Journal of Reproduction and Fertility Supplement **49**, 3–13

Oviduct proteins in fertilization and early embryo development

C. D. Nancarrow and J. L. Hill

CSIRO Division of Animal Production, Cunies Ross Street, Prospect, NSW, Locked Bag 1, Delivery Centre, Blacktown, 2148, Australia

The oviduct controls the environment in which the gametes are transported and fuse, and in which embryonic development begins. The ultrastructural topography of the ampulla and isthmus is similar, consisting of ciliated and secretory cells, but a different array of proteins is secreted by each segment along with various serum components. Amino acids are selectively secreted by the oviduct; these amino acids probably interact with the gametes or embryo to facilitate the processes of fertilization and development. An oviduct-specific glycoprotein is synthesized by the ampulla of sheep and cattle in response to oestrogen and secreted mainly from day −1 to day 3 of the ovarian cycle. This oestrus-associated glycoprotein (EGP) has a variable molecular mass of 80–97 kDa and a pI value ranging from 4.7 to 5.5. The bovine (b) and ovine (o) EGP genes are 95.5% identical and consist of 1560 base pairs encoding 519 amino acids containing one *N*-linked and several *O*-linked glycosylation sites. The terminal glycosides are *N*-acetylglucosamine and galactose-*N*-acetylgalactosamine for bEGP, and fucose, galactose and sialic acid residues are also identified for oEGP. EGP binds to zona pellucida and blastomere membranes, but evidence for EGP binding to sperm membranes is equivocal. After *in vitro* fertilization the proportion of sheep oocytes cleaving was increased in the presence of oEGP, but when single-cell embryos were cultured with oEGP, these cleavage rates were reduced. In addition, consistent positive effects of oEGP were observed on blastocyst formation. Elaboration of the mechanism of synthesis of EGP, its action and its role in fertilization and embryo development is important for our understanding of the events of early pregnancy.

Introduction

The ruminant oviduct is essential for gamete transport, fertilization and embryo transport and provides an environment for the early development of embryos. Apart from transport and the provision of a medium for culture *in vivo*, it is still uncertain whether any specific components secreted by the oviduct are essential for fertilization and early embryo development. The strongest candidates for the latter function are vitamins, amino acids, growth factors and proteins. Both vitamins and amino acids are supplied in conventional culture media or in serum additives to these media and when such artificial media are used in oocyte maturation *in vitro*, fertilization and culture, resultant embryos can implant in surrogate dams and give rise to offspring. This fact indicates that the provision of growth factors and specific proteins by the oviduct is not obligatory to fertilization and embryo development. However, the occurrence of extended gestation duration, increased lambing masses and lamb mortality (Walker *et al.*, 1992) associated with cultured or micromanipulated embryos does suggest that there may be facilitative actions of oviductal components and that these components should be included in culture media for optimal development.

Table 1. Characteristics of major secretory glycoproteins of the ruminant oviduct

kDa	pI	Source	Secretory response	Other properties	References
Sheep					
80–90	4.7	Fluid	Day −1 to day 6 To oestrogen *in vivo*	Glycosides: fucose, galactose, *N*-acetylgalactosamine	Sutton *et al.* (1984a, 1985, 1986)
92	5.0	Epithelial cells ampulla	Days 0–5	Bound to zona and blastomeres Immunochemical localization to secretory granules of epithelial cells	Gandolfi *et al.* (1989, 1991)
90–92	7.5 8.5	Explants ampulla	To oestrogen *in vivo*	2 Proteins	Buhi *et al.* (1991)
90		Flushings	To oestrogen *in vivo*	Progesterone modulated	Murray (1992, 1993)
92		Explants ampulla		2 Proteins	
		Fluid	Day −1 to day 3	Secretion throughout cycle	J.L. Hill and C.D. Nancarrow (unpublished data)
		Explants ampulla		Immunocytochemical location throughout secretory cell	
Cattle					
85–97	5.0	Explants	Day 0		Malayer *et al.* (1988)
97	5.5–8.1	Flushings Explants	Oestrus	Present throughout cycle 2 Proteins Immunocytochemical localization to secretory granules of epithelial cells	Boice *et al.* (1990)
80–95		Fluid	Oestrus		Gerena and Killian (1990)
97	4.7	Explants ampulla	Oestrus	Glycosides: *N*-acetylgalactosamine *N*-acetylglucosamine 2 Proteins	Wegner and Killian (1992)
85–97		Flushings	Follicular phase	Immunocytochemical localization in ampullary and fimbrial secretory cells	Abe *et al.* (1993a, b)
Various		Epithelial cells Flushings	Not recorded	Generalised polyclonal antiserum used Cells attracted immunocytochemical staining	Joshi (1988)

Reproduced with permission from Salamonsen and Nancarrow (1994).

