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Progesterone Downregulated the Gene Expression of Insulin-Like Growth Factor–I Receptor in Human Decidua of Early Pregnancy

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ABSTRACT

BACKGROUND: The present study assessed the effects of progesterone on the gene expression of insulin-like growth factor-I receptor(IGF-IR) in human decidua of early pregnancy in vivo and in vitro.
Methods: The expression of IGF-IR in human decidua from 54 women at 5-7 weeks of gestation were detected by immunohistochemical assay, and their serum progesterone level were measured by radioimmunoassay. Semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) was applied to determine the levels of IGF-IR mRNA in human decidual cells in vitro after cultured with different concentrations of progesterone for 72 hours.
Results: The expression of IGF-IR was mostly in decidual cells(DC),and those in glandular epithelium(GE) and luminal epithelium(LE) were weaker during early pregnancy. The expressions of IGF-IR in decidua showed significant negative-correlation with serum progesterone level (rDC=–0.748, P<0.01; rGE=–0.870, P<0.01; rLE=–0.935, P<0.01, respectively). The levels of IGF-IR mRNA in decidual cells in vitro were also down-regulated by progesterone, and showed significant negative-correlation with the concentration of progesterone (r=–0.680, P<0.001).
Conclusions: Progesterone may play an important role in fetal and maternal tissue growth and differentiation by down-regulating the gene expression of IGF-IR in human decidua of early pregnancy. These satisfy both the need for endometrium decidualization and the requirement of IGF-I for embryo growth and development.

Keywords: Progesterone. Insulin-Like Growth Factor-I Receptor. Decidua

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The insulin-like growth factor (IGF) autocrine/paracrine system consists of IGF-I and IGF-II, type I and type II IGF receptors,and IGF binding proteins(IGFBPs) that regulate the availability of the IGFs to their receptors. IGFs are believed to to play a role in endometrial differentiation and trophoblast growth. The mitogenic, differentiative, and antiapoptotic properties of IGFs and their binding proteins, as well as their spatial and temporal expression, suggest that they play important roles in endometrial, decidual, and trophoblast physiology [1,2].The IGF peptides (IGF-I and IGF-II) mediate their effects mostly through the type I IGF receptor (IGF-IR). In this study, we investigated the effect of progesterone on the gene expression of

IGF-IR in human decidua of early pregnancy in vivo and in vitro.

METHODS

Tissue collection and serum progesterone Decidua tissues were obtained from 20-30 years old pregnant women with gestation of 5 to 7 weeks undergoing induced abortion (n=54), all participating patients gave a written consent and the study was approved by the Hospital Ethical Committee. Blood were collected for serum progesterone at the same time. Serum progesterone level were measured by radioimmunoassay (RIA),each was repeated at least three times.

Immunohistochemical assay

IGF-IR expression was detected by immunohistochemical assay using the streptavidin-biotin peroxidase complex(ABC) method.Non-specific binding sites were blocked with 10% rabbit serum for 30 minutes.when the serum was removed,the tissue was incubated with polyclonal rabbit anti-human IGF-IR antibody at 4°C for 24 hours and goat anti-rabbit IgG+IgM at 37°C for 30 minutes. Then the tissue was treated with DAB-H_2O_2 and counterstained with haematoxylin.Negative controls were conducted by replacing the anti-human IGF-IR antibody with PBS.

Cell culture

Fresh decidua tissues were processed by a modification of previous method described as below[3,4]. Decidua tissue was incubated in RPMI 1640 solution containing 0.125% trypsin(Sigma) and 0.1% collagenase-II(Sigma) at 37º C for 15 minutes. The supernatant was filterd into RPMI 1640 containing 20% fetal calf serum (FBS) using a 100-mesh and 200-mesh screen sieve, while the residue was digested two more times with fresh enzyme solution. Cells were washed twice and plated in culture flasks ($5x10^5$/ml), unattached cells and debris were removed 2 hours later. After monolayers became confluent, cells were digested by RPMI 1640 solution containing 0.125% trypsin and 0.01% EDTA(Sigma),then plated in 60-mm petri dishes and cultured at 37º C in a 5% CO_2 environment. After monolayers became confluent, cells were rinsed twice and incubated in RPMI 1640 for 72 hours without progesterone, or with 0.05, 0.1, 0.2 and 0.4μmol/L of progesterone respectively.The final ethanol concentration was 0.01%. Each experiment was done in triplicate and was repeated at least three times.

