REVIEW

Growth factor expression and function in the human and mouse preimplantation embryo

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Abstract

There is increasing evidence that even before implantation, human development is regulated by embryonically and maternally derived growth factors. Studies in other mammalian species have shown that growth factors and their receptors are expressed by the preimplantation embryo and the reproductive tract. Furthermore, a number of growth factors have been shown to affect rate of embryo development, the proportion of embryos developing to the blastocyst stage, blastocyst cell number, metabolism and apoptosis. Growth factor ligands and receptors are also expressed in human embryos and the maternal reproductive tract, and supplementation of culture medium with exogenous growth factors affects cell fate, development and metabolism of human embryos *in vitro*. Autocrine, paracrine and endocrine pathways that may operate within the embryo and between the embryo and the reproductive tract before implantation are proposed.

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Preimplantation development

The preimplantation development of eutherian mammals, including the human, is remarkably similar and spans cleavage of the fertilized ovum, compaction and morula formation, and, finally, cavitation with the formation of a blastocyst (Figs 1 and 2). During this phase of development cells divide, differentiate and, at later stages, die. However, there is little or no cell growth, and the large oocyte successively cleaves into smaller and smaller cells, which achieve the same size as somatic cells in the blastocyst. The first cleavage divisions are under the control of maternal mRNA laid down during oogenesis in the ovary. The embryonic genome begins to be transcribed at the 2-cell stage in the mouse and the 4- to 8-cell stage in the human, after which maternal transcripts are steadily degraded (Fig. 1). After the third cleavage division in the mouse and the fourth in the human, cells maximize their intercellular contacts and compaction occurs. When the embryo has around 32 cells, a fluid-filled cavity appears, with the development of the blastocyst. Blastocyst formation marks the differentiation of the epithelial trophectoderm (TE), which is specialized for implantation, and the establishment of the pluripotent inner cell mass (ICM), from which the fetus is derived (Fig. 2). At the blastocyst stage the embryo loses its protective glycoprotein coat, the zona pellucida (a process known as hatching). The exposed TE cells are now able to attach to the endometrial epithelium lining the uterus, initiating the process of implantation.

Human embryo development *in vitro*

The advent of *in vitro* fertilization (IVF) in 1969 (Edwards *et al.* 1969) has provided a unique opportunity to study human preimplantation development *in vitro* (Fig. 2). Experience with IVF, embryo culture and embryo transfer (ET) suggests that there is significant embryonic loss during the first few weeks of human development. Even when two or three embryos are transferred, pregnancy rates are low (around 20% per IVF treatment cycle) and only around 14% of these embryos give rise to a baby (Human Fertilisation and Embryology Authority 2000). Observations of human preimplantation embryo development *in vitro* show that around 50% of human embryos stop developing before they reach the blastocyst stage (Hardy 1993, Langley *et al.* 2001), when implantation would take place *in vivo*. Poor development and implantation could be due to chromosomal abnormalities (Plachot *et al.* 1987, Jamieson *et al.* 1994, Munné *et al.* 1995), inadequate nuclear or cytoplasmic maturation during oogenesis (Moor *et al.* 1998), a suboptimal culture

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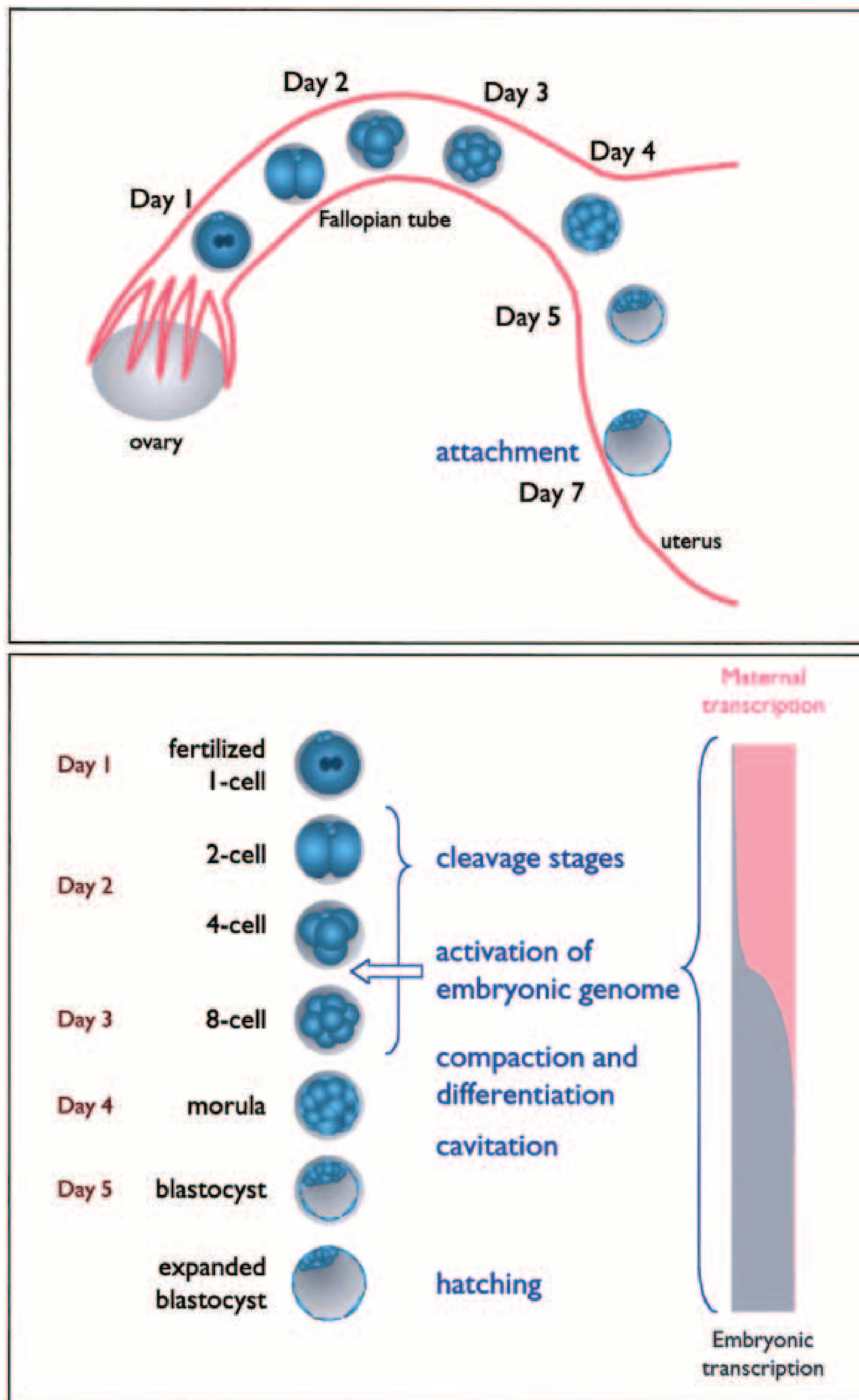

Figure 1 Diagram outlining human preimplantation development *in vivo* (upper panel), and the major cellular and molecular events that occur during this time (lower panel).