Several proteins have been identified as oviduct specific and the oestrus-associated glycoprotein first identified in sheep (oEGP) by Sutton *et al.* (1984a) and a similar glycoprotein described in cattle (bEGP) by Malayer *et al.* (1988) have been studied most thoroughly. Humans, baboons and pigs secrete similar proteins and these, together with oEGP and bEGP, represent a group with potentially similar function (Salamonsen and Nancarrow, 1994). While this review will present current knowledge about the biology of EGP in ruminants, we do not wish to overlook the possible developmental importance of growth factors and their receptors, which may be provided by the oviduct or the developing embryo (Watson *et al.*, 1994a). Demonstrations that amino acids are secreted in oviduct fluid at quite different concentrations from those in plasma (Nancarrow *et al.*, 1992) and that amino acids together with bovine serum albumin provide an exceptional culture medium (Moore and Bondioli, 1993; Gardner *et al.*, 1994; Walker *et al.*, 1995) provide strong evidence for the contention that the oviduct maintains an optimal environment for fertilization and embryo development.

Synthesis and Secretion of EGP

The secretion of EGP has been the subject of several studies using different techniques and sources of oviduct proteins (Table 1). Initial studies reported a molecular mass of 80–90 kDa for oEGP in ovine oviductal fluid (Sutton *et al.*, 1984a) when analysed on SDS-PAGE gels and 92 kDa (Gandolfi *et al.*, 1989) for ^{35}S-labelled oEGP produced by oviduct epithelial cells *in vitro*. We found at that time that oEGP was present in the oviduct lumen as a very large conglomerate or multimer, able to be reduced to its monomeric form by reduction with mercaptoethanol, and proposed that it binds to ova whereby it facilitates fertilization and embryo development (Sutton *et al.*, 1984b). oEGP is excluded from most fractionating gels but Sephacryl® S-400 (Pharmacia LKB, Uppsala) with an exclusion of 8000 kDa has been used to demonstrate that the native M_r of the protein is about 4000 kDa. The broad, diffuse M_r range following denaturing electrophoresis was attributed to variability in the pattern of glycosylation (Sutton *et al.*, 1984a). *In vitro* incorporation studies have suggested the presence of two oestrus-associated glycoproteins in ewes, one with an M_r of 90 kDa and the other of 92 kDa (Buhi *et al.*, 1991), although only one protein of 90–92 kDa was found by Murray (1992, 1993). Several studies in cattle support secretion of a pair of proteins (Table 1).

The protein of cattle has a slightly greater molecular mass (80–97 kDa) but a similar acidic pI value to that of oEGP (Table 1). For sheep and cattle, the greatest concentration of EGP was found in oviduct fluid collected at or just after oestrus (Sutton *et al.*, 1984a; Gerena and Killian, 1990) or in conditioned media from cultures of epithelial cells (Gandolfi *et al.*, 1989) or tissue explants (Malayer *et al.*, 1988; Boice *et al.*, 1990; Buhi *et al.*, 1991; Murray, 1992; Wegner and Killian, 1992) taken on day 0 of the cycle or after oestrogen induction of ovariectomized animals.

When oviductal fluid was sampled daily during the ovarian cycle and oEGP detected using a monoclonal antibody (MAC 264) raised against the culture-derived oEGP (sOP 92: Gandolfi *et al.*, 1989), it became obvious that oEGP is secreted throughout, but in a cyclic manner (J. L. Hill and C. D. Nancarrow, unpublished). A western blot gave similar patterns when the monoclonal antibody or soy bean agglutinin was used in detection (Fig. 1). The latter recognizes both terminal galactosyl (Gal) and *N*-acetylgalactosamine (Gal*N*Ac) residues on glycoproteins. This study confirmed the previous findings and showed that the concentration of oEGP in oviduct fluid is much higher during the active secretory phase (days −1 to 3).

Analysis of conditioned medium from whole explant cultures showed that EGP is secreted by the ampullary region of the oviduct in sheep and cattle (Table 1). In some experiments, the infundibulum of sheep and cows was shown to contain or synthesize EGP (Murray, 1992; Abe *et al.*, 1993b). Hollis *et al.* (1984) suggested that the secretory granules that accumulate in the secretory cells toward oestrus are the source of oEGP but could not clearly establish a secretory pattern for these granules. Gandolfi *et al.* (1991), in sheep, and Boice *et al.* (1990) and Abe *et al.* (1993b), in cattle, demonstrated an accumulation of EGP-specific immunogold particles over these granules. Immunofluorescent studies on sections of oviducts taken from cyclic ewes demonstrated that the antigen is present in the secretory cells of the ampulla throughout the cycle (Fig. 2) and is prominent in apical protrusions of these cells just before

available to glycoproteins leading to considerable functional specificity and the resultant diversity of differentiation of organisms (see review by Varki, 1993). A few glycoproteins constitute the zona pellucida and others are present on sperm surfaces and take part in sperm–egg binding mechanisms perhaps associated with the glycosyltransferases (Shur and Hall, 1986; Wassarman, 1994). Oviductal glycoproteins such as EGP may therefore interact and be involved in the process of fertilization in mammals.

