Received: 31 March 2022 | Accepted: 12 July 2022

DOI: 10.1111/rda.14205

Reproduction in Domestic Animals WILEY

REVIEW

Role of extracellular vesicles in intercellular communication during reproduction

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Funding information
Horizon 2020 Framework Programme

Abstract

The mammalian reproduction is a process of controlled cellular growth and development regulated by constant communication between the gametes, the subsequent embryo and the maternal system. Extracellular vesicles (EVs) are involved in these communications to a significant degree from the gamete production and maturation to fertilization, embryo development and implantation. They regulate the cellular physiology and the immune reaction to bring about a favourable environment for a successful pregnancy. Deciphering the mechanisms employed in EV-mediated embryo maternal communication could improve our knowledge in mammalian reproduction and increase the efficiency of animal breeding.

KEYWORDS
embryo-maternal communication, extracellular vesicles, immune regulation, intercellular signalling, maternal recognition of pregnancy

1 | INTRODUCTION

In mammalian reproduction, every significant milestone such as gamete production, maturation of gametes, fertilization, embryo development, implantation and the development of the foetus happen under tightly controlled parameters. Communication between the maternal tissue and the gametes and embryo is thought to be one of the main mechanisms utilized in regulation of the peri-implantation microenvironment in favour of establishing a successful pregnancy.

An important aspect of embryo maternal communication is immune modification. Since the embryo is a semi-allograft, containing unique antigens transcribed from the paternal genome, the maternal immune system should be rejecting the implantation. However, in a unique instance of acquired immune tolerance, the maternal immune system not only ignores the embryo, but also facilitates the implantation and in some species, the subsequent invasion. These actions are thought to be initiated by embryo-maternal communication (Fair, 2016).

Conventionally, embryo maternal crosstalk is thought to be achieved using endocrine, paracrine or juxtracrine mechanisms utilizing various hormones and chemical signals produced by the embryo and the maternal tissue. While there are decades of rigorous research corroborating various signalling pathways used in embryo-maternal communication, the consensus of the scientific community

is that the overall mechanism of the system is not yet fully elucidated. Intercellular signalling mediated by extracellular vesicles (EVs) is increasingly identified as a novel facet of the embryo-maternal dialogue. The ability of EVs to transfer labile molecules such as miRNA in a safely contained system is hypothesized to be a key component in EV-mediated intercellular communication.

2 | EXTRACELLULAR VESICLES

Extracellular vesicles are nano-sized semi-spherical membrane-bound structures (Kurian & Modi, 2019) produced by almost all types of cells by different means of biogenesis. They are broadly classified as exosomes (40–100 nm), microvesicles (100–1000 nm) and apoptotic bodies (1–2 μm) (Raposo & Stoorvogel, 2013). EVs are enclosed by a lipid bilayer and contain lipids, proteins, RNAs (lncRNA, mRNA, small non-coding RNA, rRNA and miRNA) and DNAs (dsDNA, ssDNA and mtDNA) (Chivet et al., 2014). The composition and concentration of EVs largely depend on the physiological and environmental conditions. EVs can regulate different physiological and pathological conditions through epigenetic and phenotypic modifications in recipient cells (de la Canal & Pinedo, 2018) and participate in different biological activities. They can be utilized as candidate biomarkers of health and disease and as potential targets for therapeutics (Gould & Raposo, 2013).

EVs regulate different reproductive events such as sperm maturation (Caballero et al., 2010), sperm viability, capacitation and acrosome reaction (Hasan et al., 2021), oocyte maturation (Silveira et al., 2014), recognition of conceptus in implantation (Ruiz-González et al., 2015), maintenance of pregnancy and parturition (Salomon et al., 2018). Moreover, a growing body of evidence indicate that EVs are also involved in pathological conditions such as early pregnancy loss, polycystic ovaries, endometriosis, gestational diabetes mellitus, hypertension and preeclampsia (Rooda et al., 2020). This review highlights the potential roles of EVs in selected aspects of mammalian reproduction.

