Immunochemical detection, physicochemical characterization and levels of pregnancy-associated endometrial α_2 -globulin (α_2 -PEG) in seminal plasma of men

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Summary. Pregnancy-associated endometrial α_2 -globulin (α_2 -PEG), the major secretory protein of the human uterine endometrium during the luteal phase of the menstrual cycle and early first trimester of pregnancy, has been detected by immunochemical techniques in seminal plasma. Biochemical analysis and immunoblotting has verified that immunoreactive α_2 -PEG in seminal plasma exhibits properties identical to those of endometrial α_2 -PEG, i.e. Concanavalin A-binding dimeric glycoprotein of native M_r 56 000, subunit M_r 28 000, average pI 4.7 and of α_2 -mobility. Concentration of α_2 -PEG in seminal plasma was 22.13 ± 2.82 $\mu\text{g/ml}$ (mean $\pm$ s.e.m., $n = 110$) which compared to 12.02 ± 1.65 $\mu\text{g/ml}$ (mean $\pm$ s.e.m., $n = 48$) found in amniotic fluid at 11–20 weeks of pregnancy, to 4.29 ± 1.66 $\mu\text{g/ml}$ (mean $\pm$ s.e.m., $n = 15$) in uterine luminal fluid in women during the luteal phase and to 0.245 ± 0.025 $\mu\text{g/ml}$ (mean $\pm$ s.e.m., $n = 10$) in sera at 10 weeks of pregnancy. This distribution is very different from that observed for pregnancy-associated placentally-derived serum proteins detected in seminal plasma. The source of α_2 -PEG in seminal plasma is unknown but is unlikely to be the testis because of the normal levels observed in vasectomized men. In the endometrium α_2 -PEG synthesis and secretion appears to be related to progesterone-dependent differentiation of the glandular epithelium. Therefore these observations suggest that a different mechanism of regulation of the gene for α_2 -PEG operates in the male reproductive tract.

Introduction

Pregnancy-associated endometrial α_2 -globulin (α_2 -PEG) is the major protein synthesized *de novo* and secreted *in vitro* by the human endometrium during the menstrual cycle and early first trimester of pregnancy (Bell, 1985, 1986a; Bell *et al.*, 1985a, b, 1986a). α_2 -PEG is synthesized by the secretory glandular epithelium, a tissue whose differentiation is primarily dependent upon progesterone. During the menstrual cycle, α_2 -PEG appears to be secreted into the uterine lumen (Bell & Doré-Green, 1987) and during pregnancy into the amniotic fluid (Bell *et al.*, 1986b) although the mechanism by which it is transported across the amniochorion is unknown. α_2 -PEG isolated from culture medium of pregnancy endometrium (Bell *et al.*, 1985b) or the cytosolic fraction of pregnancy endometrium (Bell, 1986b) appears as a dimeric glycoprotein of native M_r 56 000 and subunit M_r 28 000 and of α_2 -mobility. α_2 -PEG was originally identified in its subunit form and was referred to as endometrial protein 15, EP15 (Bell *et al.*, 1985a). Recent studies suggest that α_2 -PEG may be analogous to progestagen-dependent endometrial protein, PEP, isolated from the endometrium (Joshi, 1983), placental protein 14, PP14, isolated from term placenta (Bohn *et al.*, 1982), and chorionic α_2 -microglobulin, CAG-2, isolated from first trimester placenta (Tatarinov *et al.*, 1980).

However, the 'placental proteins' may be derived from placental tissue contaminated with endometrial tissue or represent the trophoblast-bound forms of α_2 -PEG/PEP (see Bell & Bohn, 1986).

Several human placental proteins have been identified immunochemically in seminal plasma albeit at low concentrations relative to pregnancy serum levels (Lee *et al.*, 1983), including placental protein 5, PP5 (Ranta *et al.*, 1981), and pregnancy-associated plasma protein A, PAPP-A (Bischof *et al.*, 1983), although in the male the tissue site of origin of these proteins has not been determined. Bell *et al.* (1986a) have suggested that α_2 -PEG may represent the human analogue of the progesterone-dependent rabbit uteroglobin or blastokinin, and since uteroglobin has also been detected in seminal plasma (Kirchner & Schroer, 1976), we have examined whether human seminal plasma also contains α_2 -PEG.

