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Review

Seminal exosomes – An important biological marker for various disorders and syndrome in human reproduction

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ABSTRACT

Background: Exosomes are nano-sized membrane vesicles, secreted by different types of cells into the body's biological fluids. They are found in abundance in semen as compared to other fluids. Exosomes contain a cargo of lipid molecules, proteins, phospholipids, cholesterol, mRNAs, and miRNAs. Each molecule of seminal exosomes (SE) has a potential role in male reproduction for childbirth. Many potential candidates are available within the seminal exosomes that can be used as diagnostic markers for various diseases or syndromes associated with male reproduction. Also these seminal exospmes play a major role in female reproductive tract for effective fertilization.
Aim: The aim of this review is to focus on the advancement of human seminal exosomal research and its various properties.
Methods: We used many databases like Scopus, Google scholar, NCBI-NLM and other sources to filter the articles of interest published in exosomes. We used phrases like "Exosomes in human semen", "Composition of exosomes in human semen" and other relevant words to filter the best articles.
Results: Seminal exosomes play a major role in sperm functions like cell-to-cell communication, motility of the sperm cells, maintaining survival capacity for the sperm in the female reproductive tract and spermatogenesis. Also, seminal exosomes are used as a carrier for many regulatory elements using small RNA molecules. miRNAs of the seminal exosomes can be used as a diagnostic marker for prostate cancer instead of prostate specific antigen (PSA). Epididymosomes can be used as a biomarker for reproductive diseases and male infertility.
Conclusion: Seminal exosomes could be used as biological markers for various reproductive disorders, male infertility diagnosis, and it can be used in anti-retroviral research for the identification of novel therapeutics for HIV-1 infection and transmission.
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1. Introduction

Usually, the secreted proteins by a cell at specific conditions can encode at least 10% of the total human genome found in the body fluids (Anfossi et al, 2018). The secretory proteins fall under soluble proteins, transmembrane proteins, and the total proteome released by either type of microvesicles and considered to be exosomes (Cosme et al., 2017). Exosomes are very minute, membranous, nano-sized (ranges from 50 to 500 nm) and probably released into the extracellular space through exocytosis (Caponnetto et al, 2017). Exosomes contain a cargo of lipids, miRNAs, mRNAs, and proteins. Many cells have the capacity of producing exosomes like B-cells, T-cells, dendritic cells, and platelets (Samanta et al., 2018a, 2018b). They are found in the body fluids like plasma, amniotic fluid, saliva, breast milk, vaginal fluid, and semen. Exosomes secreted under both physiological and pathophysiological conditions (Foster et al, 2016). Exosomes bind with the target cells via fusion and influence the host cells to perform better functions (Vyas & Dhawan,2017). Exosomes in general monitor various biological functions like immunomodulation, cell-to-cell communication, cell migration, and cell differentiation (Caruso Bavisotto et al, 2019). These exosomes bind with the target cells and transfer lipids, proteins, transcription factors to enhance the physiological functions (Meldolesi, 2018). The composition, quality, and quantity of the exosomes are the reflective index of their origin organ and this can act as a biological marker for both diagnosis and prognosis for various diseases or syndromes including cancer and male infertility (Simon et al, 2018). Human semen is a complex mixture of both spermatozoa and seminal plasma (Morgan & Watkins, 2020). The immunosuppressive property of semen may arise from the seminal plasma as it promotes the tolerance against the invading paternal antigens (Samanta et al., 2018a, 2018b). Seminal plasma constitutes a high concentration of lipid based microparticles called prostasomes which is secreted by the epithelial cells of the prostate gland (Vickram et al, 2020). Some authors have proved that other than the prostate gland, these particles might originate from the various cellular complexes of the male genital tract (Le Tortorec et al, 2020). Prostasomes are officially seminal exosomes that have proven functions like immunosuppressant, role in the inhibition of lympho-proliferative responses, and natural killer functions (Vickram et al, 2020). The seminal exosomal cargo mediates many potential effects on the target or host cells that include promoting growth factors, cytokines, membrane proteins, mRNA, and miRNA. miRNAs present in the exosomes regulate various biological functions via inhibition of more specific mRNA targets (Kalluri & LeBleu,2020). Exosomes also carry the non-coding RNAs that act as positive regulatory elements for various biological functions of reproduction (Zhang et al, 2019). The role of seminal exosomes has been well studied related to spermatozoa protection in the female reproductive tract. The role of exosomes might be correlated with their nature of origin (Wu et al, 2020). Seminal exosomes either function in blocking or enhancing the replication of sexually transmitted diseases or pathogens like HIV-1 (Welch et al., 2020a, 2020b). As we know, semen is the primary source or vector for HIV-1 infection, it requires a high volume or number of exposures. It was evidenced that post coitus, the exposure of the female genital tract to the semen might result in a lot of immunomodulatory processes; these indicate that semen contains many factors that could abate the HIV-1 infection (LeCureux, 2016). Seminal exosomes can be internalized by the host cells and then block the HIV-1 replication. It involves the destruction of viral RNA reverse transcription. Exosomes in the semen effectively reduce the efficacy of HIV-1 transmission (Welch et al., 2020a, 2020b).