3 | THE ROLE OF EVs IN MALE GAMETE MATURATION

Spermatozoa produced by the mammalian testicles are not completely matured and lack motility and fertilizing ability. Spermatozoa gain functionality while transiting through male and female reproductive system (Robaire et al., 2002). Studies showed that EVs produced from the male reproductive tract, including epididymosomes and prostasomes, play vital roles in the maturation process of spermatozoa (Saez et al., 2003; Wu et al., 2017). Spermatozoa undergo several physical and biochemical changes during their journey towards oocyte starting from the anterior vagina after coitus (Reshi et al., 2020). These modifications possibly occur due to the crosstalk between the spermatozoa and the female reproductive tract leading to functional maturation.

EVs isolated from uterine and oviductal fluids of mice contain sperm adhesion molecule 1 (SPAM1) and plasma membrane calcium pump (PMCA4), which are vital for sperm maturation (Griffiths et al., 2008). Furthermore, in vitro studies have confirmed the transfer of fertility regulating proteins from EVs to the sperm. Oestrogen hormone is believed to regulate the expression of these macromolecules (Al-Dossary et al., 2015). Therefore, Uterine EVs are possibly responsible for spermatozoa capacitation, membrane stabilization and final maturation via miRNA transfer. The presence of SPAM1 protein in EVs suggests the possible role of inhibiting a premature acrosomal reaction during the uterine transit of the spermatozoa (Griffiths et al., 2008; Martin-DeLeon, 2016). On the other hand, oviductal EVs carry αV integrin, CD9, heat shock proteins A8, lactadherin, oviductal specific glycoprotein (OVGP), lipids, SPAM1, RNAs and miRNAs (Al-Dossary & Martin-Deleon, 2016) which are involved in several spermatozoa functions such as sperm viability and motility (OVGP) and acrosome reactions (SPAM1), reducing polyspermy, inducing the protein phosphorylation, modulating fertilization and embryo development (Alminana-Brines, 2015; Avilés et al., 2015; Martin-DeLeon, 2016; Saccary et al., 2013; Zhao et al., 2016).

4 | THE ROLE OF EVs IN FEMALE GAMETE MATURATION

The development and maturation of the ovarian follicles and oocytes are highly associated and important for subsequent proper embryo development upon the fertilization. These complex processes are regulated by intercellular communication within the follicular microenvironment and structural transformation of different cell types constituting the follicle (Eppig, 2001). In addition, the crosstalk between oocytes and theca and granulosa cells are mainly done through gap junction proteins (Eppig et al., 2002).

The presence of EVs in follicular fluid has been well established (Hasan et al., 2020, 2021; Reshi et al., 2021). EVs play vital roles in intercellular communication related to follicular development and oocyte quality. Concentration of EVs negatively correlates with the size of follicles (Hasan et al., 2021). Bovine follicular fluid derived EVs affects the transcriptomes of oocytes, adjacent granulosa cells and oviductal epithelial cells, playing essential roles in oocyte maturation and embryo development (Dalanezi et al., 2017; Hasan et al., 2020). Similarly, supplementation with FF EVs during in vitro maturation (IVM) enhances the cumulus cell expansion (Hung et al., 2015), the proliferation of granulosa cells (Hung et al., 2017) and enhance blastocyst development rates (Silveira et al., 2017).

EVs indirectly affect the competence of oocytes by improving the function of cumulus cells (Rodrigues et al., 2019). During the early stages of oocyte maturation, transzonal projections extend from the cytoplasm of cumulus cells and mediate RNA transfer between cumulus cells and the oocyte (Macaulay et al., 2014; Silveira et al., 2017). In addition to RNA, exosomal cytokines can regulate various physiological aspects, including proliferation and differentiation of cells, survival or atresia of follicles and maturation of oocytes

(Field et al., 2014; Zolti et al., 1991). Follicular fluid exosomes possess cytoprotective effects against stress and under the stresses the cells experience an increased secretion of EVs that enhances the defence system preventing the cell death (Carver & Yang, 2016; Rodrigues et al., 2019). During oxidative stress, the granulosa-cells-derived EVs contain higher proportion of antioxidants and other substances associated with cellular defence compared with normal conditions (Saeed-Zidane et al., 2017). Treatment of oocytes with follicular-fluid-derived EVs reduces the apoptosis of cumulus cells and damage to oocytes caused by heat shock (Rodrigues et al., 2019; Saeed-Zidane et al., 2017).

