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Regulation of polypeptide growth factor synthesis and growth factor-related gene expression in the rat and mouse uterus before and after implantation

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Introduction

During the preimplantation period the rodent uterus undergoes a complex programme of cell proliferation and differentiation that concludes with the uterus becoming receptive to the blastocyst for implantation. At the site of trophectoderm invasion the uterine epithelium breaks down and the underlying stroma undergoes decidualization. These decidual cells and the invading trophoblasts rapidly proliferate and differentiate and, together with the allantois and chorion, form the haemochorial placenta. Although many of the uterine processes, both pre- and post-implantation, are under the regulation of female sex steroid hormones, the ubiquity of growth factors in the regulation of cell proliferation and differentiation in other systems (Metcalf, 1989), coupled with experiments largely with steroid responsive cells in culture (Sirbasku, 1978; Ikeda & Sirbasku, 1984; Tomooka *et al.*, 1986; Dickson & Lippman, 1987), suggest that polypeptide growth factors may act as mediators of these steroid actions in the uterus.

The conceptus is also a semi-allograft which raises questions of immunoregulation. The finding of potent haematopoietic growth factors in the uterus during pregnancy (Bradley *et al.*, 1971; Bartocci *et al.*, 1986; Hunt, 1989) sugests that these may have an immunoregulatory role during gestation. Furthermore, Athanassakis *et al.* (1987) and Wegmann (1988), by suggesting that the activation of a T-cell mediated immune response by the allogeneic fetus at the maternal–fetal interface results in the synthesis of lymphokines which stimulate placental cell proliferation, have proposed that the immunological and growth-promoting functions of these lymphokines may be intimately related.

In this article I shall, emphasizing my own research and that of my colleagues, review the evidence of a role for steroid-regulated uterine-synthesized growth factors in preparing the uterus for implantation and in the formation of the uteroplacental unit.

Uterine preparation for implantation

The uterus is a complex tissue divided into a myometrium, of longitudinal and circular muscle, and an endometrium consisting of an epithelial layer surrounding a central lumen branching into glands which penetrate the underlying stroma. The stroma contains many cell types, including mesenchymal, endothelial and blood cells. Each of these cell types has its own response to steroid hormones and indeed the constitution of the stromal layer can dramatically alter after steroid hormone treatment (Tchernitchin, 1983). It is therefore important, when considering steroid hormone and growth factor action in the uterus, to draw a clear distinction between the responses of these different cell types.

It is also necessary to differentiate between the developmental response of the immature uterus and the cell specificity of the response to sex steroid hormones in the adult uterus preparing for pregnancy. In the immature rat uterus there is a high rate of cell proliferation until Day 15 after birth. During this time uterine cells are not fully responsive to oestradiol-17β even though the cells contain oestrogen receptors. After Day 15 the intrinsic rate of proliferation drops and the cells become competent to respond to oestradiol but until puberty is reached, unlike the epithelial restriction of proliferation of the mature uterus (Martin *et al.*, 1973a), all the cell types respond by proliferation (Kaye *et al.*, 1972). Furthermore, at Day 5 the epithelium, even in the complete absence of any oestrogen receptors, responds transiently to oestrogen by an increased rate of DNA synthesis (Bigsby & Cunha, 1986). In these animals the oestrogen, although requiring an intact pituitary axis (Quarmby *et al.*, 1984), acts directly upon the uterus since oestrogen applied to one uterine horn induces proliferation in that horn but not in the other (Stack & Gorski, 1984). These results suggest that uterine development is regulated by a set of systemically produced hormones or growth factors and that the proliferative response to exogenously supplied oestrogens is mediated through other hormones or growth factors, produced locally in response to the oestrogen (Cunha *et al.*, 1983) and acting in concert with these systemic polypeptide hormones.

In mature rats and mice, however, cell proliferation is in response to oestrogen synthesized at oestrus and is restricted to the luminal and glandular epithelia (Martin & Finn, 1968; Clark, 1971; Martin *et al.*, 1973a). This proliferation is not dependent on an intact pituitary axis (Quarmby *et al.*, 1984). These findings imply a different level of control in mature animals. In addition, if copulation occurs after ovulation and progesterone is synthesized, then the epithelial proliferation ceases and the cells differentiate to assume a secretory aspect (Martin *et al.*, 1973a). Subsequent oestrogen production fails to induce epithelial cell proliferation but instead stimulates it in the underlying stroma (Martin *et al.*, 1973a).

