

## The oviductal sperm reservoir in domestic mammals

### Reservorio de espermatozoides en el oviducto de mamíferos domésticos

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#### SUMMARY

In all mammalian species studied to date, sperm cells are arrested in the caudal isthmus before ovulation to form an oviductal reservoir. When ovulation occurs, discrete numbers of sperm are released from this region and ascend to the ampulla where fertilization normally occurs. Experimental evidence indicates that the sperm reservoir is instrumental in delivering the appropriate number of sperm in the correct physiological state for normal fertilization. In this paper, we present a comprehensive review of the sperm reservoir formation and functions in mammals. Sperm reservoir manipulation as a potential tool to improve reproductive performance is also discussed.

*Key words:* sperm reservoir, sperm transport, oviduct, domestic animals.

*Palabras clave:* reservorio de espermatozoides, transporte de espermatozoides, oviducto, mamíferos domésticos.

#### INTRODUCTION

It is clear that the oviduct plays a central role in successful reproduction in domestic mammals. The oviduct not only participates in ovum and sperm transport to the site of fertilization, but also provides the microenvironment where the process of conception and early embryonic development occur. Studies *in vivo* and *in vitro* (Chian and Sirard, 1995; Pacey *et al* 1995b, Pollard *et al* 1991, Suárez 1998, Suárez *et al* 1997, Thomas and Ball 1996, Thomas *et al* 1994b) indicate that sperm cells accumulate in the isthmus around the time of ovulation, constituting an oviductal sperm reservoir. After ovulation, sperm cells are gradually released from this region and reach the site of fertilization to meet the female gamete. A low sperm number at the site of fertilization is a constant finding in domestic species (sperm:oocyte ratio close to 1) (reviewed in Hunter, 1996); this phenomenon reduces the risk of polyspermy, a pathological condition that invariably leads to developmental failure of the zygote in eutherian mammals.

Since oocyte maturation, fertilization and embryo development can be achieved completely by *in vitro* procedures, the oviduct might be considered a non-essential organ. In addition, pregnancies have been reported in animals with oviductal dysfunctions (e.g., lack of oviductal cilia). However, in the context of animal production, successful reproduction must be considered

in terms of a population rather than an individual basis; from this perspective, the oviduct and its functions are essential to achieve high reproductive performance.

#### ANATOMICAL BASES OF SPERM RESERVOIR

The mammalian oviduct can be divided into three different anatomical regions: infundibulum, ampulla, and isthmus, each associated with distinct physiological functions. The fimbriated infundibulum, the rostral portion of the oviduct, is responsible for oocyte transport into the tube after ovulation. The ampulla represents a dilated tubular region in which the process of fertilization is completed. Finally, the isthmus is involved in gamete and embryo transport and considered the anatomical base of the sperm reservoir (Hunter 1984, Suárez 1987). The oviductal wall is comprised of three distinctive layers: an external serosal mesosalpinx, an intermediate double-layered miosalpinx and an internal endosalpinx. The latter, also called oviductal mucosa, consists of one layer of columnar epithelial cells. This epithelium contains ciliated (most cells) and non-ciliated (secretory) cells. The oviductal mucosa is arranged in folds which increase in complexity from the utero-tubal junction to the ostium.

Using a surgical approach of oviductal ligation and transection at different times after mating, Hunter (1984) and Willmut *et al* (1984) have demonstrated that the caudal isthmus and utero-tubal junction have the function of sperm storage in pigs and cattle. In the horse, the isthmus may also function as a site for sperm storage, since more sperm were found attached to explants from the isthmus than those from the ampullary region (Thomas *et al*

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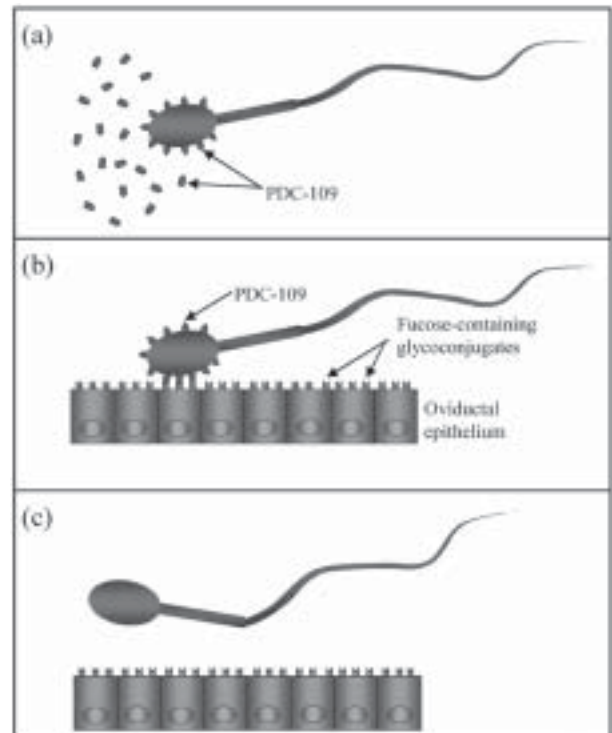
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1994a). Suárez (1987) observed more sperm in the isthmus compared with those present in the ampulla of excised mouse oviducts. The physical obstruction caused by the narrowness of isthmus lumen along with deep furrows formed by complex folds of the mucosa may contribute to sperm entrapment in this region (Hunter 1995). In addition, these features might enhance the probability of association between specific molecules present on sperm and oviductal cells, and therefore increase the number of sperm attached to oviductal epithelial cells (OEC).

