In placental (eutherian) mammals, a number of important events take place within the oviduct including the pre-fertilisation maturation of gametes (including sperm storage), sperm-egg interactions, egg activation and early embryonic development. Many of these events involve interactions of glycoconjugates; both on the surface of the gametes and with the secretions of the oviductal epithelium and these have best been studied in eutherian mammals. In marsupials, however, while the oviduct is known to produce the extracellular egg coat, the mucoid layer, that comes to surround the zona pellucida, its role in the maturation of gametes is only now being elucidated, particularly in the oocyte. This review emphasises what is known of the structure and function of the oviduct and its secretions in marsupials and briefly compares it with data from eutherians. In particular, knowledge of oviductal glycoconjugates in the structure of the post-ovulatory oocyte and its vestments around the time of fertilisation in Australian marsupials is outlined.

Key words: Oviduct, Marsupial, Oviductin, Glycoconjugates, Fertilisation

Introduction

In order for normal fertilisation and early embryonic development to occur, there must be an appropriate environment for these processes to occur. The specific molecular interactions that take place in vivo between sperm and eggs and how the microenvironment of the oviduct influences these events are poorly known, even for the most extensively studied eutherian species (for recent reviews see Suarez, 2008; Töpfer-Petersen et al., 2008). Whilst the oviduct provides an optimal environment “in terms of temperature, pH, osmotic pressure, nutrients, oxygen tension and other factors” (Buhi et al., 1997; for other reviews see Hunter, 1988, 2005; Leese, 1988), certain proteins and factors are actively synthesised and secreted which facilitate the events leading up to, and following, fertilisation and early embryonic development (Buhi et al., 1997, 2000; Buhi, 2002). In eutherians many of these events can be studied in vitro, but in Australian marsupials even repeatable in vitro fertilisation has yet to be achieved for even the most extensively studied species, nor is it clear at this stage whether sperm capacitation occurs. While the effect of oviductal secretions on pre-fertilisation maturation of sperm may be important (Sidhu et al., 1999; Mate et al., 2000), the possible role of secreted oviductal proteins on the post-ovulatory maturation of the oocyte has not been investigated. Using the brushtail possum (Trichosurus vulpecula) and fat-tailed dunnart (Sminthopsis crassicaudata), as marsupial models (Breed, 1994, 1996; Chapman and Breed, 2004), we have undertaken a histological and immunocytochemical study on the secretions of the oviduct around the time of ovulation to determine their possible contribution to the ovulated oocyte and the postovulatory egg coats (Menkhorst and Selwood, 2008). Here we review the role of the oviduct in gamete maturation of marsupials, and we propose an active involvement of oviductal glycoconjugates in sperm-egg interactions.

General morphology of the oviduct

Eutherians

The mammalian oviduct is classically divided into three regions: the infundibulum, ampulla and isthmus. The infundibulum, and in particular its finger-like...
processes the fimbriae, is the most distal region and generally lies in close proximity to the ovary and functions in the capture of ovulated oocytes. The isthmus is the most proximal part which passes into the utero-tubal junction and is generally narrow. It is the site for sperm storage prior to ovulation in many mammalian species (for reviews see Suarez, 2001, 2008). The ampulla, which lies between the isthmus and infundibulum, is generally the widest region of the oviduct, at least in eutherians, and is the site where fertilisation takes place (Hunter, 1988).

Marsupials

In marsupials, however, some differences appear to be evident in the gross anatomy of the oviduct compared with those of eutherian mammals (Fig. 1), even though Rodger and Bedford (1982) in their studies of the Virginian opossum *Didelphis virginiana* did not differentiate between regions of the duct into separate morphological regions, except for the fimbriated infundibulum. In dasyurid marsupials, as reflected in the fat-tailed dunnart, it appears that, in contrast to eutherians, the ampulla is the narrower part of the oviduct. Due to its proximity to the ovary, this region has been referred to as the "ovarian segment" (Bedford and Breed, 1994). Each oviductal region displays its own unique ultrastructural and histological features that relate to its biosynthetic and functional properties (Roberts and Breed, 1996a; Buhi et al., 1997).