Many researchers are working in the field of identifying minimally invasive or completely non-invasive biomarkers for cancer diagnosis (Qin et al, 2017). Seminal exosomes could be the choice for such identification in the field of precision medicine (Assadi et al, 2020). These can be applied in place of screening, prognosis and diagnosis, early detection, and prediction of metastasis. Prostate cancer is the most common cancer among the reproductive age group in men and currently, PSA (prostate specific antigen) is the choice of diagnosis (Tanase et al, 2017). Many contradictions were there in using PSA for the diagnosis of prostate cancer. Now, seminal exosomes in the seminal plasma of sperm may be used to classify biomarkers for prostate cancer detection or diagnosis (Hendriks et al, 2017). Prostasomes secreted by the epithelial cells of the prostate gland (exosomes) had proven biomarkers for prostate cancer diagnosis. Thus, the use of liquid biopsy in precision medicine for prostate cancer will improve diagnosis strategies (Valentino et al, 2017).

Other than these, exosomes also play a major role in the diagnosis of azoospermic conditions of male infertility. The expression levels of seminal exosomes miRNAs in the semen of defective spermatogenesis process or vasectomized men can be used as the choice of non-invasive biomarkers, these might be helpful for the diagnosis of azoospermia (Sullivan & Mieusset, 2016). In general, we can differentiate the obstructive and non-obstructive type of azoospermia conditions. The use of these types of exosomal biomarkers in azoospermic conditions helps in determining the pathological and physiological reasons for the absence of sperm in the ejaculate or it can help us to identify the presence of sperm in the testes for testicular extraction (Agarwal et al, 2020).

In this review, we have summarized the composition of exosomes in the semen, its importance in the biological functions such as immunomodulation, anti-retroviral property, potent biomarker for prostate cancer, a potent biomarker for diagnosis of azoospermic conditions, exosomes regulatory elements, prostasomes, and epididymosomes in diagnostic markers with advanced concepts and practical knowledge.

We searched the most important articles published through Scopus, Google Scholar, NCBI-NLM related to exosomes in semen.

We searched by the words "Exosomes in human semen", "Composition of exosomes in human semen", "microRNAs in human seminal exosomes", "Prostasomes", "epididymosomes", "exosomes as diagnostic markers", "seminal exosomes", "seminal exosomes and azoospermia", "exosomes in male reproduction", "exosomes and antiretroviral activity", "exosomes and HIV-1 interactions". We filtered 135 research and review articles that are coming under our objective of the current review article. All articles published between 2010 and 2020 have been taken for summarizing the recent advancement in exosomal research.

2. Composition of seminal exosomes

Human semen consists of cellular components (spermatozoa), and non-cellular components (seminal plasma). Seminal plasma is the mixture of various organ secretions like prostate, epididymis, and seminal vesicles (Jodar et al, 2017). Because of its well-documented ability to suppress the immune system, sperm can carry pathogens. Seminal plasma is a mixture of proteins, lipids, polysaccharides, transcription factors, trace elements, fructose, glucosidase, and other energy suppliers (Druart & de Graaf, 2018).

The seminal plasma plays a major role in sperm cell maturation, capacitation, and acrosome reaction. The exosomes present in the seminal plasma are known to positively impact all the sperm functions for effective fertilization (Du et al, 2016). Seminal exosomes in semen contribute around 3% of the total associated seminal plasma proteins. The major components of seminal exosomes are prostasomes and epididymosomes that are secreted by prostate and epididymis respectively (Del Giudice et al., 2016). Prostasomes role in male fertility was effectively summarized by us in the previous work done by our team. The proteomic analysis of human exosomes shows the presence of 1474 proteins. Many bioinformatic works revealed the importance of these exosomal proteins in the biological process such as cell growth, metabolism, internalization of the target cells, carrier for the regulatory functions, and so on (Sun et al, 2018). Human seminal plasma exosomes contain a repertoire for small non-coding RNAs which helps in the modulation of the female reproductive tract and supports embryonic development (Morgan & Watkins, 2020).

