Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1*

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Summary. This paper demonstrates that a group of proteins, representing a major secretory component of cattle conceptuses, is immunologically related to ovine trophoblast protein-1 (oTP-1), a principal product of culture Day 13 to 21 sheep conceptuses. Conceptuses from cows (Day 17–18) and ewes (Day 16–17) were cultured for 24 h in the presence of L-[³H]leucine. By using a rabbit antiserum to oTP-1 and Ouchterlony double-immunodiffusion analysis it was shown that material in the bovine conceptus culture medium was serologically related, but not identical, to oTP-1. A solid-phase radiobinding assay indicated that the cross-reacting bovine secretory component had an affinity for anti-oTP-1 antibody similar to that of oTP-1. Anti-oTP-1 antiserum specifically immunoprecipitated a group of 6–8 polypeptides from culture medium of cow conceptuses which, when analysed by two-dimensional gel electrophoresis, fell into two major molecular weight classes (22 000 and 24 000) with isoelectric points between 6·5 and 6·7. These immunoprecipitated polypeptides, defined as bTP-1, constituted the major secretory products of Day 16–25 cow conceptuses. They were larger and more basic than oTP-1 polypeptides (M_r about 18 000; pI 5·4–5·7). Anti-oTP-1 antiserum also recognized the major translation product of Day 17 bovine conceptus mRNA, a polypeptide significantly smaller ($M_r \sim 18 000$) than the secreted protein. It is suggested that oTP-1 and the homologous bovine protein may play similar roles in the phenomenon of maternal recognition of pregnancy in the two species.

Introduction

Embryo transfer experiments have established that maternal recognition of pregnancy in the ewe occurs 12–13 days after onset of previous oestrus (Moor & Rowson, 1966a, b). The corresponding period for the cow is Day 15–16 (Betteridge *et al.*, 1978; Northey & French, 1980). In both species the oestrous cycle can be extended significantly if extracts of conceptuses (Rowson & Moor, 1967; Martal *et al.*, 1979; Ellinwood *et al.*, 1979; Northey & French, 1980) or trophoblast tissue (Martal *et al.*, 1984a, b; Heyman *et al.*, 1984) are introduced into the uteri of nonpregnant recipients just before this critical period during which maternal recognition of pregnancy occurs. Evidence has accumulated that in the sheep the active substance is proteinaceous and produced for only a limited period, not extending beyond Day 21–23 of pregnancy (Rowson & Moor, 1967; Martal *et al.*, 1979).

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Attention has focussed on one particular secretory protein of the sheep conceptus, ovine trophoblast protein-1 (oTP-1) (Godkin *et al.*, 1984a, b; Hansen *et al.*, 1985). This polypeptide is released as a major product by cultured sheep conceptuses between Days 13 and 21 and is produced maximally around Days 15–17 (Godkin *et al.*, 1982; Hansen *et al.*, 1985). It causes an extension of luteal lifespan when introduced in purified form into the uteri of nonpregnant recipient ewes (Godkin *et al.*, 1984b). The protein consists of a group of 3–4 isoelectric variants (pI 5.4–5.7) of molecular weight $\sim 17\,000$ (Godkin *et al.*, 1982). Evidence has been presented to indicate that oTP-1 may be a hormone-like substance which acts, in a paracrine manner, on the maternal endometrium (Godkin *et al.*, 1984a).

The conceptuses of cows also release a group of acidic, low molecular weight polypeptides when cultured *in vitro*. As with oTP-1, their synthesis is limited to a short 7–10-day period (Days 16–26) and coincides with the time at which maternal recognition of pregnancy occurs (Bartol *et al.*, 1985). Introduction of total unfractionated proteins released by cultured bovine conceptuses (bovine conceptus secretory protein; bCSP) into nonpregnant recipient cows causes a significant extension of the interoestrous interval (Knickerbocker *et al.*, 1986). Since the low molecular weight acidic protein is the major component of bCSP, Bartol *et al.* (1985) have suggested that it may be homologous to oTP-1. In addition, Martal *et al.* (1984a, b) and Heyman *et al.* (1984) have shown that transfer of trophoblastic vesicles, comprised of trophoderm and extraembryonic endoderm derived from Day 11–13-sheep conceptuses, to Day 12 recipient cows resulted in extension of luteal lifespan in a significant number of animals. They also found that reciprocal interspecies transfer of bovine trophoblastic tissue to recipient ewes had a similar effect. Together these results suggest that the trophoblast of ewes and cows produces a functionally similar substance which is recognized by either species. The object of the experiments described in this paper was to determine whether oTP-1 cross-reacts immunologically with component(s) of the bCSP.