*Functional histology of the oviduct*

**Eutherians**

The epithelium of the mammalian oviduct consists of two main cell types: the ciliated cells (CC) and non-ciliated secretory cells (NCC) (Fig. 2). The number, height and synthetic activity of these cells is dependant upon the circulating levels of steroid hormones, in particular oestradiol (Hunter, 1988; Leese, 1988). After ovariectomy the oviductal epithelium changes from a normally active simple (or pseudo-stratified) columnar...
epithelium containing the two cell types, to a single-layered cuboidal epithelium consisting of a cell type that appear morphologically different from either the CC or NCC (for reviews see Hunter, 1988; Leese, 1988; Buhi et al., 2000).

In the rabbit, probably the most analogous eutherian to compare with marsupials due to the similar accumulation of a thick mucoid postovulatory egg coat, considerable histological differences occur between the different regions of the oviduct. For example, in the rabbit infundibulum CC are rare, but in the isthmus they increase in abundance where they reach their maximum (Greenwald, 1958; Jansen and Bajpai, 1982). The NCC of the rabbit synthesise and store mucin in response to oestrogen, while progesterone causes the mucin to be discharges where it comes to surround the ovulated oocytes as a mucoid coat (Greenwald, 1958).

Marsupials

Marsupial ovulated oocytes also become surrounded by a mucoid coat that varies in thickness across the various species (see Selwood, 2000; Menkhorst and Selwood, 2008). Unlike in rabbits, however, the deposition of this component of the postovulatory egg coat around marsupial oocytes occurs very rapidly after ovulation (and entry of oocytes into the oviduct), whereas in the rabbit mucin is not released until 10-12 hours after ovulation (Greenwald, 1958). These temporal differences may reflect the difference in transit of the eggs through the oviducts, which is very short in marsupials (between 4-7 h: Menkhorst et al., 2007; up to 15-20 h: Rodger and Bedford, 1982), but takes 3-4 days in rabbits (Harper, 1982). Like eutherians, marsupial oviducts also display morphological and ultrastructural changes in response to change in levels of steroid hormones (Arnold and Shorey, 1985; Roberts and Breed, 1996a).

A specialised and localised regional epithelium of the marsupial oviduct, at least in the dasyurids and didelphids, lines the mucosal crypts of the isthmus. These small out-pockets are connected to the oviductal lumen by narrow openings and are lined by cuboidal, or low-columnar, epithelium of characteristic morphological appearance (Rodger and Bedford, 1982; Breed et al., 1989; Taggart and Temple-Smith, 1991; Roberts et al., 1994; Taggart, 1994; Taggart et al., 1998). In eutherians, similar isthmic crypts have been described for species of the shrews, where there appear to be deep isthmic crypts in the genus Crocidura, while ciliated ampullary crypts appear to exist in the genera Blarina, Cryptotis, Sorex, and Myosorex (Bedford, 1996; Bedford et al., 1998, 2004). In marsupials, the isthmic crypts, where they occur, act as sperm storage sites with some of the sperm that pass through the uterotubal junction coming to reside deep within them from the time of mating until ovulation which can be up to 15 days post-coitus (Hill and O’Donoghue, 1913; Woolley, 1966; Selwood, 1980).

Oviduct-specific glycoproteins

Biosynthesis of oviduct-specific glycoproteins

Eutherians

The oviduct is a highly synthetically active structure with many proteins being synthesised and secreted by its epithelium, while many components that enter the lumen are also derived from the serum as a transudate, including albumin and transferrin (Hunter, 1988, 2005). Proteins that are synthesised by the epithelial cells include oviduct-specific glycoproteins (OGP), which have been found in a number of mammalian species and are collectively termed "oviductins" (Bleau and St-Jacques, 1989; Malette et al., 1995). These are a secreted form of mucins (Muc9) (Paquette et al., 1995; Hendrix et al., 2001). Many of the proteins that are secreted have been found to be stage- and/or region-specific, and are thus only secreted at a specific time of oestrous cycle and/or in a localised region of the oviduct (for review see Gandolfi, 1995).