Terminal glycosides are identified by specific lectin binding to proteins transferred to nitrocellulose membranes after SDS-PAGE electrophoresis. Glycosides of oEGP were initially described as Gal, Gal*N*Ac, fucose and the dimeric galactosyl (β1,3)*N*-acetylgalactosamine (Gal-Gal*N*Ac) (Sutton *et al.*, 1985). Although wheat germ agglutinin (WGA) bound to oEGP in this experiment, this does not constitute irrefutable proof of identity of the sugar moieties as it could not be completely displaced by 0.2 mol Glc*N*Ac l^{-1}. Glucose, mannose and sialic (*N*-acetylneuraminic) acid were not detected. We have now found that a combination of 0.4 mol Glc*N*Ac l^{-1} and 0.2 mol sialic acid l^{-1} inhibits binding of WGA, suggesting that both of these glycosides are represented on oEGP. Gal-Gal*N*Ac and Glc*N*Ac were reported to be present in bEGP, and WGA binding was completely inhibited by 0.5 mol Glc*N*Ac l^{-1} (Wegner and Killian, 1992). Perhaps this indicates species differences in the function of ovine and bovine EGP. Murray (1993) found that labelled Glc*N*Ac incorporation in cultured ampullary explants was decreased, or inhibited, in explants taken during the progestational phase and suggested that there is endocrine control over glycosylation of the core protein. This requires further investigation.

The need to characterize these terminal glycosides is important for an understanding of how EGP acts and what form of EGP is best to use in *in vitro* fertilization and *in vitro* culture media if a positive effect on fertilization and embryo development can be demonstrated. A source of EGP will be needed and this could be provided through genetic engineering. Glycosylation patterns of recombinant glycoproteins are unpredictable, but there are specific interactions between the type of glycosylation pattern favoured by the host cell, the glycotype, and the individual polypeptide (Warren, 1993). However, modifications to established glycosylation patterns usually reduce bioactivity *in vivo*. These aspects need to be considered when production of oEGP is directed by transfection of recombinant genes into cultured mammalian cells and production of transgenic animals.

Molecular Characterization of EGP

Molecular cloning techniques are necessary for the production of sufficient quantities of purified oEGP to determine whether it plays a role in fertilization or embryo growth and subsequent development. There are questions that relate to the action of oestrogen in initiating the sequence of events in the synthesis of oEGP (Salamonsen and Nancarrow, 1994) that could be answered using molecular probes. The gene sequence for the bEGP has recently been published (Sendai *et al.*, 1994) and we have used this information to initiate cloning procedures for oEGP. The bovine sequence consists of 1612 base pairs (bp) coding for a protein with 537 amino acids. However, the first 18 amino acids of the amino-terminal end represent a partial signal sequence, the core protein consisting of 519 amino acids with an estimated molecular mass of 57.684 kDa. The human glycoprotein in contrast has a higher molecular mass and its open reading frame is 1962 bp encoding 633 amino acids with a predicted molecular mass of 70.5 kDa (Arias *et al.*, 1994). Bovine EGP contains one *N*-glycan site and numerous potential *O*-glycan sites; Arias *et al.* (1994) found four potential *N*-glycan sites for the human protein. Hence the increase in molecular mass of the glycoprotein from 95 to 120 kDa between humans and cows appears to be attributable to the larger molecular mass of the core protein (57.7 to 70.5 kDa) and to an increase in *N*-glycosylation.

There is high sequence similarity between these proteins, accounting for the similar physiological characteristics so far described. The 3′ cDNA end of the human sequence exhibits 92% identity with 1195 nucleotides from the partial cDNA sequence of baboons (Donnelly *et al.*, 1991) and a 91% amino acid identity. Sequence similarity of the 534 amino acids of humans and cows was less at 72.5%, whereas that between cows and baboons was 71% (Sendai *et al.*, 1994). Identity between the 1560 bp of the oEGP gene and the bEGP sequence was 95.5% (J. T. A. Marshall, A. G. Brownlee and C. D. Nancarrow, unpublished).

