Characterization of proteins secreted by sheep oviduct epithelial cells and their function in embryonic development

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Summary

The role in early development of proteins secreted by oviduct epithelial cells has been investigated. Secreted proteins devoid of serum contamination have been produced by the surgical removal and immediate incubation of oviduct cells in [35S]methionine-containing medium. After electrophoretic separation, secreted polypeptides could be divided into those that were secreted uniformly throughout the oestrous cycle and a second class that showed a cyclical pattern of secretion. The first class of proteins represented a small proportion of total output whilst the predominant second class was composed mainly of polypeptides of Mr 92 and 46×10^3, respectively. Both of these polypeptide species, referred to as sheep oviduct proteins 92 and 46 (SOP 92, SOP 46), are detected only during the first 4 to 5 days after oestrus when the embryos are located in the oviduct. Oviduct cells collected at oestrus and maintained thereafter in culture secrete the same pattern of proteins and follow the same time course as their counterparts in vivo.

The interaction between the oviduct proteins and the developing embryo was studied firstly by determining whether any of the secreted proteins bound to the zona pellucida. The results of iodination studies showed that two polypeptides of Mr 92 and 46×10^3, respectively, were bound to the zona pellucida of eggs removed from the oviduct but were absent from eggs that had not had contact with the oviduct epithelium. That these newly acquired proteins represent SOP 92 and 46 is suggested by their electrophoretic mobility and their ability to bind to the zona of follicular eggs when added in vitro and by the fact that they both disappear from the zonae of embryos after exit from the oviduct. The collection of unlabelled secreted proteins enabled us to produce a monoclonal antibody, which was used in the second series of experiments on oviduct-embryo interactions. The results confirmed that SOP 92 binds to the zona pellucida and moreover showed that this protein crosses the zona and becomes associated with the individual blastomeres of the developing embryo. These findings provide evidence that the mammalian oviduct probably plays a direct role in supporting embryonic development through specific polypeptides produced by its epithelium.

Key words: oviduct, secreted proteins, zona pellucida, embryonic development, monoclonal antibody, sheep.

Introduction

The oviduct provides the correct environment for ovum transport, fertilization and early development. Attempts to reproduce any of these events outside its lumen have invariably met with a sharp drop in efficiency and/or viability.

Despite the lack of indisputable evidence, it is nevertheless widely thought that the oviduct environment exerts an active functional role in some of the early events of embryonic development (see Bavister, 1988 for a review). In particular, the protein fraction seems to play an active role especially since it binds to the zona pellucida. This is particularly evident in the rabbit where a 60 μm thick layer is deposited within 48 h after ovulation (Kane, 1975) but it has been shown also in the mouse (Kapur & Johnson, 1985), the pig (Brown & Cheng, 1986) and the hamster (Lévêillé et al. 1987, Oikawa et al. 1988). In the mouse, one protein is also found in the perivitelline space thus providing a specialized microenvironment during fertilization and early embryogenesis (Kapur & Johnson, 1986).

Oviducal proteins derive from two main sources: the serum and the secretory cells lining the lumen. The first source accounts for most of the protein content of the oviducal fluid and consists mainly of albumin and immunoglobulins (see Leese, 1988, for a review). The passage of proteins from the serum into the oviduct lumen, although not understood in detail, is thought to be a case of selective transudation with the ability of the
protein to enter the lumen being inversely proportional to its molecular size (Oliphant et al. 1978). It is suggested further that endocytosis plays a major role in this selective transudation process (Parr et al. 1988). Secretions directly from the oviduct epithelial cells provide the second major source of intraluminal proteins in a wide variety of species (rabbit: Barr & Oliphant, 1981; sheep: Sutton et al. 1984; mouse: Kapur & Johnson, 1985; baboon: Fazleabas & Verhage, 1986; human: Verhage et al. 1988). The analyses so far carried out on these proteins show that they are glycosylated (Sutton et al. 1985; Oliphant, 1986; Robitalle et al. 1988) and that these proteins selectively interact with the embryo (Kapur & Johnson, 1986; Robitalle et al. 1988).