The ampulla appears to be the major source of OPG in many species including laboratory mice (Kapur and Johnson, 1986, 1988), rabbits (Hyde and Black, 1986), pigs (Buhi et al., 1989, 1990, 1992), sheep (Murray, 1993) and cows (Wegner and Killian, 1992), whereas in the hamster, the OGP is produced by the NCC along the entire length of the oviduct (Roux and Kan, 1995; Martoglio and Kan, 1996), but predominantly by cells in the isthmus (Abe and Oikawa, 1990a,b, 1991). The isthmus is also responsible for the secretion of an OGP in the baboon (Verhage et al., 1990).

The OGP of the pig (Buhi et al., 1996), cow (Sendai et al., 1994), baboon (Donnelly et al., 1991), human (Arias et al., 1994), macaque (Verhage et al., 1997a), hamster (Suzuki et al., 1995), mouse (Sendai et al., 1995) and rabbit (Yong et al., 2002) have been partially, or completely, sequenced. Comparisons between species of the deduced amino acid sequences show a high level of conservation amongst mammalian OGP. For example, the amino acid sequence of the rhesus macaque OGP is 98% homologous to that of the olive baboon and 92% homologous to the human OGP (Verhage et al., 1997b). Less closely related species, however, display only slightly less homology with the protein sequence of porcine OGP exhibiting a significant identity (65-78%) and similarity (78-87%) to OGPs from the cow, sheep, human, mouse and hamster (Buhi et al., 1996). Apart from eutherian mammals, OGP have also been found to be present in the oviducts of amphibians (Lindsay et al., 1999) and chickens (Mann, 2008). This suggests that these glycoproteins are also likely to be present in marsupials. A search of the recently published opossum (Monodelphis domestica) genome on the Ensembl database (http://www.ensembl.com) using the mouse OGP gene sequence (termed Ogvp1: ENSMUSG000000074340) as a template found a putative opossum OGP gene homologue.
(EMSMODG00000001338) that shared 57% homology to the lab mouse sequence, around 60% identity to the human, chimpanzee, orangutan, and macaque sequences, and 63% identity to the platypus sequence. The putative opossum OGP gene is located on chromosome 2 and may transcribe a precursor peptide of 368 aa of around 42kDa with one potential N-linked glycosylation site (position 272).

Apart from divergence in protein sequences of mammalian OPGs, much interspecific variation in the structure of these molecules may be due to differences in their carbohydrate components. The number of potential N-linked glycosylation sites in OPGs, as deduced from amino acid sequence data, varies widely between species ranging from only 1 in the rabbit, cow, baboon and macaque to 8 in the golden hamster. Lectin-binding experiments have demonstrated that oligosaccharides of the OGP of the mouse, cow and hamster are WGA-reactive providing evidence of terminal α-D-NeuNAc (sialic acid) and/or non-terminal β-D-(GlcNAc)₂ residues (Malette and Bleau, 1993), whereas the cow OGP contains Gal(β1-3)GalNAc residues, thus indicating that the 1 potential N-linked glycosylation site deduced from its amino acid sequence is indeed utilised (Wegner and Killian, 1992). Nevertheless, potential N-glycosylation sites do not always reflect the N-linked glycosylation of the mature protein. As indicated above, the hamster OGP has 8 potential N-linked glycosylation sites but in vitro metabolic labelling studies suggest the presence of only one or two N-glycan chains of around 10kDa (Malette and Bleau, 1993). The mature post-translated hamster OGP, however, appears to be differentially glycosylated dependent upon the stage of the oestrous cycle and the utilisation of potential N-linked glycosylation sites may vary (McBride et al., 2004).

Marsupials

Most of the work on oviductal secretions of marsupials have been derived from investigations into the origin and composition of the postovulatory egg coats (Roberts et al., 1994, 1997; Roberts and Breed, 1996a,b; Casey et al., 2002; Menkhorst and Selwood, 2008). Histologically, it has been determined that in the brushtail possum and fat-tailed dunnart, the mucoid material secreted by the epithelium of the oviduct is a sulphated and acidic glycoprotein as demonstrated by positive staining with Alcan blue, pH 1.0 - pH 2.5 and Periodic Acid-Schiff’s Reagent (see Hughes, 1977). Histochemistry using fluorescently conjugated lectins has shown that there is a similarity in the binding of lectins between the ampullary and isthmic oviduct epithelial cells of the possum, with the NCC of both these regions reacting positively to seven out of the nine lectins used (see Table 1). The greatest intensity of binding in the NCC was found to be for β-Galactose (β-Gal) and N-acetylgalactosamine (GlcNAc) and/or sialic acid indicating that these glycoconjugates are major constituents of the oviductal secretions in this species.