## SPERM RESERVOIR FORMATION

**FACTORS INVOLVED IN SPERM RESERVOIR FORMATION.** Since Suárez and colleagues first defined the ligand-receptor-like nature of sperm-OEC attachment (Demott *et al* 1995, Lefebvre *et al* 1997, Suárez 1998, Suárez *et al* 1998), it has been hypothesized that direct sperm-OEC contact is the major mechanism underlying sperm reservoir formation (Suárez *et al* 1990). This hypothesis is further supported by studies in which sperm remained attached even after energetic washing (Smith and Yanagimachi 1990) or proteolytic enzyme treatment (Raychoudhury and Suárez 1991). Although this could be true, the relative contribution of each factor in the oviductal reservoir formation remains unclear. Mucus secretions, chemical properties of oviductal fluid, temperature gradients, patency of the lumen, and sperm binding to OEC can all contribute, to different degrees, to establishment of the sperm reservoir.

*Biochemical nature of oviduct-spermatozoon interaction.* Oviduct-sperm binding is a reversible process that appears to involve oligosaccharide moieties on the epithelial cell membrane (Demott *et al* 1995, Dobrinski *et al* 1996a, Suárez 1998, Suárez *et al* 1998) and a  $Ca^{2+}$ -dependent lectin on the sperm surface (Suárez *et al* 1998). In all species studied to date, different sugars have been demonstrated to participate in this phenomenon (Demott *et al* 1995, Dobrinski *et al* 1996a, Lefebvre *et al* 1997, Suárez *et al* 1998). In hamsters, sialic acid and fetuin (a sialoglycoprotein) reduced the number of sperm adherent to oviductal explants suggesting that sperm-oviductal interaction is mediated, at least in part, by these molecules (Demott *et al* 1995). Molecules involved in equine sperm-OEC interaction *in vitro* have also been investigated by Dobrinski *et al* (1996a). In this study, addition of galactose or glycoproteins with exposed galactosyl residues to equine sperm-OEC cocultures inhibited sperm attachment to the cells. In pigs, formation of the sperm reservoir appears to involve the interaction of mannosyl-oligosaccharide ligands on the oviductal cells with binding molecules on the sperm surface (Topfer-Petersen *et al* 2002). Experimental data indicate that fucose is the particular carbohydrate moiety that constitutes the binding site on bovine oviduct epithelia (Lefebvre *et al* 1997,



**Figure 1.** Proposed model to explain bull sperm attachment to and release from oviductal epithelium (adapted from Suárez, 2002). (a) PDC-109, a protein secreted by the seminal vesicles, associates with sperm's plasma membrane. Excess PDC-109 present in seminal fluid may preclude sperm binding to lower portions of the female reproductive track. (b) In the oviduct, spermatozoa PDC-109 recognizes fucosylated residues present on epithelial cells contributing to sperm entrapment in the lower portion of the oviduct to form a sperm reservoir. (c) Loss or modification of PDC-109 from spermatozoa surface associated with capacitation facilitates sperm release from the reservoir.

Modelo propuesto para explicar la asociación y posterior liberación de los espermatozoides bovinos desde el epitelio oviductal (adaptado de Suárez, 2002). (a) PDC-109, una proteína secretada por las vesículas seminales, se asocia a la membrana plasmática de los espermatozoides. La presencia de PDC-109 libre en el plasma seminal impediría la unión de los espermatozoides a porciones posteriores del tracto reproductor de la hembra. (b) En el oviducto, el PDC-109 ligado a la superficie de los espermatozoides reconoce residuos fucosilados presentes en las células del oviducto. Esta interacción permite la adherencia de los espermatozoides al epitelio contribuyendo de este modo a la formación del reservorio. (c) Pérdida o modificaciones de la proteína PDC-109 como consecuencia de la capacitación espermática facilita la liberación de los espermatozoides del reservorio.