In previous experiments, we demonstrated that the positive effect exerted by the oviduct on embryonic development is mimicked in vitro by coculture with oviducal epithelial cells but not with other cell types (Gandolfi & Moor, 1987). This strongly suggested that embryotrophic factors in sheep are produced selectively by the epithelium itself without the requirement for serum components.

In the present study, the proteins secreted by the sheep oviduct have been analysed and their changes, particularly during the period of embryonic passage early in the cycle, have been identified. As an extension of the study, we have studied the interactions that occur between the secreted proteins and the embryo both during development in vivo and in culture.

**Materials and methods**

**Source and culture of cells and embryos**

Cells and embryos were collected as described in detail by Gandolfi & Moor (1987). A minimum of three sheep, of which at least one was not superovulated, were used on each of days 0 (oestrus), 1, 2, 3, 4, 5, 6 and 10 of the cycle as donors of oviduct epithelium. Embryos were collected on days 2, 3, 4 and 5 to correspond with their time in or immediately after their passage through the oviduct. Flushing of the oviducts was performed using Dulbecco's phosphate-buffered saline solution (PBS) supplemented with 10 % v/v newborn calf serum (Sera Lab Ltd, Crawley Down, UK) with pH adjusted at 7.4 and osmolality at 280 mOsm kg$^{-1}$. Histological examination of some oviducts after the collection procedure showed that the oviduct cells were obtained from the epithelial layer of the ampulla region (data not presented). The exclusive epithelial origin was confirmed by the fact that none of the long-term cultures (over 15 days) showed signs of overgrowth by other cell types. The oviduct cells obtained from each sheep were allowed to sediment for 10 min. Thereafter, the supernatant was discarded and a 0.5 ml sample of the packed cell pellet was taken for analysis. Single cell suspensions were avoided because of the finding that enzyme treatment sharply reduces the number of normal ciliated cells and increases the degree of vacuolization of oviduct cells (Gandolfi & Moor, 1987). A standard packed cell volume of 100 μl was used for direct radiolabelling whilst the remaining 400 μl of packed cells were prepared for culture. These were resuspended in 4 ml TCM 199 with Earle's salts (Gibco Europe Ltd, Paisley, UK) supplemented with 10 % fetal calf serum (Sera Lab Ltd), 2·2 g l$^{-1}$ of sodium hydrogen carbonate (‘Analara’ BDH, Poole, UK) and 75 mg l$^{-1}$ of kanamycin (Sigma Ltd, Poole, UK). The resuspended cells were plated into two 4-well dishes in 0·5 ml samples (Nunc, Gibco Europe Ltd) and incubated at 38°C in a humidified atmosphere of 5 % CO$_2$ in air. Embryos were cultured only on cells collected on day 2 of the cycle as described previously (Gandolfi & Moor, 1987).

**Labelling of oviduct-secreted proteins and zonae pellucidae**

Epithelial cells (100 μl packed cell volume) were transferred to 250 μl of methionine-free MEM (Gibco) containing 0·1 % (w/v) polymethyl-alcohol (Sigma) and 100 μg ml$^{-1}$ of [35S]-methionine (Amersham Ltd) within 30 min of removal from the oviducts and labelled thereafter for 4 h at 38°C in a humidified atmosphere. We have used the protein profiles obtained after direct labelling (t = 0) as an indication of the secretory capacity of the cells in vivo. This assumption is based on the short interval between collection and labelling, on morphological evidence of no cell damage during the rapid labelling procedure and on the similarity between our profiles and those obtained by studying oviduct secretions in vivo (Sutton et al. 1984, 1985).

Cells maintained as long-term explants were similarly labelled at the end of the culture period using exactly the same protocol. Mechanically isolated zonae were labelled as previously described by Brown & Cheng (1986). Briefly, to each group of 5–10 isolated zonae in 10 μl PBS was added 2·5 μCi of N-succinimidyl 3-(4-hydroxy-5-[35S]-iodophenyl) propionate in 20 μl 0·1 M-borate buffer pH 8·5 (Bolton & Hunter, 1973). After 15 min at 25°C the zonae were washed three times in PBS and dissolved individually in sample buffer for electrophoresis. Zonae were either labelled within 30 min of their collection or after a period in culture varying from 24 to 96 h; each zona incorporated up to 200 000 cts min$^{-1}$ depending upon its category. We analysed a minimum of 10 embryos at each selected stage by immediate radiolabelling (t = 0) or after culture.