The largest regional differences in lectin staining was with soybean agglutinin (SBA), which is specific for α-D-GalNAc/α-D-Gal residues. Fluorescence was found to occur in the secretory cells of the ampulla but not elsewhere. This difference in binding was quantified using lectin immunocytochemistry with significantly more gold labelling with SBA on the secretory granules of the ampulla than in the isthmus (Fig. 3; Table 2). Greater density of gold labelling of the secretory granules within the ampulla was also found for Pisum sativum agglutinin (PSA) suggesting differential

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Table 1. Intensity of lectin binding of non-ciliated secretory (NCC) and ciliated (CC) epithelial cells from the oviduct of the peri-ovulatory brushtail possum.

<table>
<thead>
<tr>
<th>Lectin and sugar specificity</th>
<th>Ampulla</th>
<th>Isthmus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCC</td>
<td>CC</td>
</tr>
<tr>
<td>PNA: βGal-(1-3)-GalNAc</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>ECA: βGal-(1-4)-GlNAc</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Con A: α-D-Man, α-D-Glc</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LCA: α-D-Man, α-D-Glc</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RCA-I: β-D-Gal</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>WGA: [β-(1-4)-D-GlcNAc]₂, NeuNAc</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SNA: α-D-GalNAc,(2-6)-Gal/GalNAc</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>SBA: α-D-GalNAc, α-D-Gal</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>LTA: α-L-Fuc</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Fluorescence was qualitatively scored as: -, negative; +, mild; ++, strong; +++, intense. PNA: peanut agglutinin; ECA: Erythrina cristagalli agglutinin; Con A: Concanavalina ensiformis agglutinin; LCA: Lens culinaris agglutinin; RCA-I: Ricinus communis-I agglutinin; WGA: wheat germ agglutinin; SNA: Sambucus nigra agglutinin; SBA: soybean agglutinin; LTA: Lotus tetragonolobus agglutinin. Fuc: fucose; Gal: galactose; GalNAc: N-acetylgalactosamine; Glc: glucose; GlcNAc: N-acetylglucosamine; Man: mannose; NeuNAc: N-acetyleneuraminic acid (sialic acid). From Wu et al., 1988.

Table 2. Gold labelling density of secretory granules of the NCC in the ampulla and isthmus of the brushtail possum of the peri-ovulatory oviduct.

<table>
<thead>
<tr>
<th>Lectin and sugar specificity</th>
<th>Ampulla</th>
<th>Isthmus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSA: α-D-Man, α-D-Glc</td>
<td>21.8±1.11</td>
<td>9.9±0.59³</td>
</tr>
<tr>
<td>RCA-I: β-D-Gal</td>
<td>51.7±3.12</td>
<td>55.0±3.93</td>
</tr>
<tr>
<td>WGA: [β-(1-4)-D-GlcNAc]₂, NeuNAc</td>
<td>23.4±2.89</td>
<td>39.6±3.47</td>
</tr>
<tr>
<td>SBA: α-D-GalNAc, α-D-Gal</td>
<td>95.3±8.03</td>
<td>0.7±0.22³</td>
</tr>
</tbody>
</table>