RT-PCR detection

IGF-IR mRNA expression was investigated by RT-PCR analysis by a modificated method described in one step RT-PCR kit (Takara,Japan). In brief, total RNA was isolated from decidual cells growing in 60-mm culture dishes in different groups, using SV total RNA isolation family (Promega,U.S.A) and the manufacturers protocol. 1 mg of total RNA from each preparation was reverse transcribed into cDNA and then amplified by PCR in a reaction volume of 50μl. Primers for IGF-IR was 5'-GGG AAT TCC CCG ACC TCG CTG TGG GG-3'(sense)and 5'-GGA AGC TTG GAA CAG CAG CAA GTA CTC-3'(antisense)[5], and _-actin was used as an internal control for RNA degradation and RT inefficiency. The reactions were carried out in a themocycler as follows:30 minutes at 50° C; 2 minutes at 94° C;then 30 seconds at 94° C, 30 seconds at 55° C, 1 minutes at 72° C , for 35 cycles. No RT and no total RNA controls accompanied all RT-PCR reactions.The RT-PCR products were electrophoresed on 2% agarose gel containing 0.1μg/ml ethidium bromide. The visualized bands were analyzed semiquantitatively using image scanning densitometry.

Statistical analysis

Data are presented as the mean+SD(standard deviation). Statistical analysis was performed with one way ANOVA analysis for multiple comparisons. The relationship between IGF-IR and progesterone was examined by rank-order correlation analysis.

RESULTS

Human serum progesterone reached peak level during 5-6 weeks of gestation,then declined to the early stage of 5 weeks of gestation level at 7 weeks of gestation,which was significant lower than the peak level($P<0.05$). The expression of IGF-IR was mostly in decidual cells(DC),and those in glandular epithelium(GE) and luminal epithelium(LE) were weaker. The expression of IGF-IR in decidua was high at the early stage of 5 weeks of gestation,then decreased gradually to the low level at the middle stage of 6 weeks of gestation($P<0.05$),afterward increased to the level of 5 weeks of gestation at the late stage of 7 weeks of gestation. And the expressions of IGF-IR in decidua showed significant negative-correlation with serum progesterone level ($rDC=–0.748$, $P<0.01$;$rGE=–0.870$, $P<0.01$;$rLE=–0.935$, $P<0.01$,respectively).
See Table 1, Fig.1 and Fig.2.

Fig.3 demonstrates IGF-IR mRNA expression in human decidual cells of early pregnancy in vitro with or without progesterone by RT-PCR. Progesterone treatment resulted in statistically significant decreases in IGF-IR mRNA verus control($P<0.001$),but there were no significant difference between the three groups of 0.1, 0.2 and 0.4μmol/L of progesterone treatment($P>0.05$). The levels of IGF-IR mRNA in decidual cells in vitro were down-regulated by progesterone,and showed significant negative-correlation with the concentration of progesterone

(r=–0.680, P<0.001) (Fig.4).

DISCUSSION

Uterine endometrium is a dynamic tissue whose components undergo cyclic changes under the influence of steroid hormones which may act primarily or secondarily through mediators such as growth factors,and the IGFs are believed to be involved in endometrial differentiation as well as trophoblast growth[6,7]. IGF-I is important in endometrial proliferation and in the regulation of embryo/fetal tissue growth, and IGF-II participates in the regulation of endometrial decidualization,angiogenesis[8], trophoblast invasion, differentiation and placenta formation. Estrogen stimulates IGF-I gene expression in the endometrium, and IGF-I is assumed to mediate estrogen action[9]. IGF-II gene expression is associated with endometrial differentiation.

IGFs generally exerts their function via IGF-IR.In human, IGF-IR has been identified in proliferative and secretory endometrium,both in stroma and epithelium,but relatively more abundant in endometrial epithelium [10]. In our study, the expression of IGF-IR was mostly in decidual cells,and those in glandular epithelium and luminal epithelium were weaker during early pregnancy. IGF-IR may be involved in the proliferation of stromal cells,occurs early in decidualization. In fully differentiated decidual cells,IGFs may regulate cellular metabolism and IGFBP-1 production via a receptor-mediated mechanism. Studies have shown that trophoblast-derived IGF-II and decidua-derived IGFBP-1 provide autocrine/paracrine enhancement of trophoblast invasiveness largely by stimulating migration, an essential step in invasion [11,12]. The changes of IGF-IR level in decidua is coincident with the need of decidualization and embryo growth and implantation.