**Gel electrophoresis**

The incubation medium was collected at the end of the radiolabelling period and centrifuged at 5000 revs min$^{-1}$ for 10 min to avoid cell contamination. 50 μl samples of the supernatant were freeze-dried and resuspended in an equal volume of sample buffer consisting of 62·5 mM-Tris-HCl pH 6·8, 2·3 % (w/v) SDS and 10 % (w/v) glycerol in case of one-dimensional analysis or 9·5 % urea, 2 % (w/v) Nonidet P40 (Sigma) 5 % β-mercaptoethanol and 2·28 % Phasmalites (Pharmaecia Ltd, Milton Keynes, UK) when two-dimensional electrophoresis was to be performed. A 6 μl sample was removed from each sample for the measurement of incorporation into synthesized proteins by TCA precipitation as described by Moor et al. 1981. The TCA analyses were used firstly to calculate total levels of radiolabelled protein secreted into the medium. Second, the TCA results were used to calculate the volume of sample required to ensure that equal numbers of TCA-precipitable counts (10 000 cts min$^{-1}$) were loaded onto each lane of the SDS gels described in this paper. Labeled zona pellucidae were electrophoresed individually to enable a comparison of both distributive and quantitative uptake of label i.e. each track received the same amount of zona pellucida, usually 0·25, containing up to 50 000 cts min$^{-1}$. Both 2D and 1D samples were separated on a standard 8–15 % linear gradient SDS–polyacrylamide slab gel following the technique of Laemmli (1970). Two-dimensional analysis of the oviduct proteins were performed on 4 % polyacrylamide rod gels as described by O’Farrell (1975) but with a modified pH gradient ranging from 2·5 to 8. The samples were run at 400 V for 18 h, very briefly equilibrated and sub-
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Subsequently loaded on a standard 8–15% linear gradient SDS–polyacrylamide slab gel as above. Unless otherwise stated all the chemicals were BDH analar grade.

Both one- and two-dimensional gels of 35S samples were subjected to fluorography following the method of Bonner & Laskey (1974) and then exposed to a preflashed Kodak X-Omat-S X-ray film (Laskey & Mills, 1975). Dried gels of 125I samples were directly exposed to the preflashed film.

Source of unlabelled proteins
Secreted oviduct proteins were obtained after 24 h incubation of cells collected on day 1 or 2 of the cycle in protein-free TCM 199. The conditioned medium was then assessed for protein concentration by the Coomassie Blue-G dye binding method (Read & Northcote, 1981), extensively dialysed at 4°C against water, freeze-dried and resuspended in the volume of fresh TCM 199 necessary to reach a protein concentration of 2mgml~1. Alternatively, proteins at a standard concentration were resuspended in sample buffer, separated by one-dimensional gel electrophoresis as described above and revealed by silver stain (Henkeshoven & Dernik, 1985) or by periodic acid–Schiff (PAS) stain for the identification of glycosylated proteins (Dubray & Bezard, 1982).

Production and use of monoclonal antibodies
Female rats (LOU/C) were injected intramuscularly with 200ng of oviductal protein emulsified in complete Freund’s adjuvant followed, 23 days later, by a boost of the same amount of protein in incomplete Freund’s adjuvant administered subcutaneously. Specific antibody production was identified 10 days thereafter by the immunoblotting technique of Burnette (1981). The presence of antibodies was identified using peroxidase-conjugated rabbit anti-rat IgG (ICN Biochemicals) and chloronapthol (Sigma) in the presence of H2O2 (BDH) as substrate. The rat giving the strongest response was boosted on day 47 with an intravenous injection of 100μg of protein in saline. 72 h later, the spleen was removed and the cells fused with the rat myeloma line Y3 Agl.2.3 (Galfre et al. 1979). After preliminary screening by ELISA, positive culture supernatants were screened by immunoblot and selected positive lines were single-cell cloned in soft agar (Galfre & Milstein, 1981). Seven different hybridoma lines were finally established that produced monoclonal antibodies against the major secreted oviduct protein SOP 92. One of these monoclonal antibodies (AFRC MAC 264), typed as an IgG2a, was selected for use in all experiments designed to study the interactions between the oviduct protein SOP 92 and the developing embryo.

