

# Exploring the contributions of human seminal extracellular vesicles to reproduction and fertility

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## In brief

Deficits in sperm are an important contributor to male infertility, but other factors play an influential and underappreciated role. This review discusses seminal extracellular vesicles and their emerging links with infertility.

## Abstract

Declining reproductive health and fertility are global public health issues affecting an estimated 15% of reproductive-aged couples worldwide. The reasons for declining fertility are complex. However, a male contribution is thought to occur in ~50% of infertile couples. Deficits in sperm number and/or function are undeniably a major cause of infertility, but compelling evidence suggests that additional factors in the male ejaculate also play an influential and underappreciated role. In this review, we focus specifically on extracellular vesicles within human seminal plasma and explore their emerging roles in reproduction and fertility. These seminal extracellular vesicles (SEVs) are nano-sized membrane structures secreted by various cell lineages in virtually all regions of the male reproductive tract and exert key roles in intercellular communication. Consideration is given to the well-characterized effects of SEVs in supporting sperm as they transit through the female reproductive tract and their ability to modulate the immune environment within the female reproductive tract. Building on these important roles, we also detail the emerging links between dysregulated SEV production and male fertility status, and highlight the potential utility of leveraging these vesicles to improve fertility and reproductive outcomes in infertile couples. Altogether, this review highlights how expanding knowledge of SEVs provides a new perspective on the complexity of seminal fluid physiology and the underlying aetiology of male infertility.

**Keywords** seminal plasma, seminal extracellular vesicles, male infertility, conception, female reproductive tract

## Introduction

Infertility is a global concern that affects women and men of all reproductive ages. It is defined as a couple's inability to spontaneously conceive after a 12-month period of regular, unprotected vaginal intercourse and is estimated to affect ~15% of the global adult population (Boeri et al., 2021). The notion that the burden of infertility predominantly rests with the female partner overlooks that a couple's inability to conceive involves a male factor in almost 50% of cases (Eisenberg et al., 2023). Unfortunately, this inaccurate perception has affected the progress of male infertility research, thus limiting the number of diagnostic and therapeutic tools available for clinical use (Inhorn & Patrizio, 2015). However, epidemiological

evidence supporting a precipitous and ongoing decline in sperm counts over recent decades has drawn increasing public awareness to the plight of male infertility (Barratt et al., 2021).

The mechanisms underpinning male infertility are complex and there is extensive evidence that a combination of genetic causes, infections, injuries, anatomical disorders, exposure to environmental toxins, and lifestyle factors all make substantial contributions (Inhorn & Patrizio, 2015). Currently, male infertility diagnoses rely on the evaluation of semen quality (i.e., sperm count, motility, morphology, vitality), but there is also clinical value in assessing additional sperm-associated factors including DNA integrity and chromosomal deletions (Aitken, 2020). This reflects the fact that routinely assessed sperm parameters are relatively

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poor indicators of a male's fertility status. Indeed, up to 20% of infertile men present with normal semen parameters and thus are classified with unexplained infertility (Boeri et al., 2021). Taken together, these data demonstrate that our current understanding of the underlying pathophysiology of male infertility is limited, and highlight the urgent need to identify the spectrum of factors that contribute to male reproductive health and fertility status in men. One area of emerging interest in male fertility research is seminal plasma, which is now recognised as playing an important role in influencing fertility status and pregnancy outcomes.

Conventionally, seminal plasma has been assumed to have just a single function in reproduction, to support the delivery of sperm into the female reproductive tract (FRT) and facilitate their transit to the site of fertilization. However, seminal plasma also influences sperm integrity and function as well as the FRT immune environment. In animal models, perturbation of seminal plasma composition exerts effects on conception, pregnancy progression, and even offspring health (Bromfield et al., 2014; Poon et al., 2009; Schjenken et al., 2021a; Watkins et al., 2018; Wong et al., 2008; Wong et al., 2007). This influence has been linked to an array of signalling molecules that reside in both the soluble and extracellular vesicle fractions of seminal plasma (Noda & Ikawa, 2019; Robertson & Sharkey, 2016; Schjenken & Robertson, 2020). It follows that altered seminal plasma composition has been linked to reduced fertility in men (Robertson & Sharkey, 2016), raising the possibility of a causal contribution to impaired fertility and adverse pregnancy outcomes in humans.

In this review, we discuss the roles of seminal plasma at conception, placing particular emphasis on the importance of seminal extracellular vesicles (SEVs) for driving functional changes in both spermatozoa and the FRT. By extension, we consider the emerging body of evidence illustrating the influence that SEVs can exert over male fertility status. We take a clinical focus, drawing primarily on human studies, but complementing this with research on model species as appropriate. To end, we explore the potential clinical uses of SEVs and how they may aid in the reproductive field.

## Role of seminal plasma at conception

The ejaculate, or seminal fluid, is comprised of spermatozoa and seminal plasma. Seminal plasma plays a fundamental role in delivering sperm to the FRT in preparation for fertilizing an oocyte. However, beyond acting as a delivery vehicle, seminal plasma also supports reproductive health through exerting influence over sperm function and the immune response within the FRT, to affect the likelihood of conception, embryo implantation, and healthy pregnancy progression. While these functions of seminal plasma are best characterized in rodent models, they appear to be highly conserved across mammalian species, including humans (Schjenken & Robertson, 2020).

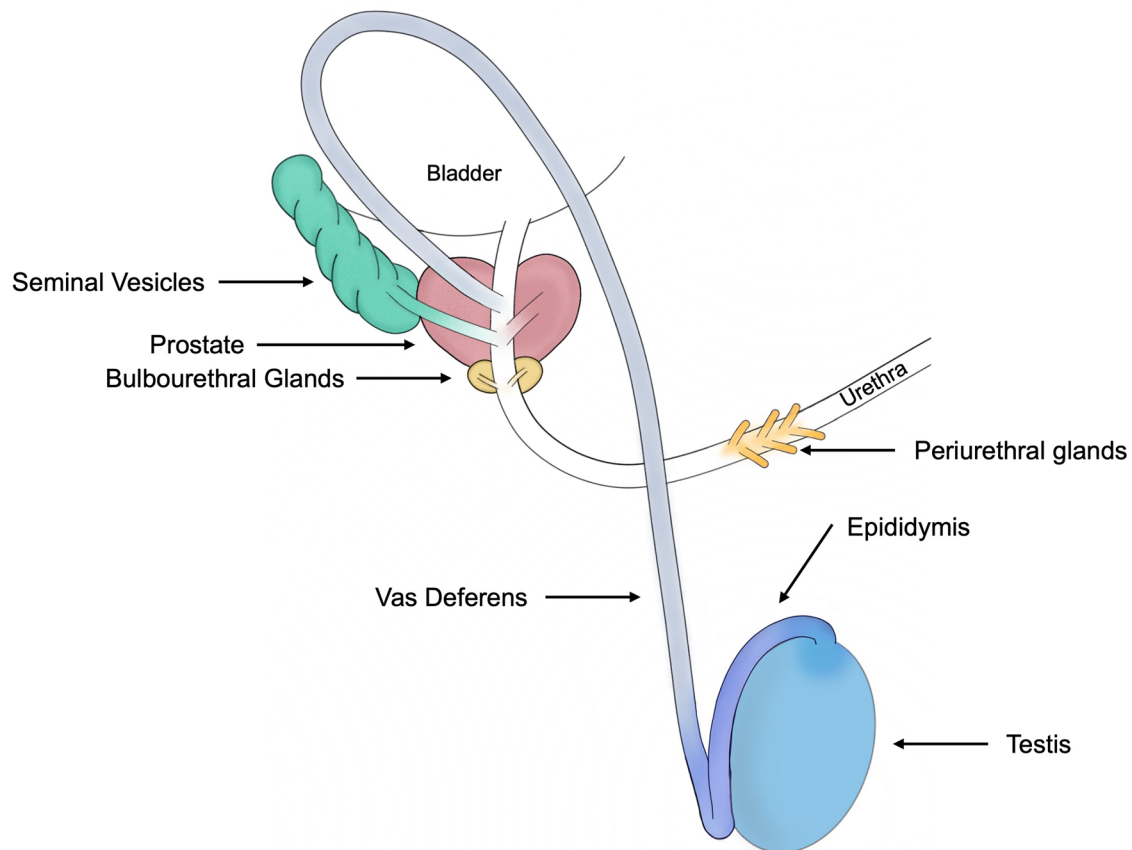
Given emerging recognition of the important functions of seminal plasma in contributing to both male and female reproductive processes at conception, there has been extensive research into identifying its complement of influential signalling mediators. Seminal plasma components originate from essentially all regions of the male reproductive tract including the testes,