Values are shown as the number of gold particles per secretory granule; mean values ± SEM. PSA: Pisum sativum agglutinin; RCA-I: Ricinus communis-I agglutinin; WGA: wheat germ agglutinin; SBA: soybean agglutinin. a: The labelling densities of secretory granules using these lectins was significantly less in the isthmic NCC than in the ampullary NCC (p<0.001); b: The labelling densities of secretory granules using this lectin was significantly less in the ampullary NCC than in the isthmic NCC (p<0.001). Gal: galactose; GalNAc: N-acetylgalactosamine; Glc: glucose; GlcNAc: N-acetyleglycosaminic acid (sialic acid). From Wu et al., 1988.
Fig. 3. Lectin histochemistry (A, B) and immunocytochemistry (C, D) of the ampullary (A, C) and isthmic (B, D) oviductal epithelium from the brushtail possum stained with soybean agglutinin (SBA). Note that the apical regions of the secretory cells of the ampullary epithelium (E) are labelled with FITC-SBA (white arrows, A), while the secretory granules (black arrow, C) of the ampullary secretory cells are heavily labelled with gold particles. The secretory cells (B) and secretory granules (black arrow, D) of the isthmus remain unstained. Some fluorescence of erythrocytes can be seen within the lamina propria (LP) (A, B); lumen (L). Scale bars: A, B, 45 µm; C, D, 0.5 µm.
localisation of α-D-Mannose residues, while significantly greater labelling of GlcNAc and/or sialic acid was found with wheat germ agglutinin (WGA) in the isthmus compared to ampullary secretory granules (p<0.05). Only β-Gal had a similar labelling intensity in the secretory granules of the ampulla and isthmus. As in eutherians (for review see Abe, 1996), these differences probably reflect the regional differences in function of the duct epithelium.

**Functions of oviduct-specific glycoproteins**

**Eutherians**

Formation of a sperm reservoir within the oviduct is thought to be brought about by binding of spermatozoa to the glycoconjugates on the surface of the isthmic epithelial cells (Demott et al., 1995; Lefebvre et al., 1997; Suarez, 1998, 2001, 2002, 2008; Suarez et al., 1998; Green et al., 2001; Talevi and Guaitieri, 2001; Wagner et al., 2002; Hunter, 2008). This binding appears to reduce the incidence of polyspermy by selectively releasing limited numbers of sperm around the time of ovulation (Hunter, 1973). Binding to the oviduct epithelium may also act to maintain sperm viability (Pollard et al., 1991; Smith and Nothnick, 1997), as well as forming a selective barrier to physiologically 'abnormal' sperm (e.g. those sperm that have undergone premature capacitation and acrosome reaction) (Fazeli et al., 1999). Sperm, in turn, influence the biosynthetic activities of the oviduct, with the finding that oviduct epithelial cells modify their gene and protein synthetic activity following interaction with sperm (Fazeli et al., 2004; Georgiou et al., 2005).

OGP also facilitate pre-fertilisation maturation of ovulated oocytes. For example, oviductal exposure is commonly associated with an increase in penetrability of zonae pellucidae and fertilisability of oocytes (Boatman et al., 1999; King et al., 1994; Kito and Bavister, 1996), although in the laboratory mouse it does not appear to be essential for *in vivo* fertilisation (Araki et al., 2003). OGP have been localized to the membranes and/or perivitelline spaces of oocytes, eggs, embryos and their vestments in a number of species (for reviews see Bleau and St.-Jacques, 1989; Malette et al., 1995; Buhi et al., 2000). This binding of OGP to the gametes and embryos is thought to be facilitated through an OGP-binding protein that is similar in structure to non-muscle myosin heavy chain (Kadom et al., 2006).

In eutherians, carbohydrates play an important role in the events leading up to sperm-egg interactions at fertilisation (for reviews see Benoff, 1997; Shalgi and Raz, 1997; Tulsiani et al., 1997; Dell et al., 1999; Topfer-Petersen, 1999; Topfer-Petersen et al., 2008; Jiménez-Movilla et al., 2009). Once sperm migrate to the site of fertilisation, initial binding of sperm to the zona pellucida (ZP) is generally thought to take place by way of the ZP glycoconjugates, while the high degree of homology of the ZP genes and proteins between eutherian species means that it is likely the oligosaccharide components of the glycoproteins may be responsible for a high level of specificity of this interaction, if this occurs (Florman and Wassarman, 1985; Wassarman, 1988, 1990, 1995; Blei and Wassarman, 1988; Thaler and Cardullo, 1996; Ozgur et al., 1998; Takasaki et al., 1999; Litscher and Wassarman, 2007).

