Epididymosomes: transfer of fertility-modulating proteins to the sperm surface

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A variety of glycosylphosphatidylinositol (GPI)-linked proteins are acquired on spermatozoa from epididymal luminal fluids (ELF) during sperm maturation. These proteins serve roles in immunoprotection and in key steps of fertilization such as capacitation, acrosomal exocytosis and sperm-egg interactions. Their acquisition on sperm cells is mediated by membrane vesicles (epididymosomes, EP) which were first reported to dock on the sperm surface, and by lipid carriers which facilitate the transfer of proteins associated with the membrane-free fraction of ELF. While the nonvesicular fraction is more efficient, both pathways are dependent on hydrophilic interactions between the GPI-anchor and the external lipid layer of the sperm surface. More recently proteomic and hypothesis-driven studies have shown that EP from several mammalian carry transmembrane (TM) proteins, including plasma membrane Ca\(^{2+}\)-ATPase 4 (PMCA4). Synthetic in the testis, PMCA4 is an essential protein and the major Ca\(^{2+}\) efflux pump in murine epididymosomes. Delivery of PMCA4 to spermatozoa from bovine and mouse EP during epididymal maturation and in vitro suggests that the docking of EP on the sperm surface precedes fusion, and experimental evidence supports a fusogenic mechanism for TM proteins. Fusion is facilitated by CD9, which generates fusion-competent sites on membranes. On the basis of knowledge of PMCA4’s interacting partners a number of TM and membrane-associated proteins have been identified or are predicted to be present, in the epididymosomal cargo deliverable to spermatozoa. These Ca\(^{2+}\)-dependent proteins, undetected in proteomic studies, play essential roles in sperm motility and fertility, and their detection highlights the usefulness of the hypothesis-driven approach.

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INTRODUCTION

The epididymis, the major component of the posttesticular pathway, is the organ where spermatozoa mature and gain progressive motility and fertilizing ability. Its role in sperm maturation is known to be mediated via secretory proteins that are delivered to the sperm surface during their transit\(^{1,2}\) which may be for a protracted period of time such as 5–10 days in mice.\(^{3}\) These secretory proteins have been identified in the epididymal lumen in the absence of spermatozoa following efferent duct ligation,\(^{4}\) in the luminal fluid,\(^{5,6}\) as well as in conditioned media of cultured epididymal epithelial cells.\(^{7}\) Initially, the secreted epididymal proteins identified were predominantly glycosyl phosphatidylinositol-(GPI)-linked such as CD52, CD59, CD73, which are proteins that play a role in immunoprotection.\(^{8,10}\) Later, the GPI-linked proteins identified were those that play a role in fertilization such as sperm-egg interaction, e.g., P34H\(^{11,12}\) and Sperm Adhesion molecule 1 (SPAM1).\(^{13}\) In the epididymal luminal fluids (ELF) secreted, GPI-linked proteins have been shown to exist partly in the soluble and insoluble fractions,\(^{5,13,15}\) which consists of extracellular membrane vesicles that are known to play a key role in intercellular cross-talk.\(^{16}\) These vesicles, termed epididymosomes, have been well-characterized and are known to transfer proteins to the sperm plasma membrane.\(^{16,18}\) This review will focus on epididymosomal proteins identified to play a role in fertility and the mechanism by which they are acquired by spermatozoa.

TRANSFER OF GPI-LINKED SPERM PROTEINS IN THE EPIDIDYMAL SECRETOME – EPIDIDYMOSOMES AND A LIPID CARRIER COMPRISE DUAL PATHWAYS

