo I as well as Juno crosstalk promotes the adhesion among the sperm a as well as oocyte needed for fertilization[124].

Paracrine Cross Talk between Embryos as well as Extra Cellular Vesicles

Despite being a little controversial,it has been documented that in vitro culture of embryos achieves greater success when embryos are kept in large groups at the time of whole culture duration[126,127]. Embryos might develop their own microsurroundings by liberating growth factors, that consists of a 'secretome' with both autocrine as well as paracrine

actions [128,129]. Embryos that have been cloned are cultured with porcine parthenogenetic embryos demonstrated a significant enhancement in their generational competency (like enhanced number of blastomeres as well as better blastocyst generation) as compared to Embryos that have been cloned getting cultured by themselves. Evaluation of culture media from porcine embryos cultured alone found30-120mm vesicles varying in sizes as per the embryos age (<40mm in cultures from 2 celled embryos as well as <120mm in cultures from blastocysts). These vesicles express the exosomal marker CD9 as well as possess miRNAs,that are OCT4,SOX2 as well as KLF4 that differ as per the stages of embryo generation. More work has demonstrated that these exosomes / micro vesicles can have the capacity to get through the ZP as well as get internalized by the blastomeres[130]. The probable functional part of ECV's in embryos formation has got to be shown. In case of humans to the reproducing the outcomes from porcine studies have been difficult in view of the culture media utilized in IVF possesses lots of ECV's-obtained from the synthetic serum substitutes which get utilized for supplementing the generating embryo[131]

Endometrial- embryos Interaction during Implantation as well as Extra Cellular Vesicles

For getting a succesfull embryos Implantation, good coordination among the endometrium as well as – embryos is needed along ECV's might take a part in the Interaction needed (figure4). It has been pointed that the endometrial epithelium liberate ECV's which are implicated in the shifting of signalling miRNAs along with adhesion molecules either to the blastocyst or the adjacent endometrium into the uterine cavity, that in turn influences receptivity of the endometrium as well as Implantation. ECV's that possess exosomal marker CD63 as well as HSP70 have been identified from the uterine luminal fluid (ULF) of cyclic as well as pregnant sheep [132]. Evaluation of these follicles demonstrated miRNAs as well as proteins expressed via both trophectoderm of the conceptus along with endometrium epithelium,like cathepsin L1 as well as prostaglandin synthase(PGS2). In the sheep, endogenous retroviruses (enJSRV's)have a key part in controlling trophectoderm of the conceptus generation as well as placental growth[132,133], In vitro studies have documented that ECV's that have been identified from the ULF of pregnant sheep [can shift RNAs(that are enJSRV's RNAs)to other cells. These observations point that ECV's existing in ULF are probably possess a biological part in the crosstalk among embryos as well as endometrium. In case of human studies ECV's have got identified from uterine fluid from women in various phases of the menstrual cycle. Both, luminal as well as glandular apical surfaces of endometrium epithelial cells express CD9 as well as CD63 exosomal markers,thus demonstrating that the endometrial epithelium are probable resources of the exosomes observed in the uterine cavity[134]. The isolation of ECV's in the uterine fluid point to a probable part in shifting information from the endometrium during Implantation, although this posit still needs to have evidence. Embryo implantation failure is considered a leading cause of infertility and asignificant bottleneck for in vitro fertilization (IVF) treatment. Confirmed factors that lead to implantation failure involve unhealthy embryos, unreceptive endometrium, and asynchronous development and communication between the two. The quality of embryos is further dependent on sperm parameters, oocyte quality, and early embryo development after fertilization. The extensive involvement of such different factors contributes to the variability of implantation potential across different menstrual cycles. An ideal approach to predict the implantation outcome should not compromise embryo implantation. The use of clinical material, including follicular fluid, cumulus cells, sperm, seminal exosomes, spent blastocyst culture medium, blood, and uterine fluid, that can be collected relatively non-invasively without compromising embryo implantation in a transfer cycle opens new perspectives for the diagnosis of embryoimplantation potential. Zhou & Dimitriadis[135], on reviewing a Compositional comparison of these samples between fertile women and women or couples with implantation failure identified both quantitative and qualitative differences in the expression of microRNAs (miRs) that hold diagnostic potential for implantation failure. Here,they reviewed current findings of secreted miRs that have been isolated as potentially of use in avoiding implantation outcome using material that can be collected relatively non-invasively. Developing non-invasive biomarkers of implantation potential would have a major impact on implantation failure and infertility. [135].