These cellular dynamics can be mimicked in ovariectomized mature mice by administration of steroid hormones (Martin *et al.*, 1973b, c). Such studies have laid the foundation for hormonal regimens to establish pseudopregnancy in ovariectomized mice and to study cell proliferation *in vivo* (Finn & Martin, 1972; Finn & Porter, 1975). Because the uterine luminal epithelium can be removed from the underlying tissue with a great deal of purity (Fagg *et al.*, 1979) we have concentrated on this epithelial tissue to study the steroid regulation of gene expression and cell proliferation in mature animals, with particular emphasis on the dual action of progesterone on cell proliferation. In these experiments 50–100 ng oestradiol-17β given to ovariectomized mice 6 days after oestradiol priming resulted in a synchronized wave of cell proliferation only in the luminal and glandular epithelium (Martin *et al.*, 1973b). The proliferative response can be modified by treatment of the ovariectomized mice with a low potency oestrogen such as oestriol which results first, in stimulation of epithelial cell division, followed immediately by a synchronized wave of cell death (Martin *et al.*, 1976). Cell death is a normal part of the rodent oestrous cycle and this experimental variation of using a low potency oestrogen has enabled us to study the hormonal regulation of cell viability (Pollard *et al.*, 1987b). Treatment of ovariectomized mice with 0·5–1·0 mg progesterone for 3 days switches the proliferative response to oestradiol from the uterine epithelium to the stroma. Progesterone also inhibits the first wave of oestradiol-induced epithelial proliferation if given concurrently with oestradiol but fails to inhibit it if given 3 h afterwards (Das & Martin, 1973). Progesterone therefore acts early in the G_1 stage of the cell cycle. Its effects are apparently mediated through its receptor since the receptor binding progestagen antagonist, mifepristone, entirely suppresses the action of progesterone on cell proliferation (Cullingford & Pollard, 1988).

The simplest hypothesis to explain the anti-proliferative action of progesterone on the uterine epithelium is that it inhibits the metabolic response to oestradiol by preventing binding of oestradiol to its receptor. Progesterone, however, neither quantitatively nor qualitatively influences oestradiol receptor binding (Quarmby & Martin, 1982a, b). Furthermore, the oestradiol stimulation of epithelial protein and rRNA synthesis is not inhibited by progesterone (Smith *et al.*, 1970;

Pollard & Martin, 1975; Cheng *et al.*, 1985). In fact, by 12 h after oestradiol administration, the time of maximal DNA synthesis, the oestrogen-induced increase in cell size, measured by protein or RNA content per cell, is identical, whether or not the animals had been pre-treated with progesterone (Cheng *et al.*, 1985). Apart from the universal stimulation of protein and RNA synthesis, polypeptide growth factors also stimulate a common signal transduction pathway that includes induction of many proliferation-related proteins, such as ornithine decarboxylase (ODC) and cyclin, as well as the activation/induction of many proto-oncogenes. We investigated the effects of oestradiol-17β and progesterone on ODC in the luminal epithelium, since the activity of this enzyme had been shown to be stimulated by oestradiol in the immature uterus (Kaye *et al.*, 1971), as well as the family of oncogenes known to be induced at the mRNA level by mitogenic stimulation of fibroblastic cells in culture. In the luminal epithelium oestradiol stimulates ODC both at the mRNA and enzyme level (Cheng & Pollard, 1986). Oestradiol-17β also induced *c*-fos and *c*-myc mRNA levels to reach a peak at 2–4 h (Weisz & Bresciani, 1988; T. Cullingford & J. W. Pollard, unpublished observations) and *c*-ras^{H} mRNA levels to peak at 12 h after subcutaneous injection (Cheng & Pollard, 1986). Induction of both *c*-myc and *c*-fos by oestradiol-17β in the immature uterus has also been documented (Murphy *et al.*, 1987b; Loose-Mitchell *et al.*, 1988). Pre-treatment of the mature animal with progesterone, however, failed to inhibit the oestradiol induction of ODC and of *c*-myc, *c*-fos and *c*-ras^{H}. In fact, progesterone even enhanced the stimulation of *c*-fos mRNA levels (T. E. Cullingford & J. W. Pollard, unpublished observations). In the uterine luminal epithelium, therefore, oestradiol-17β induces many of the biochemical responses characteristic of cultured cells stimulated to enter into cell proliferation by growth factors such as platelet-derived growth factor (PDGF), but of these, only the entry into S-phase and mitosis are inhibited by progesterone. These data strongly suggest that there is another pathway activated by progesterone early in G_1 which is able to over-ride the oestradiol signal for cell proliferation or that the induction of this 'PDGF family' of genes is more related to cell growth than to cell proliferation. Analysis of the biochemical pathways in the epithelium induced by oestrogens and inhibited by progesterone should allow a definition of those events that are causally related to cell proliferation and thus enhance our understanding of the regulation of cell division.