Polypeptides from intact embryos collected on day 2 of the cycle or from mechanically isolated zona pellucida and blastomeres were separated by SDS–PAGE and transferred to nitrocellulose by Western blotting. Immunoreactive polypeptides were detected by the immunoblotting procedure described above. Whole-mount embryo preparations were sequentially incubated with supernatant containing MAC 264 and FITC-conjugated rabbit anti-rat IgG (ICN). Both unrelated monoclonal antibodies and follicular oocytes which had not been exposed to oviduct proteins were used as negative controls.

Fig. 1. 35S-labelled proteins synthesized and secreted by the oviduct epithelial cells between oestrus (day 0) and day 10 of the cycle. SOP 92 (a) and SOP 46 (b) represent the major secretory products. The former is secreted until day 4 while the latter until day 5. The secretion of both coincides with the passage of the embryo through the oviduct.
Results

Protein secretion patterns by cells immediately after removal from the oviduct in vivo

As shown in Fig. 1, the proteins secreted by the oviduct epithelium are numerous and can be divided into 2 major classes: those that are secreted at a constant level throughout the oestrous cycle and those that undergo cyclical variations. The proteins of the first class range in $M_r$ from 200 to $10^3$ and represent a small proportion of the total secretions. On the other hand, two major secretory products are present only during the first 4 days of oestrous cycle and decline thereafter. One of these proteins migrates as a broad band of $M_r$ around $92 \times 10^3$ (SOP 92) while the other group appears as a triplet with a $M_r$ of approximately $46 \times 10^3$ (SOP 46). More detailed two-dimensional gel electrophoretic analyses showed that SOP 46 consists of a group of about 15 polypeptide isoforms with isoelectric points ranging from 4 to 5.2. The appearance of SOP 92 on two-dimensional gels (Fig. 2), the width of the band on one-dimensional gels and PAS staining of the unlabelled protein imply that SOP 92, on the other hand, consists of a single polypeptide core with different degrees of glycosylation. A second glycosylated protein with an $M_r$ of over $200 \times 10^3$ was also detected by PAS staining (Fig. 5).

While SOP 92 undergoes a sharp decrease on day 5, when the embryo enters into the uterus, SOP 46 has its peak between day 3 and 5; thus its timing is not as synchronous with the embryo transit. It should finally be noted that superovulation does not affect either the polypeptide profile or time course of secretion SOP 46 and 92. Moreover, silver-stained gels of the unlabelled proteins showed a protein pattern similar to that of the labelled products.

Parallel studies were carried out on the synthesis and secretion of proteins by ovine embryos when cultured in
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Fig. 3. \(^{35}\)S-labelled proteins secreted on each of the first 7 days of culture by oviduct epithelial cells obtained from animals on day 1 of the cycle. SOP 92 (a) is secreted in culture for 4 days and then decreases sharply in a manner similar to that observed in vivo. Similarly, SOP 46 (b) is secreted until day 5 and then decreases more gradually. Low molecular weight proteins (c) not secreted in vivo, appear in culture possibly as a consequence of cell adaptation in vitro.

medium alone, on fibroblast feeder cells or on oviduct epithelial cells. Neither SOP 92 nor SOP 46 appeared as secretory products in any of the cultures excepting for those in which the appropriately staged oviduct cells were themselves secreting the proteins. Our experiments do not eliminate entirely the formal possibility that SOP 92 and SOP 46 are secreted by embryos but only when induced to do so by oviduct cells that are themselves secreting the two proteins. That this is highly unlikely is suggested from studies of the intracellular profile of polypeptides in embryos cultured on oviduct cells (Crosby et al. 1988).