Suárez *et al* 1998) (figure 1). Further characterization of this interaction (Suárez *et al* 1998), established that a Lewis-a trisaccharide-like molecule is involved in sperm-oviductal epithelial cell binding. This oligosaccharide, which has a fucose molecule in its composition, had greater ability to inhibit sperm attachment to OEC than fucose alone (Suárez *et al* 1998).

Structural and functional evidence indicate that the fucose-binding lectin present on bull sperm is a seminal

plasma protein secreted by the seminal vesicles called PDC-109 (BSP-A1/A2) (Gwathmey *et al* 2003, Ignotz *et al* 2001). Upon ejaculation, this protein associates with the sperm's plasmatic membrane enabling it to bind to the oviductal epithelium (figure 1). This proposed mechanism explains the limited binding capacity of sperm that have not been in contact with seminal fluids i.e., epididymal sperm (Gwathmey *et al* 2003, Petrunkina *et al* 2001). Furthermore, excess PDC-109 in seminal plasma may block sperm binding sites on posterior portions of the female reproductive tract facilitating sperm transport to the oviducts (Suárez, 2002).

*Mucus secretions.* Overstreet and Cooper (1975) first observed that rabbit sperm collected from the isthmus of does before ovulation were immotile or weakly motile. This observation is interpreted as a transitory inhibition or suppression of sperm flagellar movement, since motility could be restored by dilution of immotile sperm with medium or ampullar fluid (Overstreet *et al* 1980). During sperm storage in the cauda epididymides, a proteinaceous secretion that increases the viscosity of epididymal fluid inhibits sperm flagellar movement. A mucus secretion has been described in cattle (McDaniel *et al* 1968), rabbits (Jansen and Bajpai 1982), pigs (Hunter 1995) and the human isthmus (Jansen 1978). This secretion may suppress sperm motility in an analogous manner to viscous epididymal secretions. Suárez and coworkers have observed sperm entrapped in a mucus-like secretion produced *in vitro* by oviductal explants from cows (Suárez *et al* 1990) and gilts (Raychoudhury and Suárez 1991). However, in these studies the motility of sperm entrapped in mucus was not thoroughly characterized. In scanning microscopy microphotographs, the oviductal mucus can be seen distributed on the sperm head forming relatively large spheres or granules (Hunter *et al* 1991). Suárez *et al* (1998) observed the narrow isthmus lumen filled with mucus secretions in histological sections of bovine oviducts fixed by rapid freezing to preserve the tissue architecture. Similarly, intraluminal fluid in preovulatory porcine oviducts contains abundant glycosaminoglycans which obliterate the narrow lumen of the sperm reservoir segment (Rodríguez-Martínez *et al* 2001). Hyaluronan is one of the mucopolysaccharides produced by the isthmus epithelium which reaches maximum concentration in the luminal fluid during estrus (Rodríguez-Martínez *et al* 2001). This observation in conjunction with the known ability of hyaluronan to modulate sperm capacitation *in vitro* (Suzuki *et al* 2000, Suzuki *et al* 2002), has led to the hypothesis that mucopolysaccharides may have a role in oviductal sperm transport (Rodríguez-Martínez *et al* 2001). It is probable that narrowness of the isthmus in conjunction with mucus secretions present during the preovulatory stage can impede or retard sperm transit, contributing to sperm reservoir formation (Suárez *et al* 1997).

*Chemical properties of oviductal fluid.* The oviductal fluid represents the aqueous milieu in which female and male gametes and embryos are suspended during oviductal transit. This fluid is a complex mixture of ions and macromolecules solubilized in water. Some constituents are derived from the plasma, while others are synthesized in the epithelia and secreted into the lumen (Leese, 1988). Since the ionic composition of oviductal fluid is different from that of plasma, it is assumed that a differential or selective ion transport is present in the oviductal wall. For example, oviductal fluid composition is characterized by a high potassium and low calcium concentration compared to plasma levels of these ions (Borland *et al* 1977). Studies in the rabbit indicate that low concentrations of potassium can inhibit sperm motility, a suppression that can be reversed by pyruvate (Burkman *et al* 1984). Data of Murdoch and White (1971) suggest that potassium inhibition of sperm motility is mediated by reduction of oxygen consumption and glycolysis. Differences in oviductal fluid composition during the estrous cycle (Jansen, 1984), and between ampulla and isthmus regions have been reported (Nichol *et al* 1992). In pigs, glucose and lactate have been shown to be present in isthmus fluid at different concentrations from those in ampullar fluid (Nichol *et al* 1992). These regional differences in ionic and macromolecular composition of oviductal fluid can promote preovulatory sperm arrest in the caudal oviduct.