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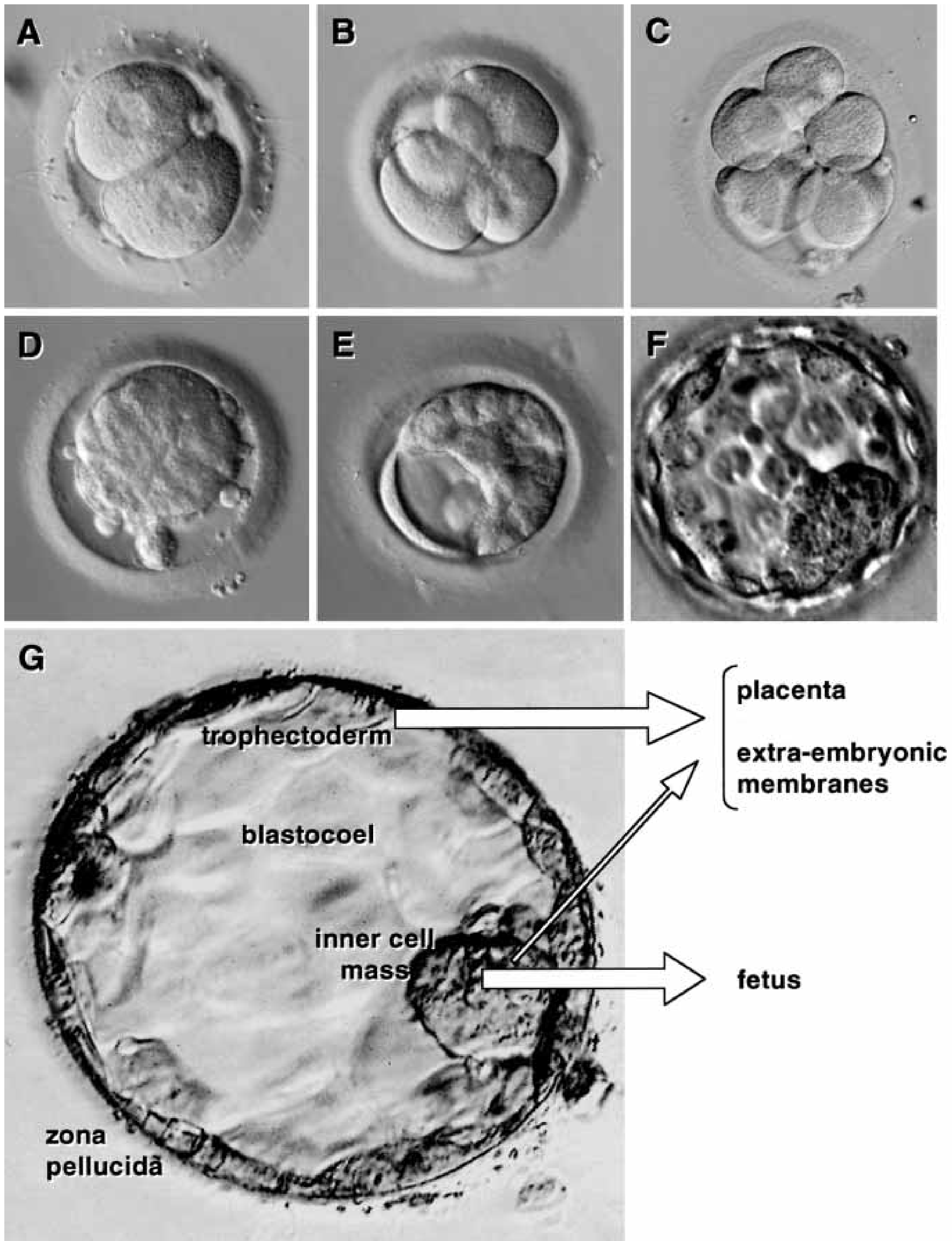

Figure 2 Human embryo development *in vitro*. (A) 2-cell embryo two days after fertilization. (B) Day two 4-cell embryo. (C) Day three 8-cell embryo. (D) Day four morula, where the cells have compacted onto each other and maximized their intercellular contacts, and the cell outlines are indiscernable. (E) Day five early blastocyst with a small fluid filled blastocoel cavity. (F) Day six expanded blastocyst. (G) Expanded blastocyst showing the trophectoderm, which will give rise to the placenta and extraembryonic membranes, and the inner cell mass, which will give rise to the fetus. The embryo is surrounded by an acellular glycoprotein coat, the zona pellucida, important for sperm binding and embryo integrity.

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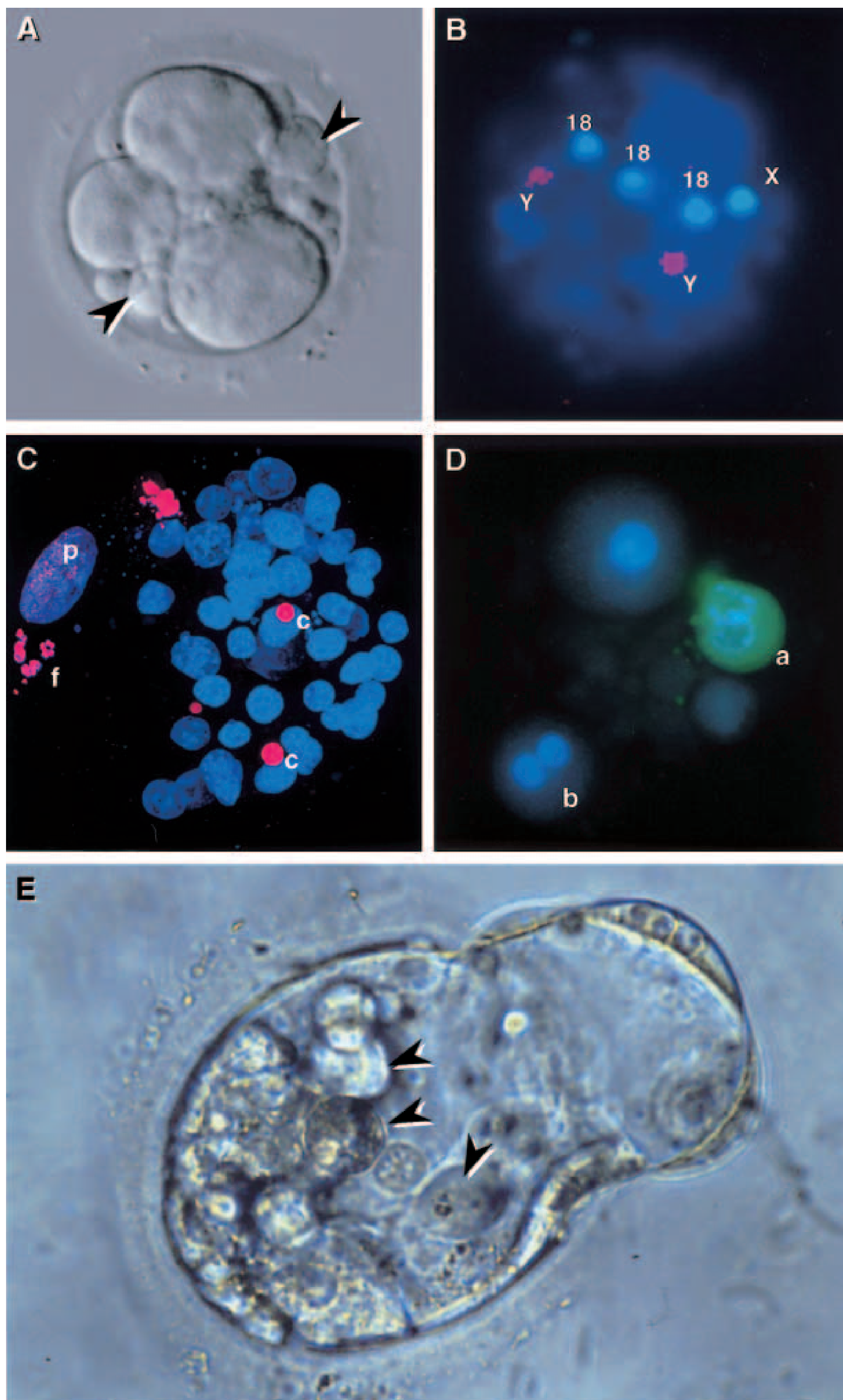