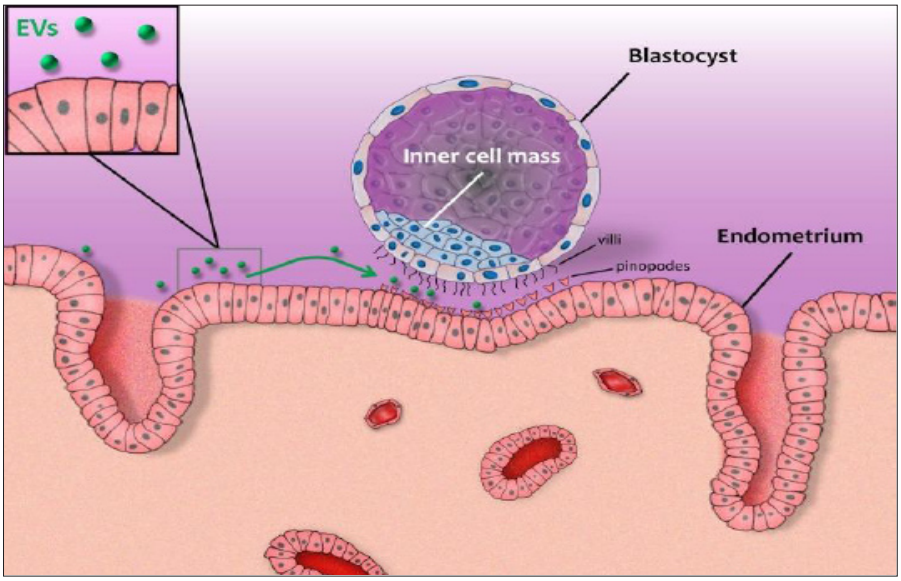

Fig4. *Extracellular vesicles and the cross-talk between the blastocyst and endometrium during implantation.*

Probable Future Clinical Relevance

Studying ECV's in reproduction can escalate our insight regarding the normal physiology of reproduction like isolating sperms along with oocytes belonging to high quality or pathological problems like Implantation failure. particularly ECV's possess the potential of isolation of non invasive biomarkers as well as generation of innovative treatment for enhancement of reproductive success.

A lot of work has been done in the previous decade for generation of non invasive biomarkers for evaluation of oocytes quality, embryos along with blastocysts generation of the highest quality as well as of isolation of the correct embryo(s) for transfer to be able to achieve a succesfull pregnancy. Newer methods like morphokinetic parameters, as well as efforts to prove proteins or liberated matabolomes in a culture medium have been enriched either clinically or by experimental means to the classic morphological parameters for selection of the embryos, Nevertheless, birth rates following ART have continued to be practically unaltered[136,137]. Various studies,recently have concentrated on miRNAs in the FF or the culture medium as probable biomarkers for escalating the IVF success rates[138]. ECV's encapsulated miRNAs, specifically, are protected from breaking down,having marked stability in biological fluids. This characteristic might markedly promote the translation of the escalating insight of miRNAs biology for clinical applications. Evaluating the ECV's encapsulated miRNAs further have crucial biological benefits over evaluation of total miRNAs. Whereas total miRNAs in human bio fluids or supernatants from cell cultures might be liberated from apoptotic cells or cell debris , ECV's miRNAs are actively liberated by viable cells are anticipated to depict an active method of crosstalk among cells as well as tissues both locally along with systemically. Particularly ECV's encapsulated miRNAs might possess a separate part as compared to miRNAs in biological fluids,since they shift biological knowledge to recipient cells.

A specific query that carries attention is if ECV's contain along with shift DNA via generating embryos as well as the fetus to the maternal circulation. In the last some years, non invasive prenatal examination utilizing cell free DNA(cfDNA) in maternal plasma has changed the clinical paradigm of prenatal screening for the usual aneuploidies as well as Y chromosome. In case cfDNA were to be transported by ECV's, along with embryonic as well as /or fetal DNA can be isolated either in the culture medium or maternal circulation. ECV's could be utilized in the form of Y chromosome-particular DNA. Moreover ECV's could be utilized for non invasive prenatal genetic diagnostic methods for finding out aneuploidy prior to ET or during the very initial stages of pregnancy,rather than by embryo biopsy like done these days[139-41].