*Temperature gradients.* Another factor that may be involved in sperm storage in the oviduct is a temperature gradient along this organ. Hunter and Nichol (1986) have measured the temperature along the oviduct of sows before and after ovulation. They found that the isthmus was 0.75°C cooler than the ampulla during the preovulatory stage, but this difference was no longer detected after ovulation. David *et al* (1971) have reported similar regional temperature differences in the rabbit oviduct. These temperature variations may reflect differences in regional vascular and lymphatic flux that in turn can affect the oviductal fluid composition. Increased contractility of the myosalpinx in the ampullar region during the preovulatory period may also be responsible for temperature gradients. Although of low magnitude, these temperature differences between ampulla and isthmus may directly or indirectly inhibit sperm motility and contribute to sperm reservoir formation.

*Patency of the oviductal lumen.* The mucosal surface of the isthmus is arranged in complex folds that reduce the patency of the organ. A distinctive characteristic of the isthmus is a well-developed smooth muscle layer located between mucosa and serosa (El-Banna and Hafez 1970). The smooth muscle in the isthmus region is profusely innervated by cholinergic fibers from the sympathetic nervous system that control tonic muscle contraction

(Hunter 1995). In addition, high numbers of adrenergic receptors are present on the cells of the circular smooth muscle layer in the isthmus (Brundin 1965); stimulation of these receptors can significantly reduce the oviductal lumen. High levels of estrogen during estrus can increase the tonic smooth muscle contraction (Hunter 1996), an effect that is probably mediated through adrenergic receptors mentioned above. Moreover, estrogen enhances oviductal obstruction by increasing the height of mucosal epithelium (McDaniel *et al* 1968) and by stimulating edema of the oviductal wall (Boyle *et al* 1987). All these factors, plus changes in direction and/or intensity of ciliar beating seem to be involved in the buildup of sperm in the caudal isthmus prior to ovulation.

**FACTORS AFFECTING SPERM ENTRAPMENT AND RELEASE FROM THE SPERM RESERVOIR.** After ovulation, sperm cells are gradually released from the reservoir in the caudal isthmus and ascend to the ampulla, ensuring a sperm/egg ratio close to 1 at the site of fertilization. Capacitating-like changes in sperm plasma membrane seem to be responsible for sperm release from oviductal epithelium (Revah *et al* 2000). Sperm exposed to capacitating conditions have limited ability to attach to oviduct cells *in vitro* (Lefebvre and Suárez 1996). Uncapacitated sperm initially bind to OEC both *in vivo* (Smith and Yanagimachi 1991) and *in vitro* (Fazeli *et al* 1999). This initial attachment is followed by induction of capacitation and release of capacitated sperm (Fazeli *et al* 1999; Smith and Yanagimachi 1991). We have demonstrated that heparin, a glycosaminoglycan known to induce bull sperm capacitation *in vitro*, is a fast and highly effective inductor of sperm release from OEC *in vitro* (Bosch *et al* 2001). The heparin-induced sperm release reported in our and another study (Talevi and Gualtieri 2001) may reflect change or loss of binding molecules on the sperm plasma membrane, which in turn promotes sperm release. Additionally, development of sperm hyperactive movement (Pacey *et al* 1995a) and the hormonal shift from follicular to luteal phase could participate in this process (Hunter 1995). Changes in the sperm membrane associated with capacitation, acquisition of sperm hypermotility and modification of the female endocrine status may all influence sperm-oviduct cell dynamics. However, the mechanisms that regulate timely sperm liberation from the site of storage after ovulation are not yet fully understood.

**IS SPERM RELEASE FROM THE OVIDUCTAL RESERVOIR REGULATED?** One of the most striking features of the vascular anatomy of the female reproductive tract in ruminants and swine species is the close apposition and extensive contact between ovarian and uterine vessels (Ginther 1976, 1974). Regulatory substances with low molecular weight such as hormones can pass from vein to artery and vice versa without the necessity of direct

vascular connections; in this way, a bi-directional communication between ovary and uterus can be established. In the sheep, it has been shown that not only estrogen, progesterone and testosterone (McCracken *et al* 1984) but also oxytocin (Schramm *et al* 1986) is interchanged between arteries and veins in the ovarian pedicle. Furthermore, the local venoarterial pathway that follows prostaglandin  $F_{2\alpha}$  produced by the non-gravid uterus to cause luteolysis has been extensively demonstrated in both sheep and cattle (for review see: Ginther 1974).

