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Review

Exosomes – Spectacular role in reproduction

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ABSTRACT

Exosomes are nano-sized structures that are found in semen, epididymal –fluid, endometrium, as well as in follicular fluid. They are responsible for transporting bioactive cargo- proteins, lipids, and nucleic acids. Exosomes have been proven to influence processes in both female and male reproductive systems, including gametogenesis, acrosomal reaction, sperm capacitation, and embryo implantation in the endometrium. Exosomes are made of the same particles as the cells they come from and are secreted by normal and pathological cells. Therefore, exosomes can reflect the physiological state of cells. Moreover, due to the transportation of biomolecules, they participate in intercellular communication and can be used as biomarkers of many diseases, including ovarian, endometrial and prostate cancer. Identification of exosomes as biomarkers could contribute to a better understanding of genital dysfunction and fertility disorders.

1. Introduction

Extracellular vesicles (EVs) act as a vehicle for cell-to-cell communication [1]. EVs are secreted by cells into the extracellular space [2]. Extracellular vesicles are: microvesicles, ectosomes, membrane particles, exosome-like vesicles, apoptotic bodies, and exosomes [3]. In turn, the best described are three groups – exosomes (EXO), microvesicles (MV), and apoptotic bodies (AB) [4]. The differences between the individual groups are mainly based on biogenesis, release pathways, content, function and size [2]. Table 1 below shows the differences between the individual EV groups.

Currently, more attention is focused on exosomes, which are bilayer extracellular membrane microvesicles secreted by most eukaryotic cells derived from the multivesicular body (MVB) [1,4,5,8]. These vesicles are surrounded by a membrane and are typically 40–160 nm in diameter, and their density ranges from 1.13 to 1.19 g / mL [5,9]. Exosomes are released during exocytosis, during which multivesicular bodies fuse with the cell membrane [10].

Originally, exosomes were referred to as 'membrane fragments' and defined as 'cell waste' [1,5]. With the development of science and

Abbreviations: AB, apoptotic bodies; AEC, amniotic epithelial cells; BC, breast cancer; BMSC, bone mesenchymal stem cells; CAF, Cancer-associated fibroblasts; CAT, catalase; cBSA, cationic bovine serum albumin; CC, cervical cancer; COL5A2, type V collagen alpha 2 protein; COX-1, cyclooxygenase 1; CRISP1, cysteine-rich secretory protein 1; ELSPBP1, epididymal sperm binding protein 1; ErbB, the growth factor receptor pathway; EV, extracellular vesicles; EXO, exosomes; FF, follicle fluid; GCs, granulosa cells; GPX, glutathione peroxidase; hAEC, human amniotic epithelial cells; hFF, human follicular fluid; HGF, hepatocyte growth factor; Hh, Hedgehog protein; HOSEPiC, a human ovarian surface epithelial cell line; HPV, human papillomavirus; HSP70, heat shock protein70; HUVEC, human umbilical vein endothelial cells; IFNT, interferon-tau; IL-6, interleukin 6; ILV, intraluminal vesicles; LDH-C4, C4 lactate dehydrogenase; LGALS3BP, galectin-3 binding protein; lncRNA, long non-coding RNA; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; MIF, migration inhibitory factor; miRNA, microRNA; MSC, mesenchymal stem cells; MV, microvesicles; MVB, multivesicular body; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OC, ovarian cancer; PA, phosphatidic acid; PC, phosphatidylcholine; PCOS, polycystic ovary syndrome; PDCD4, Programmed Cell Death 4; PE, phosphatidylethanolamine; PGF2α, prostaglandin F2α; pGSN, plasma gelsolin; PI, phosphatidylinositol; POF, premature ovarian failure; PRDX1, peroxyredoxin; PSA, specific antigen for the prostate; PTX, Paclitaxel; ROS, reactive oxygen species; sACY, sperm-specific adenylate cyclase; SCNT, somatic cell nuclear transfer; SEMG, semenogelin; siRNA, short interfering RNA; SKOV-3, ovarian cancer cell line; SOD1, superoxide dismutase; SP, semen plasma; SPAM1, sperm adhesion molecule 1; Tat, Trans-Activator of Transcription; TCs, theca cells; TEX, Tumor-Derived Exosomes; TGF-ß, transforming growth factor ß; THBS2, thrombospondin-2; TNF, α, tumor necrosis factor alfa; TSP1, thrombospondin-1; TXN1, thioredoxin; ULF, uterine lumen fluid.

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research techniques, it has been found that exosomes constitute a type of intercellular communication in many physiological and pathological processes that serves to maintain the body's homeostasis and are involved in the transport of biomolecules [1,5,9]. Exosomes transport bioactive cargo such as nucleic acids, including RNA, DNA, proteins, lipids, and enzymes [1,5].

Endosome biogenesis takes place by invagination of the cell membrane (endocytosis) (Fig. 1A, I), then early endosomes are formed (Fig. 1A, II). This is followed by the formation of the late endosomes by selecting the content of the early endosomes (Fig. 1A, III). The late endosomes form multi-vesicle bodies (MVBs) (Fig. 1A, IV) that can bind to the cytoplasmic membrane, whereby proteins are incorporated into the membrane and the components of the cytosol are absorbed and locked into intraluminal vesicles (ILV), which leads to the release of vesicle content, called exosomes (Fig. 1A, V) [5,6,8,9]. Biogenesis and structure of exosomes along with the structure of the cell membrane and intracellular effectors necessary to abscission exosomes during biogenesis are shown in Fig. 1.

Fig. 1 A shows the process of exosome biogenesis and secretion- I endocytosis, II early endosome, III late endosome, IV MVBs, V exocytosis. Fig. 1B shows schematically the structure of the exosome with cardo, surface markers.

The structure of exosomes is shown in Fig. 1 (Fig. 1B) and shows schematically the different families of proteins, lipids, and nucleic acids, including DNA, RNA, miRNA, and other non-coding RNA. Release of exosomes is supported by proteins such as Rab, ALIX, TSG101 [3].

Exosomes are made of the same molecules as the cells from which they originate. They contain nucleic acids, proteins, enzymes, lipids, or cytokines. Exosomes include proteins such as tetraspanins (CD9, CD 63, CD81, CD82), which play a role in cell penetration, invasion, and fusion [6]. The CD81 protein, which is the most abundant, is used as an exosome marker [16]. Rab protein are the main proteins involved in exosome biogenesis, and it has been suggested that these proteins are involved in exosome transport and maturation [16]. Another type of exosomes protein is heat shock proteins like HSP70, HSP90, which are part of the stress response and are involved in antigen presentation [6]. Cytoskeletal proteins (e.g. tubulin, actin) and proteins involved in MVB biogenesis (e.g. clathrin, Alix), which play a role in exosome release, and proteins responsible for membrane transport, annexin, have also been identified in exosomes. In turn, proteins such as flotillin and TSG101 are involved in the biogenesis of exosomes [6,15]. Exosomes also contain metabolic enzymes, such as ATPase, aldehyde reductase, and aspartate aminotransferase. Specific exosome marker proteins are TSG101, HSP70, or CD81 [6]. Moreover, exosomes contain various types of RNA, including long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs), which are involved in tumor development [6]. The exosome membrane contains phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), cholesterol, ceramides, sphingomyelin, glycine [15,16]. It has been shown that the content of exosomes and the composition of their membranes vary depending on various factors – including diet, expression of pathogenic genes, or hormones [16] (Fig. 1B).

Exosomes are secreted by all normal and pathological cells, which may reflect the physiological state of donor cells [4,8]. As a result, exosomes can be used in the diagnosis of many diseases [8]. These structures are found in blood, semen, saliva, plasma, urine, cerebrospinal fluid, amniotic fluid, breast milk, epididymal fluid, and in tears [1,2,6].

2. Functions of exosomes

Exosomes can participate in cellular regulation as well as physiological and pathological processes by activating specific receptors on the cell surface by means of protein ligands and bioactive lipids, or by fusing their transported cargo, such as proteins, or miRNAs, into the cytoplasmic membrane of the cell [9,17]. A growing body of evidence indicates that EVs are important mediators of intercellular communication, therefore they play a significant role in physiological and pathological processes, in terms of stem cell maintenance, tissue repair, immune modulation and tumor growth [18–21].

2.1. The role of exosomes in physiological and pathological states

It is well known that cell metabolites flow through diffusion, channels and connections between cells or their active secretion into the extracellular space [22]. It has been found that the flow of metabolites or harmful substances between cells can occur using exosomes [22]. Exosomes are recognized as mediators of intercellular communication in both physiological and pathological states [14]. EVs exhibit the ability to transport RNA, proteins, enzymes and lipids, thereby influencing physiological and pathological processes in a variety of diseases including cancer, neurodegenerative diseases, infections and autoimmune diseases. Therefore, their special importance in various biological processes such as angiogenesis, antigen presentation, apoptosis, coagulation, cell homeostasis, inflammation and intercellular signaling should be emphasized.

EVs can transfer misfolded protein, prions, or neurotoxic proteins-amyloid β can take place with the help of exosomes and macrovesicles [14,22]. Exosomes can transfer building blocks between cells, such as amino acids, and lipids, that can transfer to different places in the body. These structures are also responsible for changing the microenvironment by increasing the availability of substances for energy production, as is the case with glucose-deprived heart muscle. Then the cardiomyocytes secrete exosomes with glucose transporters (Glu1 and Glu4) that reach endothelial cells, which increase the absorption of glucose transporters and metabolism of glucose. Pyruvate is released as an intermediate product of glucoses metabolism, which is used by

Table 1
Differences between groups of extracellular vesicles.