Materials and Methods

Materials. All materials used were supplied by vendors as noted by Godkin *et al.* (1984a), Bartol *et al.* (1985) and Hansen *et al.* (1985).

Animals. Adult crossbred ewes, primarily of Rambouillet breeding, were checked twice daily for oestrus with vasectomized rams. Ewes were mated at behavioural oestrus (Day 0) to two intact rams. Pregnant ewes were anaesthetized and reproductive tracts were exposed by midventral laparotomy. Intact conceptuses were flushed from uteri on Day 16 of pregnancy with a modified minimum essential medium (MEM, see below) at 37°C and collected in sterile serum bottles.

Cows and heifers of Holstein, Jersey and crossbred beef breeding from the University of Florida research herds were utilized for collection of bovine conceptuses. The cattle were maintained on pasture and checked for oestrus by using visual observation and bulls with penile shunts. All animals were mated at first observation of oestrus (Day 0) to an intact Brown Swiss bull and artificially inseminated about 12 h later.

Cows and heifers were slaughtered on Day 17 or 18 after oestrus. Reproductive tracts recovered after exsanguination were placed in plastic bags and transported on ice to a sterile, laminar flow hood where they were trimmed of excess tissue, including the ovaries and oviducts. The cervix was closed by applying a large, curved, Rochester-Ochsner forcep to the anterior cervix. A plastic, 50-ml syringe fitted with a 16-gauge needle was then used to inject 40 ml sterile minimal essential medium (MEM) into the uterine lumen through the tip of the uterine horn contralateral to the ovary bearing the corpus luteum. The anterior tip (about 1 cm) of the horn ipsilateral to the corpus luteum had been removed to provide a enlarged opening. Conceptuses were flushed through this opening into sterile, plastic, culture dishes.

In-vitro culture of conceptuses. After collection, conceptuses from cows and ewes were transferred to sterile plastic culture dishes containing 15 ml fresh MEM and 100 $\mu\text{Ci L}^{-1}$ [^3H]leucine. Conceptuses were cultured for 24 h in a controlled atmosphere chamber (Model number 7741-10010, Bellco Biological Glassware, Vineland, NJ, U.S.A.), flushed for 10 min with 50% N_2 :47.5% O_2 :2.5% CO_2 and maintained at 37°C in the dark on a rocking platform. Incubations were terminated by removing conceptuses from culture medium. The medium was frozen at -20°C until used in subsequent studies.

Purification of oTP-1. Ovine TP-1 was purified by the method of Godkin *et al.* (1982). Purity was confirmed by polyacrylamide gel electrophoresis (Godkin *et al.*, 1982).

Medium preparation. A modified MEM was prepared according to the method of Basha *et al.* (1979) and used as described by Godkin *et al.* (1982) for the culture of conceptuses.

Preparation of conceptus culture medium for analysis. Media from cultured conceptuses were centrifuged at 12 000 *g* for 10 min to sediment any cellular material present. Supernatant fractions pooled from several cultures were then dialysed (*M*, cutoff about 1000) extensively (4 litres, changed 4 times) against 10 mM-Tris-HCl buffer (pH 8.2) to remove low molecular weight compounds, e.g. salts and unincorporated radiolabelled precursors. Whenever the protein concentration of a sample was too low for immediate analysis, samples were dialysed (*M*, cutoff about 1000) against double-distilled water, lyophilized and resolubilized in an appropriate buffer.

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Immunodiffusion techniques. Immunodiffusion plates containing 5 ml 1% (w/v) agarose in 0.07 M-sodium phosphate buffer (pH 7.4) were prepared in each well of 6-well tissue culture plates (diameter 35 mm) according to the method of Ouchterlony & Nilsson (1973). Antibody specificity was determined by placing antiserum raised against oTP-1 in rabbits (Godkin *et al.*, 1984a) in the centre well of each plate. Various dilutions of ovine CSP and bovine CSP were introduced into the outer wells. The plates were placed in a container to maintain a moist atmosphere. Resultant precipitation bands were observed at 24–48 h at room temperature. Plates were extensively washed in Dulbecco's saline (PBS; 0.2 g KCl/l; 2.16 g KH₂PO₄·7 H₂O/l, 8 g NaCl/l). (Dulbecco & Vogt, 1954) to elute nonprecipitated material, and the bands were stained with Coomassie blue. After destaining the gels were photographed.