5 | ROLE OF MATERNAL EVs DURING FERTILIZATION

Fertilization is a series of coordinated events taking place in the oviduct. This includes sperm capacitation, sperm-zona pellucida binding, acrosome reaction, zona penetration by sperms, sperm-oocyte binding and fusion, cortical reaction, oocyte activation and meiosis resumption (Georgadaki et al., 2016). Several studies have shown the contribution of EVs in facilitating these processes.

The protein PMCA4a is known to maintain the Ca^{2+} homeostasis in the sperms (Al-Dossary et al., 2013), which is crucial for its progressive and hyperactivated motility and fertility. The murine sperms receive their PMCA4a via EVs from the oviductal and uterine luminal fluids (Al-Dossary et al., 2013). In pigs too the co-incubation of sperms and EVs deriving from seminal plasma increased the acrosome reaction (Siciliano et al., 2008). Barraud-Lange et al. (2007) showed that oocytes transfer proteins, via EVs, to the sperm that has already entered the perivitelline space indicating that EVs are crucial for sperm membrane re-organization and fertilization (Barraud-Lange et al., 2007). The interactions between the Izumo1 protein of the sperm membrane and the Juno protein of oolemma plays a vital role during fertilization (Bianchi et al., 2014). Following the fertilization, the prevention of polyspermy is a crucial requirement. It is shown that Juno is removed from the oolemma and sent out via EVs where binding of these EVs to spermatazoa can block acrosome reacted spermatozoa, hence preventing polyspermy (Bianchi et al., 2014).

Following the fertilization, the early embryo gradually develops when passing through the oviduct into the uterine lumen for further implantation. Oviductal fluid is the first micro-environment to which early mammalian embryos are exposed (Saint-Dizier et al., 2020). Embryo-maternal communication at the oviduct is vital for the subsequent embryonic development, and any errors in this dialogue are found to be detrimental for the prospective implantation. During its free-floating transport in the uterus, the embryo communicates with the mother using various mediators, including EVs (Nakamura et al., 2020). Uncovering embryo-maternal interactions during the pre-implantation period may help to answer questions related to reproductive issues, such as recurrent implantation failure and ectopic pregnancy.

6 | EMBRYO MATERNAL COMMUNICATION IN THE OVIDUCT

Delineating the embryo-oviduct cross talks is difficult; thus, many in vitro models have been employed to understand the embryo-maternal communication at the oviduct (Kölle et al., 2020). The addition of oviductal EVs to the embryo culture media improved the bovine embryos produced in vitro and increases blastocyst rate (Almiñana et al., 2017), trophectodermal and total cell number and better cryo-survival post-vitrification (Lopera-Vasquez et al., 2016). Moreover, EVs isolated from different regions of oviduct show differential impacts on embryonic development. EVs isolated from isthmic oviductal fluid increased survival rate, development and better-quality blastocysts; however, EVs from ampullary oviductal fluid had no impact (Lopera-Vasquez et al., 2017). Though the mechanisms are unknown, it is possible that the transfer of embryo-tropic factors from the maternal tract to the embryos via the EVs (Kurian & Modi, 2019) since internalization of EVs by embryos has been demonstrated (Almiñana et al., 2017; Pavani et al., 2019). In a similar study, addition of oviductal fluid derived EVs to the embryo transfer media in mice significantly increased live birth rates (Qu et al., 2019). These indicate the possible translational value of maternal tract EVs in improving the embryo transfer efficiency during ARTs. From a mechanistic point of view, supplemented EVs may impart effects via changing the embryonic gene expression and previous studies demonstrated that the oviductal EVs can modulate the expression of certain key genes related to the early embryonic development. Furthermore, the supplementation of frozen–thawed oviductal EVs to the bovine embryo culture resulted in 221 differentially expressed genes (DEGs) compared to the control while 28 DEGs in the case of fresh oviductal EV supplementation vs control (Bauersachs et al., 2020). Based on an integrative bioinformatic analysis of oviductal EV mRNA and miRNA identified altered mRNA transcriptome in response to oviductal EVs, indicating that oviductal EV cargo may mediate their effects on embryos via multiple mechanisms. Possibly, (1) increased delivery of transcripts to the embryos, (2) protein translation from the delivered mRNA that alter the gene expression by embryos, (3) miRNA-based gene silencing. Overall, these studies show the positive paracrine effects that the oviductal EVs can exert on preimplantation embryos.