epididymis, vas deferens, seminal vesicles, prostate, bulbourethral (Cowper's) glands, and periurethral (Littre) glands (Samanta et al., 2018) (Figure 1). In most mammalian species, the dominant source of seminal plasma is the secretions of the seminal vesicles, a relatively poorly understood male accessory gland (Schjenken et al., 2025). Beyond the seminal vesicles, the diverse cellular origins of seminal plasma remain unresolved. However, extensive efforts are now being directed to define its molecular composition. Indeed, seminal plasma contains a wide array of bioactive moieties, including cell-free DNA, mRNAs, non-coding RNAs, proteins, metabolites, electrolytes, sugars, and steroid hormones (Llavanera et al., 2022).

Upon deposition into the FRT, one of the primary functions of seminal plasma is to support sperm in traversing the FRT and enable their transit to the oviduct where oocyte fertilization occurs (Samanta et al., 2018). Seminal plasma achieves this through signalling factors that provide nutrients and energy to spermatozoa and support sperm function and survival in the female reproductive tract (Samanta et al., 2018). All of these functional changes are supported by seminal plasma's ability to coagulate, in humans mediated through the actions of the semenogelin (SEMG) family. This coagulation prolongs the contact of spermatozoa with seminal plasma components, increasing the length of time for potential interactions to occur (Schjenken et al., 2025).

An important contribution that seminal plasma provides to sperm as they traverse the FRT is protection against the local environment to ensure sperm survival. This first occurs upon ejaculation, where alkaline seminal plasma counteracts the acidic FRT fluids to provide an optimal pH environment for sperm survival (Samanta et al., 2018). Seminal plasma also provides further support for sperm survival through the presence of a rich array of antioxidants that convey protection against oxidative stress encountered by spermatozoa in the FRT (Lewis et al., 1997; Smith et al., 1996). Additionally, studies have shown that components of the semen coagulum play an important role in sperm survival in the FRT. These functions are best characterized in mice where the protein seminal vesicle secretory protein 2 is postulated to coat spermatozoa and protect against uterine spermicides (Kawano et al., 2014), with the human orthologue SEMG1 appearing to hold similar functions (Sakaguchi et al., 2020).

In addition to these actions, seminal plasma signalling agents have been shown to regulate sperm motility and ensure the precise timing of capacitation and the acrosome reaction (Samanta et al., 2018). Initially, the coagulum ensures the transient restriction of sperm motility via seminal plasma motility inhibitor, a restriction that is reversed once liquefaction occurs (Yamasaki et al., 2017). In mice, other copulatory plug-associated proteins also play an important role in regulating sperm motility, with seminal vesicle autoantigen shown to suppress sperm motility while prostate- and testis-expressed protein 4 appears to promote motility (Huang et al., 1999). Following liquification, other seminal plasma molecules are thought to influence motility. Fructose and inorganic moieties such as potassium, bicarbonate, and magnesium, are present in seminal plasma and are used as an energy source for sperm metabolism and motility (Schjenken et al., 2025). Other molecules including E-series prostaglandins, also highly abundant in seminal plasma, enhance sperm motility while simultaneously promoting sperm survival (Colon et al., 1986; Gottlieb et al., 1988).



**Figure 1** Cellular origins of seminal plasma. The ejaculate is comprised of fluids secreted by various male reproductive tract organs. In most mammalian species, seminal plasma is primarily produced by the seminal vesicles, with further major contributions from the prostate and epididymis. Additional male reproductive tract regions that provide minor contributions to seminal plasma include the testes, bulbourethral (Cowper's) glands, periurethral (Littre glands), and the vas deferens.

Seminal plasma also helps regulate capacitation and acrosome reaction on spermatozoa. In mice, a series of seminal plasma molecules have been identified as decapacitation factors, including seminal vesicle secretory protein 2, serine protease inhibitor Kazal-type like, and serine protease inhibitor, clade E, member 2. These molecules are thought to interact with sperm immediately after ejaculation and dissociate as sperm enter the oviduct. They act to block cholesterol efflux and calcium influx, and regulate intracellular cAMP generation (Noda & Ikawa, 2019). In humans, the orthologue of the seminal vesicle secretory protein family, SEMG, has been shown to play a similar role and prevent capacitation and acrosome reaction from occurring prematurely in the FRT (De Lamirande et al., 2001). In contrast to decapacitation factors, seminal plasma also contains factors that promote capacitation. For example, soluble cluster of differentiation 38 (CD38) protein acts to enhance sperm capacitation through interactions with the receptor CD31, resulting in the induction of acrosomal exocytosis and sperm hypermotility (Kim et al., 2015b). Together, these studies highlight how seminal plasma provides spermatozoa with the appropriate signals to allow them to reach the oocyte and undergo fertilization. It also demonstrates how seminal plasma molecules facilitate the timing of these changes to maximize sperm survival.

In addition to the role of seminal plasma in influencing sperm function, there is also evidence supporting this fluid as playing

an integral role in preparing the FRT for pregnancy (Robertson & Sharkey, 2016). This process is best characterized in rodent models, where seminal plasma induces proinflammatory cytokines and chemokines at the site of semen deposition, which promote the recruitment of leukocytes, ultimately leading to the expansion of populations of tolerogenic regulatory T (Treg) cells (Schjenken et al., 2021b; Shima et al., 2015). These changes promote the establishment of an immune environment that facilitates successful embryo implantation and sustains tolerance toward fetal antigens to support robust placental development (Robertson & Sharkey, 2016; Schjenken & Robertson, 2020). In humans, the mechanisms that drive this tolerogenic environment are not yet fully understood, but current evidence aligns with observations in the mouse model, where immune changes are triggered upon exposure of the FRT to seminal fluid at the site of semen deposition—the anterior vagina near the opening of the cervix (also called the cervical os) (Sharkey et al., 2012a; Sharkey et al., 2007; Sharkey et al., 2012b). In vitro studies have shown that seminal plasma induces inflammatory cytokine production not only in vaginal epithelial cells (Sharkey et al., 2007) but also in the cervix (Sharkey et al., 2012a; Sharkey et al., 2007) and endometrium (Gutsche et al., 2003).