	Exosomes (EXO)	Microvesicles (MVs)	Apoptic body (ABs)	Literature
Diameter	40–160 nm	100–1000 nm	1–5 μm	[2,5]
Content	Proteins, lipids, nucleic acids, enzymes	Proteins, lipids, nucleic acids	Content like cytosol - intact cellular organelles, chromatin, small amounts of glycosylated proteins	[2,6]
Function	Cell transport, protein release, storage, cell-to-cell communication, immune stimulation, support for myelin formation in the nervous system, neurite growth, tissue regeneration	Cell transport, cell-to-cell communication, blood coagulation, adhesion	–	[2,7]
Origin	Multivesicular body (MVB)	Cell membrane	Cell membrane	[4,6]
Location	All cell types	Erythrocytes, thrombocytes, lymphocytes, vascular endothelial cells	Dying cells that release apoptotic bodies into the extracellular space	[2,6]
Possibilities	Use of neurodegenerative diseases biomarkers as carriers. Better understanding of exosome function could lead to the development of new cancer therapies	A better understanding of MV function could lead to the development of new cancer therapies	–	[2]

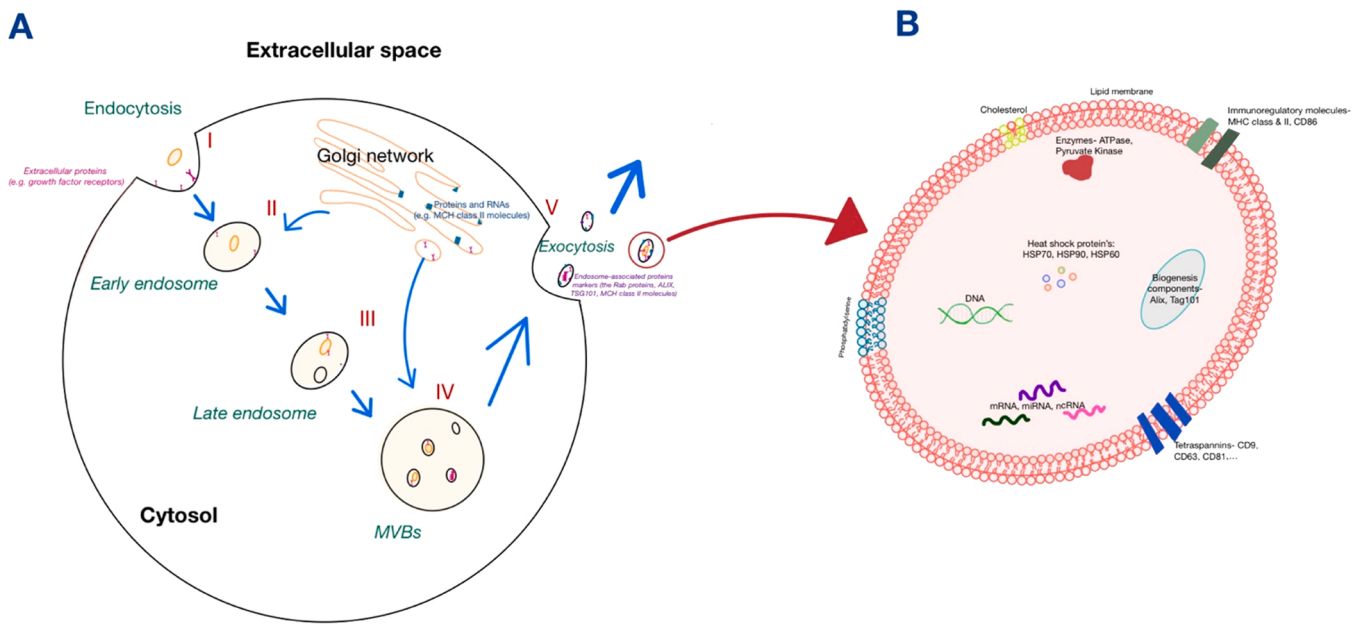

Fig. 1. Biogenesis and structure of exosomes (Figure based on: [3,11–15]).

cardiomyocytes for oxidative metabolism. It has been suggested that exosomes interact with endocrine and paracrine processes that maintain homeostasis [22]. Also, exosomal release of miRNAs is a rapid means of regulating gene expression. miR-23 and miR-182 are released by exosomes during induced muscles atrophy. The release of such components may reduce cellular stress [14].

Exosomes are also involved in angiogenesis, cell differentiation (including regulatory T cell differentiation), immunomodulation, metabolic balance, eradication of obsolete molecules, antigen presentation [1,5,9]. Extracellular vesicles that can regulate angiogenesis, or the growth of the placenta by secretion of the matrix metalloproteinase inducer (EMMPRIM) are released by trophoblast cells [23–25]. Research conducted by Grange et al. (2011) [26]has shown that microvescles from tumors can be involved in the process of metastasis and angiogenesis. A subset of cells with the CD105 antigen as a marker of mesenchymal stem cells has been identified in human kidney cancer. Moreover, CD105-positive has the ability to modify the tumor microenvironment and induce angiogenesis [26]. I has been proven that progenitor cells release exosomes involved in inducing migration of endothelial cells of blood vessels, as well as cell proliferation, and the process of angiogenesis [25–27].

A new biomarker of inflammatory diseases and conditions may be the content of exosomes during inflammatory processes. Neoplastic exosomes have been shown to be characterized by a high miRNA content, inhibition of T-cell proliferation and differentiation, and induction of apoptosis via the FasL and MARK1 pathways, promoting immune system avoidance by the tumor [28,29]. Moreover, research conducted by Fabbri et al. (2012) [30] have shown that tumor exosomes containing miR-21 and miR-29a induce a promestatic inflammatory response and are associated with cytokine secretion by binding to Toll-like 8 receptor and its activation in immune cells leading to activation NF-κB.

Exosomes also participate in the natural aging process. Aging is associated with the acquisition of Senescence-Associated Secretory Phenotype (SASP), which may facilitate phagocytic cell removal of senescent cells. The Wnt secretory molecule, whoch is responsible for maintaining the canonical homeostasis of the cellular pathway and is involved in the aging process, is abundantly present in exosomes [31, 32]. Therefore, it is suggested that the exosomes are a SASP signaling molecules. They are released from aging fibroblasts or epithelial cells. There is also an altered exosome profile in the elderly- e.g. galectin-3, which is essential during the maturation of bone cells, is significantly reduced. In exosomes obtained from the elderly, which may result from loss of bone stem cell function [31].

Exosomes are secreted from the reproductive system. The place of origin of exosomes is endometrial cells, follicular cells, follicular fluid, uterine body, embryos produced in vitro, Fallopian tubes [8].

2.1.1. Female reproductive system

The processes taking place in the female reproductive system, including pregnancy, are influenced by follicle growth, oogenesis, implantation, embryo development and proper fertilization, which are related to proper cellular communication [8]. Exosomes play a crucial role in this communication [33].

Exosomes are released from, among others, the uterus, oviduct epithelium, endometrium, pre-implantation embryos, and the play an important role in modulating transcriptional and translational activity, granular cell proliferation and differentiation, cumulus expansion, gametogenesis, or oocyte maturation, embryonic development, and implantation. They participate in the communication necessary for the fertilization process and just after it, as well as placental exosomes support angiogenesis and promote the migration of endothelial cells of blood vessels by participating in the establishment of fetal-maternal circulation [33]. Transforming growth factor beta (TGF- β) is important during follicular maturation, and mitogen-activated protein kinase (MAPK) is crucial in oocyte maturation, which is carried by exosomes [33].

miRNA-containing exosomes in the female reproductive system have been shown to be able to influence granule cell proliferation, gametogenesis, oocyte maturation, as well as embryo development and fertilization by targeting signaling pathways associated with re-meiosis and ovulation or follicular maturation [8,32–34]. miRNAs are essential for oocyte maturation, implantation and early embryonic development [8].

In the female reproductive system, exosomes play the role of regulators of uterine function and may help to maintain pregnancy. According to Koh et al. [35], boar sperm exosomes modulate the expression of resistance genes in the uterus of pigs, and reduce the expression of genes related to the process of steroid biosynthesis. In the other hand, sheep embryos contain exosomes with interferon, which may increase the level of interferon-stimulated genes in the uterus [35].

Research by Almuqhlliq et al. [36] reports that exosomes are

involved in the postnatal adaptation of cows to lactation. Moreover, any changes in circulating exosomes can be markers of disease [36]. Exosomes also participate in maternal-fetal communication during pregnancy [37]. These structures have also been reported to be involved in the timing of the cell signaling during labor induction. Uterine myositis cells exposed to amniotic epithelial cells (AEC) exosomes in vitro showed induction of a contractile phenotype by activation of NFκB and proteins associated with COX-2 contraction. In the same mouse research, it was proved that the exosomes labeled with dyes and injected intra-amniotically into pregnant mice passed into the plasma and kidney of maternal mice. In this way, it has been proven that exosomes can cross the placenta and spread through the circulatory system [38].

2.1.1.1. Exosomes in the ovarian follicles. A mature ovarian follicle contains an oocyte that is surrounded by several cell populations. The closest layer to the oocyte is the Zona pellucida surrounded by corona radiata granulosa cells and cumulus granulosa cells and mural granulosa cells (GCs) that surround the antrum of a follicle filled with follicular fluid. (FF). The outermost layer of the follicular cells are theca cells (TCs) with distinctions for the outer and inner layers [32]. The follicular fluid of plasma and follicular cell secretion contains a variety of components, including proteins, hormones, and exosomes [32,34]. FF is involved in communication between the oocyte and cumulus cells, which influences oocyte maturation and modulates meiosis in the female gamete, and also affects conception and the possibility of embryo implantation [32]. Moreover, exosomes contained in the follicular fluid affect the quality of female gametes, limiting apoptosis of cumulus cells and supporting conception [15]. The above-mentioned processes are affected by the transport of cargoes in the form of miRNA and mRNA by exosomes, which affect signaling pathways. These include mitogen-activated protein kinase (MAPK), transforming growth factor ß (TGF-ß), ErbB, Wnt, and ubiquitin-mediated pathways [32,33].

TGF-ß plays an important role in follicular maturation, and the mitogen-activated protein kinase may mediate luteinizing hormone-induced oocyte maturation. It has also been proven that the Wnt signaling pathway is necessary during ovarian development and ovarian tissue function in female. It regulates follicle development, oocyte maturation and steroidogenesis. In turn, the growth factor receptor (ErbB) pathway, insulin signaling pathways, and ubiquitin-mediated pathways are essential for cellular activities such as oocyte meiosis resumption or early embryonic development [33].

Table 2 shows the signaling pathways involved in follicular communication with exosomes.

Fertility disorders that progress with the age of females can be diagnosed by exosomes. Twenty-year-old mares showed significantly higher levels of exosomes miRNA of alveolar origin than younger mares. Higher levels of expression were obtained for miR-513a-3 P, miR-181A and miR-375, which affect the TGF ß pathway by inhibiting it, resulting in a disruption of oocyte maturation [33].

2.1.1.2. The role of exosomes in paracrine activity between cumulus cells and oocytes. Through the networks of the ovarian capillaries, a plasma filtrate is formed in the form of a follicular fluid, which is rich in exosomes, through which communication between the cumulus cells and oocytes can take place [39–41]. Communication between the cumulus cells and the eggs is important in the content of reproduction of mammals, mainly during oocyte maturation [40,42]. The mechanisms of this communication include bidirectional transfer of ions and molecules through gap junctions between the somatic cells of the follicle and the cumulus and oocyte cells [40,42]. Paracrine signaling is also related to signaling around ovulation [42]. Exosomes, through the content of miRNAs, take part in the process of RNA silencing and regulation of gene expression. A positive correlation of changes in miRNA expression with the synthesis of progesterone in the follicle fluid in cattle has been demonstrated. The presence of extracellular vesicles in the cytoplasm of cumulus cells and ootyces in cattle has also been reported [40]. In research conducted by Hung et al. (2015) [39], authors proved that exosomes can influence the function of the ovaries by supporting the expansion of the cumulus and modifying the expression ot their genes in vitro. Similar conclusions were obtained by Matsuno et al. (2017) [41], claiming that in cattle, exosomes may support the expansion of cumulus cells in vitro. In turn, exosomes in human follicle fluid containing miRNAs are involved in the regulation of biological pathways critical for dominant follicle growth and oocyte maturation [41]. This may indicate that these structures act as a mediator in cell signaling [39,40].