Existence of a local counter-current transfer of gonadal hormones from the ovarian vein into the tubal branch of the ovarian artery has led to the hypothesis of ovarian endocrine regulation of sperm progression to the site of fertilization (Hunter 1995, 1996). Through this mechanism, relatively high concentrations of ovarian hormones can reach the oviductal wall compared to systemic levels (Hunter 1995). Gonadal steroids could coordinate gamete transport to the site of fertilization by changing the composition and physical properties of oviductal fluid and mucus, gas tension, number of sperm binding sites and pattern of contractile activity (Hunter 1996). Since ovarian steroids can diffuse into the oviductal lumen, a direct effect of these hormones on sperm function and morphology cannot be excluded.

While estrogen can increase the number of spermatozoa bound to the oviductal epithelium, progesterone produces the opposite effect. The number of sperm attached to porcine oviduct explants was not different when oviducts were collected at estrus or midcycle (Suárez *et al* 1991). However, a significantly greater number of sperm were observed attached when estradiol had been added to the culture medium (Raychoudhury and Suárez 1991, Suárez *et al* 1991). Also in pigs, administration of progesterone as either local microinjections in the caudal portion of the isthmus (Hunter 1972) or systemically (Day and Polge 1968) increased the number of sperm released from the reservoir and, therefore, the incidence of polyspermia. In cattle, neither the female hormonal state nor the region of the oviduct seem to affect the number of sperm attached to oviductal explants *in vitro* (Lefebvre *et al* 1995) but the direct effect of gonadal steroids on sperm-oviduct interaction has not been thoroughly investigated (Suárez *et al* 1990).

## FUNCTIONS OF THE SPERM RESERVOIR

**SPERM CAPACITATION, ACROSOME REACTION AND MOTILITY.** Capacitation is a complex phenomenon that involves profound modifications in sperm plasma membrane organization, structure and concentration of proteins, steroids and phospholipids (Yanagimachi 1990). Normally, capacitation is initiated after mating, once the sperm are in contact with the female reproductive tract.



However, the exact site where this process begins varies according to the site of semen deposition. In species such as the bovine in which the semen is deposited in the anterior vagina, capacitation begins during sperm migration through the cervix. In species with uterine ejaculation including pigs (Hunter 1980), the oviduct seems to be the major site of sperm capacitation. Although sperm capacitation is not site specific (e.g., uterine environment can fully support sperm capacitation), the primary organ where capacitation is completed is the oviduct. Studies *in vitro* have shown that sperm binding to oviductal cells (Chian *et al* 1995; Chian and Sirard, 1995) and soluble product(s) present in conditioned medium (Chian *et al* 1995) are both necessary for sperm capacitation. Parrish *et al* (1989) reported that the oviduct produces a potent capacitating factor that is present in the oviduct fluid. Interestingly, the highest activity of this factor was observed in oviductal fluid collected at estrus. Similarly, Chian *et al* (1995) reported that a capacitating factor was present in medium conditioned by OEC. The capacitating ability was maximal in medium conditioned by OEC collected one or two days after ovulation, or when estradiol-17 $\beta$  was added to the culture medium. In conjunction, these data suggest that sperm capacitation in the oviduct is influenced by hormonal changes associated with the estrous cycle. In addition to the capacitating factor, studies in human (Kervancioglu *et al* 1994), equine (Ellington *et al* 1993b), bovine (Chian and Sirard, 1995; Guyader and Chupin, 1991), ovine (Gutiérrez *et al* 1993) and porcine species (Fazeli *et al* 1999) have demonstrated that sperm contact with OEC is involved in the capacitation process. In the bovine species, Chian and Sirard (1995) reported a higher oocyte penetration rate when sperm cells were cocultured with OEC compared to those observed in the control group without cells.

The experimental evidence presented so far suggests that sperm-OEC contact and secretions from oviductal epithelia enhance sperm capacitation. Sperm capacitation is associated with reduction of life span of sperm cells (Hunter 1987). Then, if sperm-OEC interaction promotes capacitation, it is difficult to explain one of the major functions of the sperm reservoir which is to extend sperm fertilizing ability (Boquest and Summers 1999, Pollard *et al* 1991). As a consequence of modifications in sperm membrane composition, a concomitant influx of Ca<sup>2+</sup> is observed during sperm capacitation (Dasgupta *et al* 1993). Dobrinski *et al* (1996), using a fluorescent calcium indicator, reported that sperm in contact with OEC maintained a low intracellular calcium concentration when compared to both free-swimming sperm and sperm in the control group without oviductal cells. Furthermore, it has been suggested that key pro-capacitation molecules like bicarbonate might be kept at lower levels in the sperm reservoir compared with other oviductal segments retarding in this way sperm capacitation (Rodríguez-

Martínez *et al* 2001). According to this, sperm-OEC contact and the isthmic environment seem to retard rather than induce sperm capacitation, thus prolonging sperm fertilizing capacity.

