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Extracellular vesicles and domestic animal reproduction

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ABSTRACT

Embryo implantation is a complex process in which significant changes occur continually in both the corpora lutea and in the endometrium of females and which varies depending on the embryonic, pre-implantation, or fetal stages. However, at all stages, correct maternal–embryonic communication is essential. In the last few years, a new intercellular communication tool, mediated by extracellular vesicles (EVs), has emerged. Many authors agree on the relevant role of EVs in correct communication between the mother and the embryo, as a fundamental system for the pregnancy to reach term and embryonic development to occur correctly. This review analyzes current information on known EVs, their main functions, and their role in implantation and embryonic development in domestic animals.

Abbreviations

EVs
extracellular vesicles
MVs
microvesicles
ABs
apoptotic bodies
IL
interleukin
VEGF
vascular endothelial growth factor
TFG
transforming growth factor
OVGP1
oviduct-specific glycoprotein precursor 1
ZP
zona pellucida
ARTs
assisted reproductive techniques;
IFNT
interferon tau

1. Introduction

Pregnancy is a complex process that depends on various events happening correctly, such as oocyte fertilization, embryonic development, and implantation. For correct embryonic development and subsequent implantation, maternal–embryonic communication is essential, and, mediated by hormones, this allows the expression of some molecules fundamental for these processes to occur (Llobat, 2020). After fertilization, communication between the oviduct and the embryo begins, the oviduct playing a fundamental role in early embryonic development (Besenfelder et al., 2012; Leese et al., 2008). Different studies have shown that the oviduct generates a microenvironment necessary for the correct early development of the embryo in different species, such as cow, sheep, pig, or rabbit (Besenfelder et al., 2012; Leese et al., 2008). In *in vitro* cultures, a higher percentage of blastocyst development and an improvement in cryotolerance has been demonstrated when the medium is supplemented with oviductal fluid (Banliat et al., 2019; Hamdi et al., 2018). Subsequently, during embryonic implantation, a series of events occurs in which the uterus plays a fundamental role. The endometrium must be synchronized before and during the arrival of the embryo, and three fundamental steps must occur: 1) transformation of the endometrium in order to be receptive, 2) response of the embryo to the endometrium leading to implantation, and 3) invasion of the endometrium by embryos (Ashary et al., 2018). For these events to occur, it is necessary to exchange molecules between the endometrium and the embryo that modify the gene and

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protein expression of both (Ashary et al., 2018; Llobat, 2020; Modi et al., 2012). Therefore, for the embryo to develop correctly, both in the early embryo stage and during pre-implantation and implantation, it is essential that there is correct maternal–embryonic communication.

In recent years, the fundamental role of extracellular vesicles (EVs) in cell-to-cell communication has been discovered (Machtinger et al., 2016). The EVs have the ability to transport different molecules, such as proteins, lipids, and nucleic acids, indicating that they are essential for intercellular communication in all organisms, both prokaryotes and eukaryotes (Keerthikumar et al., 2016; Yáñez-Mó et al., 2015). EVs are lipid membrane particles and can be classified as exosomes, ectosomes, microvesicles, small size microvesicles, microparticles, and apoptotic cell-derived extracellular vesicles (Berezin and Berezin, 2020). However, the Executive Committee of the International Society of Extracellular Vesicles defined EVs as mixture particles ranging from 30 to 2000 nm in diameter, and they include exosomes, microvesicles (or ectosomes), and apoptotic bodies (Lötvall et al., 2014). Recently, this classification has been made according to size, so EVs have been classified as large (L-EVs, more than 200 nm in diameter) or small (S-EVs, less than 200 nm). L-EVs include microvesicles (or ectosomes) and apoptotic bodies, whereas S-EVs include exosomes (Théry et al., 2018). The first study linking EVs to the reproductive process was from 2013, where oviductal fluid EVs were isolated from mice for first time (Al-Dossary et al., 2013). Currently, we know their fundamental role in early embryonic development and implantation as a cell-to-cell communication mechanism.

This review analyzes the cargoes presented by EVs and their possible relationship in embryonic development and implantation in different domestic animals.