Heat stress to which animals are exposed, for example cattle, cause oocyte damage, induction of apoptosis, mitochondrial dysfunction, and an increased generation of reactive oxygen species (ROS). Exosomes, by transferring cargo, deliver it to target cells by internalization into the cytoplasm or by interaction with cell surface membrane receptors [43]. These structures increase the expansion of the cumulus during oocyte maturation, but also enhance the competence of mature oocytes in vitro to form blastocyst despite heat stress conditions. It has been shown that the exosomes contained in the follicular fluid can protect the oocyte from heat stress. However, according to the authors, the cytoprotective effect is not limited to heat stress [43]. Research conducted by Saeed-Zidane et al. (2017) [44] revealed that bovine granular cells exposed to hydrogen peroxide released exosomes enriched with mRNAs encoding genes needed for antioxidant protection (CAT- catalase, SOD1-superoxide dismutase, PRDX1-peroxyredoxin, TXN1-thioredoxin) into the medium. Granulat cells subjected to oxidative stress in vitro showed exosome uptake and reduction in the effect of hydrogen peroxide on cell proliferation, and their activity [44].

Communication between the ovarian follicle and the cumulus cells is crucial for the follicle maturation, and to produce an oocyte capable of fertilization and supporting subsequent embryonic development [43].

2.1.1.3. Exosomes in the uterus. The implantation of the embryo in the uterus depends mainly on the proper communication between the endometrium and the blastocyst. prostaglandins, interleukins. Endometrial reactivity and readiness for embryo implantation also result from the participation of exosomes in the communication process originating from the endometrium and the embryo [32,34]. There are exosomes in the uterine fluid that contribute to increasing the proliferation of uterine endothelial cells at the site of implantation [32]. Some miRNAs, such as miR-200c and miR-30d, contained in exosomes are involved in implantation by regulating gene expression during this process [45]. It has also been shown that exosomes contain interferon-tau (IFNT), which in the pre-implantation period shows the ability to regulate the expression of genes responsible for implantation, and IFNT promotes the synthesis of progesterone to establish pregnancy [46]. Interferon-tau acts on the endometrium, inhibits luteolytic processes and causes the synthesis of progesterone [47]. Exosomes in the uterine fluid are characterized by the ability to modulate the endometrium and support the embryo attachment [32].

2.1.1.4. Exosomes as biomarkers of pregnancy. Proper communication between the mother and the embryo is essential for the establish of

Table 2
Signal pathways involved in communication in the ovarian follicle.

Signal pathways	Significance	Literature
TGF-ß	Follicle maturation	[33]
WNT	Ovarian development, ovarian follicle development, oocyte maturation, steroidogenesis, ovarian tissue development	[32,33]
MAPK	Oocyte maturation	[33]
Pathway via ubiquitin	Resumption of oocyte meiosis, early embryonic development	[32,33]
Insulin signaling pathway	Resumption of oocyte meiosis, early embryonic development	[33]

pregnancy. This communication is mediated by exosomes released from the uterine endometrium that transport signaling molecules such as miRNAs [48]. This is crucial during implantation and embryonic development. In a research by Qiao et al. (2018) [48] it evaluated the effect of bovine uterine lumen fluid (ULF) exosomes on embryonic development with somatic cell nuclear transfer (SCNT). Exosomes were obtained from the uterus in the early luteal phase. The conducted research confirmed that the molecules carried by exosomes are needed for communication with the embryo. They contribute to an increase in the interferon-tau content, which is associated with pregnancy diagnosis in cows. Moreover, the ability to improve the development of SCNT embryos in vitro was confirmed [48].

Turner et al. (2021) [49] in the publication indicate that in dairy cows, exosomes with miRNA act as an epigenetic regulator of signaling pathways, including inflammatory reactions. May affect the development of pregnancy in cattle and reproduction [49]. There is also the possibility of using specific miRNAs as a pregnancy biomarker [50]. It has been proven that the expression level of miRNAs is different in pregnant cows and in cows that are not or have lost pregnancy. The miRNA-bta-miR 140 has been determined and can be used as a marker of early pregnancy in high yielding dairy cows [50].

Seminal exosomes have been shown to contain small non-coding RNAs that support embryonic development by modulating the female reproductive system [10,51].

The period after calving is associated with the occurrence of inflammation. During this time, hormonal, immunological, and metabolically changes occur. In the event of an imbalance, dysfunction and inflammation may occur [36,37]. During the inflammatory response in the endometrium, there is an increase in the production of cytokines and proinflammatory chemokines, which may impair endometrial receptivity, reduce fertility, and negatively affect uterine function [35]. It has been shown that exosomes obtained from the plasma of low-fertile heifers can alter the expression of inflammatory mediators, resulting in inflammation of the uterus and reduced fertility [35].

An indicator of the fertility status may be exosomes, which may be markers of uterine, metabolic diseases [36]. Exosomes of cows with induced endometrial infection decreased the production of PGF2α (prostaglandin F2α) in *in vitro* research, which influenced the immune response of the endometrium and inhibition of the luteolytic pathway and further on the diagnosis of pregnancy by the mother [36].

Changes in the content of exosomes and their intercellular communication have been marked as a biomarker of pregnancy and all-pregnancy-related pathologies in humas. An increase in the content of exosomes in the placenta and circulation of women with eclampsia and pre-eclampsia compared to non-dysfunctional women was detected. This suggest that early detection of complications and pathologies of pregnancy by examine exosomes may be a prophylaxis and may support the implementation of treatment In the case of infertility, the exosome profile should be established [33]. Examination of the exosome profile can provide a picture of health status, as well as an increased or decreased susceptibility to disease [36].

Research suggests that exosomes isolated from the amniotic fluid contain mRNAs that are valuable tools in prenatal diagnosis. In a study by Keller et al. [52] proved that CD24 vesicles are exosomes that most likely come from the kidney and are present in the urine of newborns and in the amniotic fluid of pregnant women. In addition, it has been shown that CD24 exosomes are also found in the urine and amniotic fluid of mice. In studies in mice with a CD24 knockout, CD24 exosomes were proven to be of fetal origin. These results indicate that the secretion of exosomes into the urine and amniotic fluid is a biological process that may play a role in embryogenesis as well as later in life [52].

The importance of exosomes in the female reproductive system is summarized in Fig. 2.

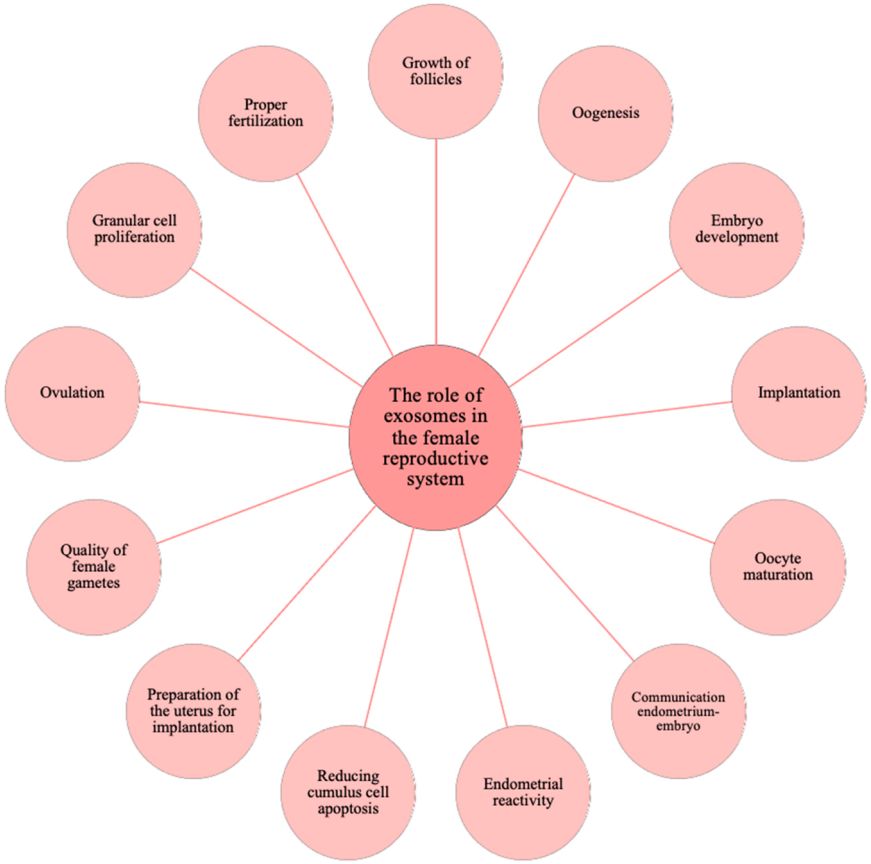

Fig. 2. The role of exosomes in the female reproductive system.

2.1.2. Male reproductive system

Semen is a mixture of secretions from the testicles, epididymis, prostate, seminal vesicles, bulbourethral glands and periurethral glands in which there are sperm cells and seminal plasma (SP). In the semen plasma there are, among others lipids, sugars, growth factors [10,51,53]. Semen plasma is a key factor for sperm maturation, capacitation, acrosomal reaction and fertilization [10]. Moreover, SP contains exosomes [51].

Prostasomes are the most abundant exosomes in the semen plasma that are secreted by prostate epithelial cells [15,51,54]. Semen exosomes have important functions related to sperm functions, e.g. they induce the regulation of sperm motility [10,15].

Seminal exosomes, which account for about 3% of semen plasma proteins, protect sperm in the environment of the female reproductive system by modulating sperm activity in response to the acidic environment of the vagina [15,51]. Moreover, prostasomes have the ability to modulate sperm motility, capacitation or acrosomal reaction, and also prevent premature sperm capacitation due to the fact that they carry cholesterol and sphingomyelin [15,54]. The participation of proteasomes in the capacitation of sperm in the female reproductive system results from their ability to increase cAMP generated in the capacitation process. During capacitation, the sperm undergoes changes in the membrane by modifying glycoproteins, which is stimulated by bicarbonate. This bicarbonate activates sperm-specific adenylate cyclase (sACY), which then contributes to an increase in cAMP levels and the phosphorylation of serine, threonine, and tyrosine-containing proteins [54].