3. Exosomes as carrier of regulatory functions

Seminal exosomes have already proven to be an immunosuppressive agent and play a role as natural killer cells. This proves that the immunosuppressive nature of seminal plasma might originate from the exosomes fraction for atleast 70% of the cases (Sousa et al, 2017). Semen might carry pathogens, because of its well documented property of being an immunosuppressant. This can influence the infection for new hosts, probably the life partner (Wigby et al, 2019). Seminal plasma contains wide components, some might enhance and some may inhibit the viral infections. Small RNAs present in exosomes of semen affect the target cells, with potential immunomodulatory functions (Lyu et al, 2019). Many researchers proved that during cell culture conditions, intracellular communications can be mediated through the miRNA of exosomes (Huang-Doran et al, 2017).

These miRNA can probably interrupt immune responses, viral infections, and cancer cell progressions. Exosomes in semen can be present in very high concentrations and delivered to a very small anatomical area, probably the recipient female reproductive tract (Robertson & Sharkey, 2016). Phagocytic cells of the genital mucosa take up the exosomes for further physiological functions. Phagocytes in the female genital tract are well exposed to the seminal exosomes and uptake them multiple times triggering the physiological functions (Pang & Teow, 2020). It is already evidenced that seminal exosomes were derived from all the tissues associated with the male reproductive tract, connected and termed as a semen carrying system. Some seminal exosomes can be derived from spermatozoa too (Barcelo et al, 2018). A small subset of miRNAs can be packed in the seminal exosomes and nearly 175 such miRNAs were found in the semen. When compared with breast milk, saliva, dendritic cells, and blood plasma, seminal exosomes share the 3 most abundant miRNA out of 10 which is remarkably high (Welch et al, 2019). Let-7b miRNA is proven to be the most common and exists in abundance in the semen. miR-30 d is also present in abundance and mediates the major physiological roles needed for human reproduction (Salas-Huetos et al, 2020).

Exosomes in human semen may exhibit a unique miRNA profile (with 175 sets) and control the major functions (de Lima Kaminski et al, 2019). miRNA in the seminal exosomes plays a major role in immune functions in target cells of host genital mucosa. miRNAs were targeted by the miRNAs (abundance) carried by the seminal exosomes (de Lima Kaminski et al, 2019). Malproduction or any alteration in the production of any of the proteins associated with seminal exosomes that exposed the genital tract especially leukocytes resulted in altered immune responses against pathogens (Ronquist et al, 2016). The Let-7 family is abundant in the semen and monitors the key functions of IL10, IL-3, and T-cell maturation (Thammaiah & Jayaram, 2016). Researchers wondered if the role of miRNAs delivered by seminal exosomes to genital mucosa will be sufficient or not and the mechanism was also unknown (Ouattara et al, 2018). But miRNAs delivered by seminal exosomes can play an important role in monitoring the authority of immune responses and prolong the viral infections in the target cells (Xia et al, 2019).

4. Exosomes miRNAs as a non-invasive biomarker for prostate cancer

As per male urology, the prediction of prostate cancer at a very early stage will help during the prognosis period (Cucchiara et al, 2018). PSA is now being used as a biomarker to diagnose prostate cancer at an early stage; this has been standard practise for the past 30 years. Nevertheless, there are some limitations to PSA mediated identification (Chistiakov et al, 2018). So clinicians looked for a better biomarker for diagnostic and therapeutic purposes for effectively segregating between the PCA tumour and cancer (Fabris et al, 2016). The abundance of miRNA expression might be the reason for cancer in prostate cells. miRNAs in any of the body fluids have been very useful biomarkers in the identification of various diseased conditions especially cancer (Salehi & Sharifi, 2018).

Semen also a biofluid, is an ideal fluid to be evaluated with its properties for identifying the diseases associated with the male reproductive system (Longo et al, 2018). Only a few researchers were working on the expression level of miRNAs in semen. Particularly the seminal plasma (secreted by prostate and other glands) has importance for the identification of prostate cancer (Vlaeminck-Guillem, 2018). miRNAs have been protected by the exosomal membrane as well as not getting degraded; This shows the proven capability of miRNA in exosomes that can be used as a biomarker for tumour diagnosis (Zhai et al, 2018). Prostate biopsies are required for screening of elevated PSA levels, but by using miRNA signature, the invasive procedure can be skipped. There is a need to focus on the dysregulated miRNAs in prostate cancer patients for the identification of biomarkers by comparing them with healthy individuals (Shukla et al, 2017).