The impacts of embryo on the oviduct have been studied extensively (García et al., 2017; Maillo et al., 2015; Schmaltz-Panneau et al., 2014), however, the effects of embryonic EVs on the oviduct are less studied (Dissanayake et al., 2021). Maillo et al. (2015) showed that the coculture of bovine embryos with bovine oviductal epithelial cells (BOECs) downregulated specific genes of the Bone Morphogenetic Protein (BMP) signalling pathway. Interestingly, the role of embryonic EVs on the gene expression of oviduct was recently investigated and the supplementation of good quality day 5 embryo derived EVs could alter the gene expression (25 DEGs) in BOECs. However, the degenerating embryo-derived EVs did not have such effects (Dissanayake et al., 2021). Among the seven upregulated genes in BOECs with quality embryo-derived EVs;

interferon-stimulated genes (ISGs) such as ISG-15, MX1, OAS1Y and LOC100139670 are of particular significance. The interferon-tau (IFN-τ) is a type 1 interferon and is the major the pregnancy recognition molecule of the ruminants, and it is possible that bovine embryos can transfer IFN-τ to the maternal tract via EVs (Nakamura et al., 2016). Moreover, two independent studies reported that these ISGs were upregulated in the oviductal epithelial cells in vitro and in vivo (Schmaltz-Panneau et al., 2014; Smits et al., 2016), in the presence of embryos. Thus, it is likely that preimplantation embryos use EVs as a mediator to notify the mother about their presence or quality while maternal signals also communicated to embryo via EVs (Figure 1). Hence, further studies on EV-mediated embryonic effects on the oviduct and oviductal EV effects on embryos would help us to understand the overall mechanisms behind embryo-maternal communication in the oviducts.

7 | INVOLVEMENT OF EVs IN EMBRYO-ENDOMETRIAL INTERFACE DURING IMPLANTATION

Embryo implantation is recognized as the most critical step in early stages of pregnancy. Majority of assisted reproduction technologies (ART) failures occur because of implantation failure. Implantation mechanisms are highly species-specific with different types of placentation (Green et al., 2021; Johnson et al., 2021). However, there are common events that occur in every mechanism of successful implantation. The bidirectional communication between the embryo and the endometrium is one such commonality (Es-Haghi et al., 2019; Østrup et al., 2011; Paulson & Comizzoli, 2021) where many including us have reported the presence and possible actions of EVs in the embryo-maternal interface.

Recognition of the embryo by the maternal immune system is crucial for a successful implantation. The primary maternal recognition signal in ruminants, IFN-τ is secreted by the elongating conceptus and acts primarily on the endometrium shutting down the $PFG_{2\alpha}$ mediated luteolytic pathway. It is reported that sheep endometrial epithelial EVs are enriched with endogenous retroviral mRNA that can act through Toll Like Receptors in trophectoderm to induce the secretion of IFNτ (Burns et al., 2016). Ovine trophoblast EVs are also enriched in IFNτ and can induce altered expression of ISGs in in vitro endometrial models, suggesting a significant involvement of EVs in the recognition of conceptus by the ewe. Similarly, in cows embryonic EVs from uterine flashings were enriched with IFNτ, and they upregulated the expression of apoptosis-related genes and adhesion molecules in endometrial epithelial cells suggesting that EV-mediated communication might be utilized in animals with similar placentation (Kusama et al., 2018; Nakamura et al., 2016, 2019). In animals with epitheliochorial placentation such as pigs, the crosstalk is deemed of the highest significance because of the lack of embryo invasion. In sows EVs are reported to be important in recruiting the natural killer (NK) cells and T-cells to the uterine microenvironment and maintaining the proinflammatory status (Bidarimath et al., 2017).