Currently, the influence of exosomes on the male reproductive system, and in particular their influence on the development of gametes, is of great interest [10]. The spermatozoa in the epididymides mature undergoing morphological and biochemical changes through the optimal microenvironment of the epididymides, which is provided by the exosomes contained in the epididymal lumen fluid [55,56]. They are involved in the incorporation of cholesterol into sperm membranes, making the male gametes more stable. Moreover, epididymal exosomes (epididymosomes) are responsible for acquiring the ability to fertilize, protect against sperm oxidative stress and participate in the regulation of motility [10].

In the bovine epididymis, two groups of epididymosomes are distinguished - first group contains tetraspanin proteins, such as CD9, CD26, CD224, which are characterized by the ability to interact with live sperm [10,54]. The second group, on the other hand, interacts with dead gametes and possesses epididymal sperm binding protein 1 (ELSPBP1). The first group of proteins (CD9-positive epididymosomes) is associated with the maturation of sperm and has proteins responsible for the key properties of male gametes, i.e. motility and oocyte recognition [10,56]. The motility-related proteins are MIF, AKR1B1, and the p25b and GliPriL1 proteins are involved in sperm-oocyte interactions. Epididymosomes enriched in ELSPBP1 are associated with zinc ion (Zn^{2+}) - dependent sperm cells, which is associated with post-death reactions. Dead sperm produce reactive oxygen species (ROS) that induce oxidative stress, while the ELSPBP1 protein in the presence of Zn^{2+} is responsible for the removal of ROS, which protects the rest of the cells from oxidative stress [10,56].

In the epididymis, the cargo is transferred to the sperm cells [54]. The transfer involves enzymes, proteins, such as cysteine-rich secretion protein 1 (CRISP1) that regulates calcium channels in sperm membranes, sperm adhesion molecule 1 (SPAM1), which is associated with the adhesion of the male gamete to the Zona pellucida of the oocytes. Another transferred factor is the macrophage migration inhibitory factor (MIF), which is responsible for sperm motility [54]. The epididymosomes also contain the enzyme glutathione peroxidase (GPX), which prevents premature acrosomal reactions and protects sperm from oxidative stress [54,56].

Table 3 presents a summary of the proteins contained in sperm exosomes.

Table 3
Proteins that are transferred from epididymides to sperm and their function in males of different species.

Protein	Function	Literature
ELSPBP1	Protection against oxidative stress, ROS removal, sperm capacitation	[10,54,56]
GPX	Prevents premature acrosomal reaction	[54,55]
MIF, AKR1B1, p25b, GliPriL1	Related to sperm motility, involved in sperm-oocyte interactions	[10,54]
CRISPP1	Regulation of calcium channels in sperm membranes	[54,55]
SPAM1	Interaction of sperm with the oocyte	[56]
GPX5, PSA, KIF5B, ANXA2, KLK2	Fusion with Zona pellucida	[51]
Ubiquitin	Elimination of defective sperm	[56]

Semen may contribute to the transfer of pathogens by the fact that the seed exosomes are immunosuppressive [51]. The action of exosomes is antiviral and antibacterial. Moreover, seminal exosomes have been shown to be able to inhibit HIV-1 replication. The exact mechanism of action against retroviruses is unknown, but it is suggested that it neutralizes the enzyme viral reverse transcriptase RNA and is associated with effective reduction of HIV-1 transmission [51].

2.1.2.1. Acrosomal reaction. Oocyte exosomes have also been proven to play an important role in the acrosomal reaction that occurs when the sperm comes into contact with the oocyte. These exosomes contain CD9 tetraspanins, which are found in the membranes of the oocytes and thanks to them the gametes are fused. Oocyte exosomes also contain CD81, which is responsible for CD9 transfer. Both tetraspanins are involved in the gamete fusion process and act independently of each other [15]. Seminal exosomes have also been shown to participate in the fusion of the oocyte with the sperm. The main proteins of human sperm exosomes involved in the fusion are GPX5, SPAM1, PSA, KIF5B, ANXA2, KLK2. Any abnormalities in the expression of these proteins affect sperm function and fertilization [51].

The importance of exosomes in the male reproductive system is shown in Fig. 3.

2.2. Exosomes as biomarkers

Exosomes, due to their small size and relative durability, show the ability to pass from their place of origin and can accumulate in body fluids, such as saliva, and urine. Moreover, they can influence the behavior of adjacent cells to the cells from which the exosomes have been released. They can also affect other cells or the microenvironment of the parental cell. Due to the possibility of exosomes passing into body fluids, pathological changes can be detected [57] Promising diagnostic markers are exosomes, and more specifically exosome membrane proteins [16,17,57]. Moreover, exosomes secreted from different cells may be subjected to various factors, such as stress during the pathological state, then they may contain various cargo, which allows for the identification of, for example, a cancer, contagious disease, or neurodegenerative [16,57]. Exosomes are crucial in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis through their ability to cross the blood-brain barrier [16]. According to Doyle and Wang (2019) [2], exosomes detected in the cerebrospinal fluid containing alpha-synuclein were associated with Parkinson's disease.

These extracellular vesicles are also important in autoimmune diseases and tumor progression by promoting angiogenesis and tumor cell migration during metastasis [1,9]. Exosome-carrying molecules (including proteins, mRNA, non-coding RNA) have been shown to regulate tumor growth, tumor metastasis, and angiogenesis during tumorigenesis. It has been reported that exosomes are important in shaping the tumor inflammatory microenvironment by stimulating the

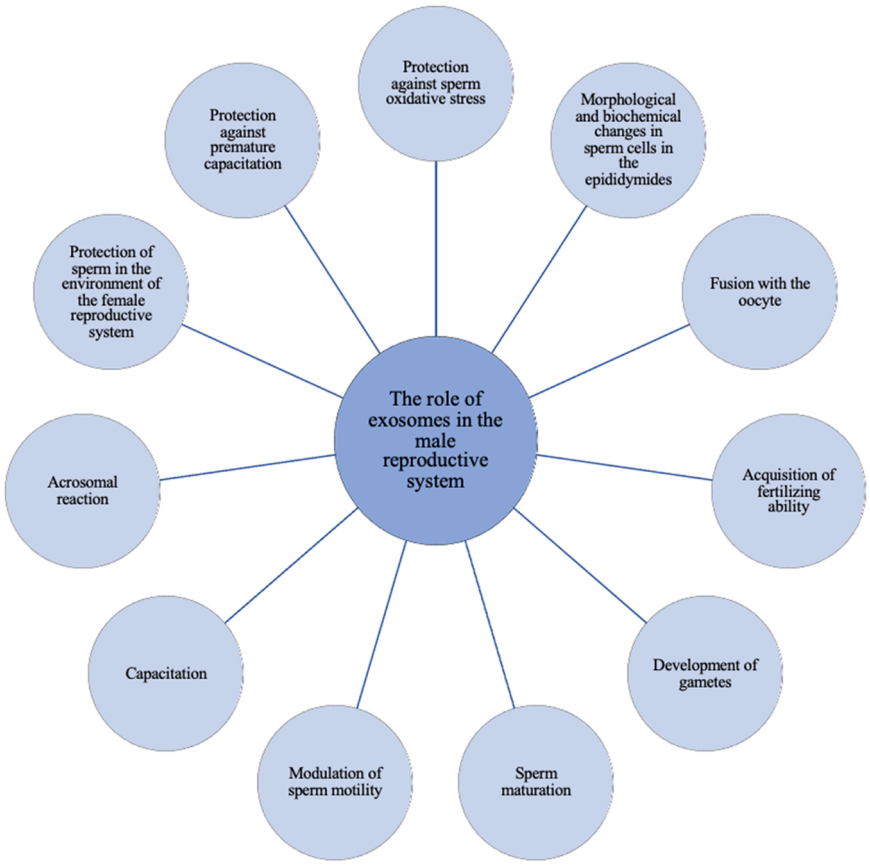

Fig. 3. The role of exosomes in male reproductive system.

activation of e.g. the NF-κB pathway in macrophages, which leads to an increase in the level of pro-inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor alfa (TNFα) during stomach or breasts cancer. Moreover, it has been proven, that exosomes also participate in the metastasis process on MiR-21 and miR-29a enclosed in exosomes secreted by the tumor, and induce a pro-metastatic inflammatory response mediated by the Toll-like receptor [11]. They can be used as biomarkers for the prediction or classification of cancer patients [5]. Exosome molecules are described in the literature as potential biomarkers of neoplastic diseases such as pancreatic cancer, prostate cancer, pheochromocytoma, stroke and other diseases [7]. Moreover, analyzing vesicle-borne molecules released from tumors may be useful in the recognition of biomarkers and mechanisms of carcinogenesis, and in monitoring tumors [4]. Tumor-derived exosomes (TEX) are important in the process of cancers formation [5].

Exosomes are specific to the cells from which they originate – healthy cells release exosomes with membrane protein expression profiles other than those from cells during tumor transformation or during infection [6,57]. For this reason, exosomes can be helpful in the diagnosis and treatment of female and male reproductive disorders, such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF), endometrial cancer, cervical cancer, ovarian cancer, male infertility, prostate gland cancer [8,51].

MicroRNAs (miRNAs) are a group of non-coding RNAs associated with biological activity that is crucial in cellular processes related to the cell cycle, apoptosis or proliferation [8,58,59]. Moreover, the miRNA is encased in an exosome membrane which protects it from degradation and therefore may be a suitable biomarker for many diseases [51].

2.2.1. Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome is an endocrine disorder that is associated with impaired ovulation and can cause female infertility [60]. Studies have shown a higher level of expression of miRNAs, such as, for example, miR-25–3p, miR-143–3p, miR-193b-3p, and a lower level of expression, for example, miR-10a-5p, miR-23b-3p in human exosomes of follicular fluid (hFF) in PCOS patients compared with healthy patients. The cited miRNAs are related to the amino acid metabolism pathways. PCOS alveolar exosomes have been shown to express miRNAs at different levels compared to the control. Different levels of miRNA expression have been suggested to be involved in different functions, such as the MAPK signaling pathway, and oncogenesis [8]. Also, miR-323–3p has been reported to promote proliferation and inhibit apoptosis of cumulus cells in PCOS patients by targeting programmed cell death 4 (PDCD4) [61]. As a result, it has been shown to alleviate the symptoms of polycyclic ovarian syndrome [8]. In turn, exosomal miR-27a-5p may induce the development of endometrial cancer in PCOS patients [61]. Other studies showed that the levels of miR-6087, miR-199a-5p, miR-143–3p, miR-483–5p, miR-23b-3p and miR-200a-3p differed in a PCOS patient compared to healthy controls. The authors indicate that the reported miRNAs can be used as PCOS markers [60].