Solid-phase radiobinding assay. Bovine conceptus secretory proteins, purified oTP-1 and bovine serum albumin were each adjusted to a concentration of 50 µg/ml in PBS. Anti-oTP-1 antiserum was enriched for immunoglobulins by precipitation with ammonium sulphate and subsequent fractionation on a DEAE-cellulose column (Good *et al.*, 1980). Proteins were eluted with a 150 ml linear salt gradient (0–0.25 M-NaCl in 10 mM-Tris-HCl buffer, pH 8.2) and 2.0 ml fractions were collected. The early eluting IgG-rich peak of anti-oTP-1 (fractions 3–19) was pooled and utilized in subsequent steps. Bovine CSP, purified oTP-1 or bovine serum albumin (50 µl of each per well) were allowed to adsorb passively to the wells of 96-well flexiviny plates (Falcon brand; Fisher Scientific, Orlando, FL) for 1 h at room temperature. These protein solutions were withdrawn, and plates washed once with 0.1% (w/v) bovine serum albumin in PBS (pH 7.4). Anti-oTP-1 antiserum or normal rabbit serum (NRS) was then added at increasing dilutions and allowed to stand in the wells at 4°C overnight. These solutions were removed and plates were washed with PBS containing bovine serum albumin. After washing, ¹²⁵I-labelled, sheep anti-rabbit IgG antibody (50 000 c.p.m.; sp. act. 10⁶ c.p.m./µg) which had been affinity purified was added to each well and left for 1 h at room temperature. The unbound labelled second antibody was then removed and the plates washed three times with PBS-bovine serum albumin. Wells were cut from the plates and the amount of bound ¹²⁵I measured.

Immunoprecipitation. Duplicate aliquants of ovine and bovine conceptus culture medium (80 µg and 120 µg protein, respectively) were lyophilized and redissolved in immunoprecipitation buffer [0.35 ml 0.3 M-NaCl, 0.05 M-Tris-acetate (pH 7.5), 1 mM-phenylmethylsulphonyl fluoride, 1 mM-disodium EDTA, 0.1 mg bovine serum albumin/ml, 0.02% (w/v) NaN₃ and 2% (v/v) Nonidet P-40]. Anti-oTP-1 antiserum (0.05 ml) or normal rabbit serum (0.05 ml) was added to ovine and bovine samples and the tubes were placed on a tube turner overnight at 4°C. Subsequently, 0.1 ml of a 10% (v/v) suspension of Protein A-Sepharose was added to each tube and allowed to incubate for 6 h at room temperature on a tube turner. The Sepharose suspension was centrifuged (15 000 *g* for 1 min) and washed five times with 1 ml detergent buffer [0.05 M-Tris-acetate (pH 7.5), 0.5% (v/v) Nonidet P-40, 0.1% sodium dodecyl sulphate, 0.3 M-NaCl and 0.02% NaN₃]. Protein adsorbed to the gel beads was solubilized in 0.05 ml 5 mM-Tris-HCl (pH 6.8), 15% (w/v) glycerol, 5% (w/v) sodium dodecyl sulphate and 5% (w/v) β-mercaptoethanol before one-dimensional electrophoresis, or in 0.05 ml 5 mM-K₂CO₃, 5 mg dithiothreitol/ml, 2% (v/v) Nonidet P-40 and 9.16 M-urea before two-dimensional electrophoresis. One-dimensional and two-dimensional polyacrylamide gel electrophoresis (1-D PAGE and 2-D PAGE), using 12.5% (w/v) acrylamide gels, were performed according to procedures described in detail by Roberts *et al.* (1984).