The practice of vasectomy has increased in the recent past, as it is treated as an important contraceptive method (Tan & Levine, 2018). This procedure will affect the concentration of exosomal

miRNAs in the seminal plasma as the volume from testes and epididymis will not reach the semen (Recuero et al., 2019). Hence under these circumstances, the miRNA expression can be below the normal range. The levels of some 50 miRNA in semen were found with altered conditions in prostate cancer patients when compared with normal healthy individuals. miR-142-3p, miR-223-3p, and miR-142-5p are the very common miRNAs found in elevated PSA levels and cancer patients (Izzotti et al,2016). These 3 miRNAs could play a major role in human reproduction as well.

The concentration of these three miRNAs in the seminal exosomes will be a reflectance of better prostate health. miR 223-3p is found to be up-regulated in many cases which were screened for prostate cancer. Compared to PSA data of prostate cancer patients, the expression level of miRNAs in exosomes of seminal plasma yield better results (Radtke et al, 2019). To identify the potent miR, many *in silico* work were done and 20 miRNAs were identified as the target genes involved in the prostate and prostate cancer pathogenesis (Colden et al., 2018). Similar to this, miR-142-3p and or 5p were found to be up-regulated in lung adenocarcinoma and breast cancer patients (Liu et al, 2019). These confirm that seminal exosomal miRNAs will provide separate biomarkers for various cancers based on the body fluidic origin. In another study, miR-342-3p and miR-374b-5p were proved to have tumor aggressiveness (Shrestha et al, 2017).

This miR-342-3p was found overexpressed in colon cancer. Also, researchers confirmed the quantitative changes at transcript levels of miRNAs of exosomes and were able to detect it in the seminal exosomes in any of the reproduction associated cancer (Condrat et al, 2020). These miRNA signature profiles of seminal exosomes can be the choice for the detection of prostate cancer. With these, we will also be able to detect the severity of prostate cancer during diagnosis (Fabris et al,2016). This will be much helpful for the patients and oncologists for undergoing further treatment. Overexpression of the above-mentioned miRNAs will pave the way for the effective diagnosis of cancer and further prostate health (Yang et al, 2019). With the above-said miRNAs and PCA level, an easy mechanism was proposed for distinguishing benign and malignant tumour (Singh et al, 2017). This combination of miRNAs and PCA levels for screening prostate cancer works more effectively than other available methods. For further details, a complete screening of miRNAs in the seminal plasma of prostate cancer patients will yield enormous useful data (Matin et al, 2018).

These data could be useful for both diagnoses as well as in the prognosis of prostate cancer patients. During prognosis, the overexpressed miRNAs can be blocked by a well-targeted therapy. Therefore the non-invasive biomarker identification will pave a better way for understanding the molecular mechanism of prostate cancer (Wang et al,2020). It will also reduce the cost of doing biopsies for the screening of prostate cancer and also improve the patient's outcome in a better way. These results will further pave the way to search the biomarker fitness for other prostate cancer properties like the metastasis period, during the treatment period (Butler et al, 2017).

5. Exosomes in asthenozoospermia (motility associated)

In a research conducted, seminal exosomes were purified and isolated from the patient's seminal plasma for men with normospermia and asthenospermia (Rao et al, 2019). Nearly 2136 proteins were expressed with both the categories. Mass spectroscopy evolution helped greatly in detecting the least molecular weight proteins also (Saha et al, 2017). When the prostasomes protein profile of normospermia and asthenospermia men were compared, 5 proteins were not expressed in the asthenospermia men, but among these none of them have been related to reproductive potential (Murdica et al, 2019). Seminal exosomes isolated from the normospermia men displayed overexpression of CRISP when compared with severe asthenospermia men (Samanta et al., 2018a, 2018b). CRISP is needed for the motility of sperm that will be transferred from seminal exosomes to spermatozoa (Giacomini et al, 2020). This is essential for proper fertilization. Exosomes uptake will be mediated by many conditions including sperm movement, maturation, and capacitation process (Zhou et al, 2018). Very important seminal exosome proteins like aquaporins expressed in testes and epididymis were found to be upregulated in both normospermia and severe asthenospermia. Glycodelin, an exosomal originated protein, found its expression in the asthenospermia samples and not in the normospermia conditions (Barrachina et al, 2019). But there is no proven mechanism where glycodelin transfers to sperm from exosomes. If this is identified, then much useful information and the fact that it can be used as a biomarker for asthenospermia conditions can be revealed (Pelloni et al, 2018).