Preliminary investigation of expression of the genes using probes for mRNA have confirmed that both the bovine and baboon genes are responsive to oestrogen (Sendai *et al.*, 1994; Arias *et al.*, 1994). These authors reported that, in progesterone-dominated tissues taken from the dioestrous phase of the bovine cycle or early follicular and postpartum stages of baboons, amounts of mRNA were lower. This may reflect the lack of oestrogen at these stages rather than a suppressive effect on expression by progesterone, for oEGP is always secreted in high concentrations in response to oestradiol, regardless of the progestational status of the ewes (Sutton *et al.*, 1986; Salamonsen and Nancarrow, 1994).

EGP Binding to Spermatozoa

In many species, oviductal oestrus-associated glycoproteins bind to oocytes and embryos (see Salamonsen and Nancarrow, 1994) but equivocal reports have been published on the binding of these proteins to spermatozoa. There are specific binding sites for spermatozoa on the zona pellucida (Gwatkin, 1977; Yanagimachi, 1981; Dunbar, 1983; Hartman, 1983) and critical roles have been identified for zona pellucida proteins, ZP3 and ZP2, in sperm binding in mice (Wassarman, 1994). It is possible that oviductal glycoproteins are also involved in the interaction of recognition proteins and species-specific binding of gametes that facilitate fertilization.

Evidence for the binding of oviduct proteins to spermatozoa is accumulating. Some of these reports indicate that EGP might participate in this binding (Voglmayr and Sawyer, 1986; Lippes and Wagh, 1989; McNutt *et al.*, 1992; King and Killian, 1994) and proteins of similar size have been extracted from sperm membranes. Both Sutton *et al.* (1984b) and King and Killian (1994) found that labelled oviduct proteins bind to ram and bull spermatozoa, and King and Killian reported that the molecular mass of the major component was 90–95 kDa. Voglmayr and Sawyer (1986) found that a variety of uterine and oviduct proteins accumulated on membranes of spermatozoa during passage to the site of fertilization. Other data indicate that several bovine oviductal proteins bind to spermatozoa including an 85–95 kDa glycoprotein, but the presence of bEGP could be detected only after permeabilization of the membranes with deoxycholate (King and Killian, 1994). Reuter *et al.* (1994), using an immunofluorescence assay with a polyclonal antiserum and immunoblotting after solubilizing the membranes, found that the human equivalent to oEGP (called oviductin or huOGP; hEGP) did not bind to human spermatozoa. In contrast, Lippes and Wagh (1989) demonstrated localized binding of a 54 kDa glycoprotein to the human sperm head. The molecular mass of this protein (human oviductin I) is more in keeping with that of non-glycosylated core proteins of EGP, although it may represent a dissimilar protein. However, hEGP bound to hamster oocytes and a uterine glycoprotein bound to human spermatozoa under the same conditions. When testing capacitated and uncapacitated sheep spermatozoa incubated *in vitro* or *in vivo*, we could not observe specific immunofluorescence greater than that found in negative controls (Fig. 3). Positive staining was found for oviduct epithelial cells, embryos and follicular oocytes incubated in oviduct proteins. The interpretation of the previous data as true physiological evidence for EGP binding to spermatozoa must therefore be questioned. Sutton *et al.* (1984b) reported degradation of proteins after incubation with spermatozoa at 37°C. Perhaps spermatozoa do degrade EGP and other oviduct proteins allowing the products to enter or pass through the membranes and this may account for the data presented by Sutton *et al.* (1984b) and King and Killian (1994).

There is high sequence similarity between the ovine and bovine genes indicating that the size and nature of non-glycosylated core proteins are similar. There appear to be some differences between the glycosyl residues of each, with the oEGP chains terminating in fucose, sialic acid and Gal*N*Ac in addition to the Gal-Gal*N*Ac and Glc*N*Ac reported for cows (Wegner and Killian, 1992). Nevertheless, differences in glycosylation patterns, lengths and branching of chains and number and nature of *N*- and *O*-linked glycosylation sites might result in different membrane binding properties, or there may be differences between the sperm membranes of different species.