2.2.2. Premature ovarian failure (POF)

Premature ovarian failure (POF) is associated with menstrual disorders, estrogen deficiency and infertility [8]. According to Esfandyari et al. (2021) [8], the use of miR-664–5p from bone mesenchymal stem cell (BMSC) exosomes was associated with an improvement in follicle health and a reduction in POF in mice. This miRNA has been shown to reduce apoptosis and improve ovarian function [8].

Another solution proposed by Q. Zhang et al. (2019) [62], is the use of human amniotic epithelial cells (hAECs) from the placenta that exhibit embryonic stem cell abilities. The study analyzed the effects of exosomes on ovarian function and follicle development. It was shown that hAEC-derived exosomes were able to suppress mouse granule cell

apoptosis and restore ovarian function in a mouse model. The authors suggest that this may be a new therapeutic approach to POF [62].

2.2.3. Breast cancer (BC)

Breast cancer (BC) is one of the most common malignancies, and the occurrence of metastases during the disease is the leading cause of mortality among women [63,64].

The glycoprotein important in breast cancer is TSP1 (thrombospondin1). It is a matrix glycoprotein secreted by endothelial cells, fibroblasts, smooth muscle cells. It is involved in the regulation of CD47, CD36 and TGF-β signaling pathways. Moreover, glycoprotein is a key component of exosomes, which is derived from cancer cells and plays a role in the metastasis of breast cancer cells, which is spread by exosomes [63]. In study conducted by Cen et al. (2019) [63], has been established in MDA-MB-231 and MCF7 breast cancer cell cultures that breast cancer derived exosomes can enhance endothelial migration of cancer cells. On the other hand, in an animal model of zebrafish (*Danio rerio*), it was shown that the injection of TSP1 overexpression in animals during the studies resulted in increased migration of breast cancer cells [63].

In a research conducted by Cui et al. (2020) [64] on serum samples obtained from 75 patients with breast cancer, it was shown that patients with this type of cancer are characterized by a higher level of circulating nucleic acids compared to healthy subjects. Moreover, the authors noted high expression of C4 lactate dehydrogenase mRNA (LDH-C4) in exosomes obtained from the blood serum of cancer patients at the level of 91.66%. There was also a negative correlation of LDH-C4 levels in serum and exosomes with pharmacotherapy, and positively correlated with tumor metastasis. This study shows that the analysis of LDH-C4 levels can be used to monitor and diagnose this disease [64].

According to Bai et al. (2020) [4], neoplastic exosomes are capable of inducing drug resistance, e.g. in obese women there is a possibility of circulating exosomes inhibiting the sensitivity of BC cells to tamoxifen used in the treatment of this disease. Moreover, exosomes favor the migration and aggression of breast cancer cells [4]. The levels of heat shock protein 70 (HSP70) in exosomes have also been found to be negatively correlated with treatment response for this type of cancer. HSP70 was elevated in exosomes obtained from patients with breast cancer and metastases [4].

2.2.4. Ovarian cancer (OC)

Another serious disease is ovarian cancer (OC) [65]. It is recognized as one of the most lethal and malignant neoplastic diseases in women in the world [7,58].

Currently, two markers in the diagnosis of the disease are commonly used - CA125 and HE4, but they are not adequately specific and sensitive [65]. Soltész et al. (2019) [65] propose the CD24 protein expressed in human cancer cells as another biomarker of this disease. This protein is highly expressed in bladder cancer, but also in ovarian, prostate and breast cancer [65]. The research was carried out on 21 serum samples obtained from patients with ovarian cancer, from which exosomes were isolated. The authors of the study showed a significant correlation of CD24 protein expression with the progression of ovarian cancer. The expression of the tested protein was also significantly higher in patients with malignant neoplasm than in patients with a lower malignancy [65]. The results of these studies indicate the possibility of using the CD24 protein as a biomarker of ovarian cancer [65].

In research by Cheng et al. (2020) [7] the proteins and lipids of exosomes were analyzed on the human ovarian surface epithelial epithelial cell line (HOSEPiC) and on ovarian cancer cells (SKOV-3). Differences in the levels of proteins and lipids in the exosomes from the SKOV-3 line compared to the HOSPEPiC line were obtained. Ovarian cancer cells expressed significantly higher levels of type V collagen alpha 2 protein (COL5A2) and lipoprotein lipase (LPL) expression than in ovarian superficial epithelial cells, which may indicate the possibility of using exosomal proteins and lipids in the early diagnosis of ovarian cancer [7].

Other studies have shown that ovarian cancer is characterized by the development of chemoresistance through exosomes, although the mechanism is largely unknown [66]. In research conducted by Asare-Werehene et al. (2020) [66] the ovarian cancer cell line of the HGS subtype showed a higher level of plasma expression of the calcium-dependent multifunctional actin binding protein- plasma gelsolin (pGSN) in chemoresistant subjects than in ovarian cancer cells. pGSN is secreted and carried by exosomes, which may be a marker of chemoresistance in ovarian cancer cells [66].

In the diagnosis of ovarian cancer, exosomes from biopsies and serum samples are used as biomarkers. In a study by Taylor and Gercel-Taylor (2008) [67] similar levels of 8 specific microRNAs were detected between cellular and exosomal microRNAs (showing correlations from 0.71 to 0.90). On the other hand, EpCAM-positive exosomes were detected in patients with mild ovarian disease and with ovarian cancer, and exosomal microRNAs from patients with ovarian cancer showed differences from those observed in mild disease. The results indicate that the microRNA analysis of tumor exosomes could be a potential diagnostic tool for this cancer [67].

2.2.5. Cervical cancer (CC)

Cervical cancer (CC) is a common gynecological cancer caused by the human papillomavirus (HPV) virus [68]. In cervical cancer, exosome secretion is increased and they are pro-angiogenic, which facilitates the formation of metastases [68]. Moreover, oncogenes of the HPV-E6, E7 virus influence the content of exosomes in CC cells. One component of such exosomes is the epithelial tumor signaling pathway-Hedgehog (Hh) -GLI[68]. This signaling is characteristic of CC. Moreover, the Hh protein could be a potential CC marker as it is exported to the exosomes of cervical cancer cells [68].

Exosomes have been suggested to play an important role in the progression of cervical cancer. Studies have detected higher levels of miR-21, miR-146a and miR-221 expression in samples obtained from patients with cervical cancer than in healthy subjects [8]. Moreover, miR-221–3p has been shown to be a significant inducer of local angiogenesis, and its expression is associated with an increase in vascular density in tumor microcirculation [69]. Also, studies in mice showed that the exosomes injection of miR-221–3p from cervical carcinoma into a mouse tumor increased the angiogenesis process as well as tumor development and growth in these animals [69]. Thrombospondin-2 (THBS2) has been suggested as a suppressor of the blood vessel formation process and a potential target of miR-221–3p. Overexpression of thrombospondin-2 may result in disruption of the action of said miRNA in human umbilical vein endothelial cells (HUVEC) [69]. Exosomes in cervical cancer are considered to be predisposed for standard use as biomarkers of the disease [8,69].

2.2.6. Endometrial cancer (EC)

Another cancer that is quite common in women is endometrial cancer [59]. The disease is often associated with a malignancy that is often diagnosed at an advanced stage. Recently, a molecular approach to treatment of this cancer has started to be explored [59].

miRNA is considered to be important in endometrial cancer by being involved in metastasis, as well as in the interaction between tumor cells and the tumor environment through signaling pathways and the use of exosomes that release cancer-associated fibroblasts (CAFs). CAF modulate tumor growth. Regulation of miRNAs contained in exosomes has been shown to influence CAF and tumor metastasis formation [59].

Detecting EC early in the disease is essential for treatment. In a study by Song et al. (2020) [70] elevated levels of LGALS3BP protein in plasma exosomes of endometrial cancer patients have been reported. Galectin-3 binding protein (LGALS3BP) is a glycoprotein found in nearly all body fluids [70]. This indicates that exosomes and the LGALS3BP they contain may be a marker for this disease. Moreover, LGALS3BP has been confirmed to promote tumor growth [70]. The highest level of expression of the studied proteins in exosomes was obtained in patients with

metastatic disease, and a high level of expression was obtained in women with EC [70].

2.2.7. Prostate cancer

Prostate cancer is a cancer that affects men whose biomarker is prostate specific antigen (PSA) [15]. This biomarker has some limitations due to the inability to distinguish the stage of the disease. An alternative may be obtaining exosomes from the urine or semen of men for diagnostic and therapeutic purposes [15].

Exosomes can be used in place of screening [51]. According to Kharazi and Badalzadeh (2020) [15], the prostate-specific membrane antigen and the prostate-specific antigen are present in the urine of prostate cancer patients. Moreover, exosomes have been proven to be biomarkers of prostate cancer, which is precise in diagnosis [51].

According to Vickram et al. (2021) [51], perm plasma is of particular importance in the identification of prostate cancer. The use of miRNAs, in particular miR-142–3p, miR-223–3p and miR-142–5p, as a disease biomarker is associated with faster disease detection and a lack of diagnostic invasiveness. These miRNAs have been shown to be correlated with the incidence of prostate cancer - miRNA expression increases with disease progression. The analysis of the expression of these miRNAs and the detection of their overexpression will contribute to faster diagnosis and prognosis of the disease [51].

Also, other studies have shown that the analysis of urine exosomes may be helpful in the assessment of protein biomarkers of kidney damage - aquaporin-1 or the AMP-3-dependent cyclic transcription factor. In exosomes obtained from the urine of patients with prostate cancer, biomarkers of the disease in the form of prostate-specific antigens, prostate cancer gene 3, have been established [6].

2.2.8. Astenozoospermia and male infertility

Asthenozoospermia significantly reduces sperm motility and thus sperm quality. Its main cause is oxidative stress that generates reactive oxygen species (ROS) that destroy the sperm structure [15].

By analyzing the miRNA expression of semen exosomes, it is possible to diagnose males with impaired spermatogenesis, or to diagnose asthenosis and azoospermia [51]. Studies in men with asthenospermia and normospermia showed a higher activity of CRISP needed to maintain sperm motility in men with normospermia than in men with disorders [51]. Glycodelin protein extrusion has also been reported in semen samples obtained from men with asthenospermia, but there is no described mechanism for the transfer of this protein from sperm exosomes to sperm [51]. The use of this type of markers helps in detecting the causes of the lack or significant reduction in the amount of sperm in the ejaculate [51].

Administration of exosomes to patients with asthenozoospermia has been shown to be helpful. MSC-Exos exosomes isolated from the human umbilical cord and exosomes from bovine granularity may be helpful in treatment. The main role of MSC-Exos is to reduce inflammation and the amount of ROS by secreting IL10 mRNA, which has the ability to polarize macrophages, thus changing their phenotype from M2 to M1, then Il10 limits the penetration of M1 macrophages, which contributes to the reduction of pro-inflammatory cytokines. In turn, bovine exosomes carry an mRNA molecule that codes for antioxidants and increases their expression [15].