6. Biomarkers in exosomal proteins for male infertility diagnosis

Exosomes are composed of several different types of proteins that play a vital role in sperm motility, acrosome reaction, capacitation, and fertilization process (Martin-DeLeon, 2016). Advanced integrated omics technology leads the scientific society to better understand and identify the exosomal proteins. Cell growth, proper protein metabolism, and cell communication maintenance are the primary roles of exosomes associated proteins (Bandu et al, 2019). Nearly 1474 proteins have been identified in the exosomes of normospermia men. Prostasomes are the major composition of exosomes in human semen; nearly 1285 proteins have been identified in the prostasomes (Saez & Sullivan, 2016). These prostasomal proteins play a major role in sperm motility and capacitation (vickram et al, 2020). Researchers have also found the difference in expression level and the quantity of prostasomal proteins between normozoospermic and other male infertile conditions (Panner Selvam et al, 2019). Prostasomal proteins play a vital role in energy production, bind with spermatozoa, and do the betterment for the survival of sperm at the female reproductive tract (Gilda & Gomes, 2017). These prostasomal proteins are under-expressed in non-normospermia men. GPX5, SPAM1, PSA, KIF5B, ANXA2, KLK2 are the important exosomal proteins that play a major role in human reproduction (Baskaran et al, 2020). These proteins help in the interaction of zona-pellucida and sperm, epididymal sperm maturation, membrane trafficking, and fusion as an energy producer (Leahy et al, 2020). Any aberrant expression of the above-mentioned proteins may further lead to affect the sperm functions and proper fertilization. In such cases, based on the protein expression levels by comparing all infertile and fertile categories, it is hypothesized that exosome proteins can be used as a biomarker for the diagnosis of male infertility.

7. Epididymosomes and its functions in human reproduction

The major population of epididymosomes are enriched with CD9, CD26, and CD224 and these proteins might interact with the live spermatozoa (da Silveira et al, 2018). The other major population is enriched with ELSPBP1 (epididymal sperm binding protein 1) that might interact with dead spermatozoa (Sullivan et al, 2018). CD9 is the major protein of epididymosomes that binds with the sperm and is likely to transfer proteins that lead to sperm functions. The epididymis is not only responsible for sperm maturation, but it also influences sperm cell heterogeneity (Nixon et al, 2019). When the sperm enters into the epididymis, it lacks motility and

remains to be immotile. The passage into epididymis continuously changes and the microenvironment facilitates the maturation process and then makes the sperm cell to be functional (Mabotha, 2019). Epididymal proteins behave like coating protein, an integral protein with spermatozoa for the betterment (Björkgren and Sipilä, 2019). Proteins p25b and MIF involved in sperm-oocyte interaction and sperm motility respectively, both function only in the case of positive CD9 epididymosomes (James et al, 2020). During the phase of the maturation process, sperm motility and egg recognition by sperm are the key properties of epididymosomes that are acquired by spermatozoa (Freitas et al, 2020). During the transit of epididymis, the positive CD9 epididymosomes play a major role in transferring lipids to the sperm membrane (Rowlison et al, 2018). Ubiquitin epididymosomes associated protein is transferred to the spermatozoa during the transit period for the proper fertilization (Machtinger et al, 2016). Ubiquitin is involved with the enzymatic degradation of major proteins (proteasome), which means it eliminates the defective spermatozoa out of the game. Epididymal sperm binding protein from epididymosomes interacts with dead spermatozoa mediated by Zn molecules (Zhou et al, 2019). ELSPBP1 combined with biliverdin reductase serves as an enzymatic loop and helps in scavenging ROS from the dead sperm cells and helps in protecting the live sperm cell from oxidative stress. Acquisition of this complex will help the sperm to live against the molecules produced by the dead sperm cells (D'Amours et al, 2018).

