Endothelin-1 and endothelin receptors are present in the sheep uterus and conceptus at implantation

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Abstract

Previous studies have demonstrated that endothelin is present in the ovine endometrium and increases at around the expected time of implantation. To characterize further uterine endothelin at the time of establishment of pregnancy in sheep, endothelin was measured by radioimmunoassay in uterine flushings obtained during the oestrous cycle and in pregnant ewes up to the time of implantation (day 16). During the oestrous cycle, the highest amounts of endothelin were present in uterine flushings on day 14 (1.1 ± 0.2 ng endothelin/uterus). During early pregnancy, basal levels of endothelin (0.5 ± 0.6 ng endothelin/uterus) were present in uterine flushings for the first 10 days and then increased on day 14 to levels similar to those found at the equivalent stage of the oestrous cycle. On days 15 and 16 of pregnancy, endothelin content in the uterine lumen increased to significantly (P<0.05) higher concentrations (2.9 ± 0.4 ng endothelin/uterus) when compared with the non-fertile cycle. The principal isoform present in flushings at the time of implantation was endothelin-1, as determined by reverse-phase HPLC. Endothelin was released principally by purified endometrial epithelial cells in culture, with barely detectable amounts released by endometrial stromal cells or conceptus tissue, which is consistent with the epithelium being the principal source of endothelin in the uterine lumen. Endothelin binding sites were present in endometrium and myometrium, as demonstrated by specific binding of 125I-labelled endothelin-1, which was saturable and displaced by endothelin-1. Both endothelin_A and B sub-types of receptors were present as demonstrated by the biphasic displacement of 125I-labelled endothelin-1 binding by the specific endothelin_B agonist BQ3020. These were localised principally on luminal and glandular epithelium and in the vasculature of the endometrium and myometrium as shown by autoradiography. Endothelin receptors were also present on the conceptus obtained at the time of implantation. In the day 20 conceptus, endothelin immunostaining was localised principally in the heart, in trophoblast in uninnucleate but not in binucleate cells, and in fetal membranes. This immunostaining of the conceptus may represent binding to receptor sites. It is concluded that endothelin-1 is present in the uterine lumen and may play an important role in the paracrine regulation of the conceptus and endometrium at the time of rapid embryo development, implantation and early placentation.

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Introduction

Endothelins (ETs) are a family of three 21 amino acid peptides originally described for their potent vasoconstrictor actions (Masaki 1993). They are present in the uteri of sheep (Riley et al. 1994), women (Cameron et al. 1992, Economos et al. 1992, Salamonsen et al. 1992) and rabbit (Orlando et al. 1990, Maggi et al. 1991). In sheep, the tissue content of ET-1, the principal isoform present, increases towards the end of the oestrous cycle and at the time of implantation, and is localised principally in epithelial cells at these times (Riley et al. 1994). In the endometrium of women ET-1 mRNA and peptide are localised in, and ET-1 is released by, the same cells (Economos et al. 1992, Salamonsen et al. 1992, Marsh et al. 1994). The effects of endothelins are modulated through two subtypes of receptor, ET_A and ET_B, which are members of the G-protein linked receptor superfamily (Masaki et al. 1994). The mRNA for both these receptor subtypes and specific binding sites are present in the uterus of women throughout the menstrual cycle (O’Reilly et al. 1992). In the sheep uterus the presence and localisation of endothelin receptors has not been examined. Although the functions of ETs in the uterus have yet to be clearly defined, it has been proposed that ET plays an important regulatory role in the vasculature by constriction of spiral arterioles for the initiation of menstruation (Findlay et al. 1992).

Recent studies have shown that ETs are expressed in a wide range of other cell types and possess paracrine
regulatory functions including mitogenic actions where their effects are comparable to those of cytokines (Battistini et al. 1993). In sheep and other ruminants, there is a relatively long period of preimplantation development, characterised by rapid blastocyst expansion during the 4 days prior to implantation (Bindon 1971). During the implantation period there is considerable angiogenesis (Reynolds & Redner 1992) and endometrial and trophoblast mitogenesis. Therefore, the sheep is a good model in which to examine preimplantation mitogenic stimuli.