Isolation and translation of conceptus mRNA. Isolation and translation of conceptus mRNA was accomplished essentially by the methods described by Hansen *et al.* (1985). Conceptus tissue was homogenized with 1 ml 4-M-guanadinium thiocyanate, 0.5% (w/v) sodium N-lauroyl sarcosine, 25 mM-sodium citrate buffer (pH 7.0), 0.1 M-2-mercaptoethanol and 0.1% (v/v) antifoam A, and then precipitated at –20°C with 25 µl 1 M-acetic acid and 750 µl absolute ethanol (Chirgwin *et al.*, 1979). The ethanol precipitate was collected by centrifugation (12 000 *g*), redissolved in the homogenization buffer (see above), layered over a 5.7 M-cesium chloride cushion and centrifuged for 20 h at 100 000 *g* (20°C) to harvest the total cellular RNA (Chirgwin *et al.*, 1979). Polyadenylated RNA was isolated from the total cellular RNA by two cycles of binding to (50 mM-potassium citrate, pH 7.5, 0.5 M-potassium chloride, 1 mM-disodium EDTA) and elution from (10 mM-potassium citrate, pH 7.5, 1 mM-disodium EDTA) oligodeoxythymidylate cellulose (Aviv & Leder, 1972).

Translation of conceptus poly A⁺ RNA was accomplished in a cell-free system using wheat-germ lysate (Roberts & Patterson, 1973). The translation mixture contained 0.25–1.0 µg poly A⁺ RNA, 6 µl wheat germ lysate, 48 mM-potassium chloride, 27 mM-Hepes (pH 7.5), 67 mM-potassium acetate, 2.7 mM-magnesium acetate, 1.2 mM-ATP, 100 µM-GTP, 5.5 mM-creatine phosphate, 200 µg creatine phosphokinase/ml, 80 µM-spermidine phosphate, 1 mM-BME, 50 µM each of 19 amino acids (minus methionine), 600 µCi L-[³⁵S]methionine/ml and 13.5 µg placental RNase inhibitor/ml in a total volume of 15 µl. After translation, the [³⁵S]methionine-labelled products were analysed by one-dimensional PAGE as total translation products or following immunoprecipitation with anti-oTP-1 antiserum (10 µl) as described in the preceding section.

Results

Ouchterlony double-immunodiffusion analysis

Rabbit antiserum prepared against highly purified oTP-1 gave a single immunoprecipitation band against both oCSP and bCSP (Figs 1a, b). When the ovine and bovine CSP were placed in adjacent outer wells of the immunodiffusion plate, with the antiserum in the central well, the immunoprecipitation band was discontinuous and had clearly defined spurs (Fig. 2).

Fig. 1. Ouchterlony double-immunodiffusion analysis of conceptus secretory proteins from (a) sheep and (b) cattle. Total conceptus secretory proteins (a, sheep; b, cattle) (15 μ l; 1.5 μ g) were placed in the centre well. Phosphate-buffered saline was placed in Well 2 and antiserum to oTP-1 was placed in Wells 3, 4, 5, 6 and 1 at increasing dilutions in phosphate-buffered saline (1:3, 1:7, 1:15, 1:31, 1:63) respectively.

Fig. 2. Ouchterlony double-immunodiffusion analysis of bovine and ovine conceptus secretory proteins. Anti-oTP-1 antiserum was placed in the centre well (Godkin *et al.*, 1982). Total bovine conceptus secretory proteins were placed in Well 1 (30 μ l, 6 μ g) and Wells 3 and 5 (15 μ l, 1.5 μ g). Total ovine conceptus secretory proteins were placed in Wells 2, 4 and 6 (15 μ l, 1.5 μ g; 10 μ l, 1.0 μ g and 7.5 μ l, 0.75 μ g) respectively.

Solid-phase radiobinding assay

Total bCSP was adsorbed passively to the wells of flexvinyl plates, Anti-oTP-1 antiserum was then added at increasing dilutions and bound immunoglobulin detected by means of ^{125}I -labelled sheep anti-rabbit IgG (Fig. 3). The results confirmed that the anti-oTP-1 antiserum bound to some component in bovine CSP and cross-reacted with purified oTP-1. Only at dilutions of antiserum below 1:20 was there detectable binding of antiserum to bovine serum albumin. We cannot, therefore, exclude the possibility that the antiserum did not show some cross-reactivity towards this protein. Preimmune serum tested over a similar range of dilutions failed to bind either oTP-1 or total bCSP. Half-maximal binding to bCSP and oTP-1 was detected at antiserum dilutions of about 1:80 and 1:160 respectively. The binding curves for these two protein fractions were parallel. At the initial 1:10 dilution of antiserum the wells containing adsorbed oTP-1 bound about twice as much ^{125}I -labelled second antibody as did the wells containing bCSP.