The most investigated cargo types carried by EVs are the miRNAs. The miRNA cargo in serum EV populations of pregnant and non-pregnant domestic animals significantly changed where non-pregnant mares showed significant enrichment of miRNA targeting focal adhesion molecules (FAM) (Klohonatz et al., 2019). These integrin-containing molecules are regulators of the extracellular matrix (ECM) and play a vital role in embryo adhesion indicating the potential to be used as a biomarker of receptivity (Klohonatz et al., 2019). In pigs, embryo-derived EVs carrying miR-125b were reported to induce gene alterations in implantation-linked LIF and its receptor LIFR in the endometrial epithelium (Krawczynski et al., 2015). Conditioned media used in in vitro embryo development is enriched with EVs carrying miRNA cargo of developmental stage dependent. In bovine embryos, the embryo-derived EVs are enriched with miRNA such as miR-24-3p, miR-191 and miR-2887

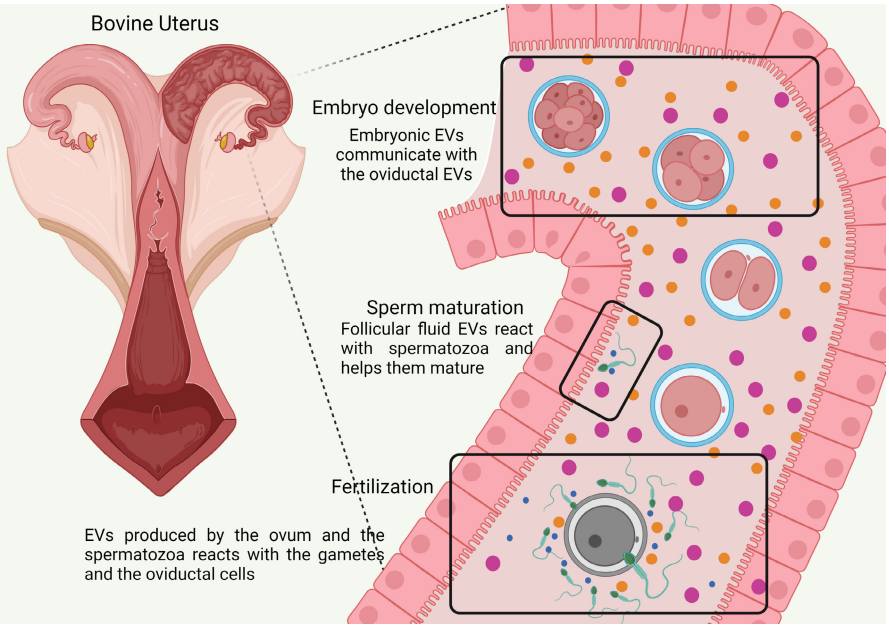

FIGURE 1 Extracellular vesicles mediated communication inside the oviduct. EVs involve in a constant regime of communication between the gametes, embryo and the maternal system. The maturation of spermatozoa is facilitated by follicular fluid EVs during their transition through the oviduct. In fertilization, EVs may be used in communication between the ovum, spermatozoa and the surrounding maternal tissue. The fertilized embryo communicates with the surrounding oviductal cells using EVs while oviductal cell derived EVs and follicular fluid EVs are used to signal the embryo.