Role of EGP in Fertilization

Oviduct-specific glycoproteins bind to the zona pellucida of oocytes and spermatozoa of several species implying a role for these proteins in fertilization or early embryo development (Gandolfi *et al.*, 1991;

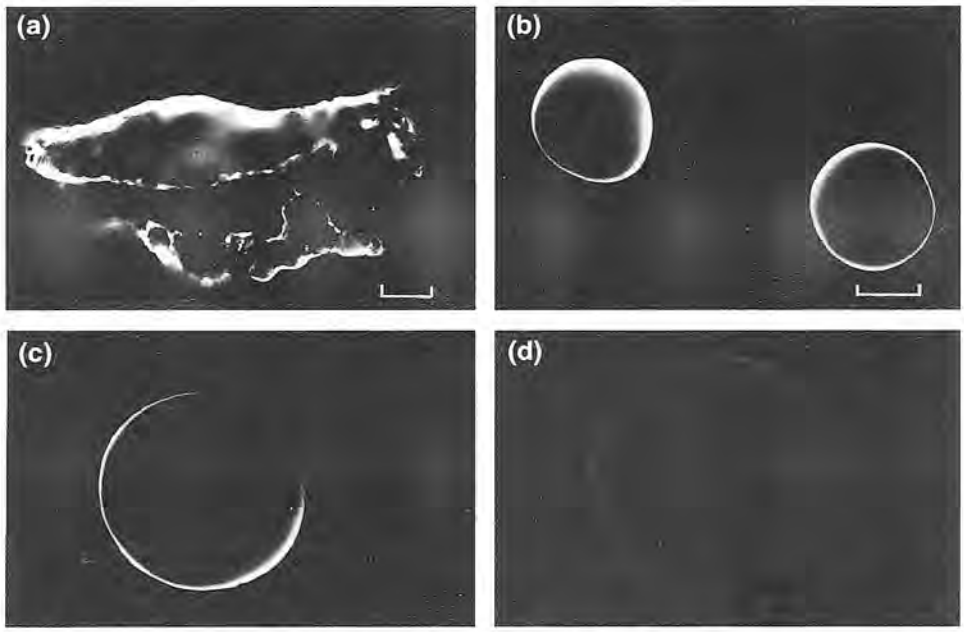

Fig. 3. Binding of ovine oestrus-associated glycoprotein (oEGP) to gametes. Three experiments were carried out in which oEGP binding to spermatozoa was tested. In the first, freshly collected and washed spermatozoa were incubated with and without oviduct fluid and fractions F1 and F2 for various times up to 12 h. F1 is the first fraction eluted from a Superose 12 FPLC column (Pharmacia, Piscataway, NS) and contained oEGP while the second (F2) was predominantly albumin. Proteins from 0.5 ml thawed oviduct fluid were eluted with 50 mmol sodium phosphate l^{-1} (pH 7.2) containing 150 mmol NaCl l^{-1}. Fractions were freeze dried, reconstituted in synthetic oviduct fluid (SOF) then dialysed against SOF for 36 h before use in culture. In the second, capacitated spermatozoa collected after swim-up were incubated as in the first experiment. The third experiment examined the binding of oEGP to spermatozoa *in vivo*, following intra-uterine insemination of freshly collected and diluted semen. Spermatozoa were recovered from different locations within the reproductive tract 2–6 h after intrauterine insemination. The presence of oEGP was determined as described in Fig. 2. Freshly collected (a) oviduct epithelial cells, (b) oviductal embryos, and follicular oocytes incubated in (c) the presence or (d) the absence of F1 were used as positive (a, b, c) and negative (d) controls. No difference between the negative control, untreated and F1-treated spermatozoa could be detected; no fluorescence was detected in either preparation.

Wegner and Killian, 1991; Boice *et al.*, 1992; King and Killian, 1994). As discussed above, evidence for a role of oEGP in fertilization by direct sperm binding is equivocal. We examined the effects of including oEGP in various IVF media (J. L. Hill, S. K. Walker, G. H. Brown and C. D. Nancarrow, unpublished). The basic IVF medium consisted of a synthetic oviduct fluid (SOF, Tervit *et al.*, 1972) either supplemented or not supplemented with human serum or sheep serum. Various amounts of a high molecular mass oEGP-enriched fraction (F1, see Fig. 3) were added to these media. In the absence of serum, fertilization, determined by the number of embryos undergoing first cleavage, was poor. Both the proportion of embryos undergoing their first cleavage and their developmental competence were significantly greater when IVF occurred in the presence of sheep serum. However, F1 added to the medium containing human serum significantly increased the proportion of cleaved embryos and the specificity of this effect was confirmed in a study in which BSA used at comparable protein concentrations to F1 failed to elicit the same positive effect. Only at a concentration of 10% did F1 increase fertilization rates in media supplemented with sheep serum. Overall, the use of F1 in fertilization medium was advantageous to the developmental competence of the resulting embryos. These data imply that there are components in sheep serum that are essential to fertilization and these might also

be present in oviduct secretions and F1. Ovine EGP cannot be detected in serum using western blot techniques (J. L. Hill and C. D. Nancarrow, unpublished); so that although F1 improved fertilization rates in the medium containing human serum, we cannot conclude unequivocally that EGP plays a role in fertilization in ruminants.