The activation of the 'PDGF-family' of cellular responses in the luminal epithelium begs the question, even in the mature animals, of whether oestradiol acts directly as a mitogen or acts via the induction of a tissue-specific growth factor(s) that acts in an autocrine or paracrine fashion or of a growth factor receptor required for the cells to progress into DNA synthesis. Such a postulated growth factor must be able to transduce the oestrogenic signal as an early function because in the immature (Stack & Gorski, 1985) and mature (Cheng *et al.*, 1985) uterus the rate of cell proliferation is directly proportional to the rate of protein synthesis which in turn is directly related to the duration and level of receptor occupancy. If oestrogen induced a growth factor or its receptor then progesterone could exert its effect on uterine cell proliferation by altering their expression appropriately.

The best candidate for a uterine growth factor is epidermal growth factor (EGF; Carpenter, 1987). Both the growth factor and its receptor have been identified in the uterus at the RNA and protein level (Mukku & Stancel, 1985a, b; DiAugustine *et al.*, 1988). In the immature uterus the levels of both were stimulated by oestradiol treatment (Gonzales *et al.*, 1984; DiAugustine *et al.*, 1988; Lingham *et al.*, 1988). EGF stimulates proliferation of cultured luminal epithelial cells from the immature mouse uterus (Tomooka *et al.*, 1986) and rabbit endometrial cells (Gerschenson *et al.*, 1979). These data are highly suggestive of a role of EGF in regulating proliferation of immature uterine cells. In the mature uterus EGF precursor was identified in luminal and glandular epithelial cells but de-novo synthesis was unaffected by oestradiol-17β (DiAugustine *et al.*, 1988). EGF receptor levels, however, are elevated at dioestrus and by oestrogen treatment of the mature uterus (Gardner *et al.*, 1989). Oestradiol might therefore act by causing the cleavage of EGF precursor into mature EGF and by stimulating synthesis of the EGF receptor, resulting in an autocrine loop for epithelial cells (DiAugustine *et al.*, 1988). The necessary cell-specific expression

of the EGF receptor has not been described, nor has the alteration of this expression by progesterone, and until this is done the role of EGF in uterine epithelial cell proliferation will remain contentious.

In cultured uterine cells of fetal guinea-pigs EGF not only causes cell proliferation but also elevates the amount of progesterone receptor (Sumida *et al.*, 1988). If similar effects occur in mature uteri then EGF could influence tissue-specific responses by inducing receptors for steroid hormones or by modifying pre-existing ones by phosphorylation (Woo *et al.*, 1986). These data hint at the complexity of the hormonal/cytokine loops that might be uncovered in the steroid control of uterine functions.

Insulin-like growth factor-I (IGF-I) has also been detected in the mature and immature uterus both at the mRNA and protein level (Murphy *et al.*, 1987a, 1987c; Murphy & Friesen, 1988). Concentrations of both are potentiated by a single injection of oestradiol-17β (Murphy *et al.*, 1987c). IGF-I is in itself only a weak mitogen in tissue culture but it does potentiate the action of other growth factors including EGF (Corps & Brown, 1988). It is possible, therefore, that EGF and IGF-I act synergistically in the mature uterus to promote proliferation. However, until the site or timing of IGF-I synthesis, and the IGF-I receptor-bearing cells have been documented conclusions to this effect cannot be drawn.