Detailed analysis of sperm plasma and its protein profile may be helpful in determining male infertility. Semen plasma proteins participate in sperm capacitation, the acrosomal reaction, but also in the mobility of male gametes. Moreover, the proteins responsible for the formation of the seed clot are semenogelins (SEMG) and fibronectin (FN1, KLK3). SEMG proteins are most abundant in semen and are the main components of the ejaculation clot that inhibits premature capacitation and provides protection in the acidic environment of the vagina [54].

Male fertility disorders are a significant problem affecting reproductive indicators. It has been shown that men with defects formed in the seminal vesicles are characterized by a lower volume of ejaculate and low levels of the main semen proteins – SEMG [54].

2.3. The role of exosomes in drug delivery in cancer

MicroRNA is important in tumor development. There is a therapeutic approach to introduce tumor suppressor miRNAs, and blockade of miRNAs can be used. The challenge that arises during these therapies is the introduction of miRNAs so that it is not degraded by endonuclease and that the organism does not respond to oncogenic miRNAs. For this purpose, the focus was on exosomes, which are promising carriers of siRNAs (short interfering RNAs) [58]. These studies examined the possibility of using exosomes as carriers of miRNA replacement therapy in ovarian cancer. Exosomes were obtained from fibroblasts of patients after surgery. During the research, suppressor miRNAs (miRNA-199a-3p) were successfully incorporated into exosomes, which showed inhibition against the spread of ovarian cancer cells in mice [71]. The results of the research suggest that exosomes obtained from OC patients can be used as drug carriers in targeted therapy, while miRNA-199a-3p inhibits several key protooncogenes, such as Met [71]. c-Met is the hepatocyte growth factor (HGF) tyrosine kinase receptor, which is encoded by the Met proto-oncogene (chromosome 7 7p31 locus) [71]. The c-Met / HGF signaling pathway regulates cellular functions such as differentiation, proliferation, angiogenesis, and metastasis. Any abnormalities in the c-Met / HGF pathway can induce mutations or cancer [71]. It has been shown that an increase in c-Met protein expression occurs in many types of cancer, including breast, ovarian, stomach and pancreatic cancer [71]. In research by Kobayashi et al. (2020) [58] has been shown that miRNA-199a-3p inhibits Met, which then inhibited c-Met phosphorylation by inhibiting c-Met proliferation and expression in an ovarian cancer cell line [58].

According to Kobayashi et al. (2020) [58], exosomes can be used to transport drugs due to their presence in the circulation, transport functions of e.g. nucleic acids and their uptake by cancer cells. Exosomes are used to deliver chemotherapeutic drugs and biomolecules in cancer treatment [4].

According to Bai et al. (2019) [4], exosomes containing the drug Paclitaxel (PTX) may inhibit the proliferation and affect the apoptosis of neoplastic cells contributing to the anti-neoplastic mechanism in breast cancer [4].

Many research have analyzed exosomes as drug carrier structures [4]. According to Zhao et al. (2020) [72], integrins of tumor exosomes are able to determine metastasis. Moreover, exosomes can be successfully used as natural exogenous RNA transporters (siRNA and miRNA) to inhibit the growth of cancer cells [72]. Due to their size, exosomes do not undergo phagocytosis, and unlike artificial carriers, they do not elicit an immune response because they come from the patient's cells [72]. siRNA has the ability to silence the expression of S100A4 proteins related to oncogenesis and therefore is able to inhibit cancer development [72]. In research by Zhao et al. (2020) [72] has been investigated the use of exosomes to suppress and reduce lung metastasis of breast cancer by encapsulating cationic bovine serum albumin (cBSA) nanoparticles from siRNA S100A4 (siS100A4). The physiological stability of such exosomes and protection of siRNA against degradation have been obtained, as well as a significant inhibition of cancer metastasis to the lung due to the accumulation of siRNA in the lung and the affinity for the tumor [72]. According to the authors, it is a promising method of cancer treatment and prevention [72].

2.4. Clinical application of exosomes – advantages and challenges

Stem cell therapy seems to be a promising therapeutic method in the case of myocardial infarction, impairment of the nervous system, or liver diseases [15,73]. In regenerative medicine, the most commonly used type of stem cells are mesenchymal stem cells (MSCs), which are characterized by their anti-inflammatory, proangiogenic properties [15,74,

75]. A significant disadvantage of MSC cells is the induction of morphological and genetic changes during passages in cell cultures, as well as induction of the recipient's immune response and induction of stress. For these reasons, other stem cell therapies have begun to develop [74]. Stem cell activity has been found to be mediated by paracrine activity through the release of exosomes that correlate with cell growth regulation and antioxidant activity [15,74]. The use of exosomes in regenerative medicine reduces the risk of unwanted side effect that have been associated with cell transplantation. Moreover, these particles are able to modulate target molecules in recipient cells, e.g. by suppressing inflammation, which makes them therapeutic modulators [15,74]. The disadvantage of using exosomes is that they can be damaged during isolation and purification [74]. The fusion of stem cells with exosomes has become a new approach in regenerative medicine and tissue engineering [15]. In research on rats, enhanced neurological regeneration was achieved after 28 days of treatment with exosomes derived from mesenchymal stromal cells at a dose of 100 μg of exosomes. Rats were characterized by greater axon density, neurite remodeling, and greater synaptic plasticity [15]. Many research have shown the lack of induction of the immune response of the organism to exosome therapy [15,35,57, 74,75].

There is now a new therapy for premature ovarian failure (POF) that may be based on exosomal stem cell therapy. Bone mesenchymal stem cell (BMSC) exosomes have been shown to improve follicular morphology in mice and rats under POF, and inhibit apopotosis. A similar effect was achieved using exosomes derived from human amniotic epithelial cells (hAEC) in mice [8]. Similar studies also took place with Asherman's syndrome [8]. This disorder s the occuttence of intrauterine adhesions that may result in infertility. The exosomes of mesenchymal stem cells have been used in studies on rats where a marked reduction in fibrotic adhesions was observed [75]. In the case of the treatment of azoospermia in an animal model, it was possible to reduce apoptosis, limit oxidative stress, and stimulate testosterone production through MSC ttransplantation into the tests end exosome secretion by stem cells. These exosomes were characterized by inducing the spermatogenesis process in infertile mice and rats [73].

Literature data show that stem cell exosomes may be a promising therapeutic approach in diseases and disorders such as POF, Asherman's syndrome, and azoospermia [8,73,75].

In order to use the possibilities of exosomes as therapeutics in various diseases and disorders, including neoplastic diseases, it is necessary to assess he properties of these structures, their size, source of origin, biodistribution in the system, as well as purification, and efficiency of administration. The lifetime of exosomes in body fluids and their stability are also an important issue [76]. The most important challenge is to get enough exosomes [77]. For this, a minimum of 10–100 μg of exosomes is required. The problem, on the other hand, is the yield from 1 mL of culture medium, which is usually less than 1 μg [78,79]. The quality of exosomes is also variable - when isolated from biological fluids, they are often characterized by impurity and low efficiency. Other factors influencing exosome retrieval are the composition, volume of the culture media, cell passage, and their viability. When acquiring exosomes, the key is to standardize the methods and optimize their production [80]. In order to produce a large number of exosomes, cultures in bioreactors are used, with short ones, even 10 times higher amounts can be obtained [81]. Disorders that can be encountered in the production of exosomes include physical, chemical and biological stress [82]. The nutrient medium may be another source of contamination. Exosomes isolated from serum-containing culture medium have few impurities, while serum-free conditions cause great stress in cells and lead to altered EV secretion [83].

In turn, obtaining a greater number of exosomes and their higher purity can be obtained by modifying standard methods, such as ultrafiltration, microflow isolation, immunoprecipitation, ultracentrifugation, precipitation [74,76]. The method of isolating exosomes is time-consuming and labor-consuming and should be selected appropriately in terms of the composition and origin of the vesicles, while these methods may negatively affect the physicochemical properties of exosomes. Another challenge may be the targeting of exosomes to treat specific conditions, such as cancer [76]. The method of isolating exosomes determines their purity and quality [77]. Ultracentrifugation and precipitation are methods that are unsuitable for therapeutic applications because the exosomes obtained in this way show different composition and size [77,83–87].

Another limitation is the storage of exosome. At room temperature, the exosomal cargo is lost, while at 4 °C the levels of exosomal proteins decreased significantly [74]. Exosomes can be stored at − 80 °C in phosphate buffered saline, and the addition of trehalose can protect them from cryodamage [83,88]. An equally important aspect is the sterility of exosomes that can succumb to viral or bacterial contamination. The main threat are viruses, like HIV, which use exosomes as vehicles to spread throughout the body [74].

At present, the use of exosomes for therapeutic drug delivery is still insufficiently research [76].

3. What else do we not know about exosomes?

Knowledge of exosomes has increased significantly in recent years [4,5]. Currently, exosomes can be used as biomarkers in the diagnosis of many reproductive diseases - PCOS, POF, ovarian, cervical and prostate cancer [4,8,51,54]. As many research indicate, exosome-related proteins are crucial in male fertility because they can serve as biomarkers of fertility disorders including sperm maturation [10].

Exosomes have been shown to be important modulators in the neoplastic process and can be used as diagnostic and prognostic biomarkers of many physiological diseases and disorders [4]. The use of cancer markers may facilitate the development of new anti-cancer therapies. Markers related to angiogenesis, which is a key phenomenon in cancer development and metastasis, are of particular importance [69].

According to Bai et al. (2020) [4], despite the development of cancer treatment techniques, there is still a need to develop mechanisms to understand the mechanisms responsible for carcinogenesis and to understand the biomarkers of these diseases. There is still a need for further research to understand the role of exosomes in the genesis of neoplastic diseases of the female reproductive system [4,63]. Few oncologists have accepted the modern prostate cancer diagnostic technique based on the detection of biomarkers from semen exosomes [51].

Exosomes show specific antiviral activity against retroviruses. It has been proven that the seed exosomes are able to inhibit HIV-1 replication by inhibiting the virus transcription factors and limiting the expression of the Tat protein (Trans-Activator of Transcription) [51]. The exact mechanism of action against retroviruses is unknown, but it is suggested that it neutralizes the viral reverse transcriptase RNA enzyme and is associated with effective reduction of HIV-1 transmission. The authors point to the need for further research on the antiretroviral properties of exosomes [51].

Identification of exosomes as markers will help to understand fertility disorders caused by dysfunction of sex organs and glands [10]. The use of exosomes in therapy as drug carriers seems to be the future of treatment [4].