Materials and Methods

Specimens

Seminal plasma was obtained from 50 vasectomized patients and from 60 men being investigated for infertility. After liquefaction the specimens were centrifuged at 1000 *g* for 20 min. Seminal plasmas were analysed for α_2 -PEG levels immediately by rocket immunoelectrophoresis and the remainder stored at -20°C . One specimen of normal seminal vesicles was obtained from one patient during excision of a bladder carcinoma to which the seminal vesicles were attached.

Antisera and antigens

Rabbit antiserum to α_2 -PEG was prepared as previously described and purified α_2 -PEG used as a standard was obtained from the cytosolic fraction of first trimester pregnancy endometrium as previously described (Bell, 1986b).

Immunochemical techniques

Ouchterlony double immunodiffusion. The technique of Ouchterlony (1958) was used with 1% (w/v) agar in 0.06 M-barbital buffer, pH 8.6 (Sigma, Poole, Dorset, U.K.).

Tandem crossed immunoelectrophoresis. This was performed in a 1% (w/v) agarose gel using 0.06 M-barbital buffer, pH 8.6, with a voltage gradient of 25 V/cm (output voltage) for 1.5 h in the first dimension and 10 V/cm for 18 h in the second dimension (Crowle, 1977). Antiserum to α_2 -PEG was used at 3.75% (v/v) in the second dimension as for rocket immunoelectrophoresis.

Rocket immunoelectrophoresis. The technique of Laurell (1972) was employed using 1% (w/v) agarose in 0.06 M-barbital buffer, pH 8.6, with a voltage gradient of 100 V (output voltage) for 18 h on 10×10 cm plates. Antiserum was used in the agarose at 3.75% (v/v) and 8 μl fractions were applied to wells. Precipitin lines were stained with Coomassie Brilliant Blue G250 after extensive washing with distilled water. For monitoring column fractions an endometrial cytosol preparation was used as a reference and assigned an α_2 -PEG content of 200 AU/ml.

Biochemical fractionation techniques

Ion-exchange chromatography. A pool of seminal plasma (15 ml) was dialysed against 0.1 M-Tris-HCl, pH 8.0, for 72 h and applied to a column (1.6×20 cm, $V_t = 40$ ml) of DE52 (Whatman). After washing with two column volumes of 0.1 M-Tris-HCl buffer, pH 8.0, to remove unbound proteins, bound proteins were removed with a 0.2 M-NaCl gradient prepared in the same buffer at a flow rate of 15 ml/h. Fractions of 2 ml were collected.

Gel filtration. A pool of seminal plasma (5 ml) was applied to a column (2.6×35 cm, $V_t = 186$ ml) of Sephacryl S-200 (Pharmacia, Milton Keynes, U.K.) equilibrated and eluted in 0.1 M-Tris-HCl, 0.5 M-NaCl buffer, pH 8.0, at a flow rate of 24 ml/h. The column was calibrated with protein standards of known molecular weight (Sigma) and 3-ml fractions were collected.

Concanavalin A-Sepharose chromatography. A pool of seminal plasma (12 ml) was dialysed against 0.5 M-Tris-HCl, pH 7.4, containing 1 M-NaCl and applied to a column (1×15 cm, $V_t = 12$ ml) of Concanavalin A-Sepharose (Pharmacia) equilibrated in the same buffer. After removing unbound material, bound glycoproteins were removed with a gradient of 0.0.2 M- α -D-methylmannoside in 0.5 M-Tris-HCl, 1 M-NaCl buffer, pH 7.4, at a flow rate of 10 ml/h. Fractions of 3 ml were collected.