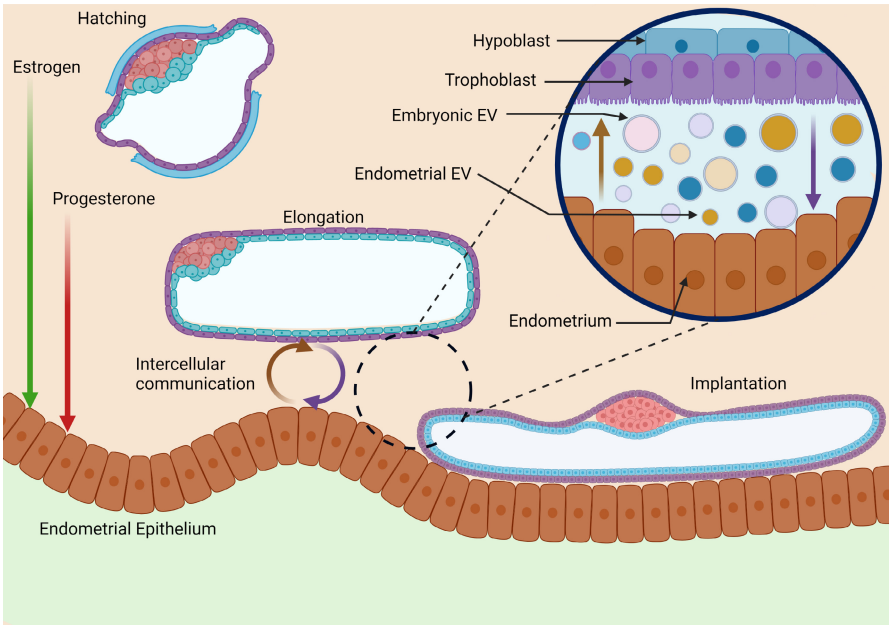

FIGURE 2 Endometrial (maternal) and embryo-derived EVs-mediated communication during peri-implantation period. In addition to the well-studied phenomenon of endocrine, paracrine, autocrine and juxtracrine signalling pathways during the peri-implantation period, EVs are extensively used for embryo maternal communication especially in the hatched elongating embryos.

that influence the endometrial transcriptome and the innate immune function (Kusama et al., 2021; Rio & Madan, 2021). The possible communication pathways via EVs in the endometrium are depicted in Figure 2.

8 | POTENTIAL USE OF EV-MEDIATED EMBRYO-MATERNAL COMMUNICATION IN "BENCH TO FARM"

In ART, which is growing ever popular in livestock management, the main challenge is deciding (1) which embryo to transfer and (2) when to transfer. Existing methods of embryo grading heavily depend on morphology, which is subjective, and embryo biopsy, which is highly invasive. Embryonic EVs have been proposed as a non-invasive embryo marker. Studies report that competent embryos produce different populations of EVs compared with degenerating embryos, and their effects on maternal system are quantifiably different (Dissanayake et al., 2020, 2021; Godakumara et al., 2021). Similarly, analysing the EVs produced by maternal tissue could also be a non-invasive method to determine the state of receptivity (Aleksejeva et al., 2022; Luddi et al., 2019). There have been efforts to use EVs as therapeutic agents in mammalian reproductive pathologies such as polycystic ovary syndrome, endometriosis and preeclampsia (Esfandyari et al., 2021). EVs can also be used as an agent of spermatozoa maturation (Hasan et al., 2021). Clearly, the true potential of EVs in diagnostics and therapeutics is just being revealed. EV research has been a very popular field of study in the recent decades. However, most of the mechanisms of action that govern the intercellular communication mediated by EVs are not yet fully understood. Whether EVs are specific to a certain type of target cell or a tissue and whether the cargo of the EVs is truly functional at the receiver cells are only a couple of fundamental questions about EVs without a clear answer at the present. Nevertheless, the field of EVs are moving steadily towards therapeutics and diagnostics. EVs are a potential goldmine for biological engineering and the best discoveries are expected soon, hopefully increasing our collective understanding of the mysteries shrouding mammalian reproduction.

AUTHOR CONTRIBUTIONS

The idea of the manuscript was created by AF. All authors contributed to the initial conception and creation structure of the manuscript. KG wrote the first draft. All authors contributed to the improvement and final edition of the manuscript.

CONFLICT OF INTEREST

Authors have no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are openly available in public domain publication repositories such as Pub Med.

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How to cite this article: Godakumara, K., Dissanayake, K., Hasan, M. M., Kodithuwakku, S. P., & Fazeli, A. (2022). Role of extracellular vesicles in intercellular communication during reproduction. *Reproduction in Domestic Animals*, *57(Suppl. 5)*, 14–21. https://doi.org/10.1111/rda.14205