This study has characterised the ET content in the uterine lumen during the oestrous cycle and early pregnancy around the time of implantation in the ewe. The cellular source of endometrial ET was determined using cultures of purified cells and the localisation of ETs in the conceptus was demonstrated using immunohistochemical techniques. The presence of specific binding sites to ET was determined in the uterus and conceptus, and localised during the peri-implantation period.

Materials and Methods

Animals

Parous Corriërdale ewes (n=80) were mated with either vasectomised or intact rams as described previously (Salamonsen et al. 1986). On the evening prior to surgery, animals were penned and food and water withheld. Ewes on days 4 (n=5 per group unless specified otherwise), 10, 12, 14 (n=7) and 16 (3) after oestrus (day 0), and on days 4, 7 (4), 8 (4), 9 (4), 10, 12, 14 (9), 15 (6), 16 (7) of pregnancy were anaesthetised, the uterus exposed through a midline incision and the cervix clamped as described previously (Salamonsen et al. 1986). Uteri were flushed with 20 ml saline and pregnancy was confirmed by the presence of a conceptus. Ewes were hysterectomised and uterine tissues were collected and frozen in embedding compound (OCT; Miles Inc., Elk hart, IN, USA) for analysis of ET binding sites. On days 17 (n=3) and 20 (3) of pregnancy, uteri were not flushed prior to hysterectomy to retain the tissue integrity of the conceptus and pregnancy was confirmed when the uterus was opened. Flushings were centrifuged (200 g for 10 min) to remove cellular debris, the supernatant collected, frozen and stored at −20 °C prior to analysis of ET content. Some conceptus tissues were collected on days 17 and 20 and either embedded in OCT or fixed in 10% neutral buffered formalin for 4 h, washed twice in Tris-buffered saline (TBS; pH 7.4; 0.05 M) and embedded in paraffin wax for immunohistochemistry. For tissue culture experiments, endometrium was collected at the abattoir from ewes in the luteal phase of the oestrous cycle, as determined by the presence of a corpus luteum. All animal experimentation was approved by the Animal Ethics Committees at the Animal Research Institute, Werribee and at Monash Medical Centre, Clayton.

Endothelin radioimmunoassay

Endothelin concentrations in uterine flushings were measured by radioimmunoassay, using a rabbit polyclonal antibody raised against a synthetic C-terminus octapeptide which cross-reacts equally with ET-1, -2 and -3, but not with big ET-1. The methods and characterisation of this assay have been reported previously (Marsh et al. 1994, Riley et al. 1994). Samples of uterine flushings (1–2 ml) were dried under vacuum and reconstituted in an appropriate volume of radioimmunoassay buffer so as to lie on the accurate portion of the ET-1 (Auspep, Melbourne, Victoria, Australia) standard curve. Data were corrected for non-specific binding (<4%) and expressed as pg ET/uterus. The sensitivity of the assay was assessed as 1·25 pg ET-1/tube, and the intra- and interassay coefficients of variation were 7·7% and 8·1% respectively.

Reverse-phase HPLC

Endothelins were analysed by HPLC as reported previously (Riley et al. 1994). Samples of uterine flushings (4–5 ml) obtained on day 15 and 16 of pregnancy were acidified, loaded on to Sep-Pak C18 cartridges (Millipore Corporation, Milford, MA, USA) and eluted with 60% acetonitrile/0·1% trifluoroacetic acid (TFA). This sample was vacuum dried, reconstituted in 0·1% TFA and loaded on to an octadecl-silica C18 column (30 cm × 2 mm). Samples were eluted for 80 min with a gradient of 0–60% acetonitrile containing 0·1% TFA at a flow rate of 0·4 ml/min. Fractions corresponding to 1 min were collected, vacuum dried and ET content was measured by RIA. The elution of ET in uterine flushing samples was compared with the elution positions of standard ET-1, -2 and -3 (Auspep). Blank runs were performed between samples and standards to confirm that there was no cross-contamination.