Messenger RNA encoding IGF-IR was more abundantly expressed in the secretory phase and during early pregnancy,compared to the proliferative phase,which shows that the expression of IGF-IR mRNA is regulated by steroid hormones[13]. In our study, the expressions of IGF-IR in decidua showed significant negative-correlation with serum progesterone level during early pregnancy,and the levels of IGF-IR mRNA in decidual cells in vitro were also down-regulated by progesterone,and showed significant negative-correlation with the concentration of progesterone. These results show that the messenger ribonucleic acid expression and protein synthesis of IGF-IR in human decidua of early pregnancy were down-regulated by progesterone. We have previously found that progesterone inhibited the expression of IGF-IR in rat endometriumperi-implantation [14].Strowitzki also reported that progesterone had some inhibitory effects on the expression of IGF-IR in human stromal cells in vitro and in turn inhibit the growth-promoting actions of IGF-I,which results in inhibition of endometrial stromal growth and acceleration cell differentiation during the secretory phase[15].

The results in our investigation indicated that progesterone may play an important role in fetal and maternal tissue growth and differentiation by down-regulating the gene expression of IGF-IR in human decidua of early pregnancy. These satisfy both the need for endometrium decidualization and the requirement of IGF-I for embryo growth and development.

Table 1. Serum progesterone concentration and IGF-IR expression in human decidual cells, glandular epithelium and luminal epithelium in early pregnancy (mean±SD)

Pregnancy days (day)	proesterone (pg/ml)	IGF-IR		
		decidual cells	glandular epithelium	luminal epithelium
35	20.30±3.51	135.28±2.26	123.88±3.41	114.36±4.51
37	23.34±2.79	136.03±4.05	122.76±2.19	107.10±2.27
39	24.05±3.24	139.99±2.99	106.70±3.13	96.24±3.15
42	32.76±4.56	135.23±3.68	95.62±2.94	92.27±4.02
45	31.38±2.58	123.93±4.32	92.29±4.27	85.37±4.98
47	21.80±2.61	127.97±2.77	87.32±3.96	86.02±3.63
49	26.94±3.24	130.26±1.86	99.99±2.22	90.19±2.08
52	19.56±3.22	136.29±4.67	103.77±3.23	101.69±3.17
54	16.91±1.91	139.07±1.82	112.21±1.81	110.79±1.26

Fig 1. IGF-IR expression in human decidual cells
(a) glandular epithelium
(b) luminal epithelium
(c) negative control
(d) in early pregnancy 400x

