ABSTRACT: The mammalian oviduct has long been recognized as an organ essential for successful reproduction. Bovine, ovine, porcine, and equine animal models have offered clear advantages for oviduct study related to gamete physiology, fertilization, and early embryonic development. Livestock species are amenable to surgical alteration of the reproductive tract, estrous cycle manipulation, gamete cryopreservation, and AI, as well as in vitro fertilization and embryo production. Although most reproductive technology developed for livestock was intended to benefit production animal agriculture, these techniques are a treasure trove of tools for researchers to better understand how the oviduct influences gamete function. Oviduct secretions obtained from in vitro tissue cultures or via indwelling oviduct catheters have been used for analyses to define the protein, lipid, carbohydrate, enzyme, and electrolyte compositions of the secretions during the estrous cycle or in response to hormone treatment. Oviduct secretions or components purified from them have also been used in in vitro assays to assess their ability to bind to sperm, influence sperm viability, motility, sperm capacitation, the acrosome reaction, sperm-egg binding, and egg penetration, as well as subsequent embryonic development. Compelling data have emerged which show that the composition of secretions differ during the estrous cycle and that their composition differs whether they originate from the ampullary or isthmic regions of the oviduct. These differences in composition are functionally relevant and associated with different responses by sperm. Evidence indicates that oviduct-specific glycoproteins, glycosaminoglycans, carbohydrates, norepinephrine, catecholamines, heat-shock protein, and osteopontin are components of the oviductal milieu that have the capacity to modulate sperm function. Future research on the livestock oviduct will likely define the role that oviduct secretions have in modulating sperm function and how these modifications ultimately affect fertilization and embryo development.

Key words: acrosome reaction, capacitation, fertility, in vitro fertilization, oviduct cannulation, oviduct region

INTRODUCTION

The oviduct is recognized as an essential component of the female reproductive system that ensures successful reproduction. In some ways it is hard to imagine how this well-vascularized tube, composed of a smooth muscle wall lined by mucosa, can have several diverse reproductive functions. The mammalian oviduct not only serves as a conduit for the transport of gametes and embryos, but also creates an environment that facilitates final gamete maturation, fertilization, and early embryo development.

It has only been during the past 60 yr that we have come to appreciate the many functions of the oviduct. Early investigators used rats and rabbits (Austin, 1951; Chang, 1951) to assess the role of the uterus and oviduct in events leading up to fertilization (Hunter and Dziuk, 1968; Bedford, 1970). They were the first to recognize that sperm needed exposure to the oviduct environment for a few hours before ovulation to be optimally prepared to penetrate an ovum in vivo. Although the physiological details involved in the transformation of sperm that occurred in the oviduct were unknown, Austin (1952) coined the term capacitation.
to describe them. Studies by Bishop (1956) showed that fluid accumulated in the rabbit oviduct lumen after in situ ligation of the ampullary and lower isthmic regions of the oviduct, leading him to conclude that secretions were produced by the mucosa.

After the early studies indicating that the oviduct influenced sperm function, there have been many studies involving gamete-oviduct interactions in livestock and other species that have been the subject of detailed reviews (Hunter, 1988, 1994; Gandolfi, 1995; Nancarrow and Hill, 1995; Hunter and Rodriguez-Martinez, 2004; Suarez, 2008). An additional comprehensive review of these studies would be redundant, and those interested in more detail should refer to the earlier reviews. The current review will highlight what has been learned during the first 45 yr of research focusing on how secretions in the oviduct lumen influence sperm function in livestock. However, for a more complete understanding of sperm physiology in the oviduct, the reader should refer to literature on the role of the oviduct epithelium in modulating sperm function (Töpfer-Petersen, 1999; Suarez, 2008; Töpfer-Petersen et al., 2008).