GPI-linked proteins uniquely possess acyl chains (Figure 1), which when inserted into the outer leaflet of the lipid bilayer of a target membrane anchor the protein and permit its lateral diffusion. Documented uptake of these proteins from the extracellular environment has been reported in red blood cells\(^{19}\) and sperm cells\(^{13,20,21}\) and has been demonstrated to occur in the absence of vesicles\(^{19,20}\) This suggests that there is a nonvesicular mechanism by which GPI-linked proteins can be transferred to spermatozoa from the ELF. This nonvesicular mechanism was investigated by using SPAM1, known to be present in the ELF of at least five species, including mice and humans,\(^{22}\) as a model. SPAM1 is a multifunctional protein which is known to perform essential roles in fertilization: (1) cumulus penetration via its neutral hyaluronidase activity, (2) secondary binding to the zona pellucida after the acrosome reaction, (3) penetration of the zona pellucida, and (4) Ca\(^{2+}\) signaling- associated acrosomal exocytosis mediated by its hyaluronic acid receptor domain.\(^{23}\) In murine ELF, SPAM1 has been shown to be present in both nonvesicular (60%) and vesicular (40%) fractions.\(^{5}\) When cauda epididymal spermatozoa were co-incubated with each of these fractions, both were able to deliver SPAM1 to the sperm surface, with the nonvesicular fraction doing so more efficiently.\(^{23}\)
SPAM1 from the nonvesicular fraction of the ELF was shown to reside in low-molecular weight monomers as well as high-molecular weight oligomeric complexes. The oligomeric complexes were incapable of delivering SPAM1 to the sperm surface, but likely served as a source of monomers (Figure 2), which effectively perform the transfer. Monomers are stabilized in an aqueous environment by hydrophobic interactions of the GPI anchors with Apolipoprotein J or clusterin (CLU) that resides in ELF. CLU is a well-known lipid carrier in a variety of biofluids and is abundantly expressed in ELF, where it is involved in facilitating sperm uptake of GPI-linked proteins, as well as their removal during the modification of the membrane, depending on its concentration in the local environment in the epididymal tract. A lipid-exchange model involving CLU or other lipid carriers (Figure 3) has been proposed for the delivery of these proteins to the sperm surface. Other fertility-modulating GPI-linked epididymal proteins that are likely to be delivered by this pathway include: GLIPR1L1 (Glioma pathogenesis-related protein 1), which is involved in sperm-zona pellucida binding, and other hyaluronidase family members, such as HYAL3, HYAL5, and HYAL2, membrane-anchored serine protease PRSS21 (testisin) and P34H/P26h/P25b.

When the mechanism of delivery of GPI-linked epididymal proteins via the vesicular pathway was studied following co-incubation of murine caudal sperm with dye–labeled epididymosomes, the label was detected over the acrosome and on the midpiece of the flagellum, suggesting the involvement of vesicles in this transport mechanism. Sullivan and Saez have proposed, from the complexity of proteins that epididymosomes carry and deliver to spermatozoa, multiple mechanisms of transfer are likely to be involved.

**Figure 1:** Diagram of glycosyl phosphatidylinositol (GPI)-linked protein showing the acyl chain, which anchors it in the outer leaflet of the lipid bilayer of a target membrane. The arrow points to the position where the phosphatidylinositol link can be enzymatically cleaved with phospholipase C.

**Figure 2:** Dual pathways exist for GPI-linked protein delivery to the sperm plasma membrane, via the vesicular and membrane-free soluble fractions of ELF. These fractions are separated by ultracentrifugation at 120,000 × g, and the supernatant can be fractionated into oligomers and monomers at 230,000 × g. The latter are inserted into the sperm plasma membrane in the presence of CLU or Apolipoprotein J via hydrophobic interactions which may also facilitate delivery from vesicles that dock in lipid rafts on the sperm membrane.

**Figure 3:** Diagram of an idealized vesicle–lipid raft–receptor fusion event. The GPI-linked protein is targeted to the lipid raft by the cytoplasmic tail, which serves as an adapter molecule. The lipid raft recruits other membrane proteins, which are likely to be delivered to the sperm surface via hydrophobic interactions. The GPI-linked protein then binds to the receptor and is internalized into the vesicle. The vesicle then fuses with the sperm plasma membrane, releasing the GPI-linked protein into the cytoplasm of the sperm.