These studies are complemented by compelling *in vivo* studies, where cervical and endometrial tissue biopsies were examined before and after seminal fluid exposure (Catalini et al., 2024;

Sharkey et al., 2012b). Consistent with evidence from in vitro studies and animal models, seminal fluid exposure led to an up-regulation of cytokine and chemokine mRNAs, and recruitment of macrophages, dendritic cells, and memory T cells into the ectocervical tissue of peri-ovulatory women. Notably, this inflammatory response did not occur in couples using condoms, supporting seminal fluid as driving these immunological changes (Sharkey et al., 2012b). A recent study utilized a similar strategy to evaluate effects of seminal plasma application on endometrial gene expression in the mid-secretory phase, and reported effects on gene pathways involved in implantation, embryo development, oocyte maturation, and angiogenesis (Catalini et al., 2024). Collectively, these effects explain the consistently reported benefit of contact with the conceiving partner's seminal fluid at the time of embryo transfer in the context of IVF, where meta-analyses demonstrate a 23–24% increase in clinical pregnancy rates (Crawford et al., 2015; Saccone et al., 2019).

In vitro studies have revealed the identity of key soluble signalling molecules carried by seminal plasma, including members of the transforming growth factor beta superfamily, as well as E-series prostaglandins (Denison et al., 1999; Sharkey et al., 2012a; Templeton et al., 1978). Whether these immune changes ultimately lead to the expansion of Treg cells for robust pregnancy tolerance in humans is unknown, but additional in vitro studies have shown that seminal plasma has immunomodulatory effects on T cells through both direct and indirect effects mediated by antigen-presenting cells (Meuleman et al., 2015).

The beneficial effects of seminal plasma exposure are thought to extend beyond embryo implantation, with studies in model species demonstrating that seminal plasma may influence placental development, fetal growth, and offspring health. By way of illustration, a subset of these studies have shown that pregnancies sired in the absence of seminal plasma can lead to altered offspring phenotypes (Bromfield et al., 2014; Poon et al., 2009). Such alterations include changes in offspring growth trajectory, disturbed metabolic function, and even altered behaviour (Bromfield et al., 2014; Poon et al., 2009). Regrettably, there are currently no comparable studies reporting the impact of seminal plasma exposure on long-term outcomes in human infants. However, an intriguing relationship exists between seminal plasma exposure and pregnancy complications, whereby repeated exposure to the conceiving male partner's seminal plasma conveys a protective effect against preeclampsia (Kho et al., 2009; Saftlas et al., 2014). These studies are consistent with a role of seminal plasma in promoting a tolerogenic maternal immune response that facilitates embryo implantation and robust placental development in human pregnancy.

## Seminal extracellular vesicles

Extracellular vesicles (EVs) are secreted, membranous structures that typically range from 30 to 5,000 nm in size. EVs are produced by virtually every cell in the body and are generally classified, based on their size, as exosomes (30–150 nm), ectosomes/microvesicles (100–1,000 nm), or apoptotic bodies (1,000–5,000 nm). By virtue of their diverse origins, EVs are heterogeneous, have a broad range of biological functions, and distinct modes of biogenesis (Welsh et al., 2024). The capacity of EVs to transmit their

cargo differs from other forms of cellular communication as the EV itself provides protection against degradation or modification that would normally occur “on the road” from one cell to another. This function is particularly important in biological fluids like seminal plasma where the lipid membrane of EVs encapsulates and protects the contents from enzymatic breakdown (Tamessar et al., 2021). This protective effect enables EVs to exert signalling capacity linked to their encapsulated cargo of lipids, nucleic acids, and proteins and thus promote a physiological function or pathological state (Welsh et al., 2024). Relevant to this review, EVs have been shown to influence not only immune function (Parra et al., 2023), but also play an important role in reproduction, recapitulating functions associated with seminal plasma (Tamessar et al., 2021).

Seminal plasma contains one of the most abundant EV populations of any bodily fluid, first observed in human seminal plasma and prostatic fluid in studies from the 1970s (Ronquist et al., 1978). Since then, SEVs have been identified in the seminal plasma of numerous mammalian species and have been shown to play an important role in the peri-conception period (Tamessar et al., 2021). Notably, SEVs were originally termed prostasomes owing to a misconception that they originated exclusively from the prostate (Aalberts et al., 2014). More recent studies suggest that SEVs have far more heterogeneous cellular origins (Batra et al., 2025; Hoog & Lotvall, 2015; Thimon et al., 2008), with all tissues that contribute fluids to the ejaculate potentially secreting EVs to influence the conception environment (Figure 1). Thus far, SEVs are thought to be exclusively from the tissues that comprise the male reproductive tract. However, the presence of EVs derived from non-reproductive regions is a possibility (Cossetti et al., 2014; Eaton et al., 2015). Studies to assess the origins of SEVs are challenging, because EVs isolated from a male reproductive tract region may not always be present in the ejaculate, and specific markers of SEVs from cells across the male reproductive tract are yet to be identified.

In humans, studies have shown that SEVs support sperm function and may potentially influence immune function in FRT epithelial cells to promote pregnancy success (Tamessar et al., 2021). These functions are supported by a complex array of EV cargo including nucleic acids, proteins, lipids, and metabolites (Parra et al., 2023). The best-characterized of these SEV cargo subtypes are proteins, which account for 3% of the total protein content in seminal plasma (Arienti et al., 1999). Indeed, >4,000 SEV proteins have been detected, although considerable variation has been a feature of the SEV protein inventories reported in these publications (Lin et al., 2019; Murdica et al., 2019a; Wang et al., 2022). This variation may be explained by the diverse human population cohorts used for analysis, as well as nuanced EV-enrichment techniques that enrich for different SEV populations (Welsh et al., 2024).

Focusing on EV-enrichment techniques, it is important to acknowledge that there is no perfect EV-enrichment method, with current methods all having limitations, and only ever enriching, and not purifying EVs. For example, approaches such as filtration and EV-precipitation methods yield a high number of diverse-sized EVs but provide limited removal of soluble contaminating proteins (Brennan et al., 2020; Sharma et al., 2020; Veerman et al., 2021; Welsh et al., 2024). Conversely, methods such as density gradient centrifugation and size-exclusion chromatography dras-