Premature acrosome reaction might be the cause for infertility; this can be stopped by GPX5 epididymosome protein (seleno-independent protein) (Agarwal et al, 2016). This GPX5 has a weaker enzymatic activity and gets transferred to the spermatozoa via fusion during the transit epididymal period and this helps in preventing the premature acrosome reaction (Maremanda et al, 2016). In the same manner, glutathione S-transferase, an epididymosomal protein secreted into the compartment of intralumen helps in preventing premature acrosome reaction (Chen et al, 2020). It protects the sperm cell from free radical damage. Another important protein is P34h which is a sperm binding protein associated with epididymosomes transferred to the sperm membrane during transit (Arenas-Ríos et al., 2017). It governs the major function of fertilization –binding of sperm to zona pellucida (Kerns et al, 2020). The major cause for immunological infertility is the protein CD52/HE5 which is secreted by the vesicles and it is an epididymosomal derived protein transferred to the plasma membrane of the sperm cell via fusion during transit of epididymal maturation (Liu, 2016). This can be the major reason for immunology mediating human male infertility (Dutta et al, 2020). Another important protein PH-20 plays a role in sperm and zona pellucida adhesion and it is also called sperm adhesion molecule 1 (SPAM1) (Saindon & Leclerc, 2018). The transfer of this protein to the membrane of the sperm is mediated by epididymosomes. An important protein called macrophage migration inhibitory factor (MIF protein) associated with epididymosomes transfer to the sperm membrane via fusion and mediate the sperm flagllar unit and the sperm motility is monitored by this protein during epididymal maturation process (Lang et al, 2018). Sperm, when it enters the epididymis, aldose reductase associated epididymosomes deprive the energy source of the sperm and keep it sluggish. But a higher activity of sorbital dehydrogenase started oxidizing the sorbitol to fructose providing the energy source required for sperm for survival and movement in the female reproductive tract (D'Amours et al, 2016).

8. Role of prostasomes (exosomes) in prostate cancer

Prostasomes are the highest constituents in exosomes of human semen (Vickram et al, 2020). Prostasomes are the only exosomes that are truly derived from the prostatic cells (Zijlstra & Stoorvogel, 2016). The prostasomes play a major role in sperm motility, the regulatory authority for sperm capacitation, acrosome reaction, and acts as an immune suppressor at the female reproductive tract (Pucci et al, 2017). The molecular composition of the prostasomes reveals the probability of prostate cancer growth and metastasis. Prostate stem cell antigen (PSCA) and TMPRSS2 confirm the prostatic origin of the vesicles (Malla et al, 2017). Researchers also confirmed the presence of anti-prostasomes antibodies in the blood sample of prostate cancer patients. Loss of cell polarity occurs for the cancer patients that leads to the release of prostasomes in the circulating environment (Wendler et al, 2017). Any antibody produced against the prostasomes might result in prostate cancer. In many cases, the antibodies against the prostasomes cannot be quantified (Fujita et al, 2017). The correlation between this antibody detection and identification of prostate cancer or metastasis is not possible. This demonstrates the poor efficacy of prostasomes antibody-based prostate cancer diagnostics (Jedinak et al, 2018). Among all other seminal exosomes, prostasomes contain the prostate cancer-specific fingerprints and this may represent the functions of the originating cells (Conigliaro et al, 2017). The presence of prostasomes in the blood can be more difficult, and hence prediction of prostasomes as a prostate cancer marker remains unfit (Urabe et al, 2020). In humans, the prostasomes are identified as 2 major filters, one with smaller vesicles, and second with larger vesicles. Major proteins like GLIPR2 and ANXA1 were able to be found in the smaller and larger vesicles respectively. Based on the expression of these 2 proteins in prostasomes, they are considered as biomarkers for male infertility and other prostate dysfunction (Boudhraa et al, 2016). Prostasomes have around 19% of structural and transport proteins. Prostasomes are rich in the annexin family which enables them to bind to spermatozoa and monitor the role of membrane trafficking (Pegtel & Gould, 2019). The fusion is mediated by Zinc and other trace elements. Both capacitation and acrosome reaction is activated only if the prostasomal cholesterol is transferred to the sperm plasma membrane during the fusion of prostasomes which is mediated by Zinc (Sharma & Kumar, 2017).