**Marsupials**

In marsupials, the role of the oviduct secretions in facilitating gamete maturation and interaction is not known, although it is clear that the oviduct is responsible for the production of at least one of the postovulatory egg coats, that of the mucoid layer (for reviews see Hughes, 1977; Breed, 1996; Selwood, 2000; Menkhorst and Selwood, 2008). While Selwood (2000) has suggested that the early deposition of mucoid might enhance sperm-ZP binding, others have inferred the opposite with suggestions that the mucoid layer reduces chances of polyspermy due to its relatively rapid acquisition and the observation of sperm trapped within it as the ovulated oocyte passes along the oviduct (Rodger and Bedford, 1982; Selwood, 1982; Breed, 1994).

In the brushtail possum proteins from the oviduct epithelium bind to the cell membrane of the sperm, and appear to maintain their viability as well as increasing their ability to undergo capacitation, binding and penetration of the ZP (Sidhu et al., 1998, 1999; Mate et al., 2000). In addition, the oviduct epithelium may give rise to the material that accumulates within the perivitelline space of the ovulated oocyte (Chapman and Breed, 2004). Previously described as a "cortical granule envelope" that accumulated following oocyte activation (Dandecker et al., 1995), the ruthenium red stained material within the perivitelline space (known as the perivitelline space matrix) has been shown to arise, not from cortical granule exocytosis, but following transit in the oviduct (Chapman and Breed, 2004). This is supported by comparing lectin immunocytochemistry of preovulatory and postovulatory egg coats of brushtail possum oocytes (see Fig. 4) where it was found that lectin gold labelling with three of the four lectins used (those of PSA, SBA and *Ricinus communis* I agglutinin [RCA-I]) of the ZP around ovarian oocytes was much lower than that of ovulated oocytes (Fig. 5). Only WGA, specific for GlcNAc and/or sialic acid, did not show a statistical significant difference in labelling. These results demonstrate that the mucoid coat surrounding the ovulated oocytes has a greater density of labelling for GlcNAc and β-Gal, and less for α-D-GalNAc/α-D-Gal and α-D-Mannose. Labelling was also evident on the microvilli and within the perivitelline space of the ovulated, but not ovarian, oocytes.

One possibility for the major increase in labelling of glycoconjugates within the ZP following ovulation is that some kind of biochemical change has taken place
which involves a rearrangement of glycoconjugates coinciding with the morphological change of the ZP following ovulation (Breed et al., 2002). Alternatively, some kind of oocyte/cumulus-specific modification may have occurred that adds to, or exposes, previously masked glycoconjugates, or glycoconjugates that had previously been inaccessible to the lectins. Reactivity to lectins has been shown to increase in ovarian ZP following removal of masking agents such as sialic acid with the use of the enzyme neuraminidase, and O-acetyl groups on sialic acids, through saponification with potassium hydroxide (Chapman et al., 2000). Neuraminidase is present, and appears to play a role in fertility, within the oviducts of non-mammalian species such as toads (De Martinez and Olavarria, 1973; De Martinez et al., 1975; Vitaio et al., 1990). It has also been suggested that in the human, the cumulus-coronal cells may secrete a factor that increases the penetrability of the ZP (Tesarik et al., 1988), while removal of sialic acid from the surface of sperm and ZP increases sperm-ZP binding (Lassalle and Testart, 1994; Banjeree and Chowdhury, 1997; Ozgur et al., 1998). Whether a similar modification occurs to the ZP following ovulation in the brushtail possum is unknown.

Another possibility is for an increase in glycoconjugate content of the possum ZP following ovulation as a result of an incorporation of OGP as demonstrated in a number of eutherian species, including the hamster (Kan et al., 1988, 1990), pig (Buhi et al., 1993), sheep (Gandolfi et al., 1991), cow (Staros and Killian, 1998) and baboon (Boice et al., 1990). In the hamster, early investigations found that the oviduct contributed a glycoprotein, termed hamster oviductin 1 (Hm OV-1), to the post-ovulatory ZP (Kan et al., 1988, 1989), and that its contribution resulted in the addition of GalNAC residues, not previously identified in the ovarian ZP but localised to the secretory granules of the ampulla (Kan et al., 1990). Similarly in the possum, α-D-GalNAc/α-D-Gal residues, which are labelled at very low levels in the ZP surrounding ovarian oocytes, were found to significantly increase in labelling following ovulation. These glycoconjugates, similar to those in the hamster, were localised to the secretory granules of the ampulla, but not the isthmus, and only appeared to be minor contributors to the mucoid coat. These findings suggest that α-D-GalNAc/α-D-Gal residues, originating from the oviducts in marsupials

