Singapore Journal of Obstetrics and Gynaecology
July 2004; 35(2): 104-111

Expression of IGF-I and its receptor in the uterus and their relationships with progesterone during peri-implantation in rat

Li Shangwei
Tan Zongjian
Han Ziyan
Huang Zhongying
Ma Qianhong
Tian Hanwen
Yan Kaixuan
Yao Xianyin

ABSTRACT

Objective: To study expressions of insulin-like growth factor (IGF-I) and its receptor in the endometrium and trophoblast and their relationships with progesterone(P) during peri-implantation in rat.

Mothods: To establish the pregnant model of rat during 3-10 day of gestation and the rat in estrus was used as control. The serum P level was measured by a Raadiommunoasay method, and expressions of IGF-I, IGF-I receptor (IGF-IR) and P receptor (PR) in the endometrium and trophoblast cells of rat was detercted by standard immunohistochemistry procedure and quantified by a computer image analysis system.

Results: IGF-I and its receptor are both expressed in luminal and glandular epithelium, stromal cells, trophoblast cells; PR is located in the same cells as IGF-I and IGF-IR. Showing no significant correlation with P and PR, the intensity of IGF-I expression in endometrium reached peak level from 5 through 7 day of pregnancy. The intensity of IGF-IR expression in endometrium appeared like a trough and reached bottom during 5-7 day of pregnancy and showed negative correlation with P and PR ($P<0.05$). The intensity of IGF-I and IGF-IR expression in the trophoblast cell increased during 8 to 10 days of pregnancy and showed no significant correlation with serum P and PR.

Conclusion : Expression of IGF-I and IGF-IR in the endometrium of rat were asynchronous during peri-implantation, that both suppresses the over proliferate effect of IGF-I on endometrium and allows decidualization and regulates the requirement of IGF-I for embryo growth and development. P and IGF-I also have a role in the modulation of trophoblast function independently.

Key words: IGF-I IGF-IR Progesterone Implantation

Reproductive & Immunological Department,
West China Second Hospital,
Sichuan University,
Chendu 610041

Correspondence :
Dr. Li Shangwei,
Reproductive and Immunological Department,
West China Second Hospital,
Sichuan University,
Chengdu 610041,
China.

INTRODUCTION

Implantation is a complex process that requires both synchrony in development and communication between maternal uterine cells and the blastocyst. The ovarian steroid hormones estrogen and progesterone prime the uterus for implantation. In mice, a secondary surge of estrogen on gestational Day 4 triggers the inductions of implantation and the production of growth factors in the uterus. These growth factors are thought to work in an autocrine/ para-crine fashion to mediate the effects of estrogen

in the uterus[1] and therefore have been termed "estromedins".[2]

Evidence suggests that the insulin-like growth factors (IGFs) are involved in estrogen-induced uterine proliferation.[3] IGF-I and the IGF-I receptor(IGF-IR) are expressed in the uterus, and estrogen up-regulates the expression of both genes.[3,4] In vitro, IGF-I acts synergistically with estrogen to induce DNA synthesis in uterine tissue.[5] These studies suggest that IGF-I may be an estromedin during the process of implantation. In this regard, the uterus is composed of heterogeneous cell-types that undergo dynamic changes to support embryo development and implantation. These changes are primarily dependent on coordinate interactions mediated by ovarian estrogen and progesterone (P). Estrogen stimulates proliferation of both uterine epithelial and stromal cells in neonatal mice, but this proliferative action of estrogen is restricted to epithelial cells in the adult mouse uterus.[6,7] In contrast, while P, is inhibitory to estrogen-mediated proliferation of the luminal and glandular epithelial cells,[7,8] P alone or a combined treatment of P and estrogen leads to uterine stromal cell proliferation. Synchronized development of the preimplantation embryo to the blastocyst stage and differentiation of the uterus to the receptive state are essential to succcessful implantation.[9,10] In the mouse, preovulatory ovarian estrogen secretion causes proliferation of the luminal and glandular epithelial cells during the first 2 days of pregnancy. On day 3, P from newly formed corpora lutea initiates stromal cell proliferation, which is further potentiated by preimplantation ovarian estrogen secretion on day 4 of pregnancy (the day of implantation).[7] On this day, epithelial cells cease to proliferate and become differentiated. This preimplantation ovarian estrogen secretion is also necessary for the increased endometrial capillary permeability at the location of the blastocyst, a prerequisite event in the initiation of implantation and subsequent decidualization of stromal cells. At the beginning of decidualization, the stromal cells immediately surrounding the implanting blastocyst proliferate and form the primary decidual zone (pdz) late on day 5. This is followed by the cessation of proliferation of stromal cells in the pdz and proliferation of stromal cells outside the pdz by day 6, forming the secondary decidual zone (sdz).[11]