9. MicroRNAs in exosomes as a marker for azoospermia

Assessment of azoospermia patients will be very crucial for the andrologists and clinicians (Esteves et al, 2020). It is mandatory to categorize azoospermia as obstructive and non-obstructive. The condition of obstructive is very easily treatable, whereas, for non-obstructive azoospermia, it is more complicated to identify the problem (Shiraishi & Matsuyama, 2017). At present, we have a limited number of parameters to access the azoospermia conditions like medical history, hormone analysis, karyotype and other genetic tests (Xie et al, 2018). In certain cases, testicular biopsy is mandatory for further diagnosis. In certain cases, FSH can be treated as markers for non-obstructive azoospermia and in certain other cases, the lower testicular volume also acts as markers (Tournaye et al, 2017). But they all remain unsatisfactory for diagnosis. As we already discussed, semen consists of many microRNAs that can be identified and screened for the potential non-invasive marker candidate for diagnosis and prognosis purpose (Deng et al, 2017). MicroRNAs present in the exosomes reflect the pathological conditions of the organ and the source of origination (Ricci et al, 2018). 23 miRNAs are associated with germline functions in the testes. miR-449a is found to be underexpressed in testicular tissues and semen samples which are usually related to a deficiency in the production of sperm (Gao et al, 2020). Researchers proved that above 37% of the total azoospermic down-regulated exosomal miRNAs were found to be located at the site of X-

chromosomes with clusters. Many of the X-linked exosomal miRNAs were expressed only in spermatogenic cells (Finocchi et al, 2020). Clusters miR-449 and miR-34b/c were found to be expressed very less in the testes and are associated with sperm maturation block in the germline itself (Reza et al, 2019). The potential of microRNAs in the exosomes should be accessed completely for its biomarker capability for identifying azoospermia conditions (Conti & Franciosi, 2018). Another challenge is to obtain the spermatozoa from the testicular biopsy (Shabataev & Tal, 2017). The miRNA profile has to be compared with each individual azoospermic conditions from different origin (Ibarra-Ramirez et al., 2020). The major diagnosis path is obstructive and secretroy azoospermia. There exists a difference in the expression level of exosomal miRNAs between obstructive and non-obstructive subjects (Majumdar & Bhattacharya, 2013). Researchers have also confirmed the role of miRNAs of exosomes in predicting the origin of azoospermic conditions and the presence of sperm at the testicular tissue (Daneshmandpour et al, 2020). The efficacy of the miRNA profile in exosomes as a biomarker can be good and on par with other diagnostic markers. miR31-5p is an important predictor for the origin of azoospermia in men. The sensitivity and specificity are also quite good for this microRNA for the prediction of the origin of azoospermia. miR-31-5p has been proven as one of the best diagnostic markers for azoospermic conditions (Kiss et al, 2016). Combined biochemical markers like FSH and microRNAs profile of exosomes can result in better diagnosis of azoospermia.

10. Exosomes as anti-HIV activity

The major function of exosomes is to deliver their cargo to the recipients and then modulate the host cell functions (Lai et al, 2013). Exosomes in semen have antiviral and antibacterial activity. Researchers are working on the exosomes to know whether exosomes in the semen are internalized by the target cells and whether exosomes possess antiviral activity against the viruses that are transmitted via semen (Madison & Okeoma, 2015). They have found that seminal exosomes can effectively internalize the target cells within 3–5 h of exposure of cells to the seminal exosomes (Kooijmans et al., 2012). Researchers have also confirmed that seminal plasma exosomes can restrict the replication of HIV-1 and other AIDS virus complexes. The study also shows that seminal exosomes are very specific in inhibiting retrovirus replication, while blood exosomes are not involved (Welch et al, 2019). Seminal exosomes did not show any antiviral effect on HSV types and this proves that seminal exosomes are very specific with antiviral activity against only retroviruses groups (Barile & Vassalli, 2017). The mechanism behind the antiretroviral effects of seminal exosomes remains unclear but most probably involved in altering reverse transcription activity and also in the composition of protein (Dias et al, 2018). We know that the HIV-1 transcription process can be regulated by the levels of positive and negative regulators in the system (Cullen, 1991). The key to control can be any factor that affects the positive regulator of transcription and lead to suppression of the proviral transcription process. Many researchers inferred the links between HIV-1 transcription and seminal exosomes (Dogrammatzis et al, 2020). Blocking the viral transcription factor can be done by seminal exosomes and repress the viral transcription factors and these may give access to the HIV-1 promoter. At many points like chromatin organization, transcription initiation, and elongation where the HIV-1 provirus regulation happens (Lenstra). All we know is that HIV is commonly transmitted through sexual intercourse and semen could be the primary vector or source of infection (Ellwanger et al., 2017). The pathogenesis of HIV- AIDS is very tough to understand, however, all these antiretroviral therapies can certainly help the host. Even though antiretroviral therapy helps, we cannot control the route of sexual transmission (Ojha et al, 2017). So, we need effective control of the HIV replication and transcription process in the host. Preferably the seminal exosomes with antiretroviral properties can be the choice of research (Ouattara et al., 2018). Researchers have confirmed that seminal exosomes protect the human immune mechanism against HIV infection proved in many in-vitro studies (Kaddour et al, 2020). Seminal exosomes inhibit the HIV-1 through the RNA expression. Seminal exosomes in the seminal plasma inhibit transcription factors like NF, Sp1 until the promoter region long terminal repeat (Lyu et al, 2019). Protein transcriptional activator (Tat) will be the HIV-1 basic protein and where seminal exosomes will target (Sadri Nahand et al, 2020). The major function of seminal exosomes here is to inhibit the binding efficacy and recruitment of Tat protein with the HIV-1 LTR complex (DeMarino et al., 2018). Through this, the mechanism can be easily understandable and we can conclude that both transcription and replication of HIV-1 can be affected by these seminal exosomes (DeMarino et al., 2018).