Chromatofocussing. A pool of seminal plasma (5 ml) was dialysed against 0.025 M-imidazole buffer, pH 7.4, and applied to a column (1×15 cm, $V_t = 12$ ml) of polybuffer exchanger PBE 94 (Pharmacia) equilibrated in the same

degassed buffer. Proteins were eluted with 12.5 column volumes of polybuffer 74-HCl, pH 4 (Pharmacia), at a flow rate of 24 ml/h. Further bound protein was removed by elution with one column volume of 1 M-NaCl. The pH of all fractions (2.5 ml) was monitored continuously and the pH readjusted to 7.4 within 30 min of elution. Fractions of 2 ml were collected.

Monitoring of column chromatographic fractions. All fractions obtained from column chromatographic separations were monitored (a) for elution of total protein by continuous absorption at 280 nm; (b) presence of immunoreactive α_2 -PEG by rocket immunoelectrophoresis; and (c) qualitative analysis of proteins by SDS-gradient polyacrylamide gel electrophoresis followed by protein dye staining.

SDS-gradient polyacrylamide gel electrophoresis (PAGE). For SDS-gradient PAGE, 50 μ l (20 track gel) aliquants of column fractions were mixed with an equal volume of buffer containing β -mercaptoethanol (10%), SDS (5%), glycerol (20%), Trisma (0.125 M, pH 6.8) and bromophenol blue (0.002%). Samples were heated at 100°C for 3 min and analysed on a 1.5-mm thick 5–15% acrylamide slab gel (containing 0.26–0.82% bisacrylamide) with a stacking gel (containing 0.12% bisacrylamide), both containing 0.1% SDS. Gels were fixed overnight in 3.5% perchloric acid–20% methanol and stained for 1–2 h with Coomassie blue G (0.04% in 3.5% perchloric acid–20% methanol). Gels were calibrated with proteins of known molecular weight, i.e. β -galactosidase (M_r 116 000), phosphorylase b (M_r 97 000), bovine serum albumin (M_r 66 000), ovalbumin (M_r 45 000), glyceraldehyde 3-phosphate dehydrogenase (M_r 36 000), carbonic anhydrase (M_r 29 000), trypsin inhibitor (M_r 20 100) and α -lactalbumin (M_r 14 200) (Sigma).

Electroblotting and antigenic detection

Protein transfer from SDS-PAGE gels to cellulose nitrate membranes (Sartorius Instruments Ltd., Belmont, U.K.) was carried out by electroblotting (Towbin *et al.*, 1979) using a Trans-Blot cell system (Bio-rad Laboratories, Watford, U.K.). The buffer used was 25 mM-Tris, pH 8.3, containing glycine (192 mM) and methanol (10%), and this was degassed on ice for 1 h immediately before use. The completed gel was placed on buffer-soaked filter paper laid on a buffer-soaked Scotch Brite pad (BioRad Laboratories, Watford, U.K.), ensuring that no air was trapped between the different layers. Cellulose nitrate membrane, wetted in distilled H₂O for 30 min, was placed over the gel and any trapped air once again removed. Finally, a buffer-soaked filter paper and a Scotch Brite pad were laid over the membrane, any trapped air removed, and the compact structure was sandwiched between the two halves of the plastic holding frame, which was then slotted into the Trans-Blot cell. Protein transfer was carried out on ice at 80 V for 2 h, the membrane being on the anodal site of the SDS-PAGE gel. After electroblotting, the protein-loaded membrane was incubated for 30 min in Tris-buffered saline (TBS), pH 7.5, containing 1% BSA and 0.5% casein, to block all unreacted protein-binding sites. The membrane was then incubated for 2 h at room temperature with rabbit antibody to α_2 -PEG diluted 1 in 100 with TBS, containing 0.05% Tween 20. At the end of the 2 h the membrane was washed for 10 min in 200 ml TBS containing 0.25% lauryl sarcosine and 0.25% Nonidet P-40 (Sigma). Finally, the membrane was incubated for 1 h at room temperature with peroxidase-conjugated goat antibody to rabbit IgG (Miles Laboratories, Slough, U.K.) diluted 1 in 100 with TBS/Tween 20.