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environment (Bavister 1995) or poor embryonic–maternal dialogue (Simon & Valbuena 1999).

The majority of human preimplantation embryos are morphologically variable, with unevenly sized cells frequently associated with cytoplasmic blebs of varying sizes (Fig. 3). Many embryos have cells and membrane-bound cellular fragments that appear to be redundant and excluded from the developmental programme. These cells either lie between the embryo and the zona pellucida, or are extruded into the blastocoel cavity (Fig. 3). Indeed, a significant feature of preimplantation human development is the intercellular variability within the embryo, with both 'normal' and 'abnormal' cells in close proximity. These abnormal cells have nuclear or chromosomal abnormalities (Hardy *et al.* 1993, Munné *et al.* 1995, Ruangvutilert *et al.* 2000) (Fig. 3), or may have ceased to undergo cell division; all these anomalies probably result from cell cycle defects within individual cells. Such 'mosaicism' is common, with 40 – 60% of human embryos consisting of a mixture of healthy and abnormal cells (Hardy *et al.* 1993, Munné *et al.* 1994, Ruangvutilert *et al.* 2000). In addition, cell death is a widespread feature, the majority of human blastocysts having one or more dying cells in both the inner cell mass and the trophectoderm (Hardy 1999) (Fig. 3). These dying cells have the characteristic morphology of cells undergoing apoptosis, including fragmenting nuclei and DNA. It is unlikely that these cellular features are an *in vitro* artefact as apoptotic nuclei are found in primate and rodent blastocysts *in vivo* (Enders *et al.* 1982, Handyside & Hunter 1986). Pyknotic, lobulated and fragmented nuclei and cytoplasmic fragmentation have also been described in human embryos *in vivo* (Hertig *et al.* 1954, Pereda & Croxatto 1978, Buster *et al.* 1985). The roles of apoptosis during preimplantation development are unclear, but could include tissue homeostasis or removal of abnormal cells.

The low pregnancy rates following IVF have provided the impetus over the past two decades for studies aimed at improving embryo culture between fertilization and transfer. It is hoped that improved culture conditions will better maintain embryo viability, leading to improved pregnancy rates. In other cell types, a variety of growth factors are known to be important in regulation of cell division and cell death. It was therefore logical first to investigate their effects during preimplantation embryogenesis in laboratory and domestic species, before extending these studies to human embryos. The effects of growth factors can be analysed in a number of ways, including assessing the proportion of embryos developing to the blastocyst stage, rate of development, metabolism, cell numbers in the blastocyst, and incidence of cell division and cell death. It has become apparent that there are both similarities and differences between species, highlighting the importance of not making assumptions about human development based on mouse studies.

Growth factors and preimplantation development

The preimplantation embryo is unique in that it develops in the absence of direct cell contact with the reproductive tract for approximately a week (in the case of the human) before implanting in the uterus. It is free-floating, lacks a blood supply and is moved continuously by the tract through a changing fluid environment. During this time, the embryo is undergoing cell division, apoptosis and differentiation. It is dependent on the luminal secretions of the oviduct and the uterus for its nutrition. Its cellular activities, including cell division, gene expression and metabolism are influenced by the environment and by factors, including growth factors, produced by the cells of the reproductive tract. Continued blastocyst development *in vivo*, implantation and the maintenance of pregnancy require an effective maternal–embryonic dialogue mediated by growth factors.

Exogenous growth factors are not essential for the completion of preimplantation development. The mouse or human embryo is relatively self sufficient and can survive and grow in isolation to the blastocyst stage *in vitro*, in a simple salt solution supplemented only with pyruvate and albumin (Whitten & Biggers 1968, Devreker *et al.* 1998). Furthermore, gene knockout studies show that, in general, embryos from mice lacking a particular growth factor are capable of completing preimplantation development and implanting. The only exceptions are mice deficient in leukaemia inhibitory factor (LIF), or epidermal growth factor receptor (EGF-R), where implantation fails (Stewart *et al.* 1992, Threadgill *et al.* 1995). Other knockout studies show that lack of a specific growth factor can have more subtle effects on development. For example, mice deficient in colony stimulating factor-1 (CSF-1) or granulocyte–macrophage colony-stimulating factor (GM-CSF) have blastocysts with reduced numbers

Figure 3 (Opposite). Common abnormalities of human preimplantation development. (A) Day 2 embryo with cytoplasmic, membrane bound fragments (arrowed). (B) Single chromosomally abnormal nucleus (blue) from an 8-cell embryo with two Y chromosomes (Y), one X chromosome (X) and three copies of chromosome 18 (18), detected using fluorescent *in situ* hybridization. (C) Confocal projection of nuclei (blue) in a day 6 human blastocyst with 53 cells showing a large polyploid nucleus (p), several fragmented nuclei (f) with fragmented DNA (detected using TUNEL, pink) which are undergoing apoptosis, and two TUNEL labelled condensed nuclei (c). (D) Embryo with two binucleate cells (a and b). In one binucleate cell (a) active caspases (green) have been labelled in the cytoplasm using fluorescently tagged active caspase inhibitor. (E) Blastocyst hatching from the zona pellucida with large excluded cells (arrowed) in the blastocoel cavity.