**APPROACHES TO STUDYING THE ROLE OF THE OVIDUCT IN MODULATING SPERM FUNCTION IN LIVESTOCK**

The use of domestic livestock to study the influence of the oviduct environment on sperm function soon followed the pioneering work in the 1950s of Austin, Chang, and Bishop. In studies to determine if ram sperm required minimal time for capacitation in the oviduct to fertilize ova, Mattner (1963) inseminated sperm into the infundibulum of the ewe oviduct by laparotomy at various intervals around ovulation. Based on rates of in vivo fertilization, he concluded that sperm needed exposure to the oviduct environment at least 1.5 h before ovulation for ovum penetration to occur.

With evidence that sperm were capacitated in vivo in an oviduct that produced secretions, additional studies would be needed to define how oviduct secretions facilitated sperm capacitation. Ideally, if secretions could be recovered from the oviduct, their rate of production and composition could be defined, and their effect on sperm could be evaluated in vitro. However, methods for continuous recovery of oviduct secretions did not exist until an innovative approach was developed by Clewe and Mastroianni (1960). Using a mid-lateral surgical incision, they secured a catheter in the rabbit oviduct infundibulum, ligated and transected the uterine end of the oviduct, and exteriorized the catheter to a collection vial mounted on the flank. This method enabled the collection of secretions for an average of 27 d postsurgery for 40 rabbits. Adaptation of this approach soon followed for cattle (Carlson et al., 1970; Kavanaugh and Killian, 1988), sheep (Black et al., 1963; Perkins et al., 1965; Belve and McDonald, 1968; Iritani et al., 1969), pigs (Iritani et al., 1974), and horses (Engle and Foley, 1975; Willis et al., 1994). Further modifications enabled continuous collection of oviduct fluid (OF) from the ampullary and isthmic regions of the same oviduct of ewes (Belve and McDonald, 1968) and cows (Kavanaugh et al., 1992).

Another approach to recover secretions from livestock oviducts utilized tissue and cell culture techniques with oviducts removed surgically at different stages of the estrous cycle or taken from animals at slaughter. This approach has been used effectively with oviduct tissues harvested from sheep, cattle, pigs (Buhi et al., 2000), and horses (Engle and Foley, 1975), among other species. Use of tissue and cell culture techniques has advantages because experiments can be conducted under controlled conditions. This method is generally less costly because it does not require special facilities to maintain experimental animals. Typically, tissues are cultured in a defined medium for a period of time and the conditioned medium containing oviduct secretions is harvested for analysis or for in vitro incubations with sperm, ova, or embryos. Using this approach it is possible to determine which components are synthesized and secreted by the oviduct. Although this is a valuable approach, it must be recognized that conditioned medium lacks components present in the oviductal milieu in situ that would have originated as serum transudate. As such, negative results should be interpreted with this in mind.

A third and least expensive approach used to recover oviduct secretions from tissues at slaughter is to flush the oviduct lumen of its contents with a known volume of saline buffer. This provides a diluted volume of oviduct secretions for compositional analyses or incubations with gametes to assess effects on function. Reasonable volumes of fluids can be recovered if several animals are available. If the stage of the estrous cycle is known when the animals are slaughtered, the effects of the estrous cycle stage can be considered in the experimental design. However, care must be taken to remove epithelial cells from the flushing to eliminate the potential contribution of the intact or fragmented epithelial cells to the results observed.

The use of livestock species as animal models to study sperm-oviduct interactions has several advantages compared with rodent and lagomorph models. Cattle, sheep, pigs, and horses are spontaneous ovulators, have lengthy, typical estrous cycles with clearly defined stages, and the daily volume of oviductal secretions collected is greater than that obtained from rabbits or rats. In addition to the intrinsic value of livestock species as animal models with potential practical applications, a significant body of literature exists covering reproduction of livestock species. Moreover, most species are amenable to estrous-cycle manipulation, gamete and embryo cryopreservation, AI, in vitro fertilization and embryo production, embryo transfer, and cell and tissue culture techniques with excised tis-
sues. This treasure trove of information and techniques is available to large animal researchers seeking to better understand how the oviduct influences sperm function in events leading to fertilization.