After washing as before, bound peroxidase activity was developed using freshly prepared 40 mg 4-chloro-1-naphthal (Sigma) dissolved in 300 μ l ethanol, added to 100 ml 5 mM-Tris. Immediately before use 100 μ l H₂O₂ were added and the solution was filtered through Whatman No. 1 filter paper (Whatman Ltd, Maidstone, U.K.). The membrane was then incubated in this solution for 15 min, washed and then pressed dry between two sheets of filter paper.

Estimation of α_2 -PEG concentrations

Rocket immunoelectrophoresis (RIE). RIE was performed as described above except that seminal plasma specimens were tested at neat, 1:4 and 1:8 (v/v) dilutions. A standard curve was included using an α_2 -PEG standard (40 μ g/ml) as a series of double dilutions to 1:16 (v/v).

Radioimmunoassay (RIA). An equilibrium RIA was used which employed α_2 -PEG purified from cytosol of first trimester pregnancy endometrium (Bell, 1986b) as a standard and for radioiodination. The assay was carried out with ¹²⁵I-labelled α_2 -PEG (16 μ Ci/ μ g) (60 000 c.p.m./20 μ l), rabbit anti- α_2 -PEG IgG (13 mg/ml) (100 μ l of 1:400 v/v) and RIA buffer (10 mM-phosphate, 0.154 M-NaCl, 50 mM-EDTA, 0.1% w/v bovine serum albumin, 0.1% sodium azide, pH 7.4) adjusted to a final volume of 800 μ l. For the dose-response curve, 100 μ l RIA buffer containing 1–1000 ng purified α_2 -PEG were included and for seminal plasma and endometrial cytosols a range of dilutions was used. Non-specific binding was determined by inclusion of excess unlabelled α_2 -PEG (2.5 μ g). After incubation at 4°C for 24 h, 200 μ l of 1:40 (v/v) goat anti-rabbit IgG were added and incubated for 18 h. After addition of 100 μ l of 14% (w/v) of polyethylene glycol and incubation of 6 h, precipitates obtained by centrifugation for 20 min at 1500 g were counted on a Packard Autoframme 500. All tubes were assayed in duplicate.

Protein estimation

The colorimetric method of Bradford (1976) was used to determine total soluble protein content, using human serum albumin as a reference standard.

Fig. 1. Immunochemical analysis of immunoreactive α_2 -PEG in human seminal plasma. **(a)** Double immunodiffusion using rabbit anti- α_2 -PEG antiserum in the centre well (A: 20 μ l neat antiserum) *versus* a range of antigen preparations including seminal plasmas (Wells 1 & 6), amniotic fluids (Wells 2 & 5), pregnancy sera (Well 3) and α_2 -PEG purified from pregnancy endometrium cytosol (Well 4). Seminal plasma (Well 6) and amniotic fluid (Well 5) did not contain immunoreactive α_2 -PEG as assessed by rocket immunoelectrophoresis. Antigen wells contained 20 μ l volumes of a 1:4 (v/v) dilution except for α_2 -PEG which was at 1:16 (v/v). **(b)** Tandem crossed immunoelectrophoresis using seminal plasma (Well 1) and α_2 -PEG purified from pregnancy endometrium cytosol (Well 2) in the first dimension (volume 8 μ l) and 3.75% (v/v) rabbit anti- α_2 -PEG antiserum in the second dimension. Arcs were of α_2 mobility. **(c)** Rocket immunoelectrophoresis with rabbit anti- α_2 -PEG antiserum (3.75%, v/v) and 8 μ l of a series of double dilutions from neat to 1:16 (v/v) of a seminal plasma (Wells 6–10) and α_2 -PEG purified from pregnancy endometrium cytosol (Wells 1–5).