Fig. 3. Solid phase radiobinding assay of conceptus secretory proteins. Anti-oTP-1 antiserum was serially diluted and tested for binding to purified oTP-1 (●—●), bCSPs (■—■), or bovine serum albumin (▲—▲). Normal rabbit serum, used in a control, was serially diluted and tested for binding to purified oTP-1 (not shown), bCSPs (not shown) or bovine serum albumin (●—●). Results of curves not shown were approximately 400 c.p.m. greater than the bovine serum albumin–nonimmune rabbit serum at a 1:10 dilution and were similar at greater dilutions. Binding was measured by means of a ^{125}I -labelled sheep anti-rabbit IgG.

Immunoprecipitation of polypeptides from ovine and bovine CSP

Sheep and cattle conceptuses were incubated in the presence of L-[^3H]leucine and cross-reacting polypeptides in the dialysed culture medium immunoprecipitated by successive addition of anti-oTP-1 antiserum and protein A–Sepharose. For oCSP, the antiserum specifically cross-reacted with polypeptide(s) with an M_r about 18 000 (Fig. 4). With bCSP, two bands of polypeptides were detected by one-dimensional polyacrylamide gel analysis. These bands had apparent molecular weights of 22 000 and 24 000 (Fig. 4).

When the polypeptides from bCSP (Fig. 5) were immunoprecipitated and analysed by two-dimensional PAGE, at least 8 polypeptides could be visualized on fluorographs (Fig. 6). The majority of these were localized in two parallel rows of apparent M_r 22 000 and 24 000. Their approximate isoelectric points ranged from 6.7 to 6.5 (Fig. 6). These polypeptides were the major components present on gels of total bCSP at Day 17 of pregnancy (Fig. 5).

Fig. 4. Analysis of immunoprecipitates from conceptus secretory proteins of cows and sheep by one-dimensional polyacrylamide gel electrophoresis and fluorography. Lanes 1 and 3 contained material from bCSPs and oCSPs respectively, which had been immunoprecipitated by means of anti-oTP-1 antiserum followed by Protein A-Sepharose. Lanes 2 and 4 were control lanes in which bCSPs and oCSPs, respectively, had been treated with normal rabbit serum followed by Protein A-Sepharose.

Fig. 5

Fig. 6

Fig. 5. Analysis of bovine CSP by two-dimensional polyacrylamide gel electrophoresis and fluorography. Horizontal scale represents pI values.

Fig. 6. Analysis of immunoprecipitable material from bovine CSP by two-dimensional polyacrylamide gel electrophoresis and fluorography. Bovine CSP was treated with anti-oTP-1 antiserum, immune complexes were collected on Protein A-Sepharose and the radioactive proteins analysed.

Immunoprecipitation of polypeptides from in-vitro translation products

Total poly (A)⁺ conceptus mRNA was translated *in vitro* in a wheat-germ translation system in which L-[³⁵S]methionine was provided as a source of labelled amino acid. The products of translation were analysed by one-dimensional PAGE and fluorography. Translation of bovine mRNA gave rise to a range of translation products with molecular weights ranging from 130 000 to 12 500 (Fig. 7). With the sheep system, the dominant translation product had an M_r of about 21 000

Fig. 7. Electrophoretic analysis of cell-free translation products of RNA isolated from cattle conceptuses. Lane 1 represents total translation products when no bovine mRNA was present. Lanes 2 and 3 show translation products when total poly (A)⁺ bovine conceptus mRNA was used as a source of exogenous mRNA. Lane 4 shows the material that was immunoprecipitated from such products by using normal rabbit serum. Lanes 5 and 6 show material that was immunoprecipitated from the total translation mixture by using anti-oTP-1 antiserum. The proteins were analysed in 12.5% polyacrylamide gels and detected by fluorography.

(Hansen *et al.*, 1985) and this component was specifically immunoprecipitated with anti-oTP-1 antiserum. For cow conceptus mRNA a dominant translation product of M_r about 18 000 was noted which cross-reacted with anti-oTP-1 antiserum.