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Czerniawska-Piątkowska, Robert Kupczyński: Conceptualization and Writing – original draft. **Alicja Kowalczyk and Marcjanna Wrzecińska** writing the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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No potential conflict of interest was reported by the authors.

References

[1] S. Gurunathan, M.-H. Kang, M. Jeyaraj, M. Qasim, J.-H. Kim, Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes, Cells 8 (4) (2019) 307.

[2] L. Doyle, M. Wang, Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis, Cells 8 (7) (2019) 727.

[3] B. Zhang, Y. Yin, R.C. Lai, S.K. Lim, Immunotherapeutic potential of extracellular vesicles, Front Immunol. 5 (2014) 518, https://doi.org/10.3389/fimmu.2014.00518/abstract.

[4] Y. Bai, J. Guo, Z. Liu, Y. Li, S. Jin, T. Wang, The role of exosomes in the female reproductive system and breast cancers, OTT 13 (2020) 12567–12586.

[5] J. Dai, Y. Su, S. Zhong, L. Cong, B. Liu, J. Yang, et al., Exosomes: key players in cancer and potential therapeutic strategy, Signal Transduct. Target Ther. 5 (1) (2020) 145.

[6] Y. Zhang, Y. Liu, H. Liu, W.H. Tang, Exosomes: biogenesis, biologic function and clinical potential, Cell Biosci. 9 (1) (2019) 19.

[7] L. Cheng, K. Zhang, Y. Qing, D. Li, M. Cui, P. Jin, et al., Proteomic and lipidomic analysis of exosomes derived from ovarian cancer cells and ovarian surface epithelial cells, J. Ovarian Res. 13 (1) (2020) 9.

[8] S. Esfandyari, H. Elkafas, R.M. Chugh, H. Park, A. Navarro, A. Al-Hendy, Exosomes as biomarkers for female reproductive diseases diagnosis and therapy, IJMS 22 (4) (2021) 2165.

[9] Z. Ni, S. Zhou, S. Li, L. Kuang, H. Chen, X. Luo, et al., Exosomes: roles and therapeutic potential in osteoarthritis, Bone Res. 8 (1) (2020) 25.

[10] S. Baskaran, M.K. Panner Selvam, A. Agarwal, Exosomes of male reproduction, Adv. Clin. Chem. 95 (2020) 149–163.

[11] L. Console, M. Scalise, C. Indiveri, Exosomes in inflammation and role as biomarkers, Clin. Chim. Acta 488 (2019) 165–171.

[12] Y. Tang, P. Zhang, Y. Wang, J. Wang, M. Su, Y. Wang, et al., The biogenesis, biology, and clinical significance of exosomal PD-L1 in cancer, Front. Immunol. 11 (2020) 604.

[13] A.S. Jadli, N. Ballasy, P. Edalat, V.B. Patel, Inside(sight) of tiny communicator: exosome biogenesis, secretion, and uptake, Mol. Cell. Biochem. 467 (1–2) (2020) 77–94.

[14] G. Desdín-Micó, M. Mittelbrunn, Role of exosomes in the protection of cellular homeostasis, Cell Adhes. Migr. 11 (2) (2017) 127–134.

[15] U. Kharazi, R. Badalzadeh, A review on the stem cell therapy and an introduction to exosomes as a new tool in reproductive medicine, Reprod. Biol. 20 (4) (2020) 447–459.

[16] S. Salarpour, M. Barani, A. Pardakhty, M. Khatami, N. Pal Singh Chauhan, The application of exosomes and Exosome-nanoparticle in treating brain disorders, J. Mol. Liq. 350 (2022), 118549.

[17] B. Yang, Y. Chen, J. Shi, Exosome biochemistry and advanced nanotechnology for next-generation theranostic platforms, Adv. Mater. 31 (2) (2019), 1802896.

[18] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J.J. Lee, J.O. Lötvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (6) (2007) 654–659.

[19] B.K. Thakur, H. Zhang, A. Becker, I. Matei, Y. Huang, B. Costa-Silva, et al., Double-stranded DNA in exosomes: a novel biomarker in cancer detection, Cell Res. 24 (6) (2014) 766–769.

[20] Q. Cheng, X. Shi, M. Han, G. Smbatyan, H.-J. Lenz, Y. Zhang, Reprogramming exosomes as nanoscale controllers of cellular immunity, J. Am. Chem. Soc. 140 (48) (2018) 16413–16417.

[21] C. He, S. Zheng, Y. Luo, B. Wang, Exosome theranostics: biology and translational medicine, Theranostics 8 (1) (2018) 237–255.

[22] P.D. Stahl, G. Raposo, Extracellular vesicles: exosomes and microvesicles, integrators of homeostasis, Physiology 34 (3) (2019) 169–177.

[23] S.S. Sidhu, A.T. Mengistab, A.N. Tauscher, J. LaVail, C. Basbaum, The microvesicle as a vehicle for EMMPRIN in tumor–stromal interactions, Oncogene 23 (4) (2004) 956–963.

[24] S. Atay, C. Gercel-Taylor, J. Suttles, G. Mor, D.D. Taylor, Trophoblast-derived exosomes mediate monocyte recruitment and differentiation: MONOCYTE education by trophoblast exosomeS, Am. J. Reprod. Immunol. 65 (1) (2011) 65–77.

[25] K.R. Vrijsen, J.P.G. Sluijter, M.W.L. Schuchardt, B.W.M. Van Balkom, W.A. Noort, S.A.J. Chamuleau, et al., Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells, J. Cell. Mol. Med. 14 (2010) 1064–1070.

[26] C. Grange, M. Tapparo, F. Collino, L. Vitillo, C. Damasco, M.C. Deregibus, et al., Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche, Cancer Res. 71 (15) (2011) 5346–5356.

[27] W. Zhu, L. Huang, Y. Li, X. Zhang, J. Gu, Y. Yan, et al., Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo, Cancer Lett. 315 (1) (2012) 28–37.

[28] A.J. Abusamra, Z. Zhong, X. Zheng, M. Li, T.E. Ichim, J.L. Chin, et al., Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis, Blood Cells Mol. Dis. 35 (2) (2005) 169–173.

[29] S. Ye, Z.-L. Li, D. Luo, B. Huang, Y.-S. Chen, X. Zhang, et al., Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma, Oncotarget 5 (14) (2014) 5439–5452.

[30] M. Fabbri, A. Paone, F. Calore, R. Galli, E. Gaudio, R. Santhanam, et al., MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response, Proc. Natl. Acad. Sci. USA 109 (31) (2012) E2110–E2116.

[31] M. D'Anca, C. Fenoglio, M. Serpente, B. Arosio, M. Cesari, E.A. Scarpini, et al., Exosome determinants of physiological aging and age-related neurodegenerative diseases, Front. Aging Neurosci. 11 (2019) 232.

[32] A. Ranjbaran, Z. Latifi, H.R. Nejabati, S. Abroon, A. Mihanfar, A.R. Sadigh, et al., Exosome-based intercellular communication in female reproductive microenvironments, J. Cell Physiol. 234 (11) (2019) 19212–19222.

[33] H. Mobarak, M. Heidarpour, F. Lolicato, M. Nouri, R. Rahbarghazi, M. Mahdipour, Physiological impact of extracellular vesicles on female reproductive system; highlights to possible restorative effects on female age-related fertility, BioFactors 45 (3) (2019) 293–303.

[34] N. Shomali, M. Hemmatzadeh, Y. Yousefzadeh, M.S. Soltani-Zangbar, K. Hamdi, A. Mehdizadeh, et al., Exosomes: emerging biomarkers and targets in folliculogenesis and endometriosis, J. Reprod. Immunol. 142 (2020), 103181.

[35] Y.Q. Koh, H.N. Peiris, K. Vaswani, F.B. Almughlliq, S. Meier, C.R. Burke, et al., Exosomes from dairy cows of divergent fertility; action on endometrial cells, J. Reprod. Immunol. 137 (2020), 102624.

[36] F.B. Almughlliq, Y.Q. Koh, H.N. Peiris, K. Vaswani, O. Holland, S. Meier, et al., Circulating exosomes may identify biomarkers for cows at risk for metabolic dysfunction, Sci. Rep. 9 (1) (2019) 13879.

[37] P. Abeysinghe, N. Turner, I. Morean Garcia, E. Mosaad, H.N. Peiris, M.D. Mitchell, The role of exosomal epigenetic modifiers in cell communication and fertility of dairy cows, IJMS 21 (23) (2020) 9106.

[38] S. Sheller-Miller, J. Lei, G. Saade, C. Salomon, I. Burd, R. Menon, Feto-maternal trafficking of exosomes in murine pregnancy models, Front Pharm. [Internet] 7 (2016) 432, https://doi.org/10.3389/fphar.2016.00432/full.

[39] W.-T. Hung, X. Hong, L.K. Christenson, L.K. McGinnis, Extracellular vesicles from bovine follicular fluid support cumulus expansion, Biol. Reprod. 93 (5) (2015) 117, https://doi.org/10.1095/biolreprod.115.132977.

[40] S.H. Lee, I.M. Saadeldin, Exosomes as a potential tool for supporting canine oocyte development, Animals 10 (11) (2020) 1971.

[41] Y. Matsuno, A. Onuma, Y.A. Fujioka, K. Yasuhara, W. Fujii, K. Naito, et al., Effects of exosome-like vesicles on cumulus expansion in pigs *in vitro*, J. Reprod. Dev. 63 (1) (2017) 51–58.

[42] D.L. Russell, R.B. Gilchrist, H.M. Brown, J.G. Thompson, Bidirectional communication between cumulus cells and the oocyte: old hands and new players? Theriogenology 86 (1) (2016) 62–68.

[43] T.A. Rodrigues, K.M. Tuna, A.A. Alli, P. Tribulo, P.J. Hansen, J. Koh, et al., Follicular fluid exosomes act on the bovine oocyte to improve oocyte competence to support development and survival to heat shock, Reprod. Fertil. Dev. 31 (5) (2019) 888.

[44] M. Saeed-Zidane, L. Linden, D. Salilew-Wondim, E. Held, C. Neuhoff, E. Tholen, et al., Cellular and exosome mediated molecular defense mechanism in bovine granulosa cells exposed to oxidative stress, PLoS One 12 (2017), e0187569.

[45] Q. Tan, S. Shi, J. Liang, X. Zhang, D. Cao, Z. Wang, MicroRNAs in small extracellular vesicles indicate successful embryo implantation during early pregnancy, Cells 9 (3) (2020) 645.

[46] A. Kowalczyk, E. Czerniawska-Piątkowska, M. Wrzecińska, in: A. Ortega (Ed.), The Importance of Interferon-Tau in the Diagnosis of Pregnancy, 2021, BioMed Research International, 2021, pp. 1–6.