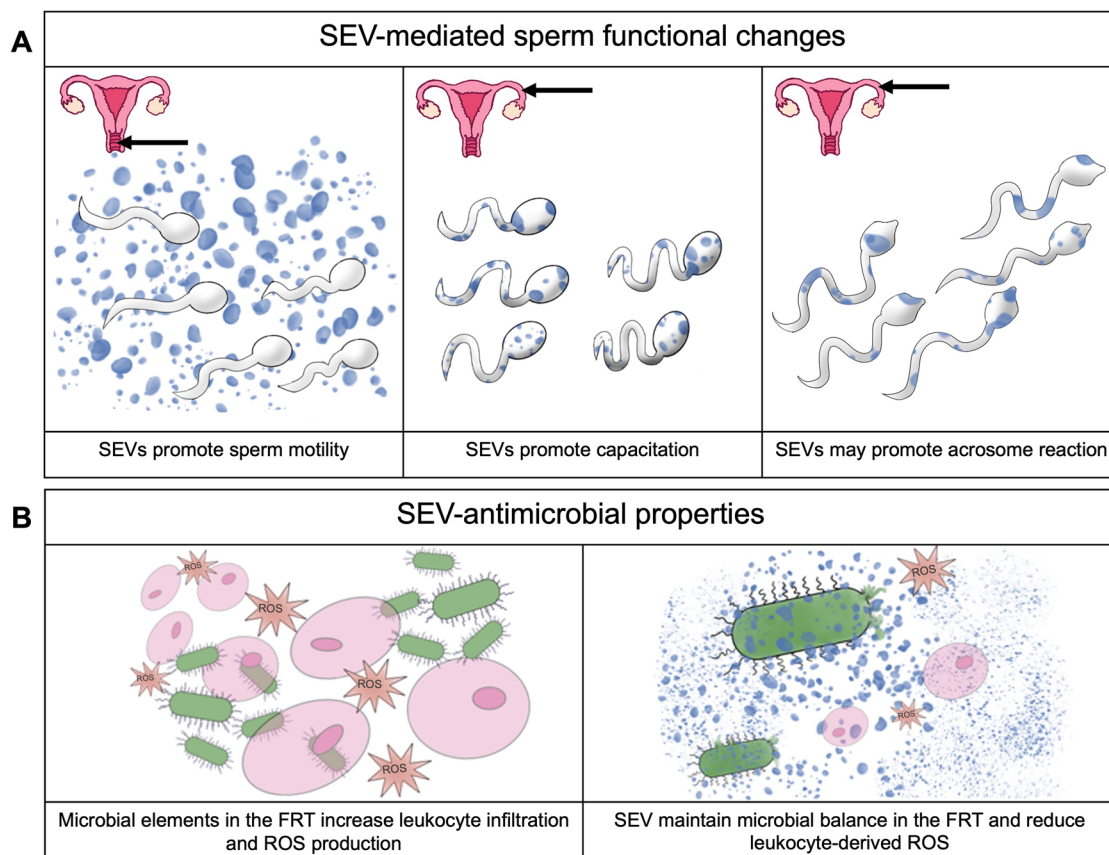
tically reduce the presence of co-isolated contaminants but yield fewer EVs (Veerman et al., 2021; Welsh et al., 2024), and may lead to the selection of EVs of different sizes/densities that can impact downstream functional assessment (Brennan et al., 2020; Sharma et al., 2020; Veerman et al., 2021; Welsh et al., 2024). In addition, studies have also begun to acknowledge that factors once considered as contaminating material may actually be part of what is termed the EV protein corona, a shell of biomolecules (like proteins, lipids, and nucleic acids) present on the surface of EVs in biological fluid that may influence EV behaviour and function (Tóth et al., 2021; Welsh et al., 2024). While an in-depth discussion of the different EV-enrichment approaches and their suitability for studies on SEVs is beyond the scope of this review, readers should refer to the articles referenced in this paragraph for a detailed overview of the challenges associated with different EV-enrichment approaches.

### SEVs influence on sperm function

SEV interactions with spermatozoa are the most comprehensively studied effect of SEVs to date. These interactions initially occur during ejaculation and continue at the site of semen deposition. In humans, this site is the upper vagina (near the ectocervix), which

features an acidic environment (pH 4.3). The alkaline composition of the seminal fluid (pH 7.2–7.8) is crucial to the survival of spermatozoa, as it creates a transient neutralization of the vaginal pH (pH 6.0–6.5) that ensures spermatozoa can traverse the cervix and reach the uterus (Caplan, 2014). It is therefore perhaps unsurprising that SEV–sperm interactions occur rapidly and are likely influenced by pH. Indeed, several studies have shown that SEV fusion with spermatozoa occurs within minutes of co-incubation and is restricted to acidic or neutral, and not alkaline, medium (Arienti et al., 1997; Park et al., 2011; Tamessar et al., 2024; Wang et al., 2022). Thus, the alkaline environment of seminal plasma may prevent SEVs from fusing with spermatozoa until the cells encounter the acidic environment within the FRT; a strategy that could enable SEVs to fine-tune sperm function as they enter the FRT in preparation for fertilization (Arienti et al., 1997).

Upon fusion, SEVs deliver cargo that have beneficial effects on sperm functional properties, including increasing sperm motility, and activating capacitation and acrosome reaction (Figure 2A) (Murdica et al., 2019b; Palmerini et al., 1999; Palmerini et al., 2003; Park et al., 2011; Wang et al., 2022). SEV proteins that modulate sperm function include CD38 and cysteine-rich secretory protein 1 (CRISP1) that activate intracellular calcium signalling in sperm cells (Murdica et al., 2019b; Park et al., 2011). However, there are



**Figure 2** Seminal extracellular vesicles (SEVs) and their influence on sperm function. SEVs support sperm function in the female reproductive tract (FRT) through several mechanisms. (A) SEVs have the capacity to interact with spermatozoa at the site of semen deposition (cervix) and deposit cargo that promotes sperm motility. Under the correct FRT conditions, near the site of fertilization (oviduct), SEVs promote capacitation and may also promote acrosome reaction. (B) SEVs possess antimicrobial properties that help clear the uterine cavity of microorganisms that can enter during intercourse. These microbial agents increase the number of leukocytes present in the FRT and therefore the amount of reactive oxygen species (ROS) secreted. SEVs can help maintain microbial balance in the FRT and reduce leukocyte-derived ROS.

conflicting results regarding SEV functional properties, with some studies showing little to no effect on capacitation and acrosome reaction (Park et al., 2011; Pons-Rejraji et al., 2011; Tamessar et al., 2024). These conflicting results may be explained by different methodological approaches to EV enrichment, which could inadvertently select for different SEV subpopulations (Welsh et al., 2024). Amongst these studies, Wang et al., 2022 demonstrated the capacity for different SEV subpopulations to exert different functions using a density gradient ultracentrifugation (seminal plasma overlaid on a 12–36% iodixanol density gradient) approach. In this study, they found that SEVs fractionated into four distinct subpopulations: high-density, medium-density, low-density, and non-vesicular material. Each of these fractions not only had unique proteomic profiles, but also had differing functional effects on sperm, with only high-density SEVs enhancing sperm motility and capacitation, while non-vesicular material enhanced sperm motility, capacitation, and the acrosome reaction (Wang et al., 2022). Alternatively, these conflicting results may reflect the complex regulatory nature of spermatozoa–SEV interactions whereby the beneficial effects of SEVs on spermatozoa may only be realized in the presence of additional signalling molecules, such as progesterone, that sperm encounter within the FRT (Park et al., 2011).