2. Types of extracellular vesicles (EVs)

2.1. Large EVs: microvesicles (MVs) and apoptotic bodies (ABs)

In addition to size, the main difference between microvesicles (MVs) and exosomes is their formation process. Whereas exosomes are intraluminal vesicles that bud into endosomes, microvesicles are large EVs that bud from the plasma membrane (Raposo and Stoorvogel, 2013). This departure of MVs from the plasma membrane is accompanied by changes in its proteins and lipids, which modify its curvature and rigidity (Kozlov et al., 2014; McMahon and Boucrot, 2015). These differences between the formation of MVs and other EVs involve their release into the intercellular space with specific cargoes (Tricarico et al., 2017). The composition of MVs ranges from membrane and structure regulatory proteins, adhesion molecules, enzymes, receptors for different molecules, lipids, molecules related to the immune system, and non-coding RNAs (Berezin and Berezin, 2020). Furthermore, their presence has been evidenced in different body fluids, such as blood, urine, and ascites (Graves et al., 2004; Piccin et al., 2007; Smalley et al., 2008).

Apoptotic bodies (ABs) are formed exclusively during programmed cell death (Akers et al., 2013) and contain chromatin fractions, a non-coding nucleus, or nucleolus RNAs (Noguchi et al., 2015). During apoptosis, cells condense their nuclear chromatin to later generate membrane blisters and finally disintegrate the cellular content in the ABs (Kerr et al., 1972). In addition to their size and their generation method, another feature of ABs compared to EVs is that they have organelles inside, that is, smaller vesicles (Taylor et al., 2008). In addition to chromatin fragments, apoptotic bodies are known to contain mitochondria, different molecules, and ligands and also non-coding DNA and RNA fragments (Jiang et al., 2017). Different studies suggest that ABs may have a role in the transfer of genetic information between cells, although the mechanism by which this occurs is not yet clear (Bergsmedh et al., 2001; Holmgren et al., 2002).

2.2. Small-EVs: exosomes

The definition of exosomes from the ExoCarta database for exosomes is “30-150 membrane vesicles of endocytic origin secreted by most cell types in vitro” (Keerthikumar et al., 2016). Exosome cargoes are very varied and include different types of interleukins (IL-1β, IL-6, IL-8), growth factors (vascular endothelial growth factor (VEGF), transforming growth factor (TFG) and hormones (aldosterone)), all essential for embryonic development and implantation in different species of domestic animals (Clayton et al., 2019; Llobat, 2020). Saadeldin et al. (2014) found mRNA related to pluripotency (Oct4, Sox2, c-Myc and Nanog) in cultured mammalian embryos (Saadeldin et al., 2014). Some papers have recently shown that the use of exosomes in the culture of fertilized mammalian oocytes produces better quality embryos with a higher development rate (Bauersachs et al., 2020; Gurung et al., 2020; Hamdi et al., 2018; Lopera-Vásquez et al., 2016).

Current information about the role exosomes and other EVs play in embryonic development and implantation in different species is discussed later in this review. Table 1 shows the main differences between microvesicles, apoptotic bodies, and exosomes.

3. Composition of EVs

3.1. Proteins

The number of proteins detected in EVs is around 1100, and they have been mainly detected in human, rat, and mouse (Pathan et al., 2019). In exosomes, the number is around 540, and most of these proteins are related to exosome biogenesis, sorting, and secretion (Keerthikumar et al., 2016). The top 10 proteins identified are related to cell death (programmed cell death 6 interacting protein (PDCD6IP)), cell metabolism (gluderaldehyde-3-phosphate dehydrogenase (GADPH), enolase 1 (ENO1)), heat shock protein 8 and 90 (HSPA8, HSP90AA1), annexin A2 and A5 (ANXA2, ANXA5), pyruvate kinase (PKM)), and cell structure and remodeling (actin-beta (ACTB)) and autocrine regulation (prostaglandin F2 receptor negative regulator (PTGFRN)) (Pathan et al., 2019). However, the number of proteins identified in EVs is increasing quickly. Thus, the database ExoCarta

Table 1
Basic characteristics of extracellular vesicle (EV) types.

Characteristics of EVs	Types of EVs		
	Microvesicles	Apoptotic Bodies	Exosomes
Diameter (nm)	More than 200 (L-EVs)	More than 200 (L-EVs)	Less than 200 (S-EVs)
Formation	Cell membrane	Apoptotic cell	Endocytic membrane
Components	Regulatory proteins, adhesion molecules, enzymes, receptors, lipids, immune system proteins	Chromatin fractions, non-coding nucleus or nucleolus RNAs, mitochondria, ligands	Interleukins, growth factors, hormones, receptors
Nuclear fragments	Non-coding RNAs	Non-coding RNAs and DNAs	mRNAs, microRNAs, non-coding RNAs
Specific markers	CD40, phosphatidylserine, integrins, selectins, ESCRT machinery proteins	Annexin V, phosphatidylserine, caspase 3, histones	CD9, CED63, CD81, ESCRT machinery proteins, flotillin-1

(Keerthikumar et al., 2016) is a great tool for determining the different components found in EVs. This catalogue has been updated on Vesiclepedia, where different authors contribute to the expansion of this large database (Kalra et al., 2012).