The mechanism(s) by which estrogen initiate implantation in the P-primed uterus is not clearly understood. It is thought that the estrogen and/or P-mediated events are accomplished by the expression of a unique set of genes in the uterus. While female sex steroid hormones directly regulate several genes including vitellogenin, PRL, uteroglobin, ovalbumin, progesterone receptor, and lactoferrin in target cells because of the presence of the steroid-responsive elements in their promoter sequences,[12] these steroids also modulate the expression of several growth factors and their receptors in the uterus in a spatiotemporal manner.[13,14]

Traditionally P is considered as the hormone of pregnancy. During early pregnancy, this hormone coordinates a series of complex events that ultimately leads to the synchronized development of the embroyo and differentiation of uterus for implantation. P acts through progesterone receptor (PR), a complex binding protein composed of two isoforms, termed PR_A and PR_B[15] originating from a single gene 16,17. PR_A lacks 164 amino acids from the N-terminal region of the full-length receptor, PR_B.[18] The relationship between the two isoforms and their biological activity still remain unclear. The consensus is that PR is induced by estrogen via the ER. Thus, many of the effects of P may be attributed to the combined effects of estrogen and P. However, recent studies demonstrate that P is essential for the induction of uterine decidualization because this process fails to occur in PR (- / -) mouse uteri.[19] In contrast, ER-alpha(- / -) mouse uteri exhibit decidualization only in the presence of P.[20,21] These results suggested that estrogenic influence via ER-alpha is minimal for the induction of decidualization process. Thus, although various complex uterine responses to ovarian steroids are mediated by differential effects of these steroids, to our knowledge no comprehensive information regarding spatiotemporal expression of these receptors in the mouse uterus during the perimplantation period is available.

Insulin-like growth factor (IGF-I) is a member of a family of insulin-like peptides.[22,23] This growth factor mediates the growth-promoting actions fo growth hormone.[24,25] Hepatic expression of the IGF-I gene is largely dependent upon serum growth hormone levels,[22,26,27] and the liver is by far the major source of circulating IGF-I. The biological effects of IGF-I are mediated via high-affinity binding to the IGF-I receptor (IGF-IR),[28] although lower-affinity binding to the IGF-II receptor[29] and insulin receptor[30] can occur and may be of physiological relevance. IGF-I induces rapid metabolic changes in target tissues and stimulated longitudinal body growth.[24,31] The effects of this growth factor on DNA Synthersis and cell proliferation have been well documented, and in several cell culture systems IGF-I is a much more potent mitogen than is insulin.[32] IGF-I is required for progression of 3T3 cells through the last 6 h of the G, phase of the cell cycle, during which commitment to DNA synthesis occur.[33,34] This growth factor has also been implicated in the control of cell differentiation [9]. The finding of growth hormone-independent expression of the IGF-I gene extrahepatic tissues[35-38] and in a variety of cultured cell types[24,31] raises the possibility that locally produced IGF-I could function as an autocrine/paracrine growth factor.

The sucessful establishment of pregnancy requires synchronized growth and differentiation of the

preimplantation embryo and the uterus. Although these events are coordinated by ovarian steroids, the molecular and cellular mechanisms by which estradiol (E) and progesterone (P) effect these changes are not clearly understood. An emerging concept is that steroid hormone actions in the uterus are mediated in part by autocrine/paracrine effect of growth factors. Recently, many researchers found that IGF-I has some role on gestation outcome by mediating decidualization of endometrium and proliferation and differentiation of trophoblast. The purpose of our study is to investigate expressions of IGF-I and its receptor in rat endometrium and trophoblast and their relationship with progesterone (P) during peri-implantation.

MATERIALS AND METHODS

Materials

Female, health, adult and virgin Sprague-Dawley (SD) rats at a body weight of 200-250g were obtained from Experimental Animal Center of Sichuan University. All animals were housed under temperature- and humidity-controlled conditions, with free access to water and food; Polyclonal rabbit anti IGF-I, IGF-IR and PR antibodies was purchased from Santa Cruz co., Streptavidin/ biotin complex and Horseradish peroxidase marked avidin was purchased from Burlingame co; Progesterone radiommunology analysis (RIA) kit using double antibody was provided by Tianjin Depu bioproduct co; Computerized graphy analysis system was provided (Sichuan University).