Embryo Coculture with Oviduct Epithelial Cells

There have been many attempts to improve the quality of embryo development by coculturing in the presence of a variety of cell types. Monolayers of oviduct epithelial cells have usually been used (see review by Nancarrow and Hill, 1994). There are two principles involved. First, cells, and in particular oviduct cells that are normally juxtaposed to early developing embryos, were thought to provide one or a range of substances that might be beneficial or even critical to development. Second, these cells might act as scavengers and absorb or metabolize embryotoxic components from the various media. In some instances, conditioned media from these cell cultures have been used. In general, development past the early blocks to cell division occurs and improved hatched blastocyst rates can be obtained, particularly in conditions of higher oxygen content, but still at suboptimal rates (Nancarrow and Hill, 1994; Watson *et al.*, 1994b). Glycine and alanine are used by embryos (Moore and Bondioli, 1993; Gardner *et al.*, 1994) and they are present in oviduct fluid at far greater concentrations than in culture media (Nancarrow *et al.*, 1992). In addition, the oviduct-specific oEGP is absent from most coculture media because synthesis and release of oEGP ceases during the 3–6 days of formation of the monolayer. Nancarrow and Hill (1994) concluded that embryo coculture might be compromised by the absence of stromal cells to mediate oestrogen induction of embryotrophic glycoproteins in epithelial cells and by too low concentrations of certain amino acids. Clearly a balance has to be reached in any culture system in that deamination of amino acids produces embryotoxic concentrations of ammonium ions (Gardner *et al.*, 1994), at least in the absence of somatic cells.

Role of oEGP in Embryo Development

Recent studies in our laboratory have examined the effect of oEGP (F1) on embryo development from fertilization or the first cleavage stage through to hatched blastocysts (J. L. Hill, S. K. Walker, G. H. Brown and C. D. Nancarrow, unpublished). Incorporation of F1 into SOF supplemented with 20% human serum was used to examine the effects of oEGP on development abnormalities that have resulted from this culture medium (Walker *et al.*, 1992). A second fraction of oviduct fluid (F2) containing mainly albumin was used as a control in the study. The addition of F1 resulted in a reduced cleavage rate of one-cell embryos flushed from the oviducts of superovulated ewes but a greater number of nuclei in newly formed blastocysts and a delay in the time taken for blastocyst formation. These effects tend to redress the influence of culture *in vitro*, compared with developmental rates *in vivo* (Walker *et al.*, 1992).

These experiments were repeated using embryos produced *in vitro* and compared four different concentrations of F1 (0–20%, v/v) (J. L. Hill, S. K. Walker, G. H. Brown and C. D. Nancarrow, unpublished). Results confirmed previous effects of the presence of F1 in the culture medium: the proportion of embryos cleaving was reduced and the proportion of cleaved embryos forming blastocysts increased. However, the day of blastocyst formation was not delayed and only in the blastocysts forming first on day 4 were there more nuclei. The consistent decrease in the number of embryos undergoing first division in the presence of F1 suggests that this oEGP-containing fraction may be involved in a process of selection. Culture of sheep embryos in control medium (SOF plus human serum) permits a greater proportion of embryos to develop to blastocysts than occurs *in vivo* (Walker *et al.*, 1992), perhaps supplanting a mechanism *in vivo* that eliminates impaired embryos. The inclusion of F1 in IVF medium but not in culture medium resulted in an increase in the proportion of oocytes cleaving (J. L. Hill, S. K. Walker, G. H. Brown and C. D. Nancarrow, unpublished), implying more than one role for EGP: one facilitating fertilization followed by a second involvement in a selection mechanism before first cleavage. Overall, these experiments consistently showed that the oEGP-enriched additive

positively affected cell division in a number of ways resulting in blastocysts that appear comparable to those developed *in vivo*.

Conclusions

A specific oestrus-associated glycoprotein is synthesized by ruminant ampullary secretory cells in response to the pro-oestrous surge of oestrogen and secreted into the oviduct lumen at about the time of ovulation and fertilization. Although at least two forms of this protein have been described, only one gene has been cloned for both cattle and sheep, and these display a high degree of identity. Nevertheless, there are some glycosylation differences that might account for possible differences in sperm binding characteristics. The role of this glycoprotein has not been elucidated, but it binds to zona pellucida and blastomere membranes and in culture appears to regulate cell division and blastocyst formation rates. Although it does not appear to be obligatory for fertilization or embryo development, it may act in a facilitatory role and in its pure form be a useful component of embryo production media *in vitro*.

The authors would like to thank our colleagues, C. Pruss, P. Mitchell, A. Dafter, J. Marshall and A. Brownlee for their various contributions to this work. We are most grateful for the professional collaboration of S. Walker and G. Brown in much of the original work presented here. This study was partially funded (JLH) by the Australian Wool Research and Promotion Organization, now the International Wool Secretariat.