The question still remains open, therefore, whether oestradiol-17β in the mature uterus acts directly in eliciting a proliferative response or via the intermediary of a uterine-synthesized polypeptide growth factor(s). However, uterine synthesis may be unnecessary for a growth factor to be involved in proliferation *per se* because one of the earliest responses of the uterus to oestradiol is vascular permeability and oedema (Szego & Roberts, 1953; Martin *et al.*, 1976) which may allow the influx of serum growth factors into the uterus. These serum-derived growth factors, given the appropriate cellular expression of their receptor, could result in cell proliferation. Support for such an idea comes from the stimulation of epithelial proliferation by uterine distension (Leroy *et al.*, 1976), but evidence against it comes from the lack of correlation between uterine oedema and cell proliferation (Grunert *et al.*, 1987). Growth factors also affect survival and differentiation of their target cells (Stanley *et al.*, 1983) and so any oestradiol-induced uterine growth factor could exert profound effects over a wide range of uterine functions other than cell division. These possibilities are now being addressed in the pre-implantation uterus.

Post-implantation uterine growth factors

The concept that uterine secretions (histotrophe) may be involved in growth of the conceptus has been extant for a number of years (see Biggers, 1988; Brigstock *et al.*, 1989). These secretions undoubtedly include growth factors which may directly influence the growth of the fetus, extra-embryonic membranes and placenta or promote uterine functions associated with pregnancy such as decidual formation or vascularization of the uterus or placental bed. Furthermore, they could also include lymphokines or cytokines that regulate immunological reactions against the semi-allogeneic fetus.

Among the best candidates for such uterine growth factors are the colony stimulating factors. These were originally shown to be at high concentrations in the pregnant mouse uterus (Bradley *et al.*, 1971; Rosendaal, 1975). The majority, if not all, of this colony stimulating activity is colony stimulating factor-1 (CSF-1) (Bartocci *et al.*, 1986). CSF-1 is a homodimeric glycoprotein growth factor originally purified as a lineage specific growth factor for mononuclear phagocytes, promoting their survival, differentiation and proliferation (Tushinski *et al.*, 1982; Stanley *et al.*, 1983; Sherr & Stanley, 1990). The CSF-1 receptor is a transmembrane glycoprotein tyrosine kinase identified as the product of the *c*-fms proto-oncogene (Sherr *et al.*, 1985; Sherr, 1988). The presence of the growth factor in the uterus and the identification of *c*-fms mRNA in the placenta (Muller *et al.*, 1983a, b) and in human trophoblasts (Hoshina *et al.*, 1985) and its product on choriocarcinoma

cells (Rettenmier *et al.*, 1986) strongly suggest a role for CSF-1 in regulating placental development during pregnancy in addition to the regulation of macrophage production.

Uterine CSF-1 concentrations exhibited a dramatic elevation throughout pregnancy in the mouse such that, at term, it was approximately 1000-fold higher than in the non-pregnant uterus (Bartocci *et al.*, 1986). CSF-1 was also found at a lower, but constant concentration in the placenta (Bartocci *et al.*, 1986) and at high levels in amniotic fluid where it reached a peak at about Day 14 of gestation (Azoulay *et al.*, 1987; Pollard *et al.*, 1990). Uterine synthesis of CSF-1 was shown by the presence of an alternatively spliced 2·3-kb CSF-1 mRNA (Pollard *et al.*, 1987a). This splice maintains the coding region but removes 3′-untranslated sequences. These sequences contain the AUUUA motif that in other lymphokines appears to code for mRNA instability (Shaw & Kamen, 1986; Ladner *et al.*, 1988), suggesting that the uterine form of CSF-1 mRNA is a more stable RNA species than that found in fibroblasts. Uterine CSF-1 mRNA was elevated by >100-fold during pregnancy to reach a peak on Day 14–16 (Arceci *et al.*, 1989) and was localized exclusively to the luminal and glandular epithelium throughout pregnancy (Pollard *et al.*, 1987b; Arceci *et al.*, 1989; Regenstreif & Rossant, 1989). The early increases in uterine CSF-1 concentration during pregnancy could be mimicked in ovariectomized mice by oestradiol and progesterone administration in regimens designed to induce pseudopregnancy. This elevated CSF-1 concentration was associated with an increase in uterine CSF-1 mRNA (Pollard *et al.*, 1987a). These data suggest that steroid hormones regulate CSF-1 synthesis through the induction of CSF-1 mRNA in epithelial cells. This hypothesis was supported by in-situ hybridization studies of pre-implantation mice which showed elevated CSF-1 mRNA levels on Day 3 of pregnancy, the time that the epithelium was first noticeably progestational (Arceci *et al.*, 1989).