**OVIDENT SECRECTIONS DURING THE ESTROUS CYCLE AND THEIR INFLUENCE ON SPERM FUNCTION**

The volume of fluid produced by the oviduct is greatest during estrus and ovulation, and minimal during the luteal phase. These differences are hormonally dependent, with maximal volume production when serum estrogen is present and serum progesterone is minimal or absent (Perkins and Goode, 1966; Killian et al., 1989). Typical volumes for cattle (Carlson et al., 1970; Kavanaugh and Killian, 1988), pigs (Iritani et al., 1963), mares (Engle and Foley, 1975), sheep (Black et al., 1968; Iritani et al., 1989), and buffalo (Vecchio et al., 2010) range from 0.5 to 7 mL. The volume of fluid production also varies by region of the oviduct within a cycle phase, with greater volumes produced by the ampulla compared with the isthmus (Belve and McDonald, 1968; Kavanaugh et al., 1992).

A logical approach to assess whether oviduct secretions have an effect on sperm function in vitro is to use the total or complete secretion recovered from the cannula, culture, or by flushing. The composite of secretions used in this approach most closely mimics the fluid environment to which sperm are exposed in the oviduct and if there is no effect there is little incentive to go further. However, in vitro studies using cannula-derived OF, conditioned medium, or oviduct flushings for incubations with sperm have demonstrated numerous effects on sperm function and have generally confirmed in vivo findings. Initial studies with ewes (Black et al., 1968), mares (Engle and Foley, 1975), and cows (Olds and Vandermark, 1957) have shown that oviduct secretions recovered at estrus stimulate oxygen uptake of sperm. Later in vitro studies showed that oviductal secretions were able to facilitate sperm capacitation, the acrosome reaction, and affect sperm-zona binding, fertilization, sperm motility, and sperm survival (Killian, 2004; Rodriguez-Martinez, 2007). Generally, the magnitude of the effect was not only influenced by the stage of the estrous cycle from which the fluid was obtained (McNutt and Killian, 1991; McNutt et al., 1994), but also by the region of the oviduct that was the source of the fluid (Anderson and Killian, 1994; Grippo et al., 1995; Way et al., 1997). Whereas these investigations generally confirmed what was suspected to be functions of the oviduct, they also raised new questions concerning the role of specific components of OF in sperm function. Given the technology available for monitoring sperm in vitro during events leading up to fertilization, it was recognized that it would be possible to test the effects of specific OF components on sperm function.

**INSIGHTS INTO HOW OVIDUCT SECRETIONS MAY MODULATE SPERM FUNCTION**

Compositional analyses have revealed that OF contains AA and proteins, enzymes, simple and complex carbohydrates, ions, lipids, and phospholipids. Oviduct fluid is composed of components derived from both the secretory epithelium and components entering the lumen as serum transudate (Leese, 1988). It is also possible that some components in the ampulla are derived from fluid entering from the peritoneal cavity, or follicular fluid at ovulation (Hansen et al., 1991). Although some components, such as albumin, serum proteins, and ions, likely originate from blood, other components are synthesized in oviduct tissue and cell cultures.

The complement of ions and energy substrates in OF is biologically consistent with what is found in mammalian serum and body fluids. Ions present include potassium, sodium, magnesium, calcium, chloride, and phosphate. However, because ion ratios in OF differ from those of serum (Leese, 1988; Grippo et al., 1992; Vecchio et al., 2010), it indicates that ion concentrations are modulated by the oviduct epithelium. Moreover, differences in total protein, pH, and ion, bicarbonate, and energy substrate concentrations in OF exist among species (Leese, 1988). Recognizing these differences among species may be important when considering media used for in vitro fertilization and production of embryos. It was recently observed for buffalo that ion, protein, and glucose concentrations of OF differed from those of bovine OF (Vecchio et al., 2010). Because the concentrations of bovine components served as the basis for formulating media used for buffalo IVF or embryo production, it is possible that formulations specifically developed for buffalo based on the composition of its OF would improve the results.