A limitation to studying sperm physiology in coculture with OEC monolayers is that it is not possible to separate the effects of direct sperm-OEC contact from those induced by soluble products released by oviductal cells. To overcome this problem, Smith *et al* (1997) developed a method to isolate apical plasma membrane vesicles (AMV) from OEC. Coculture of sperm with AMV allows researchers to study the direct effects of sperm-OEC membrane contact on sperm function since cytoplasm is not present in the system. Using this approach, it has been shown that membrane contact between sperm and AMV can modulate intracellular calcium concentration, delay sperm capacitation, and extend sperm viability (Dobrinski *et al* 1997, Smith 1998, Smith and Nothnick 1997). Though the underlying mechanism(s) is(are) not currently understood, it is conceivable that intimate contact between sperm and OEC plasma membrane might stabilize the sperm membrane, thus retarding Ca<sup>2+</sup> influx. Alternatively, recognition of specific molecules on OEC may trigger intracellular signals that lead to reduced permeability to Ca<sup>2+</sup> and/or stimulate Ca<sup>2+</sup> extrusion from the sperm.

Acrosome reaction consists of a series of morphological changes on the sperm head which ends when the sperm plasma membrane fuses with the outer acrosomal membrane and the enzymatic content from the acrosomal sac is released (Yanagimachi 1990). Capacitated sperm may undergo acrosome reaction if the appropriate stimuli are applied. It is classically assumed that the acrosome reaction *in vivo* starts when the spermatozoon passes through the cumulus cell layers or immediately after it contacts the zona pellucida. Bedford (1970) reported that two different kinds of acrosome reaction can occur: a) true acrosome reaction when an appropriate stimulus triggers a response in a previously capacitated sperm and b) false acrosome reaction represented by an unspecific loss of acrosomes in dying or dead sperm.

There does not seem to be a clear relationship between the occurrence of acrosome reaction and the presence of sperm in the oviductal sperm storage. However, most sperm cells attached to OEC both *in vivo* and *in vitro* have intact acrosomes (Ellington *et al* 1991, Gualtieri and Talevi 2000, Hunter *et al* 1991, Pollard *et al* 1991, Suárez *et al* 1991). In an electron microscopy study (Esponda and Moreno, 1998), 81% percent of adherent mouse sperm had intact acrosomes, a result that contrasted with the highly damaged acrosomes observed in the unattached sperm population.

In addition to electron microscopy studies, direct observation of sperm by light microscopy (Ellington *et al.*, 1991), chlortetracycline stain (Ellington *et al* 1993a,

Fazeli *et al* 1999), and a triple stain technique (Gutiérrez *et al* 1993) have been used to assess capacitation and acrosome status of sperm in coculture with OEC. A higher percentage of acrosome reacted sperm has been found in the unattached fraction compared to the bound sperm population (Ellington *et al* 1991, Gutiérrez *et al* 1993). This reflects the ability of OEC to selectively attach intact sperm cells and/or induce sperm capacitation. Thus, a proportion of released capacitated sperm may undergo false acrosome reaction and/or, what is called spontaneous acrosome reaction before they reach the site of fertilization.

Sperm transport in the female reproductive tract and penetration of the oocyte layers depend greatly on sperm motility. Furthermore, motility seems to be a precondition for sperm attachment to OEC since dead sperm do not interact with OEC (J. E. Ellington, personal communication). Development of reliable *in vitro* culture systems for oviductal cells (Joshi 1991, 1988, Walter 1995) has permitted the study of many aspects of sperm-OEC interactions in a system that mimics *in vivo* conditions. A few minutes after addition of a sperm suspension, actively motile sperm cells start to adhere to OEC explants (Lefebvre and Suárez 1996). Most sperm attach by their heads and have active tail movement (Chian and Sirard 1995), only a small proportion makes contact by the tail. Experimental data indicate that oviductal cells stimulate and extend sperm motility, not only through plasma membrane contact between sperm and OEC (Dobrinski *et al* 1997; Ellington *et al* 1998b, Kervancioglu *et al* 1994), but also through soluble products secreted by the cells (Abe *et al* 1995, Boquest *et al* 1999). Activation of sperm hypermotility, which is associated with sperm capacitation, occurs in sperm cocultured with OEC *in vitro* (Ellington *et al* 1993a, Ellington *et al* 1991; Lefebvre and Suárez 1996) and has been directly observed by transillumination in excised mouse oviduct shortly after mating (Suárez 1987). A change from progressive to hyperactivated motility may contribute to sperm release from the oviductal reservoir (Pacey *et al* 1995a).

