

## บทบาทของ cytokine และ growth factor ต่อกระบวนการฝังตัวของตัวอ่อน

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### Implantation: Insight to the Effects of Cytokines and Growth Factors

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#### Introduction

Implantation is the process of which the blastocyst attaches to the uterine epithelium. Generally there are two factors which determine the success of implantation. These include (1) the production of the hatched blastocyst capable of implanting and (2) the development of an endometrium that is receptive to the embryo. Overall responsibilities for the coordination of these two processes lies with ovarian hormones, estrogen and progesterone. However, it is now clear that under their influence, locally acting soluble factors secreted by the endometrium can act on the embryo to influence its development. Developing embryos, in turn, have been shown to produce soluble factors, cytokines and growth factors, that can act in an autocrine manner, or on the endometrium to influence receptivity<sup>1-2</sup>. This article will review new data indicating a critical role for cytokines and growth factors, both in preimplantation embryo development and endometrial receptivity.

#### Cytokines and implantation

Normally preimplantation embryos from several species can develop in simple defined medium but the growth rate in vitro is slower than in in vivo counterparts<sup>3</sup>. Recently several workers have shown that growing embryos with either feeder cell lines or other embryos, the so-called "co-culture", provide a better in vitro growing rate of preimplantation development<sup>4-5</sup>. These observations suggested that soluble factors secreted by the feeder cells or the embryos may play an important role in enhancing embryo development. These soluble factors are believed to be cytokines or growth factors.

Up to date several cytokines and growth factors have been reported to have influencing potentials on development of preimplantation embryos and the implantation process. These include leukemia inhibitory factor (LIF), the epidermal growth factor (EGF) family and colony-stimulating factor-1 (CSF-1). The effects of each of these cytokines or growth factors on implantation will be thoroughly discussed as follows.

#### Leukemia inhibitory factor (LIF) and implantation

LIF is a pleiotropic cytokine that was first identified through its induction of differentiation in the M1 cell line<sup>6</sup>. LIF acts on its target cells by binding to specific receptor LIF-RB. The ligand-receptor complex associates with another membrane-bound protein, gp130, resulting in signal transduction<sup>7</sup>.

In mice, LIF is upregulated in the uterine glandular epithelium on day 4, just before implantation, by the action of nidatory estrogen<sup>8</sup>. Female mice lacking a functional LIF gene are unable to support implantation. Preimplantation development appears normal since viable blastocysts are produced, which implant normally when transferred to pseudopregnant mothers<sup>9</sup>. However, pregnancies did occur in the LIF -/- females when LIF was infused into the uterine lumen by osmotic pump. These results show that maternal expression of LIF is essential for implantation; however, whether the critical site of action is the preimplantation embryo or the endometrium is not known. In situ hybridization on mouse blastocysts has shown that mRNAs encoding LIF-RB and gp 130 are

expressed only in the inner cell mass whereas mRNA encoding LIF is present in the trophoblast<sup>10</sup>. Administration of exogenous LIF improves embryo viability and hatching<sup>11</sup>. In the endometrium, LIF-R and gp 130 are expressed in luminal epithelium on day 4 and in the stroma on day 5<sup>12</sup>. These data suggest that both the preimplantation embryo and maternal endometrium are sites for action of LIF

Human embryos have been shown to express LIF-RB and gp 130 mRNAs at the blastocyst stage, but do not themselves express LIF<sup>13</sup>. Administration of LIF to preimplantation human embryos cultured in a defined serum-free system has recently been shown to have a beneficial effect on development<sup>14</sup>. Expression of mRNA encoding LIF in the endometrium is maximal during mid-luteal phase and LIF binding activity is localized to luminal epithelium of the endometrium throughout the menstrual cycle<sup>15</sup>. This suggests that in human LIF can act via an autocrine mechanism on the endometrial epithelium as in mice

In most species examined to date, there is expression of LIF in the endometrial epithelium with maximal expression in the time of implantation<sup>16</sup>. However, the regulation of LIF differs: in mice, estradiol is required for implantation and upregulated LIF; in rabbits, estradiol is not required, and LIF is upregulated by progesterone<sup>17</sup>. Similarly, while there is evidence in several species for a beneficial effect of LIF on embryo development, mouse and sheep embryos themselves make LIF, whereas human embryos do not<sup>18</sup>. These results show that the autocrine and paracrine circuits of cytokine controlling preimplantation embryo development differ between species.

### The epidermal growth factor (EGF) family

The epidermal growth factor family of growth factor consists of EGF, TGF- $\alpha$ , heparin binding EGF (HB-EGF) and amphiregulin, all of which act through the EGF receptor. Members of the EGF family are known to play an important role in the growth and differentiation of the glandular and stromal compartments of the endometrium<sup>16</sup>. The EGF receptor is also expressed on mouse and human preimplantation embryo, and reported effects of the EGFs on embryos include increased protein synthesis and improved blastocyst development<sup>1</sup>. There is also strong evidence in murids that members of the EGF family are involved in the attachment process. Intrauterine administration of EGF instead of estradiol can initiate implantation of delayed blastocyst<sup>19</sup>.

Expression of HB-EGF is induced exclusively in the luminal epithelium surrounding the blastocyst, several

hours before implantation. HB-EGF bound to heparin on the surface of luminal epithelium may be able to interact directly with EGF receptor on the embryonic trophoblast<sup>20</sup>. Thus, the EGF ligand may also be involved in signaling between the embryo and the endometrium at the time of implantation.

### Colony-stimulating factor 1 (CSF-1)

The correlation between CSF-1 and implantation was first postulated when it was found concentration of this cytokine in the mouse uterus increases by 1000 times during pregnancy<sup>21</sup>. Furthermore, the CSF-1 receptor is expressed in mouse embryos and CSF-1 enhances embryo development in vitro<sup>22</sup>. CSF-1, therefore, is believed to be another growth factor that act in a paracrine manner in influencing the implantation process and more research are now going on regarding the association between CSF-1 and implantation<sup>16</sup>.

### Conclusion

Several studies have been demonstrated recently that both cytokines and growth factors act in both autocrine and paracrine manner to regulate development of preimplantation embryos and the implantation process. It is now well established that these local growth factors mediated many of the effects of steroids on endometrium, such as proliferation, angiogenesis and secretion, as it prepares for implantation. Therefore, the assumption is made that cytokines and growth factors may involve in bringing the endometrium into a receptive state for blastocyst attachment. Although several cytokines or growth factors such as LIF may enhance preimplantation development, up to date there is no single factor that has yet been identified that is essential to such process. Further research in this area, therefore, is potentially benefits in shedding light on which growth factors are truly important in human implantation. The information regarding this aspect may, hopefully, provide us the answers to several questions regarding recurrent implantation failure commonly found in human IVF practices.

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