**Lipids in OF**

Oviduct fluid contains phospholipids and cholesterol at concentrations considerably different than those of serum. Phospholipids are known to be synthesized and secreted by cultured bovine oviduct explants (Henault and Killian, 1993). Phospholipids are 10 times more concentrated in bovine OF than in serum collected at estrus, and 4.5 times more concentrated during the luteal phase (Killian et al., 1989). On contrast, cholesterol is 6 and 5 times more concentrated, respectively, in serum collected around estrus and the luteal phase, than in OF. Phospholipids are known to be taken up by sperm (Evans and Setchel, 1978) and the phospholipid composition of the sperm membrane is altered after incubation on the uterus and oviduct (Snider and Clegg, 1975; Evans et al., 1980). For bovine sperm incubated in OF, cholesterol efflux from the sperm membrane occurs to a high-density lipoprotein acceptor (Ehrenwald et al., 1990) or albumin. Changes in
the cholesterol:phospholipid ratio have been proposed to initiate membrane changes leading to the acrosome reaction. The efflux of cholesterol from the membrane and the possible uptake of phospholipids would change the membrane cholesterol:phospholipid ratio. A change in the ratio may alter membrane fluidity, which would likely change the pH (Cross, 1998; Travis and Kopf, 2002) and alter membrane components of the signal transduction cascade to initiate other functional responses associated with sperm capacitation and the acrosome reaction (Travis and Kopf, 2002).

**Proteins in OF**

Proteins are among the most studied components of OF. Most of the proteins in fluids collected from indwelling catheters or in oviduct flushings are found in other tissues or blood. Analyses that define components of OF or oviduct flushings do not distinguish whether they have been synthesized by the oviduct, or found their way into OF as serum transudate, or both (Killian 2004). However, livestock oviduct cell and explant cultures have demonstrated that the oviduct has the capacity to synthesize and secrete an array of proteins and peptides in vitro. Major proteins and peptides shown to be synthesized by the oviducts of cows, sows, or ewes include oviduct-specific glycoprotein, osteopontin (OPN), integrins, haptoglobin, catalase, glutathione peroxidase, superoxidismutase, hyaluron, atrial natriuretic peptide A, extracellular matrix proteins, plasminogen activator and inhibitor, clusterin, IgA, Fas ligand, heat shock 70 kDa protein 8, and several growth factors (Brackett and Mastroianni, 1974; Hunter, 1994; Buhi et al., 2000; Gabler et al., 2001, 2003; Lapointe and Bilodeau, 2003; Lavery et al., 2003; Bergqvist et al., 2005a,b; Rodriguez-Martinez, 2007; Lloyd et al., 2009; Mugnier et al., 2009). Regardless of the source of the component in OF, it is prudent to assume that each has the potential to affect sperm function related to fertilization or create an environment favorable for sperm survival.

It is clear that the protein composition of OF is complex and could affect sperm function in numerous ways. Based on gel electrophoresis studies characterizing the relative molecular weight (kDa) and isoelectric point, it is evident that numerous proteins in excess of 30 kDa are present in OF, and differences in composition exist during the estrous cycle (Sutton et al., 1984; Voglmayr and Sawyer, 1986; Malayer et al., 1988; Buhi et al., 1989, 1990; Gerena and Killian, 1990) and with region of the oviduct (Grippo et al., 1992; Rodriguez and Killian, 1998). A reasonable question to ask is whether proteins originating in oviduct secretions associate with or modify the sperm membrane because one would suspect that those interacting with sperm may influence sperm function. Using different experimental approaches, it has been shown that several proteins originating in ovine or bovine OF do indeed associate with sperm (Killian, 2004). Although the array of bovine oviduct proteins associating with sperm ranged in size from 24 to 140 kDa, not all proteins were taken up, indicating that the process was selective. Moreover, evidence indicates that existing sperm membrane proteins may also be modified in OF (Voglmayr and Sawyer, 1986; Souza et al., 2008). Incubating sperm in OF may facilitate membrane alterations from uptake of oviduct proteins or by modification of preexisting proteins or a combination of both mechanisms. In addition to changes in the cholesterol:phospholipid ratio of sperm in the oviduct that could affect the sperm membrane, enzymatic alteration of the sperm surface in the oviductal environment is possible. Protease and glycosidase activities have been reported in ovine, bovine, and porcine OF (Roberts et al., 1975, 1976; Carrasco et al., 2008a,b), which could affect the protein and carbohydrate distribution on the sperm surface.