Results

Detection of immunoreactive α_2 -PEG in seminal plasma

In double immunodiffusion tests seminal plasma reacted with antiserum to α_2 -PEG to produce precipitin arcs which exhibited reaction of identity with purified α_2 -PEG and amniotic fluid (Fig. 1a). Amniotic fluid and seminal plasma specimens which did not contain α_2 -PEG as assessed by RIE failed to react in the immunodiffusion test (Fig. 1a, Wells 5 & 6). In tandem crossed immunoelectrophoresis, both purified α_2 -PEG and seminal plasma produced precipitin arcs of α_2 mobility which completely fused although the arcs with seminal plasma were diffuse (Fig. 1b). In rocket immunoelectrophoresis, as with purified α_2 -PEG, the heights of rockets obtained with a series of double dilutions was proportional to dilution, but the shapes of rockets obtained with seminal plasma were different from those obtained with α_2 -PEG and amniotic fluid, the former appearing rounded (Fig. 1c).

Fig. 2. Elution of immunoreactive α_2 -PEG in seminal plasma from (a) ion-exchange, (b) gel filtration, (c) Concanavalin A-Sepharose and (d) chromatofocussing columns. Aliquants of pooled seminal plasma (5–15 ml) were applied to the columns. Details of columns and eluting conditions are in 'Materials and Methods'. Column fractions were monitored continuously for protein at 280 nm (unmarked lines) and immunoreactive α_2 -PEG by RIE (●). Fractions were also monitored by one-dimensional SDS-gradient PAGE when duplicate gels were processed for protein staining and immunoblotting with rabbit anti- α_2 -PEG. A representative result on the gel filtration column (b) is shown in Figs 3(a) and (b). G refers to time of application of the appropriate gradient.

Fig. 3. One-dimensional SDS-gradient PAGE analysis of fractions from a gel filtration column chromatographic analysis of seminal plasma. **(a)** Aliquants of fractions from Sephacryl S-200 column chromatography of seminal plasma (see Fig. 2b) were subjected to SDS-gradient PAGE under reducing conditions (numbered tracks correspond to aliquants from every second fraction from fractions 22 to 54 in Fig. 2b). The gel was stained with Coomassie brilliant blue (see 'Materials and Methods'). Molecular weights ($\times 10^{-3}$) of marker proteins in the left-hand track are referred to along the axis. The position of authentic α_2 -PEG, migrating as its subunit, referred to as EP15, in this system is arrowed on the right. Note the presence of a stained band at this position in tracks corresponding to fraction numbers containing immunoreactive α_2 -PEG (see Fig. 2b). **(b)** A duplicate gel to that produced in Fig. 3(a) was electroblotted onto nitrocellulose paper and subjected to an immunoperoxidase technique used for an IgG preparation obtained from rabbit anti- α_2 -PEG antisera. In this technique authentic α_2 -PEG is visualized at high Ag:Ab ratios as two major bands, the lower at M_r 28 000 corresponding to the M_r 28 000 band on Coomassie blue-stained gels, i.e. EP15, and an upper band not detectable on stained gels. Immunoreactive α_2 -PEG occurs in tracks which correspond to tracks containing the stained band of M_r 28 000 (Fig. 3a) and immunoreactive α_2 -PEG detected by the RIE assay (Fig. 2b).

Physicochemical characterization of immunoreactive α_2 -PEG in seminal plasma

Experiments were performed to determine whether the immunoreactive α_2 -PEG detected in seminal plasma represented authentic α_2 -PEG. On an anion-exchange chromatography column (DE52) immunoreactive α_2 -PEG was completely bound under the conditions employed and

required a NaCl gradient for its elution. Immunoreactive α_2 -PEG eluted over a wide NaCl concentration with peak levels being observed at 0.1 M-NaCl (Fig. 2a). On a gel filtration column (Sephacryl S-200) immunoreactive α_2 -PEG eluted as a single peak with an apparent M_r of 56 000 (Fig. 2b). On a Concanavalin A-Sephacrose column immunoreactive α_2 -PEG also bound under the conditions described and required a gradient of α -D-methylmannoside for its elution (Fig. 2c). On a chromatofocussing column immunoreactive α_2 -PEG eluted over a wide pH range with peak levels being eluted at 4.7 (Fig. 2d).