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Table 1 Effects of growth factors and cytokines on mammalian preimplantation development *in vitro*

Effect	Growth factor	Species	Reference
Blastocyst formation ↑	Insulin	Mouse	Harvey & Kaye (1990)
		Cow	Matsui *et al.* (1995)
	IGF-I	Mouse	Harvey & Kaye (1992*a*)
		Cow	Matsui *et al.* (1995); Palma *et al.* (1997)
	IGF-II	Mouse	Harvey & Kaye (1992*b*)
	CSF-1	Mouse	Pampfer *et al.* (1991)
	TGF-α	Cow	Larson *et al.* (1992)
	LIF	Mouse	Lavranos *et al.* (1995)
		Sheep	Fry *et al.* (1992)
	GM-CSF	Cow	de Moraes & Hansen (1997)
		Mouse	Robertson *et al.* (2001)
	HB-EGF	Rat	Tamada *et al.* (1999)
Rate of development ↑	IGF-II	Mouse	Rappolee *et al.* (1992)
	CSF-1	Mouse	Bhatnagar *et al.* (1995)
	PAF	Mouse	Stoddart *et al.* (1996)
	EGF	Mouse	Brice *et al.* (1993)
Blastocyst cell number ↑	IGF-I & -II	Mouse	Rappolee *et al.* (1992)
	GM-CSF	Mouse	Robertson *et al.* (2001)
	PAF	Mouse	Ryan *et al.* (1990*b*)
Specifically ICM ↑	Insulin	Mouse	Harvey & Kaye (1990)
	IGF-I	Mouse	Harvey & Kaye (1992*a*)
	IGF-II	Mouse	Harvey & Kaye (1992*b*)
Specifically TE ↑	CSF-1	Mouse	Bhatnagar *et al.* (1995)
Blastocoel expansion	TGF-α	Mouse	Dardik & Schultz (1991)
	EGF		
Blastocyst hatching/attaching ↑	GM-CSF	Mouse	Robertson *et al.* (2001)
Protein synthesis (TE) ↑	Insulin	Mouse	Harvey & Kaye (1988)
	IGF-II		Rappolee *et al.* (1992)
	EGF		Wood & Kaye (1989)
Protein synthesis (ICM) ↑	TGF-α	Mouse	Dardik *et al.* (1992)
Endocytosis	Insulin	Mouse	Dunglison *et al.* (1995)
Glucose transport	Insulin	Mouse	Kaye *et al.* (1992)
	GM-CSF	Mouse	Robertson *et al.* (2001)
Metabolism	PAF	Mouse	Ryan *et al.* (1990*a*)
Gene expression	IGFs	Mouse	Shi *et al.* (1994)
	TGFs		Babalola & Shultz (1995)
Apoptosis ↓	TGF-α	Mouse	Brison & Schultz (1997)
	PAF	Mouse	O'Neill (1998)
	IGF-I	Rabbit	Herrler *et al.* (1998)
		Mouse	Brison (2000)
Apoptosis ↑	TNF-α	Mouse	Pampfer *et al.* (1997); Wuu *et al.* (1999)

of cells (Pollard 1997, Robertson *et al.* 2001), while blastocysts from transforming growth factor α (TGF-α)-deficient mice have higher levels of apoptosis compared with wild-type mice (Brison & Schultz 1998).

Furthermore, a number of investigations comparing development *in vivo* and *in vitro*, or assessing the effects of exogenous growth factors *in vitro* have shown that maternally and embryonically derived growth factors play an important role during the preimplantation phase (reviewed in Kaye 1997, Kane *et al.* 1997). Preimplantation development of mouse embryos cultured *in vitro* is retarded compared with that *in vivo* (Bowman & McLaren 1970, Harlow & Quinn 1982). Moreover, in mouse blastocysts, levels of apoptosis are threefold higher *in vitro* than *in vivo* (Brison & Schultz 1997). This suggests that the maternal tract produces factors important for embryo development. *In vitro*, reducing the volume of medium that the mouse embryo is cultured in, or culturing

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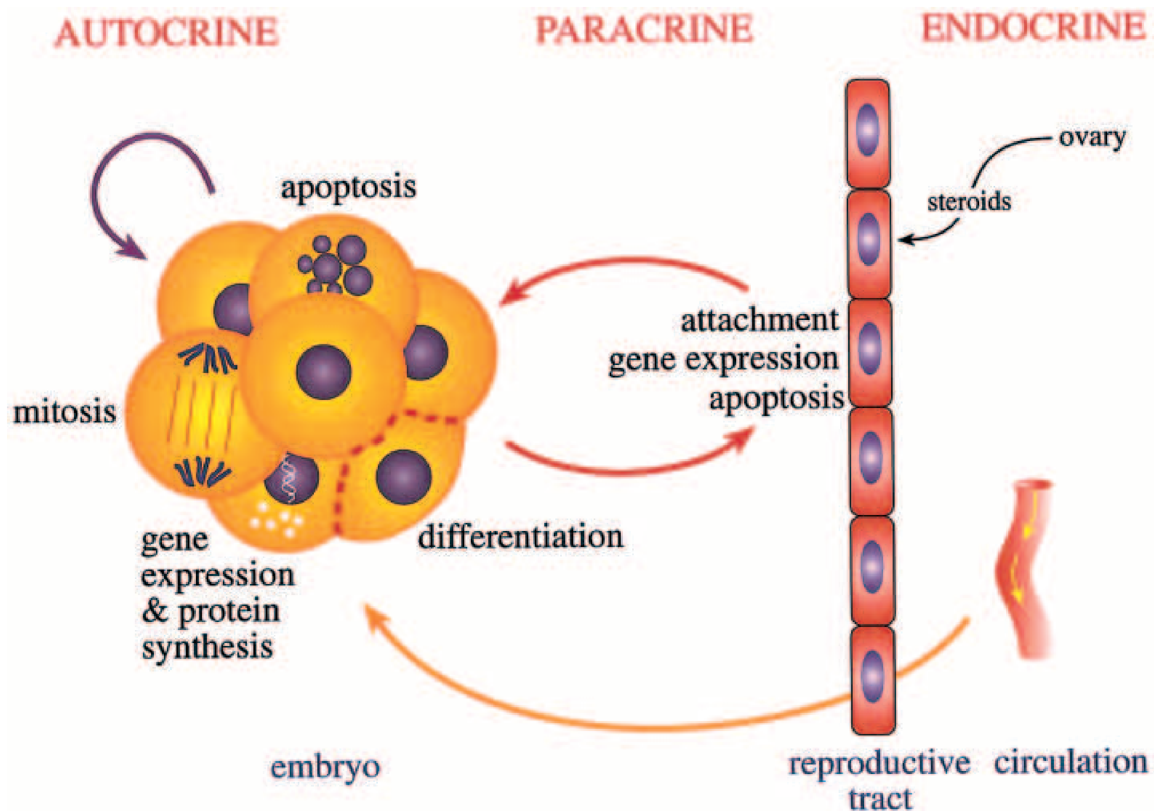

Figure 4 Diagram outlining the cellular responses of the embryo and maternal reproductive tract to maternally and embryonically derived growth factors.

embryos in groups, improves development and reduces levels of apoptosis (Wiley *et al.* 1986, Paria & Dey 1990, Lane & Gardner 1992, Stoddart *et al.* 1996, Brison & Schultz 1997, O'Neill 1998). This indicates that the embryos themselves may be producing growth factors and that there may be autocrine and paracrine pathways operating *in vivo* which are not present, or are diluted, *in vitro*.