Alterations of the sperm surface could affect the interaction between the sperm and the oviduct epithelium, or with the zona of the ovum. Indeed, after ejaculated bull sperm are capacitated in OF, their ability to bind to the zona pellucida (Way et al., 1997) or to take up zona pellucida proteins is increased (Topper et al., 1999) and there is a differential loss of carbohydrate residues from the sperm surface (Taitzoglou et al., 2007). Recently, Souza et al. (2008) showed that the distributions of 3 seminal plasma proteins [bovine seminal plasma protein 30, bovine seminal protein A1/A2, and OPN] on freshly ejaculated sperm were significantly different after sperm were incubated in ampullary and isthmic OF. A picture that emerges is that the surface of oviductal sperm is quite different from that of ejaculated sperm, which could explain functional differences between ejaculated and oviductal sperm binding to the oviduct epithelium, zona, and perivitelline membrane.

Several studies have shown that oviductal secretions maintain or facilitate sperm viability and motility (Abe et al., 1995c; Satoh et al., 1995; Boquest et al., 1999). Recent evidence indicating that post-thaw semen characteristics of Murrah buffalo are improved when oviductal secretions are included in the cryopreservation extender (Imam et al., 2008) indicates that the secretions impart a protective effect on the sperm membranes subjected to freeze-thaw. The bovine oviduct is believed to maintain the optimal balance between reactive oxygen species and antioxidants through the activity of glutathione peroxidase, superoxide dismutase, and catalase (Lapointe and Bilodeau, 2003), and catalase from OF has been shown to bind to sperm (Lapointe et al., 1998). Maintaining an ideal balance between reactive oxygen species and antioxidants is likely important for several aspects of sperm function in the oviduct, including viability and motility. Because the activity of these antioxidant enzymes reduces lipid peroxidation of phospholipids in post-thawed sperm, it may explain
the benefit of adding oviduct secretions to the semen extender (Kumaresan et al., 2006).

**Identification of Specific Oviduct Proteins That Influence Sperm Function**

Despite an impressive array of proteins that have been identified in OF or tissues, few have been purified and tested directly to evaluate how they influence sperm function in the oviduct. Two exceptions are oviduct-specific glycoproteins (OSG) and OPN. Oviduct-specific glycoproteins are exclusively synthesized and secreted by oviduct tissue and their production peaks at estrus and ovulation (see reviews by Verhage et al., 1998; Buhi, 2002; Killian, 2002). Oviduct-specific glycoproteins have been found in the oviducts of every mammalian species studied to date, and cDNA sequences for OSG indicate it is conserved among species, is related to chitinase, and exhibits chemical properties similar to mucins. For livestock species, OSG was first observed in oviducal fluid of estrous ewes (Sutton et al., 1984) and later in cow (Boice et al., 1990; Gerena and Killian, 1990), sow (Buhi et al., 1990), caprine doe (Abe et al., 1995a), and mare (Willis et al., 1994; Mugnier et al., 2009) oviducts and secretions. Studies to explore the effects of purified OSG on gamete function have involved incubating purified OSG with sperm, ova, or both. In addition to determining whether OSG became associated with the gametes in vitro, functional outcomes observed included sperm motility, viability, capacitation, the acrosome reaction, zona binding, fertilization, and subsequent rates of cleavage and embryo development. In relative terms, fewer studies have focused on determining the specific effects on sperm. It has been shown, however, that bull sperm membranes acquire OSG after incubation in OF (McNutt et al., 1992; King and Killian, 1994), sperm incubated with purified OSG have improved motility and viability (Abe et al., 1995b,c; Satoh et al., 1995), and capacitation is facilitated (King et al., 1994; McCauley et al., 2003). Increased rates of fertilization and in vitro embryo development after in vitro fertilization of ova with OSG-treated sperm have also been observed for cattle and pigs (King et al., 1994; Martus et al., 1998; Kouba et al., 2000; McCauley et al., 2003). It was also shown that exposure of either the porcine ovum or the sperm to OSG reduced rates of polyspermy without affecting rates of penetration (McCauley et al., 2003).