Protein secretion by cultured oviduct cells

Cells collected on day 1 of the cycle (ovulation) and cultured thereafter showed the same protein pattern, with SOP 92 and SOP 46 being the major secretory products (Fig. 3), as comparably staged cells labelled directly after removal of cells in vivo. The secretion of SOP 92 declines sharply after the fourth day of culture, while SOP 46 was secreted for a longer period and its drop was more gradual than that of SOP 92. By contrast, cells collected between days 2 and 5 of the cycle behaved in a different manner in culture from their counterparts labelled after removal of cells in vivo. Thus, even cells collected as late as day 4, which would in vivo have changed their secretion to the luteal pattern on the following day, nevertheless once isolated from their environment maintained the oestrous pattern for a further 4 days before switching just like cells collected on day 1 (data not shown). In addition, a few low molecular weight proteins, which were not detected from cells obtained directly from oviducts in vivo, appeared when the oviduct cells were cultured, probably reflecting the adaptation process that they have to undergo.

Oviduct–embryo interactions in vivo

The composition of the zona pellucida of follicular oocytes changes as soon as they enter the oviduct. This change is characterized by the appearance of two new proteins of \(M_r \times 10^3\) \(92 \times 10^3\) and \(46 \times 10^3\), respectively. The zona pellucida composition thereafter remains stable up to the fourth day of the cycle when the embryo is at the 8- to 16-cell stage (Fig. 4). On the following day, when most of the embryos enter the uterus, the composition of the zona again changes with the loss of the two newly acquired proteins. This pattern was found in all the embryos collected from two ewes whilst, in a third animal, the two acquired proteins were still present on day 5. The examination of day 6 embryos always confirmed the loss of SOP 92 and SOP 46. On the other hand, the examination of unfertilized eggs recovered on day 6 to 8 showed that these did not loose the two extra bands. That the newly acquired zona proteins corresponded to SOP 92 and 46, respectively, was strongly suggested both by the coincidence of molecular weight and the timing of their appearance and disappearance.
Further evidence that SOP 92 is translocated to the developing embryo has been obtained by probing Western blots of zona pellucida polypeptides with the monoclonal antibody raised against SOP 92. Fig. 5 shows that SOP 92 not only binds to the zona pellucida of embryos collected on day 2 of the cycle but also becomes associated with the embryonic membrane and/or cytoplasm. Using a FITC-conjugated second antibody on whole-mount preparations, it is possible to demonstrate that SOP 92 covered the entire zona surface (Fig. 6).

**Oviduct–embryo interactions in culture**

As illustrated in Fig. 7, when follicular oocytes are incubated in culture medium supplemented with 2 mg ml⁻¹ of concentrated oviduct proteins, the zona displays the same polypeptide composition as that of zonae from embryos removed from the oviducts in vivo. The specificity of the binding of SOP 92 and SOP 46 is underlined by the fact that none of the serum proteins in the culture medium (10% fetal calf serum) showed any zona binding whatsoever. The oviduct protein binding data have been extended by removing 1-cell embryos from the oviducts in vitro and culturing them thereafter in vitro. Fig. 8 shows that under these conditions SOP 92 is lost from the zona within 24 h whilst the lower molecular weight band (SOP 46) is maintained for two days but is lost by day 3. It should be noted that this stage in culture corresponds closely to the stage at which SOP 46 disappears from the zona in vivo; thus the spontaneous loss of SOP 46 may reflect a physiological event.