Ligand binding and autoradiography

Binding sites were measured, characterised and localised by autoradiography using the method described by Molenaar et al. (1993). Serial sections (10 μm) of full thickness myometrium and endometrium were cut and thaw-mounted onto gelatin-coated slides. Preliminary experiments established that specific binding was time- and temperature-dependent and the optimal conditions were 2 h and 23 °C as described previously. Binding assays were performed using 125I-labelled ET-1 prepared as previously described (Riley et al. 1994). In competition binding studies, labelled ligand was displaced with ET-1 and the specific ETB receptor antagonist BQ3020 (Auspep) for 2 h at 23 °C (4–6 tissue sections for each concentration of ligand). Non-specific binding was determined by displacement with 1 μM ET-1. Bound ligand was separated from unbound by three washes in Tris buffer (pH 7·4; 0·05 M).
Sections were wiped onto filter papers and the radioactivity measured by gamma counting. Non-specific binding was less than 16% of total binding. The $K_d$ was calculated as the concentration of ET-1 that occupied 50% of the binding sites. For autoradiographic localisation of binding sites, sections were incubated with 100 pM $^{125}$I-labelled ET-1 for 2 h followed by the washing procedure as described above, then sections were air dried at 4°C and exposed to X-ray film for 3–4 days.

**Immunohistochemistry**

Immunoreactive endothelin was localised in sections (6 µm) of formalin-fixed embryonic tissue using a specific rabbit polyclonal antibody that recognises all three ETs (Cambridge Research Biochemicals, Northwich, Cheshire, UK) as described previously (Salamonsen et al. 1992, Riley et al. 1994). Immunostaining was determined using the StrepAviGen super sensitive kit according to the manufacturer's instructions (Bio-Genex Laboratories, San Ramon, CA, USA) with fast red as chromagen. Sections were dehydrated, mounted and examined by light microscopy. No specific staining was observed when the primary antibody was substituted by normal rabbit serum, or when this antibody was preabsorbed by ET-1 (10 µM).

**Preparation and culture of endometrial epithelial and stromal cells**

Three separate primary cultures of purified stromal and epithelial cells were prepared from luteal phase endometrium as described previously (Cherny & Findlay 1990, Salamonsen et al. 1993). Briefly, endometrium was dissected from myometrium, finely chopped, digested in collagenase (CLS III; Worthington Biochemical Corp., Freehold, NJ, USA) and DNAse (Boehringer-Mannheim, Mannheim, Germany), followed by filtration through coarse nylon filter cloth (600 µm). The filtrate containing a mixture of cells and entire glands was washed and seeded into flasks. After 48 h, purified preparations of stromal cells were obtained from these by differential trypsinisation. Cells were plated into 24-well plastic culture plates at a density of 2 x 10^5 cells/well in Dulbecco's modified Eagle's medium with penicillin, streptomycin and fungizone (DMEM) containing 10% charcoal-stripped fetal calf serum (FCS; Trace Biosciences Pty Ltd, Castle Hill, NSW, Australia). Cells were allowed to attach for 20–24 h, then washed three times with phosphate-buffered saline and medium replaced with 1 ml DMEM with a serum-free supplement of insulin (10 µg/ml; Human Atrapid, Novo-Nordisk Pharmaceuticals Pty Ltd, Sydney, NSW, Australia), transferrin (10 µg/ml; Sigma Chemical Co., St Louis, MO, USA), selenite (25 ng/ml; Sigma), hydrocortisone (100 ng/ml; Sigma) and without or with

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Immunoreactive ET content (mean ± s.e.m.) of sheep uterine flushings during the oestrous cycle (open bars) and early pregnancy (hatched bars). Histogram bars within these two groups with the same superscript letter are not significantly different ($P>0.05$). During early pregnancy, an asterisk (*) on a histogram bar denotes the presence of significantly ($P<0.05$) more endothelin immunoreactivity when compared with the same day of the oestrous cycle.
10% FCS. Cells were further incubated at 37 °C for 48 h and the medium harvested, centrifuged (200 g for 10 min) to remove cellular debris and stored at −20 °C until ET content was determined by RIA. Cells were harvested for DNA assay (Labarca & Paigen 1980).

Culture of conceptus tissue
Conceptuses (n=12) were collected from uterine flushings on day 14 (n=3) and 15 (4), or from the uterine lumen by blunt dissection on days 17 (2) and 20 (3) of pregnancy, and maintained in culture in DMEM as specified above in a humidified atmosphere of 95% air/5% CO₂ at 37 °C as described previously (Salamonsen et al. 1984). This tissue was cultured for 24 h, which is the time period over which this tissue can be maintained under these conditions without morphological changes occurring. Medium was collected and stored at −20 °C before ET was measured by RIA.