Related to the reproduction process, Almiñana et al. (2017) analyzed protein content in EVs of *in vitro* and *in vivo* bovine oviducts with mass spectrometry. Their results demonstrated the different proteomic profile of EVs from *in vivo* and *in vitro* origin, and the most common functions of these proteins are metabolic and cellular process while presenting other relevant functions, such as immune system processes, developmental processes, and biological regulation. They identified some proteins related to gamete generation, fertilization, and embryo development, among others. Analysis of proteomic compounds of human-placenta-derived EVs has been carried out, signaling that these EV proteins are involved in internalization of vesicles, the complement pathway, and histocompatibility antigens and differ depending on EV size (Tong et al., 2016). More recently, proteome analysis of EVs derived from seminal plasma in ram has been realized, and the results suggest that EV protein cargo in the spermatozoa is related to sperm fertility and the removal of the sperm membrane proteins (Leahy et al., 2020).

3.2. Lipids

The lipid composition of EVs has been less studied. Some of these studies found different amounts of cholesterol, diacylglycerol, lactosylceramide, phosphatidic acid, and phosphatidylglycerol depending on the type of exosome-releasing cell (Skotland et al., 2019). A recent study shows the lipidomic profile of EVs in human serum in different EV fractions generated by ultracentrifugation. The authors were able to observe differences in the composition of these fractions in 48 lipids, such as lipoproteins, apolipoproteins and glycerolipids (Chen et al., 2019). However, the number of studies with lipid misidentifications is very high, so knowledge of the lipid composition of EVs is still incomplete (Simons, 2018; Wood and Cebak, 2018).

3.3. Nucleic acids

The first time that nucleic acids were detected in EVs was in 2007 by Valadi (Valadi et al., 2007). In this study, the authors observed the presence of functional mRNAs and small RNAs in exosomes from mouse and a human mastocyte cell line. They proposed to call it "exosomal shuttle RNA" (esRNA) and postulated exosomes as a means of transferring nucleic acid cells between cells. Other studies have since shown that miRNAs from exosomes are exported and can regulate gene expression in other cells (Ismail et al., 2013; Montecalvo et al., 2012; Pegtel et al., 2010). A large number of studies have demonstrated EV-mediated nucleic acid transfer. This transfer has been related to the regulation of different functions, such as cardiac regulation and vascular remodeling (Heymans et al., 2013; Luo et al., 2018; Sayed et al., 2007; Thum et al., 2008; Xiao and Chen, 2010), apoptosis (Crescitelli et al., 2013), breast cancer (Lee et al., 2013), maintenance of renewal stem cells (Koh et al., 2010), and brain function (Agnati et al., 2010; Koniusz et al., 2016).

4. Reproduction functions of EVs

Mammalian pregnancy is a complex process, and its arrival at term depends on a large number of events, both in the male and in the female. Gamete maturation, fertilization, embryonic development, and subsequent migration to the uterus, where the endometrium is invaded, are very complex processes that require continuous and specific regulation. Some studies have shown how this regulation depends greatly on cell-to-cell communication mediated by EVs in different mammalian species, and EVs have found in different mammalian reproduction tissues (Table 2).