**Discussion**

A consistent problem in the analysis of proteins in oviduct fluids has been to distinguish between those derived from the serum and the minority secreted by the oviduct epithelium. The technique used in our experiments has proved very efficient for the study of the secreted proteins for two major reasons. First, the level of sensitivity has been high because of the complete absence of background caused by serum protein contamination both after immediate incubation of in vivo derived cells and following long-term culture. Second, by using standardized procedures in all experiments, we could compare easily and precisely the behaviour of the oviduct cells obtained direct from
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Fig. 5. Characterization of oviduct proteins collected on day 2 of the cycle and their interaction with the embryo. Track A shows the polypeptide pattern of 50 μg secreted proteins as revealed by silver staining; the close similarity with the pattern of labelled polypeptides will be noted. Track B shows the PAS staining pattern of a similar quantity of secreted protein and demonstrates that both SOP 92 and a polypeptide of $M_r 200 \times 10^3$ are glycosylated. In track C 50 μg of native protein was blotted onto nitrocellulose and probed with the monoclonal antibody MAC 264 raised against SOP 92. Despite denaturation of the proteins during electrophoresis specific antibody recognition of SOP 92 occurred. The same antibody was used to probe the proteins obtained after separating the zonae pellucidae (track D) from the blastomeres (track E) of 20 2-cell embryos. The results show that SOP 92 binds to the zona and can therefore be identified as the newly acquired protein of $92 \times 10^3$ illustrated in Fig. 4. Moreover, it is clear from track E that SOP 92 crosses the zona and becomes a constituent of the developing embryo.

Oviduct in vivo and after culture. These essential features of our experiments have made it possible to demonstrate that the sheep oviduct epithelium secretes a range of polypeptides throughout the cycle rather than the single glycoprotein ($M_r 90 \times 10^3$) that had previously been described (Sutton et al. 1984). On the other hand, the chemical characteristics and cyclical pattern of secretion of this glycoprotein identified from oviduct fluid obtained by chronic cannulation (Sutton et al. 1985) is of such remarkable similarity to SOP 92 as to suggest that the same protein is being considered. This further confirms that the technique we used provides a picture, as close as it is possible to obtain, of the secretions occurring in the animal.

We emphasize that proteins secreted by the oviduct epithelium divide into a small group that are produced continuously throughout the cycle and a larger group that show a cyclical pattern of secretion. The precise timing of the cyclical secretions is particularly interesting because it corresponds with the passage of the embryos through the oviduct. In particular, SOP 92 production sharply decreases as soon as the embryo enters into the uterus, while SOP 46 decreases 24 to 48 h later having reached its peak between day 3 and 5. The analysis of proteins secreted by cultured epithelial cells obtained on day 1 of the cycle showed that the same cyclical pattern of secretion is maintained in culture although at a lower level than that from cells labelled directly after in vivo collection. Moreover, the oestrus–metestrus pattern is faithfully preserved in culture totally without further hormonal supplementation. While the use of the Mab unequivocally identified the higher $M_r$ protein bound to the zona as SOP 92, several indirect lines of evidence indicate that the lower $M_r$ protein is very likely to be the SOP 46. Thus, both the proteins that bind to the zona are almost certainly produced by the oviduct epithelium and are therefore not serum exudates. In all cases so far reported, only one protein of oviductal origin and with a $M_r$ of over $200 \times 10^3$ translocates to the zona pellucida (mouse: Kapur & Johnson, 1986; hamster: Léveillé et al. 1987) with the single exception of the pig where an extra
Fig. 7. Composition of iodinated zonae pellucidae from follicular oocytes incubated overnight in TCM 199 supplemented with 10% foetal calf serum in absence (A) and presence (B) of 2 mg ml$^{-1}$ oviductal proteins. Two new proteins are absorbed on the zonae of the tested oocytes reproducing the parameter illustrated in Fig. 4 for oviducal embryos. This provides further evidence that both the acquired proteins derive from oviduct secretions.