A reduction or inhibition of sperm motility during the phase of sperm storage has been proposed as a mechanism to extend sperm viability and fertilizing capacity (Suárez *et al* 1990); however, objective studies involving motility patterns of sperm attached to OEC have not been reported. Further investigation of sperm metabolism during oviductal transport may clarify this controversial aspect.

**SPERM SELECTION.** Indirect evidence suggests that sperm attachment to oviductal cells *in vivo* is a prerequisite for fertilization, or at least that spermatozoa released from the reservoir are more likely to fertilize an oocyte. This hypothesis is supported by a study in which only a subpopulation of motile and morphologically normal stallion spermatozoa was attached to oviductal cell monolayers *in vitro* (Thomas *et al* 1994b). In addition, in

humans, a higher proportion of morphologically normal spermatozoa were recovered from oviduct, bursa or peritoneum compared to spermatozoa found in caudal portions of the female reproductive system (Asch 1976, Mortimer *et al* 1982). Electron microscopy studies confirmed that a high percentage of sperm attached to oviductal epithelium had intact acrosomes (Esponda and Moreno 1998, Pollard *et al* 1991). Sperm morphology and motility are not the only criteria for oviductal sperm selection. Ellington *et al* (1999) have studied the chromatin quality of human sperm attached to bovine oviductal cells using the flow cytometric sperm chromatin structure assay (SCSA) (Evenson and Jost 1994). It has been demonstrated that human sperm bound to oviductal cells had higher chromatin quality than unbound sperm (Ellington *et al* 1999a). In conjunction, these data suggest that the oviduct isthmus functions as a screen for selecting a sperm subpopulation for fertilization.

**SPERM FERTILIZING ABILITY.** Perhaps the basic function of sperm storage is to ensure a successful meeting of male and female gametes in the oviduct by maintaining sperm viability and fertilizing ability (Pollard *et al* 1991). Establishment of this reservoir seems to be an important condition for successful fertilization. In the mare and other females with long estrus, a prolonged oviductal storage of sperm is required because fertilization can occur several days after mating (Day 1942). In these species, the ability of the oviductal environment to prolong fertility of sperm is of paramount importance to optimize the reproductive process. Extended sperm longevity in the oviduct may also be advantageous in species which either ovulate a large number of follicles over time or ovulate after estrus. Unlike other farm animals, cattle ovulate around 12 h after the end of the estrus; therefore, sperm are in the female's reproductive tract about 12-30 h before ovulation.

Sperm capacitation and active motility along with an intact acrosome are necessary conditions for successful fertilization. There is evidence that soluble products secreted by oviductal cells and/or direct contact of sperm with oviductal cells can extend sperm fertilizing capacity (Pollard *et al* 1991). In an attempt to mimic *in vivo* conditions, *in vitro* fertilization systems with sperm cocultured with OEC have been used (Chian and Sirard 1995; Pollard *et al* 1991). One of the main drawbacks of these systems is that it is not possible to know the real number of sperm available for fertilization. Chian and Sirard (1995) reported that the number of penetrated oocytes was similar in the coculture system compared to a standard system in which OEC were not present. Unfortunately, in this study the developmental capacity of embryos produced under these conditions was not determined. Further research to study developmental competence of embryos produced in this type of *in vitro* fertilization systems is needed.

## MANIPULATION OF THE SPERM RESERVOIR TO IMPROVE REPRODUCTIVE PERFORMANCE

Considering the central role of the oviduct in gamete transport, fertilization and embryo growth (for review see: Ellington, 1991), it seems plausible that development of methods to control oviductal physiology would be of great value for improving reproductive performance in farm animals. Rabbit and sheep oviducts have been successfully used to grow preimplantation bovine embryos produced *in vitro* from the 1- to 2-cell stage up to the morula/blastocyst stage (Behboodi *et al* 1993, Ellington *et al* 1990, Fukui *et al* 1983, Leibfried-Rutledge *et al* 1987, Trounson *et al* 1977). Moreover, as reviewed by Bavister (1988), many laboratories have reported a beneficial effect of OEC in culture on *in vitro* fertilization (IVF) and *in vitro* embryo development.