The role of growth factors in development has been supported by studies in mouse and other species showing that a range of polypeptide growth factor ligands are produced by the reproductive tract and preimplantation embryo, while many of their receptors can be detected on the embryo surface. These include members of the insulin and insulin-like growth factor (IGF) family, the epidermal growth factor (EGF) family, the fibroblast growth factor (FGF) family, the platelet derived growth factor (PDGF) family and the tumour necrosis factor (TNF) family (reviewed in Kane *et al.* 1997). The expression of a number of growth factor ligands and receptors show interesting temporal and regional changes during preimplantation development. For example, in the mouse, immunohistochemistry has shown that TGF-β_2 is expressed after activation of the embryonic genome, and at the blastocyst stage is localized only to the TE, with no expression in the ICM (Slager *et al.* 1991). mRNA for insulin receptor and IGF-I receptor is also only expressed from the 8-cell stage onwards. Immunohistochemistry shows that the protein for insulin receptor is localized on the apical surface of the TE, and on cells of the ICM (Heyner *et al.* 1989). The embryo itself is not able to produce insulin, but responds to it (Table 1), suggesting the presence of a paracrine pathway with maternal insulin inducing metabolic and mitogenic responses in the embryo. An example of an autocrine pathway is provided by the localization of EGF-R and action of the ligand TGF-α (which binds to EGF-R) in mouse embryos. TGF-α is localized to the ICM, and EGF-R is present mainly on the baso-lateral surfaces of TE cells. The ICM can respond to TGF-α, suggesting that an autocrine loop exists within the blastocyst, whereby the ICM produces TGF-α which acts on receptors within the ICM and local polar TE cells (Dardik *et al.* 1992).

Generally, a number of growth factors and cytokines have been shown to promote blastocyst formation and increase rate of development in mouse, cow, sheep and rat (Table 1) (Fig. 4). Under suboptimal conditions, such as single embryo culture in large volumes of culture medium,

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Table 2 Expression of growth factor ligands and their tyrosine kinase receptors in human preimplantation embryos and the reproductive tract

	Stage						
	1-cell	2- to 4-cell	8-cell	Morula	Blastocyst	Tract	**Reference**
Growth factor							
EGF	✙✚		✙✚		✙✚		Chia *et al.* (1995)
						✚	Moller *et al.* (2001)
						✙✚	Lei & Rao (1992)
HB-EGF						✙✚	Birdsall *et al.* (1996)
TGFα	✙✚		✙✚		✙✚		Chia *et al.* (1995)
		✚	✚				Smotrich *et al.* (1996)
				✚	✚		Hemmings *et al.* (1992)
						✙✚	Pfeifer & Chegini (1994)
						✚	Lei & Rao (1992)
EGFR	✙✚		✙✚		✙✚		Chia *et al.* (1995)
		✚/–	✚				Smotrich *et al.* (1996)
						✚	Haining *et al.* (1991); Moller *et al.* (2001)
						✙✚	Pfeifer & Chegini (1994)
						✙✚	Lei & Rao (1992)
Insulin	–	–	–		–		Lighten *et al.* (1997)
Insulin R	✙	–	✙		✙		Lighten *et al.* (1997)
IGF-I						✚	Pfeifer & Chegini (1994)
	–	–	–		–	✙✚	Lighten *et al.* (1997); Lighten *et al.* (1998)
		–	✚			–	Smotrich *et al.* (1996)
			–	–	–		Hemmings *et al.* (1992)
IGF-IR	✙	✙	✙		✙✚		Lighten *et al.* (1997); Lighten (1998)
		–	✚				Smotrich *et al.* (1996)
IGF-II	✙	✙	✙		✙		Lighten *et al.* (1997)
				✚	✚		Hemmings *et al.* (1992)
					–		Ohlsson *et al.* (1989)
IGF-IIR	✙	✙	✙		✙		Lighten *et al.* (1997)
PDGF-A	✙	–	✙	✙	✙		Osterlund *et al.* (1996)
PDGFR-α	–	✙	✙	–	✙		Osterlund *et al.* (1996)
PDGF-B	–	–	–	–	–		Osterlund *et al.* (1996)
					✚		Svalander *et al.* (1991)
						✙	Boehm *et al.* (1990)
PDGFR-β	–	–	✙	✙	–		Osterlund *et al.* (1996)
VEGF	–		✙	✙	✙		Krussel *et al.* (2000)
VEGFR						✚	Moller *et al.* (2001)
CSF-1		–	–	–	–		Sharkey *et al.* (1995)
	✚	✚	✚				Zolti *et al.* (1991)
CSF-1 receptor		✙	✙	–	✙		Sharkey *et al.* (1995)
SCF		✙	✙	✙	–		Sharkey *et al.* (1995)
ckit[a]		✙	✙	–	✙		Sharkey *et al.* (1995)

[a], receptor for SCF; ✙ mRNA; ✚ protein; Receptors in bold typeface.

EGF and TGF-α also have a beneficial effect (Paria & Dey 1990). Growth factors have been shown to increase blastocyst cell number, insulin, IGF-I and IGF-II specifically acting on the ICM and CSF-1 increasing trophectoderm TE cell number (Table 1). Effects on protein synthesis, endocytosis, glucose transport, metabolism, gene expression and apoptosis have also been reported in the mouse (Table 1; Fig. 4). Supplementation of culture medium with TGF-α, platelet activating factor (PAF) or IGF-I decreases apoptosis in mouse and rabbit blastocysts *in vitro*, indicating that these growth factors can act as survival factors during preimplantation development.