Methods

Female SD rats were assigned randomly into 9 groups based on their gestation date such as day 3 of gestation 3d), 4d, 5d, 6d, 7d, 8d, 9d and 10d, a group of rats in estrous were used as control, and there were 5 rats in each group. Female SD rat in estrous determined by vaginal smears were caged singly overnight with males of proven fertility. Mating was confirmed by the visualization of sperms in daily vaginal smears (Id). Pseudopregnancy could be excluded by visualizing the embryos in oviducts or uterine flushing before embryo implantation (approximate 3d-5d) and presence of visible implantation sites in rat uterus after embryo implantation (approximately 6d-10d). Specimen handling: Rats were killed at same time on Days 3 to 10 of gestation. Blood of rats were collected and stored in refrigerator at 4°C for 24 hours in order to separate the serum, and serum P concentration were measured by RIA. Two sections from the same lateral uterus of each rat, containing at least one fetal after embryo implantation, were rapidly removed, fixed in 4% paraformaldehyde and buried transversely and longitudinally in paraffin. Sections (5 μ m) were cut and examined after stained with haematoxylin and eosin. IGF, IGF-IR were detected by a standard immunohistochemical (IHC) procedure using a streptavidin/biotin complex and counterstained with haematoxylin. PBS, instead of primary antibody, was used as negative control. Analysis of IHC results: Stained slides could be examined when there was no specific staining in the control. Positive cells should have clear cyto-architecture, well locating positive staining and clearing background. Intensities of IGF-I, IGF-IR and PR expression were quantified by a multimedia computer image analysis system. Ten regions on each slide were selected randomly under microscopy (200X) and fed to the computer for analysis. Grayscale with arbitrary units (U) of positive cells was used to reflect the intensity of staining and the comparative content of IGF-I, IGF-IR and PR protein.

Statistics

Comparisons among mean of the groups were conducted by using Newman-Keuls Multiple Range Test. The relationship between IGF-I/IGF-IR was examined by using Linear Correlation Test. Significance level equals to 0.05.

Results

The experiment lasted 22 days. There was no significant difference in body weight between all groups before and after experiment. There was also no significant difference in the number of fetuses among all these groups.

Expressions of PR, IGF-I and IGF-IR in rat endometrium and trophoblast

PR were not expressed in stromal cells but mainly in glandular and luminal epithelium of rat endometrium in estrous. From 3d through 5d, the expression of PR in glandular and luminal epithelium was more extensive than that during estrous, and that in stromal cells can be observed. PR's expression in endometrium, being stronger in glands and lumen than in stroma, were most intense from 6d through 7d, and then declined after 8d. There was still strong expression of PR in macrostroma cells constituting the maternal parts of rat placenta in late gestation.

IGF-I and IGF-IR all located in the same cells as PR. The expression of IGF-I and IGF-IR were restricted to glandular and luminal epithelium, and could not be detected in stromal cells during estrous. From 3d through 4d, IGF-IR's expression

in glandular and luminal epithelium declined and was weaker than that in estrous, and the staining in stromal cells could be visualized. From 5d through 7d, expression of IGF-I and IGF-IR was mainly detected in stroma, staining was stronger in basal decidua than that in parietal deciduas. There is still weak staining in residual glandular and luminal epithelium. From 8d through 10d, expression in parietal decidua declined, staining in basai decidua remained strong. There was no positive staining in residual glandular and luminal epithelium.

IGF-I, IGF-IR and PR were all expressed in trophoblast cells, the intensity of staining raised with gestation date during 8 to 10 days of pregnancy.

Measurements of serum P concentration and PR, IGF-I and IGF-IR expression in rat

Serum P concentration of pregnant rats during 3d to 4d was slight but not significantly higher than that in estrous (P.0.05). P reached and remained maximum level during 5 to 8 day of gestation (P,0.05), and then declined to estrous level from 9d through 10d (P>0.05). The intensity of PR expression varied like that of P and reached peak level from 5d through 7d (P<0.05), and then declined to estrous level after 8d.