In addition to their putative roles in influencing sperm function, SEVs also convey protective properties against microbial infections and in mitigating the impact of oxidative stress (Figure 2B) (Burden et al., 2006; Carlsson et al., 2000). As an example of their antimicrobial properties, SEVs have been documented to create pores capable of perforating bacterial cell walls and causing microbial cell death (Carlsson et al., 2000). This property of SEVs aids in reducing reliance on leukocyte-mediated protection, thereby minimizing sperm damage associated with leukocyte-derived oxidative stress within the FRT. SEVs also provide an additional layer of protection from oxidative stress via their inherent antioxidant properties. Notably, these antioxidant properties contrast with those of the soluble fraction of seminal plasma in which the antioxidant constituents primarily act to scavenge reactive oxygen species (Aalberts et al., 2014). Instead, SEVs act upstream in the oxidative cascade to limit the production of reactive oxygen species by increasing the membrane rigidity of neutrophils, major contributors of oxidative stress via their superoxide anion generation (Saez et al., 1998). This membrane rigidification is theorized to be mediated by the transfer of cholesterol and sphingomyelin from SEVs to the leukocytic cells. These two lipids are known to be abundant in SEVs and are well-known membrane rigidifying agents (Saez et al., 1998). Both these antioxidant properties assist in the protection of sperm integrity and developmental competence as they navigate the FRT.

## SEVs influence on the female reproductive tract environment

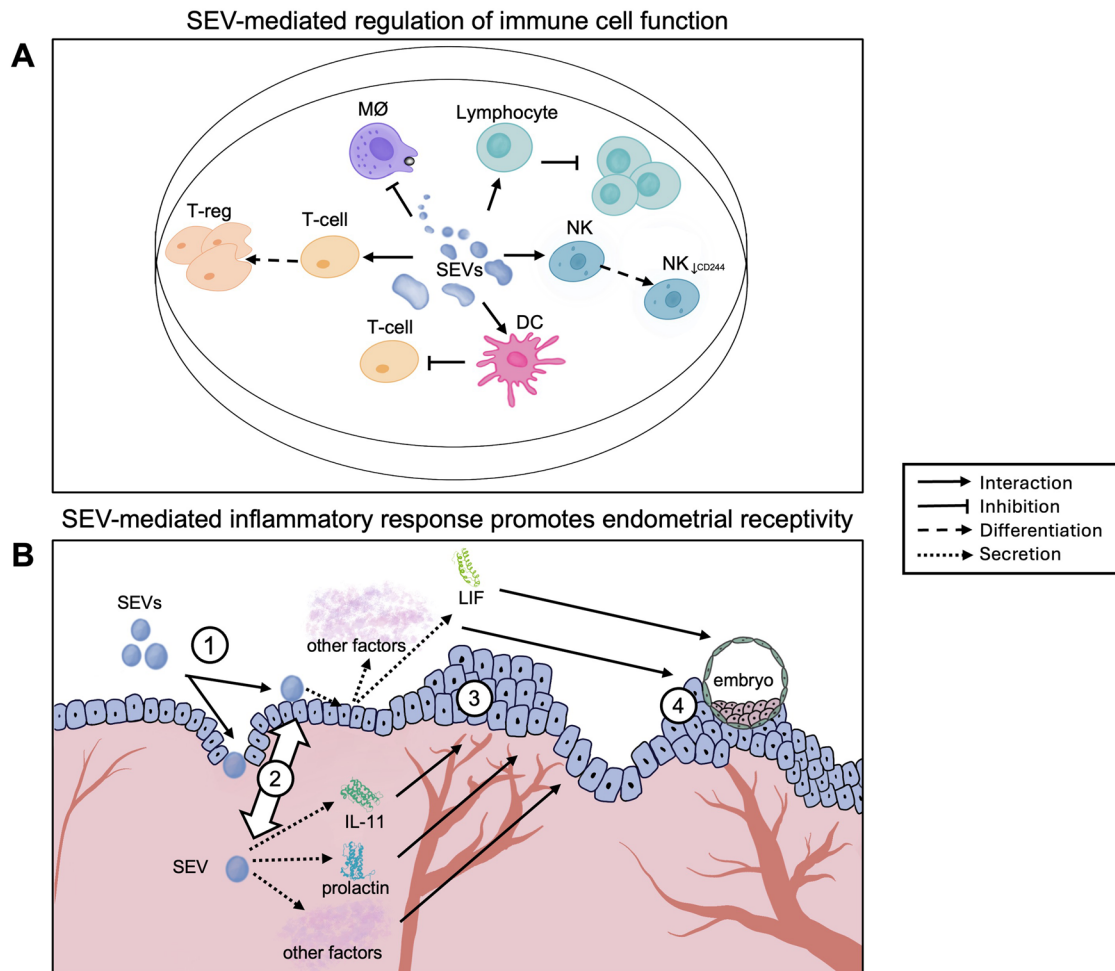
In addition to modulating sperm function, SEVs have been proposed to directly influence the FRT, most notably via regulation of the female immune response to semen (Figure 3). In humans, these functions were first discovered in *in vitro* studies demonstrating that SEVs can interact with leukocytes and inhibit lymphocyte proliferation and macrophage phagocytosis (Kelly et al.,

1991) (Figure 3A). Expanding on these early insights, more recent studies have shown that SEVs can alter the phenotype and function of a diverse range of immune cells within the FRT, including natural killer cells, dendritic cells, and T cells (Tarazona et al., 2011; Vojtech et al., 2019; Zhang et al., 2024), promoting the differentiation of these cells into a tolerogenic phenotype (Figure 3A). Amongst the changes induced by SEVs, their capacity to alter dendritic cell activation of T cell responses through an indoleamine 2,3-dioxygenase-dependent mechanism (Vojtech et al., 2019) is of particular interest. This is because a key consequence of seminal plasma exposure in mammals is the differentiation of dendritic cells into a pro-tolerogenic phenotype that promotes the expansion of Treg cells, which in turn mediate immune tolerance that is critical for successful embryo implantation (Schjenken & Robertson, 2020).

Akin to molecular changes elicited by seminal plasma, SEVs also alter peri-conceptual gene expression profiles in the epithelial lining of the FRT. Such functions were first observed in pigs, wherein SEVs provoked alterations to signalling pathways associated with chemokine and inflammatory responses, and T cell differentiation in endometrial epithelial cells. Comparable gene expression changes were also observed *in vivo* following mating, suggesting that SEVs were an important factor driving the endometrial response to pig seminal fluid (Bai et al., 2018). In humans, there is limited evidence of SEVs exerting a similar role, however it has been reported that partially purified SEVs (the SEV-enrichment method used retains soluble seminal plasma signalling factors) can elicit inflammatory gene activation in human endometrial epithelial and stromal cells *in vitro* (Figure 3B) (Marques De Menezes et al., 2020).

Beyond their capacity to induce inflammation in endometrial cells, SEVs have also been linked to the promotion of endometrial tissue remodelling and the establishment of a receptive endometrial environment (Figure 3B) (George et al., 2020; Rodriguez-Caro et al., 2019; Wang et al., 2024). Co-incubation of SEVs with primary endometrial cells has been shown to increase prolactin secretion, thereby promoting decidualization to support embryo implantation (Figure 3B) (Rodriguez-Caro et al., 2019). Studies in primary endometrial stromal fibroblast cells have provided evidence that SEVs promote decidualization through an interleukin-11-dependent mechanism (Figure 3B) (George et al., 2020). Such changes may be mediated by specific SEV subtypes, with recent studies showing that high-density SEVs induce the differential regulation of 1,274 genes in endometrial-like Ishikawa cell lines (Wang et al., 2024). Notable among these SEV-inducible genes was leukemia inhibitory factor (LIF), a key pro-implantation factor responsible for promoting adhesion of trophoblast cells to endometrial cells (Figure 3B) (Wang et al., 2024). While informative, these results should be interpreted with caution as *in vitro* conditions are an approximation of the *in vivo* environment (Zhang et al., 2024) and to our knowledge there are currently no *in vivo* studies exploring the functions of SEVs. Notwithstanding these limitations, the above data suggest that the ability of SEVs to elicit a female immune response may play an important role in establishing an appropriate endometrial environment that facilitates embryo implantation and pregnancy success.