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Table 3 Expression of cytokines and their receptors in human preimplantation embryos and reproductive tract

	Stage						
	1-cell	2- to 4-cell	8-cell	Morula	Blastocyst	Tract	**Reference**
Growth factor							
VEGF	–		✙	✙	✙		Krussel *et al.* (2000)
VEGFR						✚	Moller *et al.* (2001)
LIF		–	–	–	–		Sharkey *et al.* (1995)
	✙	✙	✙	✙	✙		Chen *et al.* (1999)
						✙	Charnock-Jones *et al.* (1994)
						✙✚	Cullinan *et al.* (1996)
LIF-R					✙		Charnock-Jones *et al.* (1994)
		–	–	–	✙		Sharkey *et al.* (1995)
	✙	✙	✙	✙	✙		Chen *et al.* (1999)
	✙	✙	✙		✙		van Eijk *et al.* (1996)
						✙✚	Cullinan *et al.* (1996)
TNF-α		–	✙	–	–		Sharkey *et al.* (1995)
			✚				Zolti *et al.* (1991)
TNF-R		✙	✙	–	–		Sharkey *et al.* (1995)
GM-CSF						✚	Giacomini *et al.* (1995)
						✙✚	Zhao & Chegini (1999)
PAF		✚					Collier *et al.* (1990)
PAF-R		–	–	–	–		Sharkey *et al.* (1995)
						✙✚	Ahmed *et al.* (1998)
IL-1	✚	✚	✚				Zolti *et al.* (1991)
IL-1β	✚	✚	✚	✚	✚		De los Santo *et al.* (1996)
IL-1R t1	✚	✚	✚	–	✚		De los Santos *et al.* (1996)
						✙	Simon *et al.* (1993)
IL-6		–	–	–	✙		Sharkey *et al.* (1995)
	✚	✚	✚				Zolti *et al.* (1991)
IL-6-R		–	–	–	✙		Sharkey *et al.* (1995)
TGFβR-T1	✙✚	–	–		✙		Osterlund & Fried (2000)
TGFβ-T2	✙✚	–	–		–		Osterlund & Fried (2000)

✙ mRNA; ✚ protein; Receptors in bold typeface; IL-1R t1, interleukin-1 receptor type 1; TGFβR-T1, TGFβ receptor type 1; TGFβR-T2, TGFβ receptor type 2.

Conversely, some growth factors, such as TNF-α, can induce apoptosis in the ICM of the mouse blastocyst.

Finally, there is increasing evidence that growth factor signalling between embryo and endometrium is important in implantation (Fig. 4). EGF receptor is expressed in the mouse blastocyst (Wiley *et al.* 1992), binding EGF, TGF-α and heparin binding-epidermal growth factor (HB-EGF). Activation of the EGF family of receptors appears to be important in implantation. Treatment of mouse blastocysts with EGF increases implantation after transfer to recipient mice (Morita *et al.* 1994). In the absence of EGF-R, the inner cell mass degenerates at the time of implantation in blastocysts from mice of a particular genetic background (CF-1) (Threadgill *et al.* 1995). Induction of localized expression of HB-EGF in the mouse uterus by the blastocyst (Das *et al.* 1994) and adhesion of mouse blastocysts to cells expressing HB-EGF (Raab *et al.* 1996) suggest that HB-EGF plays a paracrine role in implantation. Maternal LIF is also essential for successful implantation, as demonstrated by the failure of wild-type mouse embryos to implant in LIF-deficient mice (Stewart *et al.* 1992). LIF and EGF increase production of proteinases by mouse blastocysts (Harvey *et al.* 1995), indicating a paracrine signalling pathway from endometrium to blastocyst, preparing the blastocyst for subsequent uterine invasion. Treatment of mouse embryos with PAF increases blastocyst cell number and also significantly improves implantation after transfer to recipient mice (Ryan *et al.* 1990*b*). Antagonists to PAF, and also to interleukin-1 (IL-1) receptor, block implantation in mice (Ryan *et al.*

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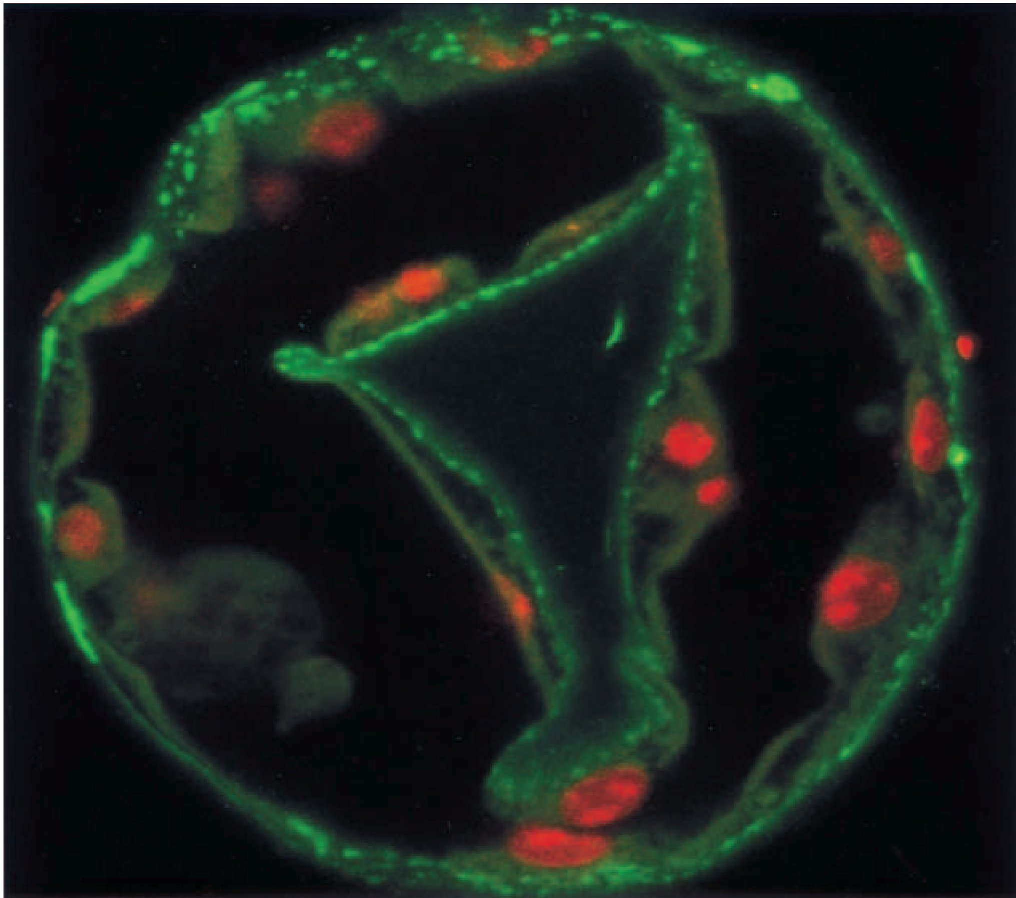

Figure 5 Confocal image showing IGF-I receptor expression (green plaques) on the trophectoderm of a human blastocyst. Nuclei have been labelled with propidium iodide (red). The slight collapse of the blastocyst following mounting on the microscope slide accounts for the invagination of the TE in the centre of the image.

1990*b*, Simon *et al.* 1994). Finally, murine GM-CSF supports the attachment and outgrowth of mouse blastocysts *in vitro* (Armstrong & Chaouat 1989), implicating it in implantation.

Thus, studies in a variety of mammalian species have clearly demonstrated that growth factors are important in blastocyst development and implantation (Fig. 4). These have provided a basis for similar studies in human development.