Fig. 4. Elution of ^{125}I -labelled α_2 -PEG incubated with seminal plasma from a gel filtration column (Sephadex SF G-100). An aliquant of seminal plasma (500 μl) (■) or phosphate-buffered saline (□) was incubated at 37°C for 18 h with ^{125}I -labelled α_2 -PEG (3×10^6 c.p.m.) and applied and eluted from a column of Sephadex G-100 SF. The column (1 $\times$ 34 cm, $V_t = 27$ ml) was eluted with 50 mM-phosphate buffer, pH 7.4, containing 0.5 M-NaCl and 0.5% (w/v) BSA, at a flow rate of 30 ml/h and 1.0 ml fractions were collected. Aliquants of fractions (20 μl) were monitored for radioactivity in a gamma counter. The void volume (V_o) and total volume of column (V_t) are marked.

To obtain further characterization, aliquants of all column fractions were analysed by one-dimensional SDS-PAGE under reducing conditions. After staining of gels, all column fractions containing immunoreactive α_2 -PEG possessed a stained band at apparent M_r 28 000, which corresponds to the molecular weight under reducing conditions of authentic α_2 -PEG, i.e. the subunit referred to as EP15 (Bell *et al.*, 1985a, b). The gel analysis of fractions from the Sephacryl S-200 column is shown in Fig. 3(a) as a representative experiment. To confirm that this band of M_r 28 000 contained the immunoreactive protein in seminal plasma, duplicate SDS-PAGE gels were electroblotted onto nitrocellulose paper and reacted with antiserum to α_2 -PEG and the antibody binding was visualized by an immunoperoxidase technique. Figure (3b) shows the results of such an experiment on the Sephacryl S-200 column fractions (see Fig. 2b; Fig. 3a), demonstrating the localization of immunoreactive α_2 -PEG to the band of M_r 28 000 although on the blot this band appeared to be composed of two bands with the lower band predominating. A second, higher molecular weight immunoreactive band coeluted with the band of M_r 28 000 and has been observed even with purified α_2 -PEG when subjected to the same technique, in spite of the absence of a Coomassie Blue-stained band at this position on gels. However, in seminal plasma this higher

Fig. 5. One-dimensional SDS-gradient PAGE analysis of ^{125}I -labelled α_2 -PEG incubated with seminal plasma. An aliquant of seminal plasma (500 μl) (b) or phosphate-buffered saline (500 μl) (a) was incubated at 37°C for 18 h with ^{125}I -labelled α_2 -PEG (3×10^6 c.p.m.) and aliquants were subsequently analysed by the SDS-gradient PAGE technique. After electrophoresis the gel was dried and autoradiography performed. After development of the X-ray film the region of the film corresponding to the lower 40% of the tracks was scanned in a densitometer, since no radioactivity was detected on the upper 60% of the gel tracks. The positions of molecular weight markers (upper axis), authentic α_2 -PEG (α_2 -PEG) (arrowed) and the gel front (GF) are marked.

molecular weight band was more predominant than in purified α_2 -PEG preparations. Similar results demonstrating the correspondence of the M_r 28 000 stained protein to the immunoreactive α_2 -PEG were obtained with all column techniques.

Behaviour of seminal plasma in a radioimmunoassay for α_2 -PEG

Dilutions of seminal plasma in a specific radioimmunoassay for α_2 -PEG produced a parallel competition curve with authentic α_2 -PEG purified from cytosol of first trimester pregnancy endometrium or crude endometrial cytosol. To determine whether any protease activity of seminal plasma interfered with the RIA, iodinated tracer was incubated with seminal plasma at 37°C for 18 h and the mixture applied to a Sephadex G100 column ($V_i = 27$ ml) and also analysed on one-dimensional SDS-gradient PAGE. ^{125}I -labelled α_2 -PEG tracer was also subjected to the same separation techniques as a control. When ^{125}I -labelled α_2 -PEG was incubated with seminal plasma, a second peak of radioactivity was eluted at the V_i of the column (Fig. 4). This partial breakdown of the tracer was confirmed by SDS-gradient PAGE analysis since in this system an increase in the radioactivity at the