Statistical analysis
Statistical differences between groups were assessed using analysis of variance with a post hoc Tukey's test, after ensuring the homogeneity of variance using Bartlett's test, or by Student's unpaired t-test, as appropriate. A difference was considered significant when P<0.05.

Results
Content and characterisation of endothelin in uterine flushings
ET immunoreactivity was present in uterine flushings throughout the oestrous cycle and during the initial 16 days of pregnancy (Fig. 1). During the oestrous cycle, the amount of ET in the uterine flushings increased on day 12, was significantly (P<0.05) higher on day 14 and decreased on day 16 to basal levels. During the initial 12 days of pregnancy, there was no significant change in endothelin content of uterine flushings, although ET content on day 10 of pregnancy was significantly (P<0.05) greater than on the same day of the oestrous cycle. On day 14 of pregnancy, ET content of uterine flushings increased as occurred during the oestrous cycle. However, ET levels continued to increase and were significantly (P<0.05) higher on day 15 and again on day 16 of pregnancy. There was a significantly greater amount of ET present on day 16 of pregnancy than on the corresponding day of the oestrous cycle. The principal isoform present in extracts of uterine flushings from day 16 of pregnancy eluted in the same fraction as ET-1 standard, as determined by HPLC (Fig. 2). ET-1 immunoreactivity in the extracts of uterine flushings diluted out in parallel with the ET-1 standard in the radioimmunoassay (Fig. 3).

Endothelin output from endometrial epithelial and stromal cells, and conceptus in culture
Endometrial epithelial cells released at least 14-fold and significantly (P<0.05) greater amounts (225±134 pg ET/well; range 612–52 pg ET/well) of ET than stromal cells (less than 40 pg ET/well), although there was considerable variation between individual uteri. In the stromal cell cultures, the ET released was just above detectable limits in two of three cultures (Fig. 4) and undetectable in the third. In two of three cultures of epithelial cells but not stromal cells, FCS significantly (P<0.05) stimulated ET output. The DNA content of wells was similar for both stromal and epithelial cells. The presence of immunoreactive ET was also examined in medium conditioned for 24 h by embryos collected between days 14 and 20 of
pregnancy. In all twelve of the samples of conditioned medium examined which had contained considerably more tissue than cells in monolayer culture, no ET immunoreactivity was detectable (data not shown).

**Ligand binding and autoradiography of 
^{125}I-labelled ET-1 in uterus and conceptus**

Specific binding sites for ET were present on sections of sheep uterine tissues at the time of implantation (days 16–20). Binding of 
^{125}I-labelled ET-1 was saturable and displaced by unlabelled ET-1 (1 µM; Fig. 5a) with a dissociation constant (K_d) of 0.7 ± 0.3 nM (n=4). Both ET receptor subtypes were present as shown by the biphasic displacement curve of 
^{125}I-labelled ET-1 by the specific ET_B receptor agonist BQ3020 (Fig. 5b). Specific ET binding sites were localised in endometrium obtained on day 16 of pregnancy by autoradiography (Fig. 6a), with the most intense specific binding on glandular epithelial cells, some binding on caruncular stromal cells and less binding on intercaruncular stromal tissues. Binding was also present in caruncular stroma. Specific binding sites were also present in the vasculature of the endometrium, in particular in caruncular regions and in the myometrium. Little specific binding was found in the smooth muscle of myometrium. No specific binding was observed in sections where 
^{125}I-labelled ET-1 was displaced with 1 µM ET-1 (Fig. 6b). ET receptors were also present in conceptus tissues (predominantly trophoblast) (Fig. 7). In all conceptus tissues examined, specific 
^{125}I-labelled ET binding was saturable and displaceable by unlabelled ET-1 (1 µM; Fig. 7), with a K_d of 0.6 ± 0.4 nM (n=5).
localised in trophoblast in uninucleate, but not in binucleate cells (Fig. 8b), in epithelial cells of the amnion and allantois, and in the vasculature in the yolk sac. No specific immunostaining for ET-1 was observed in negative control sections (Fig. 8c).