Growth factors and the human embryo

Following IVF, co-culture of human embryos on monolayers of epithelial cells has been shown to increase blastocyst formation (Menezo *et al.* 1990) and blastocyst cell number (Vlad *et al.* 1996). This is thought to be due either to the removal of embryotoxic substances from the medium, or by the secretion of factors that enhance development. However, co-cultures are labour intensive to maintain in a busy IVF unit, may carry the risk of contamination and have different nutritional needs from embryos. Therefore, attention has been focussed on developing defined serum-free media to maintain viable human embryos.

The human reproductive tract produces a number of growth factors including EGF, TGF-α, HB-EGF, IGF-I, PDGF-B, LIF and GM-CSF (Tables 2 and 3). It also expresses several receptors (R) including EGF-R, vascular endothelial growth factor (VEGF)-R, LIF-R and PAF-R (Tables 2 and 3).

Early studies measured secretion of growth factors by human embryos into the culture medium (Tables 2 and 3) (Svalander *et al.* 1991, Zolti *et al.* 1991, Hemmings *et al.* 1992). Subsequently, expression of mRNA and protein for a variety of growth factor ligands and their receptors have been investigated in single human embryos using sensitive techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR), *in situ* hybridisation and immunohistochemistry (Tables 2 and 3). These growth factors include EGF, TGF-α, IGF-II, VEGF, LIF and stem cell

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Table 4 Effects of growth factors on preimplantation human development *in vitro*

Growth-factor	Concentration studied	% of embryos forming blastocysts	Blastocyst cell number	Metabolism	Other effects
PAF[1]	0·2–1·5 μM	na	na	↑ CO_2 production from glucose	↑ Pregnancy rate following IVF/ET
LIF[2]	5–20 ng/ml	no change	na	na	
LIF[3]	1000 i.u./ml	↑ (18 to 44%)	na	na	↑ Hatching
Insulin[4]	100 ng/ml	No change (46–66%)	No change	↑ Lactate production	↑ Protein synthesis
Insulin[5]	25 μg/ml	Not applicable	Not applicable	Not applicable	↑ hCG production by day 7 blastocysts
PDGF[5]	2 ng/ml	Not applicable	Not applicable	Not applicable	↑ hCG production by day 7 blastocysts
HB-EGF[6]	1–100 nM	↑ (41 to 71%)	No change	No change	↑ hCG production
IGF-I[7,8]	1·7 nM	↑ (35 to 60%)	↑ ICM by 60%	No change	
IGF-I[9]	1·7 nM	↑ (49 to 74%)	No change (total) ICM na	na	↓ Apoptosis by 50%
GM-CSF[10]	2 ng/ml	↑ (30 to 76%)	↑ ICM by 35%	na	↑ Rate of development ↑ Attachment and TE outgrowth

na, not analysed; [1]O'Neill *et al.* (1989); [2]Jurisicova *et al.* (1995); [3]Dunglison *et al.* (1996); [4]Conaghan (1996); [5]Lopata & Oliva (1993); [6]Martin *et al.* (1998); [7]Lighten *et al.* (1998); [8]Lighten (1998); [9]Spanos *et al.* (2000); [10]Sjoblom *et al.* (1999).

factor (SCF) (Chia *et al.* 1995, Sharkey *et al.* 1995, Lighten *et al.* 1997, Chen *et al.* 1999, Krussel *et al.* 2000). Receptors for the EGF, IGF and PDGF growth factor families, and for a variety of cytokines, are expressed by the human embryo throughout development (Tables 2 and 3, Fig. 5). These studies have highlighted some significant differences between species in growth factor ligand and receptor expression. For example, EGF is expressed by human embryos (Chia *et al.* 1995), but not by mouse and cow embryos. Conversely, IGF-I ligand mRNA is not found in the human embryo (Lighten *et al.* 1997), but is found in mouse, cow and pig embryos (Kane *et al.* 1997).

As in other species, supplementation of culture medium with exogenous growth factors has been shown to be beneficial for preimplantation development (Table 4). LIF, IGF-I, HB-EGF and GM-CSF have all been shown to increase the proportion of embryos developing to the blastocyst stage (Dunglison *et al.* 1996, Martin *et al.* 1998, Lighten *et al.* 1998, Sjoblom *et al.* 1999, Spanos *et al.* 2000). Furthermore, IGF-I and GM-CSF increase blastocyst cell number specifically in the ICM (Lighten *et al.* 1998, Sjoblom *et al.* 1999). Insulin affects metabolism, increasing protein synthesis and lactate production (Conaghan 1996) and increasing human chorionic gonadotrophin (hCG) production after day 7 at the blastocyst stage (Lopata & Oliva 1993).

IGF-I provides a good illustration of the possible paracrine action of a maternal tract-derived growth factor on the human preimplantation embryo. Lighten *et al.* (1998) showed that IGF-I is produced by the fallopian tube and is present in oviduct and uterine fluid. Human embryos express receptors for IGF-I throughout preimplantation development (Fig. 5) but do not express the IGF-I ligand (Lighten *et al.* 1997, Lighten 1998). Addition of IGF-I to culture medium significantly increased the proportion of embryos developing to the blastocyst stage by 25% (Lighten *et al.* 1998, Spanos *et al.* 2000). These blastocysts had a significant (59%) increase in the number of ICM cells (Lighten *et al.* 1998). Culture of embryos with an antibody specific for the IGF-I receptor, α-IR3, negated the beneficial effect of IGF-I, demonstrating that it acted through the IGF-IR. In order to ascertain whether IGF-I was increasing human blastocyst cell numbers by inhibiting cell death, Spanos and coworkers (2000) examined the extent of apoptosis in human blastocysts that had been cultured in the presence and absence of IGF-I. Fragmenting (apoptotic) and healthy interphase nuclei were distinguished using 4′,6-diamidino-2-phenylindole (DAPI) labelling to show nuclear morphology, and apoptosis was confirmed with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) to label DNA fragmentation, a classic biochemical marker of apoptosis. IGF-I was shown to reduce significantly the percentage of apoptotic nuclei in human blastocysts by approximately 50% (Spanos *et al.* 2000). Thus the human preimplantation embryo is able to respond to growth factors produced by the reproductive tract, which, in the case of IGF-I appear to be important in cell survival at the morula and blastocyst stages.