Fig. 8. Composition of iodinated zonae pellucida recovered from embryos during a 3-day culture on oviduct cells feeder-layer. While SOP 92 is lost within 24 h the lower $M_r$ band persists for 2 days. Embryos cultured for 3 days show the same pattern of the embryos recovered from the uterus on day 5 of the cycle. Because in both cases the embryos reach the same developmental stage (8/16 cells) the loss of both proteins in culture may reflect a physiological event.
Fig. 6. Staining of a 2-cell embryo with MAC 264 (anti-SOP 92) and FITC-conjugated rabbit anti-rat IgG, showing that SOP 92 is uniformly distributed on the whole zona surface.
90×10³ polypeptide has also been identified (Brown & Cheng, 1986). On the contrary, no exceptions are reported to the glycosylation of all these proteins. The sheep conforms to these general rules although some details are different from the other species examined. First, the two proteins involved are of lower molecular weight than in the rodents (92 and 46×10³, respectively) and secondly, while SOP 92 is a glycoprotein, SOP 46 is not and is furthermore almost certainly not a subunit of the larger protein. Evidence in support of this conclusion comes firstly from the fact that the two proteins differ substantially on two-dimensional gel electrophoretic analyses. Second, on isoelectric focusing gel SOP 92 shows an extended PI characteristic of a single protein core with differing degrees of glycosylation while SOP 46 shows a restricted polymorphic pattern characteristic of a family of related proteins. Third, the cessation of synthesis and the disappearance of the two proteins from oviduct secretions occurs at different stages of the cycle. Finally, none of the monoclonal antibodies produced against SOP 92 by different clones ever recognized SOP 46. This lack of cross-reactivity was also observed with the antisera obtained from immunized rats.

Our results also provide some insight into the dynamics of oviduct–embryo interaction in this species. Binding of the two proteins to the zona pellucida appears to be a reversible process implying that in vivo their association with the embryo is to an extent in equilibrium with the immediate environment. The loss of the two proteins from the zona pellucida when the embryo enters the uterus could however be due to some local factor (e.g. enzymes) or alternatively to an inherent structural modification of the investment resulting in shedding of the proteins. Favouring the latter possibility is the fact that embryos cultured for 3 days spontaneously lose the proteins while unfertilized eggs retain them even in the uterine environment.

The analysis of the possible function of these two proteins appears, however, more problematic. Our previous experiments indicate that the oviduct epithelium, but not other somatic cells tested, were capable of supporting development in culture of 1-cell embryos without loss of viability (Gandolfi & Moor, 1987). On the other hand, our present experiments have indicated how the culture conditions we used cannot reproduce the zona–protein interactions that we found in vivo. In particular, SOP 92 is lost within 24 h and the 3 days interval between explantation and coculture that we have routinely allowed provides the embryos with a declining source of both SOP 92 and 46. The contrast between these two sets of results is even sharper if we consider that the usual protein concentration in the oviduct fluid is around 15–30 mg ml⁻¹ (Restall & Wales, 1966; Iritani et al. 1969), which suggests that the concentration of SOP 92 and 46 is much higher than that reached in our normal culture conditions (usually less than 0.2 mg ml⁻¹ at the beginning and declining thereafter). This led to the supposition that the positive effect of the oviduct cells in culture may reflect their ability to maintain a minimal functional quantity of oviduct proteins which have been accumulated during the first 24 h the embryo has spent in the natural oviduct environment. Some support for this hypothesis comes from another set of experiments in which we showed that when in vitro matured and in vitro fertilized eggs are cultured, oviduct cells can fully support their cleavage rate but cannot prevent a sharp loss of viability (Fukui et al. 1988). It is possible that this is because these embryos had not accumulated trophic factors in vivo before being placed in vitro. More convincing evidence probably comes from our observation that SOP 92 not only binds to the outside of the zona but is also directly associated with the blastomeres. Although at the moment we are uncertain as to whether the protein binds to the cell membrane or becomes incorporated within the embryo, this may represent a way that enables it to accumulate important oviducal factors. It is also an important difference from that described in other species where, as detailed in the introduction, oviduct-produced proteins have so far been found only on the outer surface of the zona pellucida. The only exception is in the mouse (Kapur & Johnson, 1986), where an oviductal glycoprotein, GP 215, accumulates in the perivitelline space while a soluble oviduct factor binds to the egg surface (Gaunt, 1985).

It has been known for a long time that nonspecific proteins such as albumin can be transported in the oviduct from the maternal serum into the embryonic cytoplasm (Glass, 1969). Our results and those in the mouse now show that proteins specifically produced by the oviduct epithelium bind to the embryo membrane and may also become internalized. Although its function is still unknown, there is evidence that this binding may be important for the development of the early embryo. Moreover, it is now probable that embryonic development is dependent upon direct maternal support earlier than was previously thought.

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References