Osteopontin is an extracellular matrix protein found in bull seminal plasma in amounts that correlate with bull fertility (Killian et al., 1993; Cancel et al., 1997). Oviduct fluid contains OPN, which is synthesized and secreted by bovine oviduct mucosa (Gabler et al., 2003). Although OPN has been detected in ejaculated bull sperm membranes (Erikson et al., 2007b) and its membrane distribution changes after incubation in OF (Souza et al., 2008), there does not appear to be a net gain in OPN associated with the membrane of sperm incubated in OF (Erikson et al., 2007a). Pretreatment of bovine oocytes with purified milk OPN improved rates of sperm binding to zona, fertilization, and embryo development in vitro, although pretreatment of sperm with OPN had no effect (Gonçalves et al., 2008). In pigs, exposure of both sperm and eggs to some doses of OPN improved the rate of in vitro fertilization, and OPN reduced the incidence of polyspermy in a dose-dependent manner (Hao et al., 2006). Osteopontin has been shown to facilitate bovine sperm capacitation and viability (Erikson et al., 2007b; Monaco et al., 2009), and OPN-treated sperm are associated with increased rates of in vitro fertilization, cleavage, and percentages of advanced blastocysts (Gonçalves et al., 2008; Monaco et al., 2009). In vitro fertilization studies with ejaculated bull sperm have shown that bovine sperm treated with OPN antibody had greater rates of binding to the zona pellucida and reduced rates of fertilization than controls (Erikson et al., 2007a). Data for the bull appear to support the observation in pigs (Hao et al., 2006) that OPN may function to reduce polyspermy and facilitates fertilization. Because OPN is a protein that is known to be involved in cell signaling and cell adhesion by binding to integrins through the arginine-glycine-aspartic acid (RGD) sequence, we explored whether or not this mechanism was important in sperm-egg binding and fertilization (Gonçalves et al., 2009). In vitro pretreatment of bovine sperm and eggs with RGD reduced the rates of sperm-egg binding and fertilization compared with untreated controls or those incubated with RGE peptides. Moreover, sperm-egg binding and fertilization rates were also reduced when oocytes or sperm were preincubated with antibodies to integrins-alphaV and -alpha5. Taken together, these studies provide evidence that the mechanism whereby OPN influences sperm-egg binding and fertilization is mediated through integrins binding to the RGD sequence of OPN.

It is somewhat surprising that OSG and OPN have several similar effects in vitro on sperm function. In the bigger scheme of oviduct physiology, redundant mechanisms to ensure successful events leading to fertilization and embryonic development are likely important, particularly when considering that knockout mouse models for OSG (Araki et al., 2003) and OPN (Rittling et al., 1998) have been shown to be fertile.

**CONCLUSIONS**

Future studies with oviduct secretions will likely evaluate the effect of other components on sperm to better understand their role in modulating sperm function in the oviduct. Because livestock species offer many experimental advantages for studying the oviduct, they will likely continue to be an important animal model. These studies are not only important to better understand the role of the oviductal milieu in vivo, but they
will also likely result in practical applications to improve reproductive technology and ultimately improve the reproductive efficiency of livestock.

**LITERATURE CITED**