CSF-1 receptor mRNA was detected by northern blotting in mouse placenta (Muller *et al.*, 1983a, b; Arceci *et al.*, 1989; Regenstreif & Rossant, 1989) and uterus before placentation (Arceci *et al.*, 1989). Detailed in-situ hybridization studies revealed a complex pattern of CSF-1 receptor mRNA expression throughout pregnancy (Regenstreif & Rossant, 1989; Pollard *et al.*, 1989; Arceci *et al.*, 1989). Initially, a high level of CSF-1 receptor mRNA was detected in the decidua starting at Day 6. This persisted in the decidua basalis during formation of the placenta but declined once the mature placenta was formed. CSF-1 receptor mRNA expression was coincidently detected in the trophectoderm (Arceci *et al.*, 1989). Throughout pregnancy expression was found in spongiotrophoblasts and in the labyrinthine layers but was highest on giant trophoblasts. This level of expression of giant trophoblasts > spongiotrophoblasts > cells of the labyrinthine layer, once established, persisted throughout pregnancy. Expression could also be detected in cells of the yolk sac (Regenstreif & Rossant, 1989; Arceci *et al.*, 1989). In the human placenta *c*-fms transcription starts from multiple sites. These transcriptional start sites were >25 kb 5′ to those found in monocytes and permitted the expression of a placental specific 5′ exon (Visvader & Verma, 1989). If the pattern of *c*-fms expression is the same during gestation in humans as found in the mouse, then alternative transcription start sites may be used such that each cell type has a different regulation of *c*-fms transcription.

The temporal relationship of uterine CSF-1 synthesis with both decidual and trophoblast cell expression of the CSF-1 receptor and placental growth strongly suggests a role for CSF-1 in placental growth and development. This conjecture is supported by the CSF-1 stimulation of proliferation of primary placental cells in culture and of established placental cell lines (Athanassakis *et al.*, 1987). A definite role for CSF-1 in placental growth, however, has still to be established. Furthermore, high level expression of CSF-1 mRNA expression in the non-proliferative trophoblastic giant cells and on spongiotrophoblasts after the placenta has finished growing also suggests other roles for CSF-1 in placental functions. In this context it is worth noting that CSF-1, apart from regulating mononuclear phagocyte proliferation, also regulates their survival and differentiation as well as inducing macrophages to synthesize other cytokines including IL-II (Moore *et al.*, 1980), G-CSF (Metcalf & Nicola, 1985), interferons (Moore *et al.*, 1984) and tumour

necrosis factor (TNF; Warren & Ralph, 1986). Such actions of CSF-1 on trophoblasts and decidual cells remain to be explored.

CSF-1 is also chemotactic for mononuclear phagocytes (Wang *et al.*, 1988) and promotes the survival of differentiated macrophages (Stanley *et al.*, 1983). The high levels of uterine CSF-1 may therefore account for the accumulation of macrophages in the uterus and placenta during pregnancy (Hunt *et al.*, 1984, 1985; Wood *et al.*, 1987). These macrophages may in turn be stimulated by CSF-1 to produce cytokines that act on the conceptus or other lymphoid cells in the uterus producing complex autocrine/paracrine loops (Hunt,1989). Uterine macrophages also appear to be important in immunoregulation since they have been shown to secrete immunosuppressive molecules (Hunt *et al.*, 1984; Tawfik *et al.*, 1986) which diminish the functions of lymphocytes with potential reactivity against the fetus. CSF-1 therefore, although not activating macrophages in a classical sense (Nathan *et al.*, 1984), may have an important immunological role during pregnancy via its effects on macrophages.

Granulocyte–macrophage colony stimulating activity (GM-CSF) also stimulates proliferation of placental cell lines (Athanassakis *et al.*, 1987). Based on this and other data Wegmann (1984) and Athanassakis *et al.* (1987) have proposed that GM-CSF and other T-cell lymphokines, produced as a consequence of T-cell activation by the allogeneic fetus, have a role in promoting placental growth. This has been termed the immunostimulatory hypothesis (Wegmann, 1984, 1988). CSF-1 has been included as one of these factors because of its actions on the immunologically important uterine macrophages and its potential role on trophoblasts as well as its synergy with T-cell growth factors in haematopoiesis (Wegmann, 1988). CSF-1, however, appears to be produced throughout the uterus under steroid regulation (Pollard *et al.*, 1990) and not at the sites of implantation. These data suggest systemic regulation of CSF-1 rather than a local activation by immunological responses to the fetus. CSF-1 can, therefore, only be considered to be a factor acting synergistically with locally produced T-cell growth factors. Furthermore, it has yet to be determined whether T-cell growth factors such as GM-CSF are produced locally at the conceptus sites or at appropriate times during pregnancy. Indeed, to date there have been conflicting reports (Burgess *et al.*, 1977; Azoulay *et al.*, 1987).