[47] K. Kusama, K. Nakamura, R. Bai, K. Nagaoka, T. Sakurai, K. Imakawa, Intrauterine exosomes are required for bovine conceptus implantation, Biochem. Biophys. Res. Commun. 495 (1) (2018) 1370–1375.

[48] F. Qiao, H. Ge, X. Ma, Y. Zhang, Z. Zuo, M. Wang, et al., Bovine uterus-derived exosomes improve developmental competence of somatic cell nuclear transfer embryos, Theriogenology 114 (2018) 199–205.

[49] N. Turner, P. Abeysinghe, P. Sadowski, M.D. Mitchell, Exosomal cargo may hold the key to improving reproductive outcomes in dairy cows, IJMS 22 (4) (2021) 2024.

[50] H.-J. Lim, H.J. Kim, J.H. Lee, D.H. Lim, J.K. Son, E.-T. Kim, et al., Identification of plasma miRNA biomarkers for pregnancy detection in dairy cattle, J. Anim. Reprod. Biotechnol. 36 (1) (2021) 35–44.

[51] A.S. Vickram, P.S. Srikumar, S. Srinivasan, P. Jeyanthi, K. Anbarasu, S. Thanigaivel, et al., Seminal exosomes – An important biological marker for various disorders and syndrome in human reproduction, Saudi J. Biol. Sci. 28 (6) (2021) 3607–3615.

[52] S. Keller, C. Rupp, A. Stoeck, S. Runz, M. Fogel, S. Lugert, et al., CD24 is a marker of exosomes secreted into urine and amniotic fluid, Kidney Int. 72 (9) (2007) 1095–1102.

[53] A. Kowalczyk, E. Gałęska, E. Czerniawska-Piątkowska, A. Szul, L. Hebda, The impact of regular sperm donation on bulls' seminal plasma hormonal profile and phantom response, Sci. Rep. 11 (1) (2021) 11116.

[54] L. Candenas, R. Chianese, Exosome composition and seminal plasma proteome: a promising source of biomarkers of male infertility, Int. J. Mol. Sci. (2020) 27.

[55] C.T. Tamessar, N.A. Trigg, B. Nixon, D.A. Skerrett-Byrne, D.J. Sharkey, S. A. Robertson, et al., Roles of male reproductive tract extracellular vesicles in reproduction, Am J Reprod Immunol. 85 (2021), e13338.

[56] N. Paul, T.R. Talluri, P. Nag, A. Kumaresan, Epididymosomes: a potential male fertility influencer, Andrologia 53 (2021), e14155.
[57] Q. Hu, H. Su, J. Li, C. Lyon, W. Tang, M. Wan, et al., Clinical applications of exosome membrane proteins, Precis. Clin. Med. 3 (1) (2020) 54–66.
[58] M. Kobayashi, K. Sawada, M. Miyamoto, A. Shimizu, M. Yamamoto, Y. Kinose, et al., Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer, Biochem. Biophys. Res. Commun. 527 (1) (2020) 153–161.
[59] N. Zhang, Y. Wang, H. Liu, W. Shen, Extracellular vesicle encapsulated microRNA-320a inhibits endometrial cancer by suppression of the HIF1α/VEGFA axis, Exp. Cell Res. 394 (2) (2020), 112113.
[60] J. Hu, T. Tang, Z. Zeng, J. Wu, X. Tan, J. Yan, The expression of small RNAs in exosomes of follicular fluid altered in human polycystic ovarian syndrome, PeerJ 8 (2020), e8640.
[61] F. Zhang, S.-P. Li, T. Zhang, B. Yu, J. Zhang, H.-G. Ding, et al., High throughput microRNAs sequencing profile of serum exosomes in women with and without polycystic ovarian syndrome, PeerJ 9 (2021), e10998.
[62] Q. Zhang, J. Sun, Y. Huang, S. Bu, Y. Guo, T. Gu, et al., Human amniotic epithelial cell-derived exosomes restore ovarian function by transferring microRNAs against apoptosis, Mol. Ther. Nucleic Acids 16 (2019) 407–418.
[63] J. Cen, L. Feng, H. Ke, L. Bao, L.Z. Li, Y. Tanaka, et al., Exosomal thrombospondin-1 disrupts the integrity of endothelial intercellular junctions to facilitate breast cancer cell metastasis, Cancers 11 (12) (2019) 1946.
[64] Z. Cui, Y. Chen, M. Hu, Y. Lin, S. Zhang, L. Kong, et al., Diagnostic and prognostic value of the cancer-testis antigen lactate dehydrogenase C4 in breast cancer, Clin. Chim. Acta 503 (2020) 203–209.
[65] B. Soltész, J. Lukács, E. Szilágyi, É. Márton, M. Szilágyi Bónizs, A. Penyige, et al., Expression of CD24 in plasma, exosome and ovarian tissue samples of serous ovarian cancer patients, J. Biotechnol. 298 (2019) 16–20.
[66] M. Asare-Werehene, K. Nakka, A. Reunov, C.-T. Chiu, W.-T. Lee, M.R. Abedini, et al., The exosome-mediated autocrine and paracrine actions of plasma gelsolin in ovarian cancer chemoresistance, Oncogene 39 (7) (2020) 1600–1616.
[67] D.D. Taylor, C. Gercel-Taylor, MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer, Gynecol. Oncol. 110 (1) (2008) 13–21.
[68] A. Bhat, J. Yadav, K. Thakur, N. Aggarwal, T. Tripathi, A. Chhokar, et al., Exosomes from cervical cancer cells facilitate pro-angiogenic endothelial reconditioning through transfer of Hedgehog–GLI signaling components, Cancer Cell Int. 21 (1) (2021) 319.
[69] M. Hashemipour, H. Boroumand, S. Mollazadeh, V. Tajiknia, Z. Nourollahzadeh, M. Rohani Borj, et al., Exosomal microRNAs and exosomal long non-coding RNAs in gynecologic cancers, Gynecol. Oncol. 161 (1) (2021) 314–327.
[70] Y. Song, M. Wang, H. Tong, Y. Tan, X. Hu, K. Wang, et al., Plasma exosomes from endometrial cancer patients contain LGALS3BP to promote endometrial cancer progression, Oncogene 40 (3) (2021) 633–646.
[71] J.H. Kim, H.J. Jang, H.S. Kim, B.J. Kim, S.H. Park, Prognostic impact of high c-Met expression in ovarian cancer: a meta-analysis, J. Cancer 9 (19) (2018) 3427–3434.
[72] L. Zhao, C. Gu, Y. Gan, L. Shao, H. Chen, H. Zhu, Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis, J. Control. Release 318 (2020) 1–15.
[73] R. Zhankina, N. Baghban, M. Askarov, D. Saipiyeva, A. Ibragimov, B. Kadirova, et al., Mesenchymal stromal/stem cells and their exosomes for restoration of spermatogenesis in non-obstructive azoospermia: a systemic review, Stem Cell Res. Ther. 12 (1) (2021) 229.
[74] A. Rezabakhsh, E. Sokullu, R. Rahbarghazi, Applications, challenges and prospects of mesenchymal stem cell exosomes in regenerative medicine, Stem Cell Res. Ther. 12 (1) (2021) 521.
[75] G.S. Saribas, C. Ozogul, M. Tiryaki, F. Alpaslan Pinarli, Hamdemir, S. Kilic, Effects of uterus derived mesenchymal stem cells and their exosomes on asherman's syndrome, Acta Histochem. 122 (1) (2020), 151465.
[76] G. Kibria, E.K. Ramos, Y. Wan, D.R. Gius, H. Liu, Exosomes as a drug delivery system in cancer therapy: potential and challenges, Mol. Pharm. 15 (9) (2018) 3625–3633.
[77] T. Yamashita, Y. Takahashi, Y. Takakura, Possibility of exosome-based therapeutics and challenges in production of exosomes eligible for therapeutic application, Biol. Pharm. Bull. 41 (6) (2018) 835–842.
[78] C. Charoenviriyakul, Y. Takahashi, M. Morishita, A. Matsumoto, M. Nishikawa, Y. Takakura, Cell type-specific and common characteristics of exosomes derived from mouse cell lines: yield, physicochemical properties, and pharmacokinetics, Eur. J. Pharm. Sci. 96 (2017) 316–322.
[79] T. Yamashita, Y. Takahashi, M. Nishikawa, Y. Takakura, Effect of exosome isolation methods on physicochemical properties of exosomes and clearance of exosomes from the blood circulation, Eur. J. Pharm. Biopharm. 98 (2016) 1–8.
[80] M.I. Ramirez, M.G. Amorim, C. Gadelha, I. Milic, J.A. Welsh, V.M. Freitas, et al., Technical challenges of working with extracellular vesicles, Nanoscale 10 (3) (2018) 881–906.
[81] D.C. Watson, D. Bayik, A. Srivatsan, C. Bergamaschi, A. Valentin, G. Niu, et al., Efficient production and enhanced tumor delivery of engineered extracellular vesicles, Biomaterials 105 (2016) 195–205.
[82] M. Harmati, Z. Tarnai, G. Decsi, S. Kormondi, Z. Szegletes, L. Janovak, et al., Stressors alter intercellular communication and exosome profile of nasopharyngeal carcinoma cells, J. Oral. Pathol. Med. 46 (4) (2017) 259–266.
[83] K.W. Witwer, E.I. Buzás, L.T. Bemis, A. Bora, C. Lässer, J. Lötvall, et al., Standardization of sample collection, isolation and analysis methods in extracellular vesicle research, J. Extracell. Vesicles 2 (1) (2013) 20360.
[84] A. Bobrie, M. Colombo, S. Krumeich, G. Raposo, C. Théry, Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation, J. Extracell. Vesicles 1 (1) (2012) 18397.
[85] J. Kowal, G. Arras, M. Colombo, M. Jouve, J.P. Morath, B. Primdal-Bengtson, et al., Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes, Proc. Natl. Acad. Sci. USA 113 (8) (2016) E968–E977.
[86] A. Lesniak, F. Fenaroli, M.P. Monopoli, C. Åberg, K.A. Dawson, A. Salvati, Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells, ACS Nano 6 (7) (2012) 5845–5857.
[87] E. Willms, H.J. Johansson, I. Mäger, Y. Lee, K.E.M. Blomberg, M. Sadik, et al., Cells release subpopulations of exosomes with distinct molecular and biological properties, Sci. Rep. 6 (1) (2016) 22519.
[88] S. Bosch, L. de Beaurepaire, M. Allard, M. Mosser, C. Heichette, D. Chrétien, et al., Trehalose prevents aggregation of exosomes and cryodamage, Sci. Rep. 6 (1) (2016) 36162.