Most of our knowledge about OEC-sperm interactions comes from studies in which sperm cells were cocultured with oviductal cells *in vitro* (Chian *et al* 1995, Chian and Sirard 1995, Dobrinski *et al* 1996b, Ellington *et al* 1998a, Ellington *et al* 1991, Lefebvre *et al* 1995, Suárez *et al* 1990). These studies have rendered invaluable information about the molecular basis of sperm-OEC attachment and sperm physiology in coculture with OEC. However, it should be pointed out that OEC cannot thoroughly mimic *in vivo* oviductal conditions (Walter, 1995).

Although our understanding of the molecular basis of sperm-oviductal cell interaction has increased considerably in recent years (Lefebvre *et al* 1997, Suárez 1998, Suárez *et al* 1998), the mechanisms that govern progression of sperm through the oviduct are still poorly understood. Evidence strongly suggests that ovarian steroids play a key role in controlling sperm migration to the site of fertilization in pigs (Hunter *et al* 1999, Hunter 1972, 1995, 1984, 1981). On the other hand, in cattle it has not been possible to establish a clear relationship between sperm build up in the isthmus and the stage of the estrous cycle and hormonal concentration (Lefebvre *et al* 1995). The number of bull sperm attached to oviduct explants was neither dependent on the region of oviduct (i.e., isthmus vs ampulla) nor on the stage of the estrous cycle (Lefebvre *et al* 1995).

Artificial insemination (AI) is a technique extensively used not only in dairy cattle but also in the beef industry. Since standing estrus in cattle is short (15-18 h) and insemination takes place approximately 12 h after estrus detection in the popular AM-PM breeding strategy, a proportion of females is inseminated at the early metestrus. Furthermore, spermatozoa are deposited into the uterus contrasting with vaginal deposition during natural mating. Therefore, under these AI conditions, the mechanisms that control the number of spermatozoa passing from the uterus to the site of fertilization in the oviduct may be less effective or somehow affected. Increased patency of the oviductal lumen after estrus is associated with a drop of

estrogen plasmatic concentrations and, probably, increases of progesterone concentrations. In addition, the ability of sperm to attach to oviductal cells could be altered by molecular changes at the sperm surface caused by the freezing-thawing process (Ellington *et al* 1999b) or changes in the number of sperm binding sites on oviductal epithelial cells. An augmented sperm transport may frequently be the case when the sperm suspension is deposited into the uterus during metestrus. Accordingly, the risk of polyspermic fertilization is increased under these circumstances, and can negatively affect the conception rate and the profitability of the cattle industry. Understanding the mechanisms that regulate sperm entrapment and release from oviductal sperm storage is essential to development of strategies (e.g., hormonal treatments) in order to manipulate sperm transport through the female tract to maximize reproductive efficiency under different management conditions. Of particular interest is the development of methods to improve artificial insemination with low number of spermatozoa to maximize the use of ejaculates of valuable males and sexed semen.

The success of *in vitro* embryo production programs depends almost entirely on the quality of biological material used, e.g. oocytes and sperm. The number of transferable embryos produced in these programs depends on both high quality mature oocytes and adequate numbers of good quality motile spermatozoa. Since males ejaculate a heterogeneous population of sperm, several separation techniques such as swim up and density gradients (e.g., Percoll) have been developed (Bavister 1990, Mortimer 1990). These techniques not only allow for selection of sperm with enhanced motility but may also be used to remove the extender and dead cells (up 50% of total) present in frozen and thawed sperm samples (Bavister, 1990). Potential disadvantages of these techniques are low sperm recovery and risk of both mechanical and chemical damage to sperm (Bavister 1990). Based on the fact that OEC selectively binds a sperm subpopulation with low chromatin damage (Ellington *et al* 1999a) and low morphological abnormalities (Thomas *et al* 1994b), coculture could thus be used to select the best sperm for IVF (Gualtieri and Talevi 2003) and other assisted reproductive techniques including intracytoplasmic sperm injection.

## RESUMEN

En todas las especies estudiadas hasta el presente, los espermatozoides se acumulan en la porción caudal del istmo antes de la ovulación, formando un reservorio de espermatozoides en el oviducto. Luego de la ovulación un número preciso de espermatozoides es liberado desde esta área y ascienden hacia la ampolla del oviducto donde ocurre la fertilización. La evidencia experimental indica que el reservorio de espermatozoides es importante para proveer un número apropiado de espermatozoides en condiciones fisiológicas



óptimas para fertilizar el ovocito. En este artículo presentamos una revisión exhaustiva de las características y funciones del reservorio oviductal de espermatozoides en mamíferos, como así también su manipulación como una herramienta potencial para mejorar la eficiencia reproductiva.

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