Table 1 Results of quantified of serum P concentration and PR, IGF-I and IGF-IR expression in rat

	P (pg/ml)	PR (U)		IGF-I (U)		IGF-IR (U)	
Sample	Serum	Endometrium	Trophoblast	Endometrium	Trophoblast	Endometrium	Trophoblast
Estrous	37.89 ± 7.86	139.07 ± 6.27	-	145.82 ± 10.85	-	145.95 ± 6.83	-
3d*	44.15 ± 14.89	142.95 ± 9.11	-	148.24 ± 6.72	-	137.31 ± 5.61	-
4d	54.92 ± 5.95	144.64 ± 3.20	-	146.73 ± 5.37	-	142.28 ± 10.28	-
5d	74.24 ± 11.87*	154.97 ± 5.45*	-	151.77 ± 6.22*	-	125.95 ± 9.77*	-
6d	70.65 ± 4.34*	150.83 ± 6.65*	-	154.09 ± 8.32*	-	126.55 ± 7.61*	-
7d	75.74 ± 27.62*	155.53 ± 7.16*	-	154.37 ± 7.74*	-	144.64 ± 6.65*	-
8d	64.56 ± 17.19*	138.85 ± 14.92	136.23 ± 9.23	140.27 ± 20.70	132.49 ± 11.34	137.77 ± 8.02	122.37 ± 9.37
9d	44.37 ± 12.47	143.87 ± 9.05	144.27 ± 8.49*	143.72 ± 8.98	145.49 ± 9.37*	134.44 ± 6.34	142.62 ± 13.31*
10d	48.89 ± 7.70	135.03 ± 9.68	155.19 ± 6.34*	145.51 ± 6.61	154.77 ± 10.29*	131.71 ± 7.39	150.33 ± 18.42*

✶ 3d means 3rd day of pregnancy.
★ P<0.05 when compared with Estrous group.
❖ P<0.05 when compared with 8 days of pregnancy.

Relationship between IGF-I/IGF-IR and P/PR

Our results revealed that although the expression of IGF-I in endometrium varied like P and PR during early gestation in rat, no significant relationship between them was found when examined by bivariate correlation test ($r_{IGF\text{-}I,P}$=0.201, P=0.192 $r_{IGF\text{-}I,\ PR}$=0.184, P=0.270).

We found that there was negative significant relationship between IGF-IR's expression and serum P level or PR's expression ($r_{IGF\text{-}IR,P}$=0.317, P=0.44; $r_{IGF\text{-}IR,\ PR}$=0.372, P=0.032), Expression of IGF-I or IGF-IR in trophoblast from 8d through 10d in rat showed no relationshop with P or PR (P>0.10).

Discussion

The expression and function of IGF-I and IGF-IR in endometrium and trophoblast during peri-implantation in rat.

Expression of IGF-I in endometrium from 3d to 4d was slight but not significantly higher than that in estrous (P>0.05). Like variation of PR's expression, the strongest immunoreactivity of IGF-I antibody was detected during 5 to 7 day of gestation (P<0.05), and then declined to estrous level after 8d.

Intensity of IGF-IR expression in endometrium was not same as that of IGF-I. The staining specific for IGF-IR from 3d through 4d was weaker than that during estrous (P>0.05), and declined to minimum during 5 to 6 days of gestation (P<0.05). After 8d, IGF-IR' expression resumed to estrous level and remained stable until 10d.

Expression of IGF-I, IGF-IR and PR in the trophoblasts were stronger during 9 to 10 days of gestation than that of 8d (P<0.05).

IGF-I is an important peptide growth factor that exerts its function via endocrine, paracrine and autocrine manner. Our results revealed that IGF-I had the same cellular location as IGF-IR, and it may be a proof that IGF-I exerts its function mainly via paracrine and autocrine way in endometrium of rat. It is well known that IGF-I is mainly produced by local tissue. We found that there was positive staining of IGF-I in glandular and luminal epithelium

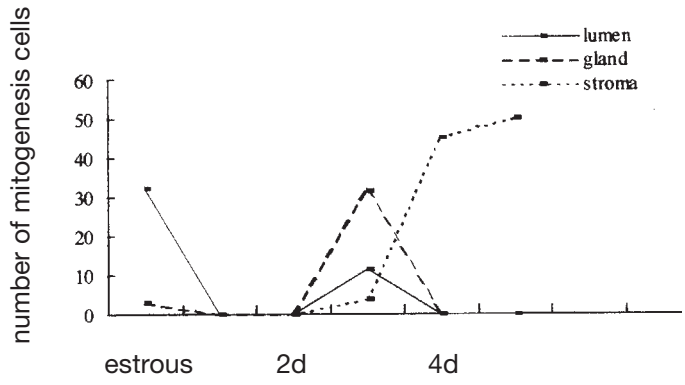
of rat endometrium in early pregnant, its intensity varied with gestation date and was consistent with the expression of IGF-I mRNA reported by Correia[39] suggesting that IGF-I is mainly synthesized by local endometrium of rat in early gestation. IGF-I was also expressed in trophoblast from 8d through 10d and was more intense with advancing gestation, indicating that trophoblast may be another source of IGF-I.