4.1. Gamete maturation

EVs were detected for the first time in human seminal fluid, presenting proteins, lipids, and nucleic acids (Ronquist et al., 2012, 2009). EV cargoes could be involved in capacitation, acrosome reaction, and fertilization signals (Carlini et al., 1997; Ikawa et al., 2010; Machtinger et al., 2016). The epididymal epithelium has been seen to secrete EVs, called epididymosomes, that contain adhesion molecules and proteins for sperm motility, fertilization ability, protection, and maturation and have been isolated in different species such as human, bovine, hamster, and ram (Fornés et al., 1995; Frenette et al., 2002, 2003; Frenette and Sullivan, 2001; Gatti et al., 2005; Sullivan et al., 2005, 2007; Sullivan and Saez, 2013; Théry et al., 2009; Thimon et al., 2008; Yanagimachi et al., 1985). In bovine, proteins associated with sperm maturation, such as macrophage migration inhibitory factor (MIF) and aldose reductase (AKRB1), have been found in epididymal fluid (Frenette et al., 2003), whereas the acrosome reaction increases porcine sperm incubated with EVs isolated from seminal plasma (Siciliano et al., 2008) and prevents premature acrosome reaction in human and mouse *via* GPX5 (Pons-Rejraji et al., 2011; Rejraji et al., 2002). Further studies showed that epididymosomes could regulate gene expression by miRNAs so that different profiles of miRNAs are released into the intraluminal fluid (Belleannée et al., 2013). Recent investigations demonstrated that the miRNA profile of epididymosomes can also influence early embryonic development and that it depends on a multitude of epigenetic changes that occur throughout transit and storage in the epididymis (Nixon et al., 2019; Trigg et al., 2019).

Gametes enter the oviduct, where both merge for fertilization to take place. The secretory cells of the oviduct secrete intracytoplasmic granules, and the oviductal fluid is significant in subsequent embryonic survival (Besenfelder et al., 2012). Gamete maturation, fertilization, and early embryonic development occur in the oviduct (Fu et al.,

Table 2
Mammalian species and reproductive tissues where EVs have been found.

Species	EV Locations	References
Humans	Oviduct, epididymal fluid, cytotrophoblast, endometrial tissue, uterine fluid, endometrial epithelial cells	(Bathala et al., 2018; Ng et al., 2013; Salomon et al., 2013; Thimon et al., 2008; Vilella et al., 2015)
Rodents	Oviduct, epididymal fluid, epididymal epithelium, endometrial epithelial cells	(Al-Dossary et al., 2013; Fornés et al., 1995; Vilella et al., 2015; Yanagimachi et al., 1985)
Cattle	Oviduct, epididymal fluid, seminal plasma, follicular fluid, oviductal fluid, uterine fluid, embryos	(Almiñana et al., 2018, 2017; da Silveira et al., 2017; Dissanayake et al., 2020; Frenette and Sullivan, 2001; Kusama et al., 2018; Sullivan et al., 2005)
Pigs	Oviduct, seminal plasma, epididymal epithelium, uterine fluid, embryos	(Alcântara-Neto et al., 2020; Bai et al., 2018; Bidarimath et al., 2017; Hughes and Berger, 2015; Krawczynski et al., 2015)
Sheep	Uterine fluid, epididymal fluid, uterine epithelium	(Burns et al., 2014; Gatti et al., 2005; Nakamura et al., 2016)
Horses	Follicular fluid	(da Silveira et al., 2012)
Dogs	Oviductal epithelium	(Lange-Consiglio et al., 2017)
Cats	Oviductal epithelium	(de Ferraz et al., 2019)

2020), and different studies showed the presence of EVs, called oviductosomes, in the oviductal fluid. Al-Dossary et al. (Al-Dossary et al., 2013) observed these oviductosomes in mice for first time and subsequent studies have demonstrated the presence of oviductosomes in different species, supporting the idea that they could be related to all the processes that occur in the oviduct, including gamete capacitation and early embryonic development (Almiñana et al., 2018, 2017; Bathala et al., 2018; Lopera-Vásquez et al., 2016; Qu et al., 2019). Almiñana et al. (2017) recently identified oviduct-specific glycoprotein precursor 1 (OVGP1) in bovine oviductosomes. This protein, capable of binding to the zona pellucida (ZP) of the oocyte, facilitates a correct early embryonic development, controlling polyspermy and stabilizing the oviductal environment (Algarra et al., 2016; Coy et al., 2008). Studies in pigs are less numerous than in cows; however, the importance of EVs as transmitting vehicles, mainly mRNAs and miRNA, necessary for fertilization, maturation of gametes, embryonic development, and implantation has also been demonstrated. The presence of EVs in the porcine epididymis has been observed, and it is also known that the formation of the apical blisters that give rise to these EVs is independent of the estradiol levels (Hughes and Berger, 2015). Extracellular vesicles have been isolated in seminal plasma of pig, and their relevance in sperm maturation, prevention of premature acrosome reaction, capacitation, and finally fertilization has been demonstrated (Machtinger et al., 2016; Skalnikova et al., 2019). More recently, Barranco et al. (Barranco et al., 2019) have shown the presence of different types of EVs, specifically MVs and exosomes, in porcine seminal plasma. Recently, Baskaran et al. (2020) published a review indicating the relevance of exosomes and their content to the sperm quality of semen and its relationship with infertility in humans. Although this relationship has not yet been demonstrated in animal species, it is very likely that the events observed in humans can also be seen in other mammalian species.