The precise SEV sub-populations responsible for conferring these immune-regulatory properties, and the key cargo they carry are yet to be fully characterized. However studies have begun



**Figure 3** Functions of seminal extracellular vesicles (SEVs) in the human female reproductive tract (FRT). In humans, in vitro exposure to SEVs in the FRT has been shown to elicit an array of female responses that are thought to promote conception and embryo implantation. (A) SEV-mediated regulation of immune cell function. In vitro studies show SEVs inhibit macrophage (MØ) phagocytosis and lymphocyte proliferation. SEVs are known to directly interact with natural killer (NK) cells, dendritic cells (DC), and T cells to regulate their function. This interaction with SEVs favours the differentiation of NK, DC, and T cells into a more protolerogenic phenotype, with NK cells having reduced CD244 expression, DC inhibiting T cells through an indoleamine 2,3-dioxygenase mechanism, and T cells differentiating into a regulatory T-cell subtype (T-reg) to promote a more immunotolerant environment crucial for successful embryo implantation. (B) SEV-mediated inflammatory response promotes endometrial receptivity. 1, SEVs are introduced into the FRT, and in vitro studies show they can interact with the stroma and epithelial cells of the endometrium and elicit inflammatory gene activation. 2, SEVs promote the secretion of interleukin (IL)-11, prolactin, other factors (that cause alterations to gene expression profiles), and leukocyte inhibitor factor (LIF). 3, IL11 and prolactin promote decidualization (epithelial thickening and increased vascularization) to support embryo implantation. 4, LIF promotes adhesion of trophectoderm cells to endometrial cells.

to explore this, with high-density seminal EVs enhancing endometrial receptivity through regulation of LIF (Wang et al., 2024). Candidate SEV signalling molecules include CD38, previously shown to influence sperm function (Park et al., 2011) but also having immune-regulatory properties promoting the differentiation of CD4<sup>+</sup> T cells into Treg cells (Kim et al., 2015a). Additionally, proteomic studies have identified that SEVs carry important immune-regulatory molecules such as SEMG, vacuolar-type H<sup>+</sup>-ATPase, and transforming growth factor beta (TGFβ) family members (Lin et al., 2019; Manouchehri Doulabi et al., 2022), all of which have been implicated as signalling agents within seminal fluid (Schjenken & Robertson, 2020).

To our knowledge, only SEV interactions with endometrial tissue have been explored in humans (Gholipour et al., 2023; Marques De Menezes et al., 2020; Rodriguez-Caro et al., 2019; Wang et al.,

2024). Whether SEVs reach the endometrium under normal physiological conditions remains unknown. However, signalling agents within seminal plasma have been proposed to reach the endometrium by virtue of peristaltic contractions following coitus, with SEVs being a plausible delivery system (Schjenken & Robertson, 2020). Alternatively, SEVs could conceivably use spermatozoa as a vector for delivery into the upper FRT to promote their interaction with the various resident cell types. Given that seminal plasma provokes gene expression changes in vaginal and cervical epithelial cells (Sharkey et al., 2012a; Sharkey et al., 2007; Sharkey et al., 2012b), an important and currently unanswered question is whether SEV interactions can evoke similar changes. Regardless, these studies show that SEVs have potential to help encourage a physiological response within the FRT that aids in initiating and sustaining a healthy pregnancy.

## SEVs and infertility

The success of IVF, where conception occurs largely in the absence of seminal plasma, suggests that seminal plasma (and SEVs) are not an absolute requirement for pregnancy success. However, the balance of experimental evidence reviewed herein provides strong evidence that seminal plasma (and SEVs) are beneficial for promoting fertility and optimal reproductive health. Perhaps unsurprisingly then, there are now many studies linking alterations in seminal plasma, or SEVs, with infertility aetiologies.

The most compelling evidence for a contribution of seminal plasma to fertility and reproductive health comes from clinical studies examining the outcomes of assisted reproductive technology procedures. In an IVF setting, clinical guidelines following embryo transfer indicate that couples should abstain from intercourse following embryo transfer, as it is believed that the potential of infection or uterine contractions outweigh the possible benefits (Robertson & Sharkey, 2016). However, meta-analyses of studies exploring the effect of seminal plasma exposure around the period of embryo transfer on pregnancy outcomes support significant increases in the clinical pregnancy rates in seminal plasma-exposed cohorts (Crawford et al., 2015; Saccone et al., 2019). The beneficial effects of seminal plasma exposure on pregnancy outcomes are also documented in studies showing an increased prevalence of gestational disorders among women who conceived after limited exposure to seminal plasma from the conceiving partner (Kho et al., 2009). Taken together, these studies highlight the important role seminal plasma plays in supporting fertility, although the identity of the seminal plasma molecules that influence these clinical parameters is yet to be fully determined. As such, the contribution of SEVs to mediating the beneficial effects of seminal plasma exposure remains an important yet unresolved question.

Given the potentially important functions of SEVs in the peri-conception period, and the compelling evidence of integral roles for EVs in health and disease in other settings (Tamessar et al., 2021), there is increasing interest in their contributions to fertility and infertility. Much of the research in this field has used -omic approaches to define the molecular composition of SEVs, with the protein and non-coding RNA content being the most comprehensively studied (Parra et al., 2023). As SEVs are best characterized in the context of their influence over sperm function, a primary goal of these studies has been to define SEV compositional changes associated with infertility diagnoses related to sperm disorders, including: asthenozoospermia (low motility) (Hong et al., 2021; Lin et al., 2019; Murdica et al., 2019a; Roy et al., 2023; Vickram et al., 2022), azoospermia (absence of sperm) (Vickram et al., 2022; Yao et al., 2021), oligospermia (low sperm count) (Vickram et al., 2022), teratozoospermia (abnormal sperm morphology) (Roy et al., 2023), oligoasthenozoospermia (low sperm count and poor motility) (Abu-Halima et al., 2016; Vickram et al., 2022), and non-normozoospermic individuals (Garcia-Rodriguez et al., 2018). Interestingly, SEV composition appears to be altered across all subtypes of infertility, pointing to roles for SEVs either in the development of these conditions, or their modification as a consequence. Regardless, as discussed below, this information has begun to be harnessed to unravel dysregulated pathways associated with these conditions that could eventually inform targeted therapeutic approaches for restoring fertility.

Amongst the spectrum of infertility aetiologies, asthenozoospermia has been the most comprehensively studied, due to the close links between sperm motility and SEV function (Lin et al., 2019; Murdica et al., 2019a; Murdica et al., 2019b). SEVs sourced from asthenozoospermic individuals appear to have reduced capacity to improve sperm motility and capacitation, compared to equivalent SEVs isolated from normozoospermic individuals (Murdica et al., 2019b), thus highlighting their potential contribution to disease. SEV proteins implicated in asthenozoospermia include CRISP1, glycodelin, and transient receptor potential cation channel subfamily V member 6, all of which have previously been linked to male infertility (Lin et al., 2019; Murdica et al., 2019a; Murdica et al., 2019b).