Besides this ECV's are further being Evaluated for their potential clinical applications, Specifically, for targeted drug delivery. Earlier for reproduction,nanoparticles were utilized for experimental ways of loading sperms with exogenous genetic material which was for the purpose of transferring to oocytes at the time of fertilization[142,143]. Nevertheless,the longterm consequences of utilizing external nanoparticles at the time of gestation are ?. With regards to this ECV's, as endogenous bio molecules,might be Specifically, beneficial. ECV's, are naturally existent in human biofluids, as well as might be engineered as well as for tissue- particular transfers,like the shifting of selected compounds into gametes as well as embryos for escalationofofreproductivesuccess[144]. Endometrial receptivity is a biosensor for embryo quality, as embryos with reduced developmental potential are rejected. However, embryo quality only accounts for an estimated one-third of implantation failures, with suboptimal endometrial receptivity accounting for the remaining two-thirds. As pregnancy progresses, a uterus continues to engage in close communication with an embryo/fetus, exchanging information in the form of endocrine, paracrine, and other cues. Given the long mammalian gestation period, this dialogue is intricate, diverse, and, currently,not fully understood. Recent progress and the availability of high-throughput techniques, including transcriptomics, proteomics, and metabolomics, has aided the simultaneous examination of multiplemolecular alterations, enhancing our knowledge in this area. This review is meant to explain the known modes of mother–embryo cross-communication gathered from animal and human studies[145]. Extracellular vesicles (ECVs) mediated intracellular communication plays an imperative role in the proper completion of different physiological events. Most of the bio-fluids are enriched with several subpopulations of ECVs including exosomes and microvesicles (MVs), with the capacity of transferring different functional molecules (lipids, proteins, and nucleic acids) to target cells. Recipient cells upon receiving the signal molecules undergo different changes that positively affect the structural and functional integrity of the cells. Yar Qamar etal tried to emphasize the part of ECVs liberated by gametes, the female reproductive tract, and the growing conceptus in the successful completion of different reproductive events related to gestation. ECVs correlated with the reproductive system are actively involved in the control of various physiological processes that are gamete maturation, fertilization, and embryo and fetal development. In the reproductive system, EVs mediated intracellular communication is not unidirectional but is rather regulated through crosstalk between the reproductive tract and the growing conceptus. These vesicles are secreted from the ovary, oviductal epithelium, endometrium, developing embryo, and the placenta. The cargo inside these vesicles exerts pleiotropic effects on bothmaternal and embryonic environments. A better understanding of the EVs-mediated crosstalk will be helpful in the development of useful tools serving both the diagnostic as well as therapeutic needs related to female fertility. [146]

Conclusions

After having reviewed the role of ECV's as potential biomarkers along with role in endometriosis and for therapeutic efficacy in diabetes mellitus [147,148] here we attempted to review the role of ECV's potentially for escalating the success in Artificial reproductive technology (ART). Work in the field of intercellular crosstalk has. escalated tremendously in the earlier decade. ECV's along with their cargo,that are proteins as well as miRNAs have been evaluated in lot of concerned body fluids. Whereas in a lot of cases,their functional part is not clear, escalated proof in the literature exists that this crosstalk might aid in sperm along with oocyte,fertilization,avoidance of polyspermy as well as embryos Implantation. Maximum studies regarding ECV's in reproduction have utilized animal models,though currently human work regarding this subject is getting unraveled. Most of the present work is just correlated. A key requirement exists for future functional along with mode of action for giving experimental proof for ECV's as well as their contents as the ones bringing about intercellular crosstalk. The biggest challenge in human reproduction is the ethical concerns that prohibit us to conduct studies which utilize these pathways to corroborate this cause as well as affect association.

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Citation: **Kulvinder Kochar Kaur, Gautam Allahbadia, Mandeep Singh.** *An Update on Future Utilization of Extracellular Vesicles in Evaluation Part of These in Maturation of Gametes, Fertilization along with Embryo Implantation for Escalating the Success of Artificial Reproductive Technology : A Systematic Review. Open Access Journal of Gynecology and Obstetrics. 2020; 3(2): 10-29.*