Various other cytokines are also produced by macrophages and T-cells (Nathan, 1987). Of these TNF and transforming growth factor-β (TGF-β) are inhibitory for placental cell proliferation in culture (Wahl *et al.*, 1988; Hunt, 1989). TGF-β, or a similar molecule, may also suppress anti-fetal lymphocyte activities (Clark *et al.*, 1988). TGF-β and TNF have been identified in human placenta (Frolik *et al.*, 1983; Jaattela *et al.*, 1988) but detailed study of their occurrence in the mouse and rat uteroplacental unit has not yet been published. IL-I producing cells, possibly macrophages, have been identified in the virgin mouse sub-epithelial uterine stroma (Takacs *et al.*, 1988). IL-I is found in human amniotic fluid (Tamatani *et al.*, 1988) human placenta (Main *et al.*, 1987), and cultured rat placental cells have a small proliferative response to IL-I (Hunt, 1989). An mRNA to the T-cell growth factor IL-II has been described in human syncytial trophoblasts (Boehm *et al.*, 1989) and IL-II has been detected in human placenta and amnion (Soubiran *et al.*, 1987) but not yet in mice or rats. It may therefore be that a cytokine dialogue is taking place between uterine lymphopoietic and myleopoietic cells and the placenta, perhaps under the overall regulation of uterine synthesized growth factors such as CSF-1. Some acting possible regulatory links through uterine macrophages are discussed by Hunt (1989).

TGF-α mRNA has been detected in rat decidual cells with a peak at Day 8 (Han *et al.*, 1987) and the protein has been identified in embryos (Twardzik, 1985). The EGF receptor, to which TGF-α binds (Massague, 1983), is present in mouse placental tissues (Smith & Talamantes, 1986), the amnion and several organs of the embryo (Adamson, 1987). Although the functions of TGF-α are not known, it seems likely that it and not EGF is binding to the extra-embryonic membranes where it may have a paracrine effect on these tissues.

IGF-I has also been detected before implantation as described above. This observation, coupled with the facts that the human placenta produces IGF-I and IGF-II (Fant *et al.*, 1986; Ohlsson *et al.*, 1989) and expresses IGF-II receptor, and that the major secreted product of human decidua is an

IGF-I binding protein (Bell, 1988), suggests that IGF-I or IGF-II may have a role in the regulation of trophoblast or endometrial growth, perhaps in synergy with TGF-α. Documentation of IGF-I or II production and identification of IGF-I receptor-bearing cells during gestation have not been performed for the mouse and rat.

Several other growth-modulating activities have been described in the human, pig, sheep and cow pregnant uterus, early embryo or placenta. Some of these, such as the oestromedins (Ikeda & Sirbasku, 1984), are unidentified whilst others are known growth factors, including fibroblast growth factor (FGF; Brigstock *et al.*, 1989) and PDGF (Goustin *et al.*, 1985), or are interferons (Stewart *et al.*, 1987; Imakawa *et al.*, 1987). Nevertheless, it is only for the interferons that a function has been proposed and a detailed description of the synthesis been presented (Godkin *et al.*, 1984; Imakawa *et al.*, 1987; Hansen *et al.*, 1988; Stewart *et al.*, 1989). Similar molecules may be identified in murids.

Summary

It is apparent that the uterus is a rich source of growth factors, the synthesis of which may be induced by female sex steroids. These growth factors appear to be involved in complex autocrine/paracrine regulatory circuits which in turn interact with steroid hormones. With the exception of CSF-1, however, detailed studies of the appearance of these growth factors and their receptors during pregnancy and under different hormonal regimens have yet to be performed. Furthermore, causative roles have not been established for any of these growth factors in uterine biology and pregnancy. In the near future we can expect that, by using in-situ techniques, the producing and responding cells will be identified. Cell culture models will also have to be established to investigate specific growth factor-induced functions. In the longer term, once more is known about the regulation of uterine growth factors, imaginative new experiments need to be designed, perhaps involving transgenic animals, to establish causative roles for growth factors in uterine biology.

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