**Figure 4:** Delivery of epididymal protein to sperm

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mainly 4a in the lower region (caudal). This shift was considered to reflect the acquisition of PMCA4a from the ELF. As the PMCA4a variant is more efficient than 4b in clearing calcium from the cytosol, the presence of increasing amounts of 4a in maturing sperm cells would ensure that they would meet the high demand for calcium efflux after hyperactivated motility, capacitation, and the acrosome reaction, which all require elevated levels of calcium. Thus, it was hypothesized that in murine spermatozoa the PMCA4a variant is expressed in the ELF and carried on epididymosomes where it can be delivered to sperm cells during their maturation and transit. Experimental evidence revealed the presence of PMCA4a exclusively in the epididymosomal fraction of the ELF. The findings also demonstrated that the bovine and murine systems differ, since in the latter both PMCA4a and 4b variants are expressed in the testis, the apical surface of the epithelia of all three epididymal regions, as well as in their secreted ELF. It should be noted that while PMCA4a is more efficient than 4b in returning Ca$^{2+}$ to resting levels, 4b plays an important role in signal transduction via its C-terminal PDZ ligand. Importantly, murine epididymosomes were able to deliver PMCA4a to caudal spermatozoa following co-incubation in vitro, consistent with its transfer in vivo which was reflected in a five-fold increase on caudal, compared with caput, sperm cells. This finding, along with the higher Ca$^{2+}$-ATPase activity in bovine caudal spermatozoa than those in the caput, supports a role of PMCA4a in epididymal sperm maturation. Acquisition of additional PMCA4a in caudal sperm cells parallels their gain in motility and their cytosolic Ca$^{2+}$ concentration, which is 2- to 6-times lower than that in caput spermatozoa. As motility is lost in mature caudal Pmca4-null murine spermatozoa where the intracellular Ca$^{2+}$ concentration [Ca$^{2+}$], is significantly elevated, physiological immotility of wild-type caput spermatozoa in the presence of increased [Ca$^{2+}$], compared with caudal ones underscores the role of epididymosomal PMCA4 in sperm maturation. It should be noted that both PMCA4a and 4b variants are present on epididymosomes and are co-immunoprecipitated with an interacting partner of PMCA4b, Ca$^{2+}$/CaM-dependent serine kinase (CASK). This interaction is PDZ domain-mediated, and in murine sperm cells involves the PDZ motif of CASK and PMCA4b’s PDZ ligand which is absent from PMCA4a. Thus, the ability to co-immunoprecipitate both Ca$^{2+}$ pump variants with CASK in the epididymosomal cargo revealed for the first time that the variants work together in a multiprotein complex, to heighten their combined impact in meeting the demands following functional sperm activities that precede fertilization. The inclusion of PMCA4a in a complex with CASK in the absence a PDZ ligand was thought to be facilitated by the formation of a heterodimer between 4a and 4b, with the latter directly interacting with CASK. The co-immunoprecipitation data indicated that CASK, a scaffolding membrane-associated protein that also exists in a soluble form, is an important component of the epididymosomal cargo and is likely to be delivered to the sperm surface during epididymal maturation along with PMCA4b when PMCA4a is also transferred. The finding that CASK is an epididymosomal protein is supported by an early study showing its presence in epididymal tissues.

The above demonstrates how knowledge of an interacting partner of a sperm protein that is present in epididymosomes can lead to the identification of other proteins in the epididymosomal cargo. Aravindan et al. showed that in murine sperm PMCA4b and junctional adhesion molecule A (JAM-A), which also has a PDZ ligand, are common interacting partners of CASK. Since CASK has a single PDZ domain, PMCA4b and JAM-A must bind sequentially and not simultaneously. As the PMCA4b-CASK interaction was shown to occur preferentially in uncapacitated spermatozoa when the [Ca$^{2+}$], is relatively low, it is likely that [Ca$^{2+}$], also dictates preferential binding of the JAM-A-CASK complex. However, regardless of the condition for binding, the existence of a JAM-A-CASK complex in spermatozoa and the presence of CASK in epididymosomes lead to the prediction that JAM-A is present in murine epididymosomes. Studies to investigate the latter are in progress.