IGF-IR is an evolutionary conserved and ubiquitous transmembrane tyrosine kinase activated by IGF-I, IGF-II or insulin at hyperphysiological doese.[40] IGF-IR is capable of regulating diverse biological processes such as proliferation, survival, transformation, diferentiation, cell-cell, and cell-substrate interactions. The functions of IGF-IR in a given cellular system rely on the induction of specific signaling pathways. Which pathways are induced depends on the number of activated IGF-IRs, availability of intracelluar signal transducers, action of negative regulators, and is influenced by extracellular modulators.[43] Activation of IGF-IR results in tyrosine phosphorylation of its cytoplasmic beta-submit, followed by recruitment of IGF-IR substrates, of which the most notable are insulin receptor substrate 1 (IRS-1) and src- and collagen-homology (SHC) protein. IGF-IR-induced tyrosine phosphorylation of IRS-1 and SHC allows them to bind several effector proteins (enzymes and/or adapters) and activate multiple downstream signaling pathways.[44,45]

The main function of IGF-I is to promote mitosis and modulation metabolize of cells. We found that IGF-I expressed intensely in glandular and luminal epithelium but not in stromal cells in estrous. When the rats became pregnant, the intensity of positive staining in gland and lumen declined until being undetectable while they were raised in stroma, it is consistent with the proliferation pattern of endometrial cells reported by Leroy,[46] and suggests that IGF-I may have some role in proliferation of endometrium of rat during early gestation. It was also reported by other researchers that IGF-I could induce proliferations of mouse endometrium[47] and human stromal[48] cells in vitro, and proliferation of endometrium is the necessary step for decidualization.

IGF-I generally exerts its functions via IGF-IR, but our results revealed that the expression of IGF-I was not always synchronized with that of IGF-IR in rat endometrium, their curves separated between 5 to 7 days of gestation, this may be important for the establishment and maintenance of early pregnancy. From 3d through 4d when embryo wandered freely in ovary tube or uterus cavity, there were intense expression of IGF-I and its own receptor in rat endometrium, and it suggests that IGF-I may have some role in proliferation of endometrium. From 5d to 7d when nidation of embryo took place and decidualization of endometrium progressed quickly, the expression of IGF-IR declined to minimum while that of IGF-I were elevated maximally and it may both suppress the proliferate effect of IGF-I on endometrium and allow decidualization and satisfy the requirement of IGF-I for embryo growth and development.

Figure 1 *Mitogensis pattern of rat endometrial cells in early gestation*



The main function of IGF-I is to promote mitosis and modulation metabolize of cells. We found that IGF-I expressed intensely in glandular and luminal epithelium but not in stromal cells in estrous. When the rats became pregnant, the intensity of positive staining in gland and lumen declined until being undetectable while they were raised in stroma, it is consistent with the proliferation pattern of endometrial cells reported by Leroy,[46] and suggests that IGF-I may have some role in proliferation of endometrium of rat during early gestation. It was also reported by other researchers that IGF-I could induce proliferations of mouse endometrium[47] and human stromal[48] cells in vitro, and proliferation of endometrium is the necessary step for decidualization.

IGF-I's concentration in swine uterus flushing was related to embryo survival and Ko[50] found a positive feedback relationship between IGF-I in swine uterus and P-450 cytochrome, a rate-limiting enzyme of estrogen in fetus.[50] During 8 to 10 days of gestation, IGF-I and its own receptor expressed less intense staining of the endometrium and was mainly located in stromal cells, indicating that IGF-I might have some role in decidualization of endometrium. Irwin[48] also reported that the co-action of IGF-I, IGF-II, EGF and P could induce the decidualization of endometrium in vitro.