**Discussion**

These studies have demonstrated that immunoreactive ET is present in uterine flushings throughout the oestrous cycle and early pregnancy in sheep. During the oestrous cycle, ET content in the uterine lumen was maximal on day 14 then levels declined by day 16, whereas during early pregnancy no change in ET content occurred at the time of hatching (day 8) but after day 12 of pregnancy ET increased, and continued to rise during the period of rapid blastocyst expansion until implantation (day 16–17). The probable source of this ET is most likely the endometrial epithelium, not the conceptus. Furthermore, binding sites for ET are localised on both the uterus and conceptus. The principal isoform identified in flushings at the time of implantation was ET-1. These findings are in agreement with our previous data in sheep that demonstrated an increase in this isoform in tissue extracts of endometrium and myometrium, and also increased intensity of ET immunostaining in the endometrium between days 12 and 20 of pregnancy (Riley et al. 1994). This is somewhat different from that in women (Cameron et al. 1993) where endometrial extracts contained all three ET isoforms and their mRNAs, and although ET-1 was the most prevalent isoform, there was no correlation with either the abundance of isoform or stage of cycle.

From *in vitro* studies on purified cell populations, it has been shown that glandular epithelial cells are the principal site of ET production by the endometrium. There was little or no release of ET immunoreactivity by stromal cells and no detectable ET was released into medium conditioned by conceptus tissues, indicating that ET measured in the uterine lumen is likely to be derived from epithelial cells of the endometrium. This is in agreement with studies in women demonstrating that cultured endometrial epithelial cells release between 5 and 20 times more ET-1 than stromal cells (Marsh et al. 1994). It remains to be established whether release of ET by ovine endometrial epithelial cells is directional, although there is evidence for the basal release of ETs by endothelial cells (Wagner et al. 1992).

The production of ET by cultured ovine endometrial epithelial cells was stimulated by FCS which contains both steroids and growth factors although the precise factor(s) that is involved remains to be defined. During the oestrous cycle, plasma progesterone concentrations have started to decline at a stage when ET concentrations in uterine flushings increase, whereas during pregnancy, progesterone levels remain high after day 14 while ET content in
uterine flushings at this time continue to increase. However, in ovariectomised progesterone-treated sheep, there was less ET immunostaining in the endometrium when compared with the very high levels of immunostaining in the uterus of ovariectomised untreated ewes (Riley et al. 1994). Transforming growth factor (TGF) β stimulates ET production from cultured human endometrial epithelial and stromal cells (Economos et al. 1992, Marsh et al. 1994), as well as from other cell types, and in the ovine conceptus expression of TGFβ1 and TGFβ2 mRNA increases around the time of implantation (Doré et al. 1994). Whether TGFβ is one of the factors in the uterine environment that regulates ovine endometrial ET production remains to be determined. Neutral endopeptidase (NEP), the membrane-bound ectoenzyme that metabolises ET, is also present in the sheep uterus in endometrial stromal cells and in smooth muscle cells of the myometrium and vasculature and is most intense when circulating progesterone levels are high during the luteal phase of the oestrous cycle and after day 10 of pregnancy (Riley et al. 1995). NEP has been demonstrated in the human endometrium, is regulated by TGFβ, and has been proposed as a key regulator in the availability of local concentrations of ET at the time of menstruation (Casey et al. 1991, 1993). The control of ET in the ovine uterus is likely to be a complex mechanism involving endocrine control by ovarian steroids and paracrine interactions within the endometrium and with the conceptus to modulate its production and metabolism.

Specific ET binding sites of both ET\textsubscript{A} and ET\textsubscript{B} receptor subtypes were present in the ovine uterus and conceptus and have similar properties to those reported in the uterus of women (Bacon et al. 1995) and other tissues (Masaki et al. 1994). ET receptors were localised principally on the endometrial luminal and glandular epithelium and the most intense immunostaining for ET was also localised at these sites (Riley et al. 1994) as expected this is the principal site of production. Specific binding was also