4.2. Early embryo development and oviduct interaction

The use of oocytes removed prematurely for assisted reproductive techniques (ARTs) is a common technique in cattle (Diógenes et al., 2017). However, in *in vitro* production systems, the quality and quantity of viable embryos is much lower than *in vivo*, and embryos produced present altered gene expression patterns and lower pregnancy rates of transferable embryos (Niemann and Wrenzycki, 2000; Pontes et al., 2009; Rizos et al., 2008). Supplementation media with EVs derived from oviductal fluid downregulates the expression of pregnancy recognition factors, such as interferon-tau (IFNT) and placenta-specific 8 (Plac8), and alters the expression genes associated with apoptosis and cell proliferations, such as *Bax*, *Oct4*, and *Bcl2* (Lopera-Vásquez et al., 2016; Qu et al., 2019). Different types of EVs have been identified in bovine oviductal fluid at different stages of the estrous cycle, showing that they are under hormonal regulation (Almiñana et al., 2018). Differences in metabolome profile of oviduct EVs recovered in different stage of estrous cycle have been shown (Gatien et al., 2019). Due to the relevance of *in vitro* embryo production in this species and the importance that EVs seem to have in maternal–embryonic communication, in recent years a large number of studies have been carried out in order to determine whether enrichment of the culture media with EVs could improve these rates and quality of blastocysts produced *in vitro*. Some studies in bovine embryo culture have shown that the presence of EVs of the oviduct in the culture improves the development and quality of bovine embryos (Lopera-Vasquez et al., 2017; Lopera-Vásquez et al., 2016; Qiao et al., 2018). Moreover, bovine embryos secreted EVs in culture, and these EVs could be captured by other embryos, improving blastocyst rates (Dissanayake et al., 2020; Pavani et al., 2018). Similar results have been found in canine oocyte culture, where EVs secreted by oviduct epithelium improve oocyte viability *in vitro* (Lange-Consiglio et al., 2017). However, in addition to the maternal oviduct and uterus, secretion of EVs by early embryos has also been demonstrated in different species, such as bovine and mouse, which could indicate the significance of these molecules in maternal–embryonic communication (Fu et al., 2020; Kim et al., 2019; Qu et al., 2019; Saadeldin et al., 2014). Characteristics of EV populations secreted by embryos depends on embryo quality (Mellisho et al., 2017). Like bovine embryos, porcine embryos also secrete EVs that improve blastocyst rates in culture by embryo–embryo communication (Bidarimath et al., 2017; Saadeldin et al., 2014). Indeed, EVs secreted by theca cells, oocytes, and non-ovarian tissues have been shown to have different mRNA profiles, suggesting important roles in early embryonic development and fertilization of EVs (Matsuno et al., 2019). These findings indicate that EVs play a fundamental role in all reproductive stages in pigs, from the maturation of gametes, both male and female, to fertilization or early embryonic development. On the other hand, EVs have also been found in the female reproductive tract of sows. EVs are present in uterine luminal fluid, and their cargoes of miRNAs reveal regulation of maternal–embryonic interaction (Krawczynski et al., 2015). Therefore, the secretion of different populations of EVs with different profiles of miRNAs related to maternal–embryonic communication by oviductal cells and the isthmus has been demonstrated (Jamaludin et al., 2019). Saadeldin et al. (2014) indicated the relevance of MVs in pig embryo communication. These microvesicles contain mRNAs essential for early embryonic development (Llobat, 2020; Saadeldin et al., 2014).

A possible explanation for functions of EVs is found in their cargoes, both of nucleic acids and proteins. miRNA profiles in EVs of follicular liquid indicated functions related to chromatin remodeling, transcriptional regulation, and DNA methylation and hydroxymethylation patterns of embryos (da Silveira et al., 2017). Furthermore, proteins and nucleic acids isolated from EVs differ with estrous cycle, and mRNA associated with histone methyltransferases, histone demethylases, and DNA methyltransferase have been identified in EVs isolated to oviductal fluid, indicating that epigenetic regulation in early embryos is controlled by EVs, and their cargoes are controlled the estrus cycle (Almiñana et al., 2018). Several relevant genes for embryo development, such as *Bcl2*, *Cdk6*, and *c-Myc*, are targets of oviduct EV miRNAs (miR-34c-5p, or miR-449b) (Fereshteh et al., 2018; Wang et al., 2017).