References

Abe H, Numazawa C, Abe M, Hoshi H and Oikawa T (1993a) Production and characterization of monoclonal antibodies specific for bovine oviductal glycoproteins *Journal Experimental Zoology* **265** 77–83

Abe H, Numazawa C, Abe M, Onodera M and Katsumi A (1993b) Immunocytochemical localization of oviduct-specific glycoproteins in the oviductal epithelium from cows at follicular and luteal phases *Cell and Tissue Research* **274** 41–47

Arias EB, Verhage HG and Jaffe RC (1994) Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein *Biology of Reproduction* **51** 685–694

Boice ML, Geisert RD, Blair RM and Verhage HG (1990) Identification and characterization of bovine oviductal glycoproteins synthesized at estrus *Biology of Reproduction* **43** 457–465

Boice ML, Mavrogianis PA, Murphy CN, Prather RS and Day BN (1992) Immunocytochemical analysis of the association of bovine oviduct-specific glycoproteins with early embryos *Journal of Experimental Zoology* **263** 225–229

Buhi WC, Bazer FW, Alvarez IM and Mirando MA (1991) *In vitro* synthesis of oviductal proteins associated with estrus and 17β-estradiol-treated ovariectomized ewes *Endocrinology* **128** 3086–3095

Donnelly KM, Fazleabas AT, Verhage HG, Mavrogianis PA and Jaffe RC (1991) Cloning of a recombinant complementary DNA to a baboon (*Papio anubis*) estradiol-dependent oviduct-specific glycoprotein *Molecular Endocrinology* **5** 356–364

Dunbar BS (1983) Morphological, biochemical and immunochemical characterization of the mammalian zona pellucida. In *Mechanism and Control of Animal Fertilization* pp 139–175 Ed. JF Hartman. Academic Press, New York

Gandolfi F, Brevini TAL, Richardson L, Brown CR and Moor RM (1989) Characterization of proteins secreted by sheep oviduct epithelial cells and their function in embryonic development *Development* **106** 303–312

Gandolfi F, Modina S, Brevini TAL, Galli C, Moor RM and Lauria A (1991) Oviduct ampullary epithelium contributes a glycoprotein to the zona pellucida, perivitelline space and blastomere membrane of sheep embryos *European Journal of Basic and Applied Histochemistry* **35** 383–392

Gardner DK, Lane M, Spitzer A and Batt P (1994) Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage *in vitro* in the absence of serum and somatic cells: amino acids, vitamins and culturing embryos in groups stimulate development *Biology of Reproduction* **50** 388–400

Gerena RL and Killian GJ (1990) Electrophoretic characterization of proteins in oviduct fluid of cows during the estrous cycle *Journal of Experimental Zoology* **256** 113–120

Gwatkin RBL (1977) *Fertilization Mechanisms in Man and Mammals* Plenum Press, New York

Hartman JF (1983) Mammalian fertilization: gamete surface interactions *in vitro*. In *Mechanism and Control of Animal Fertilization* pp 325–364 Ed. JF Hartman. Academic Press, New York

Hollis DE, Frith PA, Vaughan JD, Chapman RE and Nancarrow CD (1984) Ultrastructural changes in the oviductal epithelium of Merino ewes during the estrous cycle *American Journal of Anatomy* **171** 441–456

Joshi MS (1988) Isolation, cell culture and immunocytochemical characterization of oviduct epithelial cells of the cow *Journal of Reproduction and Fertility* **83** 249–261

King RS and Killian GJ (1994) Purification of bovine estrus-associated glycoprotein and localization of binding on sperm *Biology of Reproduction* **51** 34–42

Lippes J and Wagh PV (1989) Human oviductal fluid (hOF) proteins. IV. Evidence for hOF proteins binding to human sperm *Fertility and Sterility* **51** 89–94

McNutt T, Rogowski L, Vasilatos-Younken R and Killian G (1992) Adsorption of oviductal fluid proteins by the bovine sperm membrane during *in vitro* capacitation *Molecular Reproduction and Development* **33** 313–323

Malayer LR, Hansen PJ and Buhi WC (1988) Secretion of proteins by cultured bovine oviducts collected from estrus through early diestrus *Journal of Experimental Zoology* **248** 345–353

Moore K and Bondioli KR (1993) Glycine and alanine supplementation of culture medium enhances development of *in vitro* matured and fertilised cattle embryos *Biology of Reproduction* **48** 833–840

Murray MK (1992) Biosynthesis and immunocytochemical localization of an estrogen-dependent glycoprotein and associated morphological alterations in the sheep ampulla oviduct *Biology of Reproduction* **47** 889–902