**Figure 6.** (a) Representative autoradiograph localising \(^{125}\)I-labelled ET-1 binding sites on a section of ovine uterus collected on day 16 of pregnancy. Specific binding is localised in glandular epithelial tissue (g) of the endometrium (e) and in the vasculature of the caruncle (cv) and myometrium (mv), with little binding in the smooth muscle of myometrium (m). Scale bar=1 mm. (b) Negative control of autoradiograph on consecutive section where specific binding of \(^{125}\)I-labelled ET-1 was displaced with 1 µM ET-1. No specific binding can be detected. Scale bar=1 mm.
present on stromal cells particularly in the caruncles and the presence of ET receptors on both epithelial and stromal cells may permit paracrine regulation of ET production, for instance ET stimulates its own release in endothelial cells (Sajjonmaa et al. 1992) and also actions between cell types. ET binding sites are also localised in the vasculature and ETs can regulate blood flow in the sheep uterus (Yang & Clark 1992). The ET receptor subtypes are coupled to distinct signal transduction mechanisms (Aramori & Nakanishi 1992) and differentially regulate cellular processes, for instance the expression of fos and jun oncogenes, AP-1 transcription factor activity and mitogenesis (Simonson et al. 1992). On day 16 of pregnancy in sheep, at the time of apposition and implantation, vascular permeability increases at the caruncular implantation sites as demonstrated by the uptake of the dye pontamine blue (Boshier 1970). This effect may be mediated by ET-1 which increases microvascular permeability in rat tissues (Filep et al. 1993), an action mediated by the ETA receptor. The increasing amounts of ET-1 in the uterine lumen and in endometrial tissues at this time (Riley et al.

**FIGURE 7.** Displacement of specific 125I-labelled ET-1 binding by ET-1 in serial sections of sheep conceptus tissues (predominantly trophoblast). Values are means ± s.e.m.; n=4–6 sections of tissue/point.

**FIGURE 8.** Localisation of ET immunoreactivity in sections of day 20 ovine (a) embryo (scale bar=200 µm) and (b) trophoblast cells (scale bar=5 µm), with (c) the negative control (same magnification as Fig. 8b) demonstrating no specific immunostaining. Key: a=atrium of heart; f=fetal membranes; un= uninucleate trophoblast cells; bn=binucleate trophoblast cells.
1994) may have a role in these changes in vascular permeability during implantation.

Recent studies in mice deficient in ET-1 by gene knockout have suggested that ET-1 plays an important role in early embryo development (Kurihara et al. 1994), although it is not known whether there is some redundancy with the expression of alternative ET gene products. In the ovine embryo, immunostaining for ET was present principally in the heart, although it was not possible to determine the ET isoform detected due to the lack of selectivity of the antibody used. Positive ET staining was also present in trophoblast in uninucleate, but not in binucleate, cells and specific ET binding sites were present on the conceptus and may represent a signalling mechanism by which ET may regulate these developmental processes. No detectable ET was released by conceptus tissues maintained in culture. As ET-1 is not stored in cells in secretory granules (Emori et al. 1991, Masaki 1993), the positive ET immunostaining observed in these tissues may represent bound ET derived from the uterine lumen, although it is not known whether the antibody used in this study can recognise the ET epitope when bound to its receptor. The localisation of ET immunoreactivity in the human placenta is not clear. Mallasné et al. (1993) reported ET localised in third trimester placenta in syncytio- and intermediate trophoblast, decidua and in the vasculature, whereas van Papendorp et al. (1991) did not observe immunostaining in trophoblast. ET is released by cultured third trimester trophoblast cells and by decidual but not trophoblast cells obtained during the first trimester (Kubota et al. 1992, Mallasné et al. 1993), indicating that expression of ET may vary with gestational age. There is no information on the presence of ET in the human conceptus at the preimplantation stage.

For implantation to be successful, a high degree of synchrony between conceptus and endometrium is necessary to ensure that the uterus is receptive at the appropriate stage of blastocyst development. A large number of growth factors, cytokines and other regulatory molecules are present in the uterine lumen during the periimplantation period and it has been hypothesised that their role is to provide a suitable environment for blastocyst expansion and embryonic development and to facilitate the implantation processes (review: Findlay & Salamonsen 1991). ET-1 is present in increasing amounts in the uterine lumen at the time of rapid blastocyst expansion prior to and at the time of implantation in the ewe, and may have a role in mediating these mitogenic effects. ETs are potent mitogens both as competency and progression factors in a wide variety of tissues, including vascular smooth muscle (Hirata et al. 1989, Bobik et al. 1990), vascular pericytes (Yamagishi et al. 1993), mesenchymal cells including human placental stromal cells (Fant et al. 1992), and epithelial cells of the thyroid (Eguchi et al. 1993), either on their own or as co-mitogens with other growth factors (Battistini et al. 1993). The high concentrations of ET-1 present in the uterine lumen may have a stimulatory role in the mitogenesis associated with endometrial and conceptus remodelling and angiogenesis that occurs during implantation and early placentation.

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