It was also demonstrated that IGF-I had some role in proliferation, differentiation and metabolism of the early trophoblast. Kanai et al[51] reported that IGF-I

could promote the proliferation and migration of the trophoblast. Kniss et al[52] found that IGF-I could accelerate glucose and amino acid transportation in dose dependent manner of early pregnant trophoblast cells in vitro, Halhali and colleagues still found that IGF-I facilitated the transformation of 25-(OH)2D3 to 1,25-(OH)2D3, a more active molecule of vitamin D. Our results revealed that IGF-I and its own receptor are expressed and located in the trophoblast cells from 8d through 10d in rat. It is suggested that IGF-I might have some role in the functional modulation of rat trophoblast via paracrine / autocrine mechanisms during peri-implantation.

Modulation of IGF-I and IGF-IR expression in rat endometrium by P during peri-implantation

Murphy and coworkers[54] regarded estrogen (E2) as main regulator of IGF-I and IGF-IRexpression in endometrium. Our data indicated that although expression of IGF-I had similar variation like that of serum P concentration and PR expression, no significant relationship was found when examined by bivariate correlation test and it suggests that P has no effects on expresssion of IGF-I during peri-implantation. The similarity between them may be a coincidence or a suggestion that P modulates IGF-I expression indirectly via other mediator. Our data also revealed a negative significant relationship between IGF-IR and PR and P, and also it suggests that P inhibits the expression of IGF-IR in rat endometrium. Strowitzki el al[55] also reported that P had some inhibitory effects on the expression of IGF-IR in human stromal cells in vitro. It seemed that only high serum P concentration exerted inhibitory effects on IGF-IR's expression in rat endometrium in our experiment. Because of different modulation effects of high serum P concentration on expressions of IGF-I and IGF-IR, their curve separated during peri-implantation. It may be an important mechanism that suppreseed the proliferative effect of IGF-I on endometrium and allowed decidualization of endometrium and necessitates requirement of IGF-I for embryo growth and development during peri-implantation.

Although IGF-I, IGF-IR and PR are all expressed in the rat trophoblasts no significant relationship were found between them. It suggests that P does not effect expressions of IGF-I and IGF-IR in rat trophoblasts. IGF-I and P may modulate of trophoblast functions independently.

REFERENCES

1. *Cross JC, Werb Z, Fisher SI. Implantation and the placenta : Key pieces of the development puzzle. Science 1994: 266:1508-1518.*
2. *Sirbasku DA, Benson RH. Estrogen-inducible growth factors that may act as mediators (estromedins) of estrogen-promoted tumor cell growth. In: Sato GS. Ross R (eds.), Hormones and Cell Culture, Cold spring Harbor, NY: Cold Spring Harbor Laboratory; 1979: 477-479.*
3. *Murphy IJ, Ghahary A. Uterine insulin-like growth factor-I: regulation of expression and its role in estrogen-induced uterine proliferation. Endocr Rev 1990; 11:443-453.*
4. *Kapur S. Tamada H, Dey SK, Andrews GK. Expression of insulin-like growth factor-I (IGF-I) and its receptor in the peri-implantation mouse uterus, and cell-specific regulation of IGF-I gene expression by estradiol and progesterone. Biol Reprod 1992; 46:208-219.*
5. *Ghahary A. Chakrabarti S. Murphy IJ. Localization of the sites of synthesis and action of insulin-like growth factor-I in the rat uterus. Mol Endocrinol 1990; 4:191-195.*
6. *Martin I, Finn CA, Trinder G 1973 Hypertrophy and hyperplasia in mouse uterus after estrogen treatment: an autoradiographic study. J Endocrinol 56:133-144.*
7. *Huet-Hudson YM, Andrews GK, Dey SK 1989 Cell type-specific localization of c-Myc protein in the mouse uterus: modulation by steriod hormones and analysis of the perimplantation period. Endorcrinology 125:1683-1690.*
8. *Martin I, Das Rm, Finn CA 1973. The inhibition by progesterone of uterine epithelial proliferation in the mouse J Endocrinol 57:549-554.*
9. *Psychoyos A 1973 Endocrine control of egg implantation. In: Greep RO, Ast-wood EG, Geiger SR (eds) Handbook of Physiology. American Physiological Society, Washington DC, pp 187-215.*
10. *Paria BC, Huet-Hudson YM, Dey SK 1993 Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. Proc Natl Acad Sci USA 90:10159-10162.*
11. *Dey SK 1996 Implantation. IN: Adashi EY, Rock JA, Rosenwaks Z (eds) Re-productive Endocrinology, Surgery, and Technology. Lippincott-Raven, New York, pp 421-434.*
12. *Orimo A, Inoue S, Ikeda K, Noji S, Muramatsu M 1995 Molecular cloning, structure and expression of mouse estrogen-responsive finger protein Efp. J Biol Chem 270:24006-24413.*
13. *Das SK, Das N, Wang J, Lim H, Schryver B, Plowman GD, Dey SK 1997. Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the "window" of implantation. Dev. Biol 190:178-190.*
14. *Das SK, Chakraborty I, Paria BC, Wang X-N, Plowman G, Dey SK 1995. Amphiregulin is an implantation-specific and progesterone-regulated gene in the mouse uterus. Mol Endocrinol 9:691-705.*
15. *Schrader WT, O'Malley BM 1972 Progesterone-binding components of chick oviduct. IV. Characterization of purified subunits. J Biol Chem 247:51-59.*
16. *Conneely OM, Maxwell BI, toft DO, Scharder WT, O'Malley BW 1987. The A and B forms of the chicken*