Moving past proteomic profiling to examine other molecular features of SEVs, a study recently explored the presence of ureido post-translational modifications in SEV proteins of normozoospermic, asthenozoospermic, or teratozoospermic individuals (Roy et al., 2023). This study reported that the presence of SEV ureido protein modifications were negatively correlated with sperm morphology and progressive motility in both asthenozoospermic or teratozoospermic disorders. Further, a subset of the SEV proteins harbouring the ureido modification had previously been associated with sperm function and inflammation (Roy et al., 2023). In addition to asthenozoospermia and teratozoospermia, other studies have evaluated proteomics and phosphoproteomics of SEVs from azoospermic patients. For example, both SEV protein content and phosphorylation status are altered in azoospermic patients, with proteins and phosphosites that were differentially regulated being associated with sperm development and function (Yao et al., 2021).

Another study has attempted to assess whether there are common SEV proteome profiles in non-normozoospermic individuals. In this work, the proteome of normozoospermic and non-normozoospermic patients (the specific sperm disorders of the men with non-normozoospermia were not described) was assessed using mass spectrometry. The study reported 43 of the 1,238 SEV proteins detected as being differentially regulated, with an additional 5 proteins appearing exclusively in one of the two cohorts (Garcia-Rodriguez et al., 2018). These findings are intriguing, particularly given the diversity of pathologies underlying infertility. Amongst the 43 differentially expressed proteins altered in the non-normozoospermic cohort, proteins associated with sperm energy production, semen liquification, sperm DNA organization, and sperm histone-protamine transition were present (Garcia-Rodriguez et al., 2018), again supporting the role of EVs in influencing sperm function.

Numerous studies have also explored other SEV cargo types, including small non-coding RNAs and lipids, and their potential contribution to infertility. For example, the microRNA profile of SEVs from proven fertile men have been shown to differ from that of SEVs from oligoasthenozoospermic patients (Abu-Halima et al., 2016). Interestingly, these differentially regulated microRNAs targeted genes associated with spermatogenesis, TGF $\beta$  and T-cell receptor signalling pathways. Further expanding our knowledge of SEV-mediated gene regulation, PIWI-interacting RNA (piRNA) content is significantly decreased in the SEVs of asthenozoospermic, compared to normozoospermic men, although the functional consequence of these changes is currently unknown (Hong et al., 2021). SEV lipid profiling has revealed differences in cholesterol content and antioxidant capacity between fertile and

infertile men, with the lowest content documented in SEVs from azoospermic individuals (Vickram et al., 2022).

Beyond their influence on male infertility, it is intriguing to consider whether male partner SEVs are relevant to female infertility. Recent studies examining the protein content of SEVs in couples experiencing recurrent pregnancy loss (RPL) highlight paternal contributions to this disease (Jena et al., 2021; Jena et al., 2023). In these studies, SEVs from known fertile men and male partners of couples experiencing RPL were assessed using proteomics. While the proteins identified as being differentially expressed were associated with numerous cellular functions, the most notable among these was immune signalling pathways (Jena et al., 2021). Growth differentiation factor 15 (GDF15), a key immune-regulatory protein abundant in seminal plasma and a member of the TGF $\beta$  superfamily, was among the SEV-associated factors found to be downregulated in these patients (Jena et al., 2023; Robertson & Sharkey, 2016). In contrast, SEVs from couples with RPL were found to have increased complement component C3, which is proposed to regulate the influx of neutrophils in the uterus following seminal fluid exposure (Jena et al., 2023; Schjenken & Robertson, 2020). Given that immune aetiologies are implicated in RPL (Robertson & Sharkey, 2016), these data identify SEVs as being a male partner factor that potentially influences susceptibility to this disease.

Altogether, these studies show that SEVs are altered in a variety of different infertility aetiologies. However, whether these changes cause infertility, or are a consequence of infertility, is unknown. Additionally, it remains unclear whether particular SEV subpopulations are disproportionately responsible for driving infertility, and which regions of the male reproductive tract these vesicles originate from—important questions that future studies should consider.

## Clinical utility of SEVs

Links between SEVs and infertility raise the question of whether knowledge of SEV composition and function may have clinical utility. In this context, applications could range from using SEVs as biomarkers to assist in diagnosing disease, developing EV-based therapeutics to target the delivery of fertility promoting agents, or even using EVs to enhance sperm quality following cryopreservation (Figure 4).

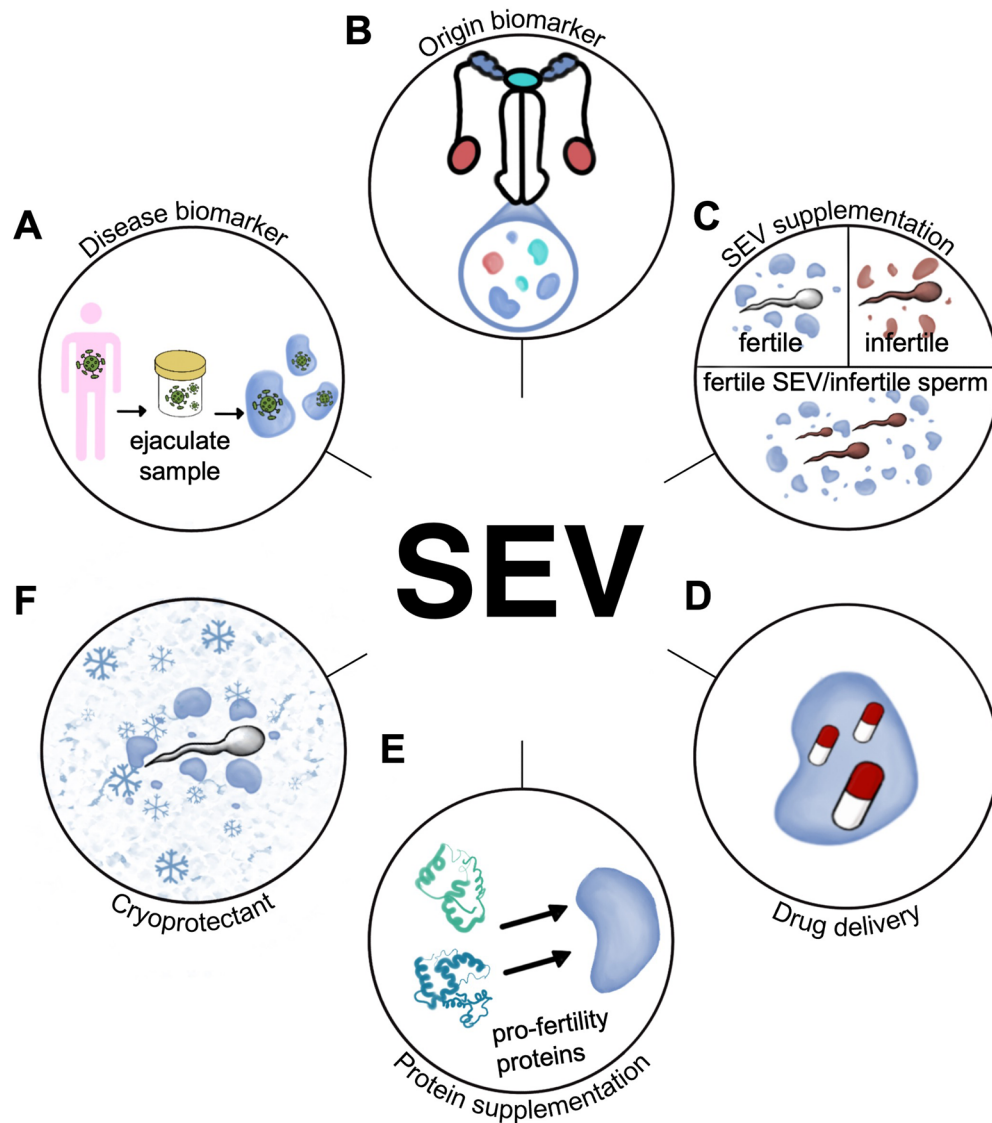
One of the most straightforward clinical applications of SEVs lies in their use as a diagnostic tool. EVs have long been considered promising candidates for disease biomarkers as their composition is known to reflect the health, or lack thereof, of their parent cells (Figure 4A). Additionally, EVs can be more sensitive than other laboratory tests as their content is encapsulated and therefore protected from potential degradation (Giacomini et al., 2020). The utility of SEVs as biomarkers has been evaluated in the differential diagnosis between obstructive and non-obstructive azoospermia, a distinction that typically requires a testicular biopsy. In this study, the SEV protein biomarkers solute carrier family 5 member 12 and H2B clustered histone 1 were identified as being highly sensitive and specific for predicting the presence of germ cells in the testis of azoospermic patients (Yao et al., 2021).