Murray MK (1993) An estrogen-dependent glycoprotein is synthesized and released from the oviduct in a temporal and region-specific manner during early pregnancy in the ewe *Biology of Reproduction* **48** 446–453

Nancarrow CD and Hill JL (1994) Coculture, oviduct secretion and the function of oviduct-specific glycoproteins *Cell Biology International* **18** 1105–1114

Nancarrow CD, Hill JL and Connell PJ (1992) Amino acid secretion by the ovine oviduct *Proceedings of the Australian Society for Reproductive Biology* **24** 71 (Abstract)

Reuter LM, O'Day-Bowman MB, Mavrogianis PA, Fazleabas AT and Verhage HG (1994) *In vitro* incubation of golden (Syrian) hamster ovarian oocytes and human sperm with a human oviduct specific glycoprotein *Molecular Reproduction and Development* **38** 160–169

Salamonsen LA and Nancarrow CD (1994) Cell biology of the oviduct and endometrium. In *Molecular Biology of the Female Reproductive System* pp 289–328 Ed. JK Findlay. Academic Press, New York

Sendai Y, Abe H, Kikuchi M, Satoh T and Hoshi H (1994) Purification and molecular cloning of bovine oviduct specific glycoprotein *Biology of Reproduction* **50** 927–934

Shur BD and Hall NG (1986) A role for mouse sperm surface galactosyltransferase in sperm binding to egg zona pellucida *Journal of Cell Biology* **95** 574–579

Sutton R, Nancarrow CD, Wallace ALC and Rigby NW (1984a) Identification of an oestrus-associated glycoprotein in oviducal fluid of the sheep *Journal of Reproduction and Fertility* **72** 415–422

Sutton R, Wallace ALC, Engel H and Nancarrow CD (1984b) Binding of sheep oviducal fluid proteins to spermatozoa. In *Reproduction in Sheep*, Eds DR Lindsay and DT Pearce. Australian Academy of Science, Canberra

Sutton R, Wallace ALC and Nancarrow CD (1985) Characterisation of a glycoprotein in oviductal fluid by two-dimensional electrophoresis and lectin binding to protein gel blots *Electrophoresis* **6** 516–520

Sutton R, Nancarrow CD and Wallace ACL (1986) Oestrogen and seasonal effects on the production of an oestrus-associated glycoprotein in oviducal fluid of sheep *Journal of Reproduction and Fertility* **77** 645–653

Tervit HR, Whittingham DG and Rowson LEA (1972) Successful culture *in vitro* of sheep and cattle ova *Journal of Reproduction and Fertility* **30** 493–497

Varki A (1993) Biological roles of oligosaccharides: all of the theories are correct *Glycobiology* **3** 97–130

Voglmayr JK and Sawyer RF (1986) Surface transformation of ram spermatozoa in uterine, oviduct and cauda epididymal fluids *in vitro Journal of Reproduction and Fertility* **78** 315–325

Walker SK, Heard TM and Seamark RF (1992) *In vitro* culture of sheep embryos without co-culture: successes and perspectives *Theriogenology* **37** 111–126

Walker SK, Hill JK, Kleeman DO and Nancarrow CD (1995) The effect of amino acids at oviductal fluid concentrations on the development of ovine embryos in a defined medium *Theriogenology* **43 345** (Abstract)

Warren CE (1993) Glycosylation *Current Opinion in Biotechnology* **4** 596–602

Wassarman PM (1994) Gamete interactions during mammalian fertilization *Theriogenology* **41** 31–44

Watson AJ, Watson PH, Arcellana-Panlilio M, Warnes D, Walker SK, Schultz GA, Armstrong DT and Seamark RF (1994a) A growth factor phenotype map for ovine preimplantation development *Biology of Reproduction* **50** 725–733

Watson AJ, Watson PH, Warnes D, Walker SK, Armstrong DT and Seamark RF (1994b) Preimplantation development of *in vitro*-matured and *in vitro*-fertilized ovine zygotes: comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere *Biology of Reproduction* **50** 715–724

Wegner CC and Killian GJ (1991) *In vitro* and *in vivo* association of an oviduct estrus-associated protein with bovine zona pellucida *Molecular Reproduction and Development* **29** 77–84

Wegner CC and Killian GJ (1992) Origin of oestrus-associated glycoproteins in bovine oviductal fluid *Journal of Reproduction and Fertility* **95** 841–854

Yanagimachi R (1981) Mechanisms of fertilization in mammals. In *Fertilization and Embryonic Development in Vitro* pp 81–182 Eds L Mastroianni and JD Biggers. Plenum, New York