progesterone receptor arise by alternate initiation of translation of a unique mRNA. Biochem Biophys Res Commun 149:493-501.

17. *Kastner P, Krust A, Turcotte B, Strupp U, Tora I, Gronemeyer H, Chambon P 1990. Two distinct estrogen-regulated promotes generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603-1614.*
18. *Tung I, Mohamed MK, Hoeffler JP, Takimoto Gs, Horwitz KB 1993 Antagonist-occupied human prgesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhbited by A-receptors. Mol Endocrinol 7:1256-1265.*
19. *Lydon JP, DeNayo Fj, Funk CR, Mani Sk, Hughes AR, Montgomery Jr CA, Shyamala G, Conneely OM,O'Malley BW 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 9:2266-2278.*
20. *Curtis SW, Clark J, Myers P, Korach KS 1999 Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor a knockout mouse uterus. Proc Natl Acad Sci USA 96:3646-3651.*
21. *Paria BC, Tan J, Lubahn DB, Dey SK, Das Sk 1999 Uterine decidual response occurs in estrogen receptor-a deficient mice. Endocrinology 140:2704-2710.*
22. *Zapf J. Froesch ER, Humbel RE. The insulin-like growth factor (IGF) of human serum: chemical and biological characterization and aspects of their possible physiological role. Curr Top Cell Regul 1981; 19:257-309.*
23. *Sheng Shuli. Current theory and application of Polypeptide hormone. Beijing: Science and technology literature publishing company. 1998;249-251(In Chinese).*
24. *Van Wyk JJ. the somatomedins: biological actions and physiologic control mechanisms. Horm Prot Pept 1984; 12:82-125.*
25. *Froesch ER, Schmid C, Schwander J. Zapf J. Action of insulin-like growth factors Annu Rev Physiol 1985; 47:443-467.*
26. *Mathews LS, Norstedt G. Palmiter RD. Regulation of insulin-like growth factor I gene expression by growth hormone. Proc Natl Acad Sci USA 1986; 83:9343-9347.*
27. *Clemmons DR, Van Wyk JJ. Somatomedins: phsiological control and effects on cell proliferation. In: Baserga R (ed),Handbook of Experimental Pharmacology. New York: Springer-Verlag: 1981; 57:161-208.*
28. *Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Hanzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramchandran J, Fujita-Yamaguchi Y. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggest structural determinants that define functional specificity. EMBO J 1986; 5:2503-2512.*
29. *Morgan DO, Edman JC, Strandring DN, Fried VA, Smith MC, Roth RA, Rutter WJ. Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 1978; 329:301-307.*
30. *Massague J. Czech MP. The subunit structure of two distnct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. J Biol Chem 1982; 257:5038-5046.*
31. *Sara VR, Hall K. Insulin-like growth factors and their binding proteins. Physiol Rev 1990; 70:591-614.*
32. *Lammers R, Gray A, Schlessinger J, Ullrich A, Differential signalling potential of insulin- and IGF-I receptor cytoplasmic domains. EMBO J 1989; 8:1369-1375.*
33. *LeofEB, Wharton W, Van Wyk JJ, Pledger WJ, Epidermal growth factor (EGF) and somatomedin C regulate G_1 progression in competent Balb/c-3T3 cells. Exp Cell Res 1982; 141:107-115.*
34. *Leof EB, Van Wyk Jj, O'Keefe EF, Pledger WJ, Epidermal growth factor (EGF) is required only during the traverse of early G, in PDGF stimulated density-arrested Balb/c-3T3 cells Exp Cell Res 1983; 147:201-208.*
35. *D'Ercole AJ, Stiles Ad, Underwood LE, Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc Natl Acad Sci USA 1984; 81:935-937.*
36. *Clemmons DR, Underwood LE, Van Wyk JJ. Hormonal control of immunoreactive somatomedin production by cultured human fibroblasts. J Chin Invest 1981; 67:10-17.*
37. *Murphy LM, Bell GI, Duckworth ML, Freisen HG. Identification, characterization and regulation of a rat complemantary deoxyribonucleic acid which encodes insulin-like growth factor-I. Endocrinology 1987; 121:684-691.*
38. *Simmen FA, Simmen RCM. Peptide growth factors and proto-oncogenes in mammalian conceptus development. Biol Reprod 1991;441-5.*
39. *Correia DSG, Bell SC, Pringle JH, Teixeira N. Expression of mRNA encoding insulin-like growth factors I and II by uterine tissues and placenta during pregnancy in the rat. Mol Reprod Dev, 1999;53(3):294.*
40. *Blakesley VA, Butler AA, Koval Ap, Okubo Y, LeRoithg D, 1999 In: Rosenfeld RG, Roberts CT, editors. The IGF system Totowa, NJ: Human Press Inc. p329-353.*
41. *Baserga R 2000. The contradictions of the insulin-like growth factor I receptor. Oncogene 19:5574-5581.*
42. *O'Connor R, Fennelly C, Krause D. 2000. Regulation of survival signals from the insulin-like growth fctor-I receptor. Biochem Soc Trans 28:47-51.*
43. *Surmacz E. 2000. Function of the IGF-IR in breast cancer. J Mammary Gland Biol Neopl 5:95-105.*
44. *Burks DJ, White MF. 2001. IRS proteins and beta-cell function. Diabetes 50:S140-S145.*
45. *Shepherd PR, Withers D, Siddle K, 1998. Phosphoinositide 3-kinase: The key swithch mechanism in insulin signaling. Biochem J 333:471-490.*
46. *Leroy F, Galand P, Chretien S. The mitogenic action of ovarian hormones on the uterine and the vaginal epithelium during the oestrous cycle in the rat: A radioautographic study. J Endocrinol, 1969;45:441.*
47. *Quamby V E, Korack K S. The influence of 17 ß-estradiol on patterns of cell division in the uterus. Endocrinology. 1984;114:694.*
48. *Irwin JC, De Las Fuentes L, Giudice LC. Growth factors and decidualization in vitro. Ann N Y c Acad Sci, 1994;734:7.*
49. *Wilson ME, Ford SD. Differences in trophectoderm mitotic rate and P450 17alpha-hydroxylase expression between late preimplantation. Meishan and York Shire conceptuses. Biol Reprod, 1997;56(2):380.*
50. *Ko Y, Choi I, Green ML, Simmen FA, Simmen RC, Transient expression of the cytochrome P450 aromatase*