![Figure 3](image-url) A lipid exchange model for the delivery of GPI-linked proteins from the soluble fraction of ELF is shown. GPI-linked proteins are stabilized by lipid carriers such as CLU or Apolipoprotein J, which transports monomers to the sperm membrane and then acts as an acceptor for cholesterol which it transfers to the epididymal epithelial lining for receptor-mediated endocytosis. Taken from Biol Reprod 2009; 81: 562–70.

![Figure 4](image-url) Transmission electron microscopy reveals docking of epididymosomes on the sperm plasma membrane to allow delivery of GPI-linked proteins to the sperm surface via hydrophobic interactions. On the smaller epididymosome SPAM1 which is immunogold-labeled is seen in the process of being transferred to the sperm membrane, while the contact of the larger epididymosomes appears to involve membrane fusion. Taken from Mol Reprod Dev 2008; 75: 1627–36.
Since PMCA4b binds to CASK preferentially at low [Ca\(^{2+}\)], it is useful to ask what is/are PMCA4’s interacting partner/s at high [Ca\(^{2+}\)] in spermatozoa. From what is known of PMCA4’s interaction in endothelial and neuronal cells, where PMCA4 has been reported to regulate negatively both endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS), it can be predicted that these interactions are also present in sperm proteins. Importantly both NOSs, which are rapidly activated by [Ca\(^{2+}\)], are present in spermatozoa where they are responsible for the production of nitric oxide (NO), which is required for a variety of sperm functions. Since excess NO has deleterious effects on spermatozoa, PMCA4’s interaction with the NOSs to regulate negatively would prevent oxidative stress, which is known to affect sperm motility as well as the integrity of the sperm genome. In light of this and the finding that in humans eNOS is expressed in the testis, spermatozoa and epididymis, it can be expected that these membrane-associated NOSs, as well as Caveolin-1 (CAV-1), a scaffold protein with which eNOS interacts, are potential epididymosomal proteins that will be transferred to sperm cells along with PMCA4.

From the regulatory relationship between PMCA4 and the NOSs, it would seem advantageous that these proteins be transferred together. This would be similar to the detected PMCA4a-PMCA4b-CASK complex that was co-immunoprecipitated from epididymosomes, indicating that the proteins are likely to be transferred as a complex. A list of transmembrane and membrane-associated proteins identified in EP or potentially present in their cargo, on the basis of a hypothesis-driven approach is seen in Table 1. The list includes PMCA1a, and b, which are murine sperm proteins and which have identical partners as PMCA4a, and 4b, and which the Martin-DeLeon Lab has detected in ELF and shown to be delivered to spermatozoa (unpublished data). Interestingly, none of these proteins appears in the list identified from the proteomic approach for human epididymosomes. This is not surprising for PMCA4, which is very low in abundance, accounting for only 0.01%–0.1% of all membrane proteins. Thus the hypothesis-driven approach, which is based on knowledge of the functional role and the interacting partners of the proteins, might be useful to detect the presence of low-in-abundance membrane or membrane-associated proteins when they exist in the epididymosomal cargo.

How are these transmembrane and membrane-associated epididymosomal protein complexes transferred to the sperm surface? Schwarz et al. analyzed the fusogenic properties of bovine epididymosomes and their involvement in the transfer of PMCA4, among other molecules, to bovine spermatozoa. Using labeled epididymosomes co-incubation experiments, they provided evidence for a fusogenic mechanism for the delivery PMCA4. More recently, studies on oviductal microvesicles/exosomes also provided support for a fusogenic pathway in the delivery PMCA4 and other transmembrane proteins to murine spermatozoa. From the use of a lipophilic dye for the exosomes/microvesicles and three-dimensional super-resolution structured illumination microscopy, sperm-EP fusion was detectable and co-localized with immunolabeled PMCA4a.

Membrane fusion is not only an effective mechanism for the delivery of transmembrane and membrane-associated proteins and their complexes, but should also mediate the delivery of GPI-linked proteins from epididymosomes. Thus the docking of epididymosomes that was detected by the delivery of SPAM1 is a step that precedes fusion. As CD9 tetraspanin has been implicated in membrane fusion, EP-sperm fusion appears likely to be mediated via CD9, which is a biochemical marker of exosomes and an adhesion molecule that generates fusion–competent sites. Consistent with this is the finding that CD9 has been detected on the murine sperm membrane over the acrosome and on the midpiece, and that CD9-positive microvesicles that fuse to the sperm membrane at these regions have been shown to transfer molecules to maturing live bovine spermatozoa in a tissue-specific manner. Further, with the use of function-blocking antibodies for CD9 there was a significant decrease in protein delivery to sperm cells, providing evidence for CD9-mediated fusion in cargo delivery of epididymosomes.