Discussion

A considerable body of evidence has accumulated to suggest that the mechanisms involved in maternal recognition of pregnancy and luteal maintenance in the ewe and cow are similar (Roberts *et al.*, 1985; Bazer *et al.*, 1986; Thatcher *et al.*, 1986). Secretory components, proteinaceous in nature, are thought to be involved as antiluteolytic substances in both species. These substances probably act locally on the uterine endometrium and, by mechanisms still not understood, reduce the pulsatile release of the presumed uterine luteolysin prostaglandin F-2 α , from the gravid uterine horn (Thatcher *et al.*, 1986; Fincher *et al.*, 1985; Knickerbocker *et al.*, 1986). Other antiluteolytic, luteoprotective and luteotrophic mechanisms may also be regulated by these unique proteins secreted by the conceptus (Thatcher *et al.*, 1986).

In both the ewe and cow, low molecular weight acidic proteins are major components of the conceptus secretions during the critical time that the corpus luteum is rescued. The question has arisen as to whether these proteins are antiluteolytic, either alone or in combination with other conceptus products. Until the present study these low molecular weight acidic polypeptides produced by the ewe and cow conceptus had not been compared.

Ouchterlony double immunodiffusion analysis clearly showed that antiserum to oTP-1 cross-reacted immunologically with some component(s) in bCSP. When ovine and bovine conceptus secretory proteins were placed in adjacent outer wells, with the antiserum to oTP-1 located in the central well, the fusion of immunoprecipitation lines was incomplete and spurs were evident. This

indicates that a protein is present in bCSP which is not identical to oTP-1 but is related serologically to it. This result was confirmed by the solid-phase radiobinding assay which showed that half-maximal binding of antiserum to adsorbed oTP-1 occurred at about twice the antiserum dilution as observed with bCSP. The protein serologically related to oTP-1 in bCSP was clearly present as a high proportion of the total protein. In addition, the binding curves obtained with increasing antiserum dilutions were parallel, a result which suggests that the relative affinity of antibody towards oTP-1 and the cross-reacting protein in bCSP is similar. The fact that the antiserum did not bind strongly to bovine serum albumin in the solid-phase radiobinding assay (Fig. 3) or to serum proteins (R. M. Roberts, unpublished results) indicated that the reaction between bCSP and antiserum was not directed nonspecifically towards some plasma component. Neither has cross-reactivity been demonstrated against polypeptides released by Day 12–14 pig conceptuses (unpublished results). The antiserum does not therefore appear to have broad cross-reactivity with trophoblast proteins from all species.

Anti-oTP-1 antiserum specifically immunoprecipitated a group of 6–8 polypeptides from bCSP which, when analysed by two-dimensional PAGE, fell into two major molecular weight classes (M_r 22 000, 24 000). A trace of an M_r 26 000 component was also present. These bovine polypeptides were slightly more basic and of significantly higher molecular weight than the previously described oTP-1 components (Godkin *et al.*, 1982). The fact that only a single molecular weight band of protein is immunoprecipitated from cell-free translation of bovine poly (A)⁺ mRNA by anti-oTP-1 antiserum suggests that the heterogeneity in molecular size of the bovine protein may be the result of some degree of post-translational processing of the initial translation product. This cell-free translation product has a molecular weight 4000–6000 less than the products immunoprecipitated from bCSP. This is in contrast to the immunoprecipitable translation product for ovine poly (A)⁺ mRNA, in which the translation product has a molecular weight of 4000 larger than the products immunoprecipitated from oCSP (Hansen *et al.*, 1985). These differences suggest that the post-translation processing of the ovine and bovine proteins is dissimilar. One possible explanation is that the bovine proteins are glycosylated whereas the ovine protein simply undergoes proteolytic cleavage, e.g. removal of a signal sequence, with no glycosylation.

In conclusion, results of this study demonstrate that the cow conceptus secretes a protein complex that is immunologically related to oTP-1. This protein complex is a major product released by Day 16–26 cow conceptuses during the critical period at which the corpus luteum has to be protected from the luteolytic action of uterine PGF-2 α (Bartol *et al.*, 1985). Because of this immunological similarity and because it is synthesized at a developmentally equivalent stage of pregnancy as oTP-1, we suggest that the protein complex secreted by the bovine conceptus which cross-reacts with antiserum to oTP-1 should be called bovine trophoblast protein-1 (or bTP-1). We predict that oTP-1 and bTP-1 may play functionally similar roles in maternal recognition of pregnancy in the two species. Finally, provided there is no cross-reactivity with plasma proteins in the culture medium, anti-oTP-1 antiserum is likely to prove a valuable reagent in further studies on bTP-1, a protein which has so far resisted attempts at purification by conventional means.

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