While the use of SEVs to diagnose infertility shows promise across all infertility pathologies, it could be particularly valuable for idiopathic and unexplained infertility. Alterations to SEVs in these conditions might not only provide insight into infertility but also identify the male reproductive tract regions that may be major contributors to this disease state (Figure 4B). Ultimately, these findings will empower researchers to develop targeted interventions and therapeutic strategies to restore fertility. Additionally, given that SEVs have been shown to be dysregulated in couples experiencing RPL (Jena et al., 2021; Jena et al., 2023), future studies could begin exploring whether SEVs may predict RPL risk in couples. Outside of fertility, SEVs could prove to be a valuable diagnostic tool for cells that require invasive procedures to access, such as prostatic tissue for cancer diagnosis (Vickram et al., 2020). However, before SEVs can be used as diagnostic tools, substantial basic research and validation are required. This is especially important due to the heterogeneity of diseases and the challenges associated with introducing new biomarkers into clinical practice.

A second clinical application could involve use of SEVs as a therapeutic strategy to enhance sperm function or improve FRT receptivity in assisted reproductive settings. For example, studies identifying SEV cargo associated with infertility (García-Rodríguez et al., 2018; Jena et al., 2021; Jena et al., 2023; Lin et al., 2019; Murdica et al., 2019a) could reveal SEV-derived fertility-promoting agents, paving the way for the development of tailored therapeutic approaches. Supplementing SEVs in infertile patients to improve sperm function is one potential strategy (Figure 4C) (Murdica et al., 2019b). An intriguing alternative approach lies in the development of nanoparticles which could deliver therapeutic medications (Figure 4D), or potentially pro-fertility EV-derived signalling agents (Figure 4E). Nanoparticles are promising carriers of therapeutic agents, and like EVs, are biocompatible, have superior targeting capacity, high cellular uptake, and low immunogenicity. Indeed, nanoparticles have been proposed to be used to support male fertility (Shandilya et al., 2020). In one example, human ferritin nanocarriers loaded with ATP were shown to improve sperm motility in a mouse model of asthenozoospermia (Pang et al., 2022).

An additional area where SEVs hold clinical promise is in sperm cryopreservation (Figure 4F). As mentioned earlier, SEVs possess antioxidant capabilities (Saez et al., 1998), with many studies now assessing the merits of incorporating antioxidants into cryopreservation media (Hungerford et al., 2023). Sperm cryopreservation elevates reactive oxygen species, leading to increases in DNA damage and impaired sperm functionality (Hungerford et al., 2023). In humans, studies have shown that spermatozoa cryopreserved in the presence of SEVs have significantly improved motility, morphology, and viability post-thaw, with the improvement in sperm quality associated with reduced levels of reactive oxygen species and DNA damage compared to conventional cryopreservation methods (Mahdavinezhad et al., 2022).

Taken together, the available evidence strongly suggests that a deeper understanding of SEV composition and function could meaningfully contribute to improving human fertility. However, further research is needed to identify the specific contributions of individual SEV-associated factors before their clinical potential can be fully realized.



**Figure 4** Seminal extracellular vesicles (SEVs) and their potential in a clinical setting. There is compelling evidence that SEVs contribute to infertility in some individuals, with studies showing altered SEV cargo across a range of male and female infertility aetiologies. This raises the question as to whether SEVs may hold clinical application in an assisted reproductive setting. This may range from (A) using SEVs as a simple minimally invasive “biopsy” for disease biomarkers, or (B) as origin biomarkers to diagnose tissue-specific pathologies. Other potential uses involve (C) approaches such as supplementing SEVs from fertile individuals to infertile patients to recover sperm function, or developing SEV-based therapeutics to support targeted delivery of fertility promoting agents, such as (D) drugs or (E) pro-fertility proteins. Another potential use involves (F) utilizing the antioxidant capacity of SEVs to support sperm cryopreservation or improve sperm quality post-thaw.

## Conclusions

Male infertility is a global health issue, but our understanding of its causes is rudimentary. The primary diagnostic tool for assessing the aetiology of infertility is a descriptive semen analysis; however, normal semen parameters do not necessarily assure fertility in men. This limited understanding of the physiology behind male fertility is reflected in the lack of comprehensive diagnostic and therapeutic options in fertility clinics, underscoring the urgent need to define novel mechanisms that underpin male fertility. While defective sperm function is an important cause of infertility, this review has focused on the emerging and important contribution of seminal plasma and its constituents. Our focus on SEVs reflects their crucial role in cell-to-cell communication and

in modulating sperm function and the FRT immune environment. We summarise evidence indicating the contribution of SEVs to fertility, highlighting that deficits in SEVs are linked not only to male, but also female infertility, identifying that the nucleic acid, protein, and lipid cargo encapsulated in SEVs likely serve important roles in defining an individual’s fertility status. This knowledge emphasises the value of continued research into the clinical, diagnostic, and therapeutic applications of SEVs to promote fertility.

## Author contributions

Elizabeth Torres-Arce (Conceptualization, Writing—original draft, Writing—review & editing), David J. Sharkey (Conceptualization, Funding acquisition, Supervision, Writing—review & editing), John

E. Schjenken (Conceptualization, Funding acquisition, Supervision, Writing—review & editing), Brett Nixon (Supervision, Writing—review & editing), Cottrell T. Tamessar (Writing—review & editing), and Sarah A. Robertson (Writing—review & editing)

## Conflicts of interest

None declared.

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