CONCLUSION

A variety of sperm proteins that are expressed in the testis are also expressed in the epithelia of the epididymis, where they are secreted into the luminal fluid and delivered to the sperm surface. Fertility-modulating proteins in the secretome may be GPI-linked, transmembrane or membrane-associated. Epididymosomes, membrane vesicles which may be exosomes or microvesicles, serve as the vehicle for the transfer of all three classes of proteins to the sperm surface, while GPI-linked proteins can also be transferred from the soluble membrane-free fraction of the ELF. This fraction exists in both oligomeric and monomeric forms, with protein transfer occurring primarily from the latter while the former serves as a source for monomers. Transfer from monomers is dependent on clusterin (CLU), a lipid carrier which stabilizes GPI monomers and delivers them to the sperm membrane via hydrophobic interactions. Epididymosomes fuse with the sperm membrane in delivering their cargo in a CD9-dependent manner, and transmembrane and membrane-associated proteins in an interactome are likely to be delivered in a complex. Further work is needed to determine the presence of the proteins predicted to reside in the epididymosomal cargo and their transfer to spermatozoa.

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COMPETING INTEREST

The author declares that there are no competing interests.

REFERENCES

1. Cooper TG. Role of the epididymis in mediating changes in the male gamete during maturation.Adv Exp Med Biol 1995; 377: 87–101.
2. Jones R. Membrane remodelling during sperm maturation in the epididymis. Oxf Rev Reprod Biol 1989; 11: 285–337.
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3 Robaire B, Hinton BT, Orgebin-Crist M. The epididymis. In: Neill J, Plant T, Pfaff D, Challis J, Kreutzer D, editors. Knobil and Neill's Physiology of Reproduction. New York: Raven Press; 2006. p. 1071–148.

4 Zhang H, Jones R, Martin-DeLeon PA. Expression and secretion of rat SPAM1 (281 or PH-20) in the epididymis: role of testicular lumicine factors. Matrix Biol 2004; 22: 653–61.

5 Zhang, H. Martin-DeLeon PA. Mouse epididymal SPam1 (PH-20) is released in the luminal fluid with its lipid anchor. J Androl 2003; 24: 51–8.

6 Zhang, H. Martin-DeLeon PA. Mouse epididymal SPam1 (PH-20) is released in vivo and in vitro, and SPam1 is differentially regulated in testsis and epididymis. Biol Reprod 2001; 65: 1586–93.

7 Cooper TG. Interactions between epididymal secretions and spermatozoa. J Reprod Fertil Suppl 1998; 53: 119–36.

8 Kirchoff C, Pera I, Der P, Yeung CH, Cooper T. The molecular biology of the sperm surface. Post-testicular membrane remodelling. Adv Exp Med Biol 2009; 424: 221–32.

9 Kirchoff C, Hale G. Cell-to-cell transfer of glycoprophosphatidylinositol-anchored membrane proteins during sperm maturation. Mol Hum Reprod 1996; 2: 177–84.

10 Yeung CH, Schröter S, Wagenfeld A, Kirchoff C, Klics S, et al. Interaction of the human epididymal protein CD52 (HES) with epididymal spermatozoa from men and cynomolgus monkeys. Mol Reprod Dev 1997; 48: 267–75.

11 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Saez F, Bignonnette F. Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional sperm motility for PMCA4. Biol Reprod 2004; 279: 28220–6.

12 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. Histochim Cell Biol 2008; 129: 331–43.

13 Cooper TG. Interactions between epididymal secretions and spermatozoa. In: Cooper TG, ed. Interactions between epididymal secretions and spermatozoa. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 417–34.