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**Fig. 4.** Density of gold labelling of the zona pellucida of the ovarian (Ov) and ovulated (Tubal) oocytes and mucoid coats (Mucoid) of the brushtail possum following incubation in four different lectins. The labelling densities are shown as the mean number of gold particles/µm² ± SEM. Asterisks (*) represent significant differences (p<0.05) in gold labeling of the same lectin from the previous stage of development. PSA: *Pisum sativum* agglutinin; RCA-I: *Ricinus communis*-I agglutinin; WGA: wheat germ agglutinin; SBA: soybean agglutinin.

**Fig. 5.** Zona pellucida of the peri-ovulatory ovarian (A) and ovulated (B) oocyte of the brushtail possum incubated with biotinylated-*Pisum sativum* (garden pea) lectin (biotin-PSA) and labelled with 10nm gold-conjugated goat anti-biotin antibody. Note sparse gold labelling of the zona pellucida (ZP) surrounding the peri-ovulatory oocyte (Oo) (A) but very dense labelling of the zona surrounding the ovulated oocyte (B) that may be localised to the zona pellucida filaments (arrow). Labelling is also evident on the microvilli (Mv) of the ovulated oocyte (B); corona radiata cell (Cr); perivitelline space (PVS). PSA binding is specific for α-D-Mannose. Scale bar: 1 µm.
from the ampullary region of the oviduct and transferred to the ZP after ovulation, may play a role in sperm-zona pellucida interactions in this species.

Likewise, labelling with PSA for $\alpha$-D-Mannose, also showed similar modifications (Fig. 5). Taking the lectin immuno-gold cytochemistry results of the oviduct, ZP and mucoid coat together, a hypothetical summary of the effect of duct epithelium secretory activity on the ovulated oocytes is presented (Fig. 6). The recently ovulated oocyte first enters the oviduct via the infundibulum and reaches the ampulla where fertilisation takes place (Selwood, 1982; Bedford and Breed, 1994; Jungnickel et al., 2000). This is also the site for the initial deposition of the mucoid coat, and any influence of the oviduct secretions on gamete interaction, either positive or negative, is likely to occur within this region. Within the ampulla, therefore, $\alpha$-D-GalNAc (and/or $\alpha$-D-Gal) and $\alpha$-D-Man appear to have the greatest effect on the post-ovulatory ZP (Fig. 6).

Furthermore, the localisation of lectin labelling to the material within the perivitelline space and on the microvilli of the oolemma, in conjunction with the findings of Chapman and Breed (2004), provide further evidence for the incorporation of oviductal glycoproteins into these areas. The exact role of these incorporated glycoproteins is unknown, but, the inability to achieve sperm-oolemma binding in vitro following pre-incubation of sperm, but not follicular oocytes, with oviduct-conditioned media suggests that they may play a role in sperm-egg fusion (Mate et al., 2000). This is further supported by recent findings that intracytoplasmic sperm injection can induce fertilisation and early embryonic development (Magarey and Mate, 2003; Richings et al., 2004) suggesting perhaps that the inability to achieve successful in vitro fertilisation may be due to an absence of an oviductal influence at the level of the oolemma.

**Concluding remarks**

The present brief review provides some evidence that secretions of the oviduct affect gamete maturation and interaction in Australian marsupials. The secretions of the oviduct not only appear to be necessary for successful prefertilisation maturation of spermatozoa and formation of the mucoid coat around the ovulated oocyte, but also for the composition of the post-ovulatory ZP to facilitate sperm binding. In addition, material also appears to accumulate within the perivitelline space. The regional variation of glycosylation of the oviductal secretions in marsupials suggests that there is indeed a complex interaction between the oviduct and gametes. While it has been previously postulated that some variation may exist for the secretion of the mucoid layer between the ampulla and isthmus (Roberts and Breed, 1996a), such variation in terms of the specific glycosylation patterns of the oviductal glycoproteins and their possible contribution to the oocyte and the post-ovulatory ZP in marsupials is suggested by these observations.

**References**


Oviducts in marsupials


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