gel front was observed in the sample incubated with seminal plasma (Fig. 5). Under the conditions described seminal plasma therefore appeared to cause a 19% breakdown of the ^{125}I -labelled α_2 -PEG tracer used in the RIA. However, this effect of seminal plasma does not account for the discrepancy in estimations of α_2 -PEG concentrations in seminal plasma by the RIA and RIE assays in which, although a high degree of correlation in the relative levels obtained was found ($r = 0.863$, $P < 0.001$), the former assay detected 2.5-fold the amounts of α_2 -PEG detected by the RIE technique. This may result from an underestimation of values in the RIE technique since different rocket morphology was obtained with seminal plasma specimens in contrast to amniotic fluid and endometrial cytosol. This was supported by the ability to detect α_2 -PEG on Coomassie blue-stained SDS gels of column fractions (Fig. 3) on which, according to RIE estimates of content, the α_2 -PEG present should have been undetectable by such a staining method.

Concentrations of α_2 -PEG in seminal plasma

In seminal plasma specimens ($n = 110$), including those obtained from vasectomized men ($n = 50$), α_2 -PEG was detectable in 101/110 (91.8%) specimens. The mean $\pm$ s.e.m. concentration was $22.13 \pm 2.82 \mu\text{g/ml}$ and values ranged from 0 to $240 \mu\text{g/ml}$ (Fig. 6). The α_2 -PEG concentration in seminal plasmas obtained from 50 vasectomized men was 22.24 ± 3.54 (mean $\pm$ s.e.m.) which was not significantly different from that obtained with the infertile group, i.e. 22.05 ± 4.27 (mean $\pm$ s.e.m., $n = 60$).

Fig. 6. Concentrations of α_2 -PEG in human seminal plasma. Values of α_2 -PEG were assayed in 110 seminal plasma specimens by the RIE technique (see 'Materials & Methods') and are expressed as $\log_{10}$ values. nd, not detectable.

Concentrations of α_2 -PEG in seminal vesicular fluid

The level of α_2 -PEG in the fluid obtained from the seminal vesicles of a patient with bladder carcinoma was $7.6 \mu\text{g/ml}$ as determined by rocket immunoelectrophoresis.

Discussion

These results demonstrate that human seminal plasma contains a protein that shares immunochemical and physicochemical properties with pregnancy-associated endometrial α_2 -globulin (α_2 -PEG), a protein synthesized and secreted by the uterine endometrium during the menstrual cycle (Bell *et al.*, 1986a) and first trimester of pregnancy (Bell *et al.*, 1985a). The protein in seminal plasma exhibited a native M_r of 56 000 and subunit M_r of 28 000, it bound to Concanavalin-A, eluted from an anion exchange column at 0.1 M-NaCl and exhibited an average pI of 4.7. Although this evidence suggests that the protein of seminal plasma is identical to endometrial α_2 -PEG confirmation awaits detailed biochemical analysis.

The source of seminal plasma α_2 -PEG is unknown but is unlikely to be the testis because of the normal levels detected in seminal plasma obtained from vasectomized men. The substantial amounts in a specimen of fluid obtained from seminal vesicles may suggest this latter tissue as the source of seminal plasma α_2 -PEG. Most human seminal plasma proteins originate from the seminal vesicles and prostate and both possess a secretory glandular epithelium (Beyler & Zaneveld, 1982; Polakoski & Kopta, 1982). However, uteroglobin was initially detected in secretions of the seminal vesicle of rabbits (Noske & Gooding, 1976) and was subsequently localized to the paraprostate gland by Muller (1983). It has been suggested that the former observation was due to the effect of anaesthesia or death, forcing the products of accessory sexual organs into the lumen of the seminal vesicle (Muller, 1983). A similar effect of anaesthesia in our single specimen of seminal vesicle glands could have occurred. In the endometrium, α_2 -PEG is derived from the secretory glandular epithelium therefore α_2 -PEG may be derived from this cellular source both in the male and female reproductive tracts. In the endometrium α_2 -PEG synthesis and secretion appears to be associated with progesterone-dependent differentiation of the glandular epithelium (Bell & Drife, 1986). In the male reproductive tract α_2 -PEG synthesis and secretion is presumably regulated by another mechanism and therefore α_2 -PEG may represent a useful marker for the study of differential regulation of gene expression in different tissues.