14 Slater GW, Miller ML, Pyne GJ, Sutliff RL, O’Connor KT, et al. Targeted ablation of plasma membrane Ca2+-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. J Biol Chem 2004; 279: 33742–50.

15 Schuh K, Cartwright EJ, Jankevics E, Bendschuh K, Liebmann J, et al. Plasma membrane Ca2+-ATPase 4 is required for sperm motility and male fertility. J Biol Chem 2004; 279: 28220–6.

16 The plasma membrane Ca2+-ATPase 4 is required for sperm motility and male fertility. J Biol Chem 2004; 279: 28220–6.

17 Cooper TG. Interactions between epididymal secretions and spermatozoa. In: Cooper TG, ed. Interactions between epididymal secretions and spermatozoa. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 417–34.

18 Cooper TG. Interactions between epididymal secretions and spermatozoa. J Androl 2003; 24: 51–8.

19 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Bignonnette F, Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional sperm motility for PMCA4. Biol Reprod 2004; 279: 28220–6.

20 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. Histochim Cell Biol 2008; 129: 331–43.

21 Cooper TG. Interactions between epididymal secretions and spermatozoa. In: Cooper TG, ed. Interactions between epididymal secretions and spermatozoa. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 417–34.

22 Cooper TG. Interactions between epididymal secretions and spermatozoa. J Androl 2003; 24: 51–8.

23 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Bignonnette F, Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional sperm motility for PMCA4. Biol Reprod 2004; 279: 28220–6.

24 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. Histochim Cell Biol 2008; 129: 331–43.

25 Cooper TG. Interactions between epididymal secretions and spermatozoa. J Androl 2003; 24: 51–8.

26 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Bignonnette F, Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional sperm motility for PMCA4. Biol Reprod 2004; 279: 28220–6.

27 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. Histochim Cell Biol 2008; 129: 331–43.

28 Cooper TG. Interactions between epididymal secretions and spermatozoa. J Androl 2003; 24: 51–8.

29 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Bignonnette F, Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional sperm motility for PMCA4. Biol Reprod 2004; 279: 28220–6.

30 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. Histochim Cell Biol 2008; 129: 331–43.
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57 Herrero MB, Gagnon C. Nitric oxide: a novel mediator of sperm function. J Androl 2001; 22: 349–56.
58 Ramya T, Misro MM, Sinha D, Nandan D, Mithal S. Altered levels of seminal nitric oxide, nitric oxide synthase, and enzymatic antioxidants and their association with sperm function in infertile subjects. Fertil Steril 2011; 95: 135–40.
59 Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. Asian J Androl 2014; 16: 31–8.
60 Zini A, O’Bryan MK, Magid MS, Schlegel PN. Immunohistochemical localization of endothelial nitric oxide synthase in human testis, epididymis, and vas deferens suggests a possible role for nitric oxide in spermatogenesis, sperm maturation, and programmed cell death. Biol Reprod 1996; 55: 935–41.
61 Dhillon B, Badiwala MV, Li SH, Li RK, Weisel RD, et al. Caveolin: a key target for modulating nitric oxide availability in health and disease. Mol Cell Biochem 2003; 247: 101–9.
62 Schwarz A, Wemmuth G, Post H, Brandenburger T, Aumüller G, et al. Vesicular transfer of membrane components to bovine epididymal spermatozoa. Cell Tissue Res 2013; 353: 549–61.
63 Al-Dossary AA, Caplan J, Martin-DeLeon, PA. The contribution of exosomes/microvesicles to the sperm proteome. Mol Reprod Dev 2015; 82: 79.
64 Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002; 2: 569–79.
65 Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009; 9: 581–93.
66 Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. Annu Rev Cell Dev Biol 2003; 19: 397–422.
67 Al-Dossary AA, Martin-DeLeon PA. JAM-A-CASK Complex Interacts with CD9 Tetraspanin and αv Integrin to Mediate Ca2+ Signaling in Capacitation and the Acrosome-Reaction in Mouse Sperm. 39th Annual Meeting of the Am Soc Andrology, Atlanta, Georgia, April 4th–8th, 2014.