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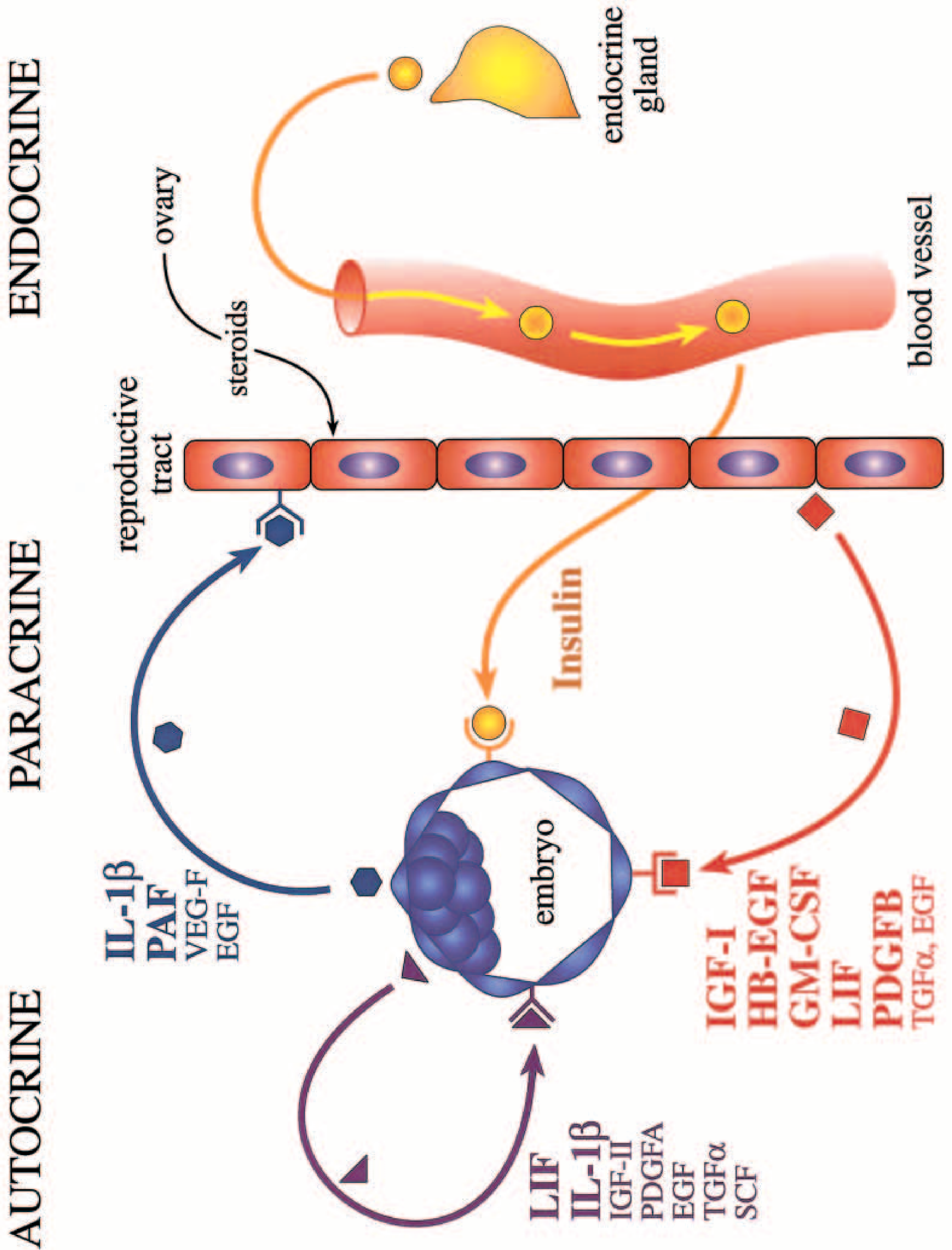

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In addition to the embryo's response to IGF-I, human embryos can only secrete IL-1α and/or β in the presence of endometrial epithelial cells (but not stromal cells). Furthermore, as in other species, LIF may well be crucial in human implantation, as LIF is produced by the human endometrium (Charnock-Jones *et al.* 1994, Cullinan *et al.* 1996) and the human embryo expresses the receptor (van Eijk *et al.* 1996, Chen *et al.* 1999).

Conversely, there is direct evidence that the endometrium can respond to the human blastocyst. *In vitro* studies have shown that the human blastocyst can regulate expression of the cell adhesion molecule β3 integrin (via production of IL-1β), the mucin MUC-1 and the chemokine IL-8 (Simon *et al.* 1997, 2000), as well as induce localized apoptosis in the endometrial epithelium (Galan *et al.* 2000). Production of VEGF by the embryo could also be important in implantation, possibly by inducing angiogenesis at the implantation site (Krussel *et al.* 2000). Following IVF, a significant reduction in peripheral platelet count has been observed in some women between embryo transfer and implantation, which is thought to result from the production of PAF by the embryo (O'Neill *et al.* 1985).

Conclusions

There is increasing evidence in a variety of mammalian species that growth factors play an important role in blastocyst development, regulation of cellular events and in maternal embryonic dialogue (Table 1). Human studies have shown that a wide range of growth factor ligands and their receptors are expressed during preimplantation development (Tables 2 and 3), and that exogenous growth factors seem to affect mainly blastocyst formation (Table 4).

On the basis of the studies described here, it is possible to suggest putative endocrine, paracrine and autocrine pathways that act during preimplantation human development (Fig. 6). Simultaneous expression of both ligand and its receptor by the embryo suggests the presence of an autocrine signalling pathway. This could be confirmed by studying the effect of blocking endogenous production of growth factor using antisense oligonucleotides, as has been done in the mouse (Rappolee *et al.* 1992). Growth factor production by the embryo, in combination with receptor expression on the oviduct or uterus, provides evidence for paracrine signalling from the embryo to the maternal tract, with these growth factors possibly being involved with preparation for implantation. Expression of growth factor ligand by the tract and receptor by the embryo suggests paracrine signalling by maternally derived growth factors to the embryo. These maternal growth factors are probably the most important candidates for improving human embryo development *in vitro*. However, it is important to assess the safety of exogenous growth factors before clinical application. Some growth factors such as IGF-I improve blastocyst formation, probably by inhibiting apoptosis (Spanos *et al.* 2000). It is possible that such growth factors may over-ride a natural checkpoint and 'rescue' abnormal embryos, transfer of which would be highly undesirable.

Further concerns arise from the observation that *in vitro* culture of embryos from domestic species leads to a high proportion of fetuses with large offspring syndrome (LOS; reviewed in Young *et al.* 1998). They are characterised by being abnormally heavy at birth. Such fetuses have an increased abortion rate, increased gestation length and frequent phenotypic abnormalities. It has been proposed that the environment to which the preimplantation embryo is exposed may alter the genomic imprinting of certain key genes involved with growth, at a stage when these imprints are being established or maintained. Recently, LOS has been associated with alterations in the genomic imprinting of IGF-II receptor, causing reduced receptor expression, hence reduced clearance of IGF-II and fetal overgrowth (Young *et al.* 2001). The observations that the culture environment can alter gene expression and genomic imprinting, and hence disturb growth factor signalling pathways leading to fetal abnormalities, counsels caution in the clinical use of growth factors for human embryo culture.

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Figure 6 (Opposite). Diagram outlining proposed autocrine, paracrine and endocrine growth factor pathways operating during preimplantation human development, based on current published data (see Tables 2, 3 and 4). Growth factors in large typeface indicate where a response has been reported, in small typeface where there is evidence for only part of the pathway.

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