Table 1. Comparison of distribution of PAPP-A and α_2 -PEG in seminal plasma and other fluids

Compartment	PAPP-A		α_2 -PEG	
	Conc. ($\mu\text{g/ml}$)	Ratio	Conc. ($\mu\text{g/ml}$)	Ratio*
Seminal plasma	1.49 ± 0.11 (30) ^a	0.0038	22.13 ± 2.82 (110) ^d	90.33
Intrauterine luminal fluid (menstrual cycle)	0.21 ± 0.08 (5) ^b (late secretory)	0.0005	4.29 ± 1.66 (15) ^e (Days 16–28)	17.5
Amniotic fluid	3.8^c (Week 40)	0.0098	16 ± 3.7 (28) ^f (Week 15)	65.3
Serum/plasma (pregnancy)	388 ± 60 (20) ^c (Week 40)	1	0.245 ± 0.025 (10) ^g (Week 10)	1

Results are expressed as mean $\pm$ s.e.m. for the no. of samples in parentheses.

References: (a) Bischof *et al.*, 1983; (b) Bischof *et al.*, 1984; (c) Bischof *et al.*, 1982b; (d) this paper; (e) Bell & Doré-Green, 1987; (f) Bell *et al.*, 1986b; (g) S. C. Bell, unpublished observations.

*Compared to that in serum/plasma.

Several pregnancy-associated serum proteins derived from the placenta have previously been detected in seminal plasma. These include hCG, human placental lactogen, SP1 (Krause, 1982; Lee *et al.*, 1983), PP5 (Ranta *et al.*, 1981; Lee *et al.*, 1983) and PAPP-A (Bischof *et al.*, 1983) although the evidence is principally immunochemical and no vigorous biochemical characterization of these antigens in seminal plasma has been performed. It has been suggested that PAPP-A is of endometrial origin (DuBerg *et al.*, 1982; Bischof *et al.*, 1982a). However, when the compartmental distribution of PAPP-A is compared with the distribution of α_2 -PEG, an unequivocally demonstrated endometrially-derived protein, PAPP-A appears to have a different origin (Table 1). The placental protein CAG-2 isolated from first trimester placental tissue (Tatarinov *et al.*, 1980) has been detected immunochemically in seminal plasma (Petrinin *et al.*, 1980). CAG-2 is immunochemically identical to PP14 (Bohn *et al.*, 1982), a protein that has been demonstrated to be related to α_2 -PEG (Bell & Bohn, 1986).

The concentrations of α_2 -PEG in seminal plasma represent the highest levels of α_2 -PEG detected in any physiological fluid (amniotic fluid, uterine luminal fluid or sera), but the function of α_2 -PEG in these compartments, whether similar or different, is unknown. The detection of α_2 -PEG in uterine luminal flushings (Bell & Doré-Green, 1987) suggests a human analogue of rabbit uteroglobin, the major protein of rabbit intrauterine luminal fluid (Beato, 1977) and also detectable in seminal plasma (Kirchner & Schroer, 1976). Another possibility is raised by the recent suggestion that a major secretory protein of the luteal phase endometrium, M_r 25 000, represents glycosylated prolactin (Heffner *et al.*, 1986). Since we have detected α_2 -PEG as the major secretory protein during the same period in a similar gel system as subunit M_r 28 000 (Bell *et al.*, 1986a), the possibility exists that α_2 -PEG is glycosylated prolactin in a dimeric form. Human glycosylated prolactin has been described (Lewis *et al.*, 1985) and its physiological and endocrinological role with respect to free prolactin has yet to be elucidated.

Studies are in progress to determine the site of synthesis of α_2 -PEG within the male reproductive tract and to determine whether levels of α_2 -PEG in seminal plasma may be related to male infertility.

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