4.3. Embryo development and uterine interaction

Oviduct EVs modified the embryo transcriptome in culture and therefore regulated development (Bauersachs et al., 2020). Endometrial cells cultured with intrauterine EVs show differential profiles of transcripts related to the immune system, so EVs could be regulated the union between conceptus and endometrial epithelium in implantation (Nakamura et al., 2019). Quality of embryos could be related to miRNAs and snoRNA profiles in EVs secreted by them (Dissanayake et al., 2020; Mellisho et al., 2019). da Silveira et al. (2017) observed that when the culture medium of somatic cell nuclear transfer is supplemented with EVs from the uterus, bovine blastocyst rates and interferon tau (IFNT) expression are improved (Qiao et al., 2018). Protein cargoes in EVs have also been studied, and it seems that maternal–embryonic communication independent of IFNT could be regulated by protein cargoes of EVs secreted by conceptus (Malo Estepa et al., 2020). Ninety-seven proteins are expressed exclusively in EVs of the oviduct *in vivo versus in vitro*, related to spermatozoa union, fertilization, and embryo development (Almiñana et al., 2017). In other species, there are fewer studies on this topic, although EVs have been found in luminal fluid from sheep uterus and in equine follicular fluid (Burns et al., 2014; da Silveira et al., 2012). In both cases, the load of miRNAs and proteins of the EVs seems to be directly related to the

development of the concept and to the maternal–embryonic interactions necessary for the establishment and maintenance of pregnancy. It seems clear that EVs secreted by both the embryo and maternal tissues play a key role in pregnancy, although many unknowns remain.

4.4. EVs and postnatal development

After parturition, development depends largely on breast milk. Maternal milk presents different compounds, including RNA and microRNA, that confer immunity to infants and are related to postnatal growth (Morton, 1954; Weber et al., 2010). Milk nucleic acids are stable in exosomes under gastrointestinal conditions, which are deleterious due to the encapsulation of these nucleic acids in EVs (van Herwijnen et al., 2016). The presence of exosomes that contain nucleic acids and proteins has been detected in human, mouse, cow, pig, and water buffalo milk (Admyre et al., 2007; Chen et al., 2017, 2020; Nakatani et al., 2006; Pieters et al., 2015). Functions of these milk exosomes have been studied. Recently, Galley and Besner (2020) published a review that analyzed the therapeutic effect of breast-milk-derived exosomes in the gastrointestinal disease necrotizing enterocolitis in human. *In vitro* studies have revealed that supplementation of exosomes derived from cow milk could enhance immune cells under inflammatory conditions, and exosomes derived from human breast milk influence immune response (Admyre et al., 2007; Komine-Aizawa et al., 2020). Analysis of the miRNA transcriptome of cow commercial-milk-derived exosomes indicated a large amount of small RNA and RNA (Benmoussa et al., 2020). The most abundant miRNA in non-processed cow milk seems to be bta-miR-148a, capable of attenuating the expression of DNA methyltransferase 1, critical in epigenetic regulation (Benmoussa and Provost, 2019; Melnik and Schmitz, 2017). Patterns of miRNA contents in milk exosomes are different between species and can be absorbed both *in vivo* and *in vitro*, suggesting that these miRNAs could have important regulatory functions in postnatal development (Lin et al., 2020).

5. Conclusions

During pregnancy in mammals, several cellular and molecular mechanisms are activated, each involving different transcription factors, growth factors, cytokines, and others, related to gamete maturation, fertilization, embryo development, implantation, placentation, vascularization and maternal–embryonic recognition. Despite extensive knowledge of these factors, the interaction between them and the metabolic pathways involved remains to be clarified. Extracellular vesicles have been proposed as a fundamental mechanism in cell-to-cell communication in different events, including those mentioned above. EV cargoes are directly related to the functions that they can carry out in domestic animal reproduction, elucidating how this communication actually occurs in order for the pregnancy to take place. On the other hand, the production of embryos *in vitro* is one of the most commonly used techniques to preserve genetic quality in species such as bovine. However, the quality and quantity of viable embryos obtained could be substantially improved. Major knowledge related to actions of EVs in cellular communication could open new doors to the more effective use of these techniques. Finally, presence of EVs in breast milk reveals its important role in regulatory functions of postnatal development, such as immune system development.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Uncited reference

ExoCarta, n.d

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