gene in elongating procine blastocysts is correlated with uterine insuline-like growth factor levels during perimplantation development. Mol Reprod Dev, 1994;37(1):110.

51. *Kani Am, Kanai Y, Kurohmaru M, Sakai S, Hayashi Y. Insulin-like growth factor I (IGF-I) stimulates proliferation and migration of mouse ectoplacental cone cell, while IGF-II transforms them into trophoblastic giant cell in vitro. Bio Reprod, 1993;48(2):252.*
52. *Kniss D A, Shubert PJ, Zimmerman PD, Landon MB, Gabbe SG. Insulin-like growth factors: their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi, J Reprod Med, 1994;39(4):249.*

52 *Halhali A, Diaz L, Sanchez I, Garabedian M, Bourges H, Larrea F. Effects of IGF-I on 1,25-dihydroxyvitamin D_3 syntheses by human placenta in culture. Mol Hum Reprod,1999;5(8):771.*

53. *Murphy LJ, Murphy LC, Friesen HG. Estrogen induces insulin-like growth factor-I expression in the rat uterus. Mol Endocrinol,1987;1(7):445.*
54. *Strowitzki T, Singer GA, Retting I, Capp E. Characterization of receptor for insulin-like growth factor type I on cultured human endometrial stromal cells: downregulation by progesterone. Gynecol Endocrinol, 1996;10(4):229.*