In the course of sexual transmission of HIV-1, the entire female genital mucous will be exposed to HIV-1 and the seminal exosomes present in the semen (Nijmeijer et al, 2019). The direct method of cell to cell transmission and cell-free virions transinfection are the means of HIV-1 propagation (Leon et al, 2016). For HIV-1, dendritic cells, submucous cells, macrophages, and T cells are the easy target and these are very susceptible (Kariuki et al, 2017). Researchers have confirmed that the seminal exosomes have the capacity of blocking the spread of HIV-1 from the female vaginal epithelial cells to any type of target cell; this research was mediated by cell to cell infection model (Coutinho et al, 2017). The same research proves that seminal exosomes suppress the transmission of HIV-1 alongside the female vaginal barrier (Sims et al, 2017). Also, the seminal exosomes restrict the intravaginal transmission of the virus. These findings suggest that a lot of potent seminal exosomes are available that can be used as antiviral agents. Seminal exosomes possess proven antiretroviral agents in the semen (Gutierrez-Valencia et al., 2017). These results provide insights for the newer therapeutic mechanism for the treatment of HIV-1 infection.

11. Conclusion

We summarized all the important aspects of seminal exosomes in male reproduction and men's sexual health. An important observation is that seminal exosomes contribute around 3% of the overall proteins present in the seminal plasma. Seminal exosomes can potentially convey the regulatory signals to the entire recipient or host mucosa through transferring small RNA molecules. Seminal exosomes contain specific antiretroviral properties, and this inhibits HIV-1 replication process. This proven research will further provide very novel opportunities and leads to new therapeutics for HIV-1 infection. Also, we conclude that the structural heterogeneity of the exosomal particles in human semen correlates with the functional diversity in male reproduction. Proteomic studies of prostasomes reveal more structural proteins and their function in male reproduction. Many researchers reveal the seminal exosomes can be the choice for the development of new and novel therapeutic regimes to combat the HIV-1 infection and transmission. Also, we conclude any short term semen freezing will not have much effect on the seminal exosomes mediated HIV-1 inhibition. Seminal exosomes can be the best choice for prostate cancer diagnosis as many contradictory research data are available for PSA mediated diagnosis. Also, the prostasomes play a major role in transferring important proteins to the spermatozoa for motility in the female reproductive tract. Overall, the seminal exosomes can be the choice

of candidate for diagnosis and prognosis of various diseases associated with human male reproduction.

12. Future insights

Many researchers already started working on the insights of seminal exosomes, especially with prostasomes and epididymosomes. Though the anti-retroviral activity of the seminal exosomes was identified, the mechanism underneath it is poorly understood. Many theoretical hypotheses support the role of exosomes in male reproduction. But there is a need for cutting edge research with each of the proteins and their associates in the seminal exosomes for identification of novel strategies in blocking HIV- transmission. The use of seminal exosomes for the diagnosis of prostate cancer is still under a dilemma, though it gives better results when compared to prostate specific antigen as a marker. Only very few oncologists were accepting the use of seminal exosomes as a non-invasive biomarker. Already a lot of research is done with prostasomes, but the research concerning epididymosomes is lagging. The overall proteomic profile of prostasomes and epididymosomes will yield better results for the identification of novel biomarkers. Already prostasomes' proteomic profile yields many markers for the diagnosis of male infertility. The same strategy can be followed in epididymosomes to better understand epididymis health. Altogether these 3 organs, prostate, epididymis, and seminal vesicles constitute the major contribution for seminal exosomes in seminal plasma. These 3 organ proteomics profiles will give insights on seminal exosomes and their functions towards male reproduction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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