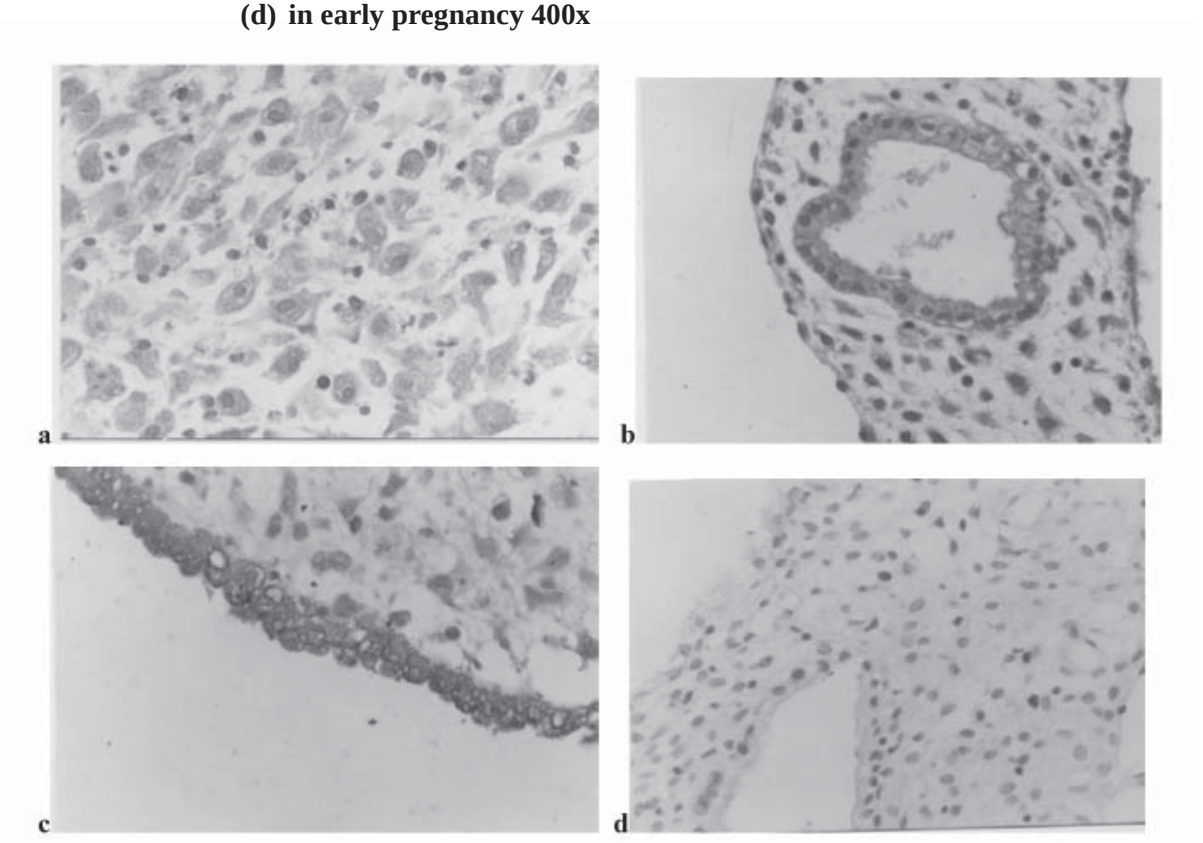


Fig 2. Serum progesterone concentration and IGF-IR expression in human decidual cells, glandular epithelium and luminal epithelium in early pregnancy

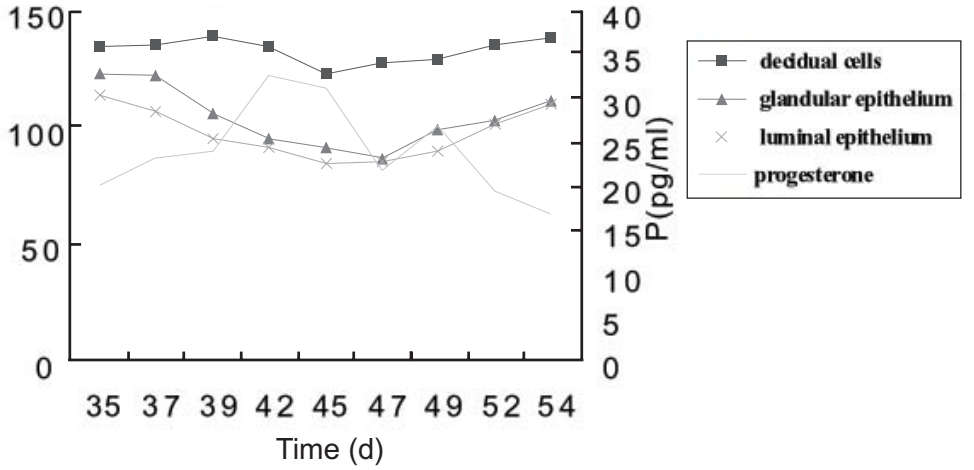


Fig 3. β-ACTIN mRNA and IGF-IR mRNA expression in human decidual cells of early pregnancy in vitro by RT-PCR (M: Marker, lane 1 to 5:cells were cultured with 0,0.05,0.1,0.2 and 0.4μ mol/L of progesterone for 72 hours respectively)

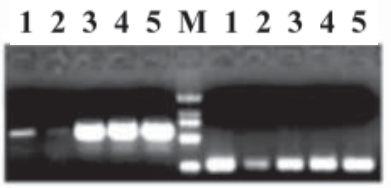
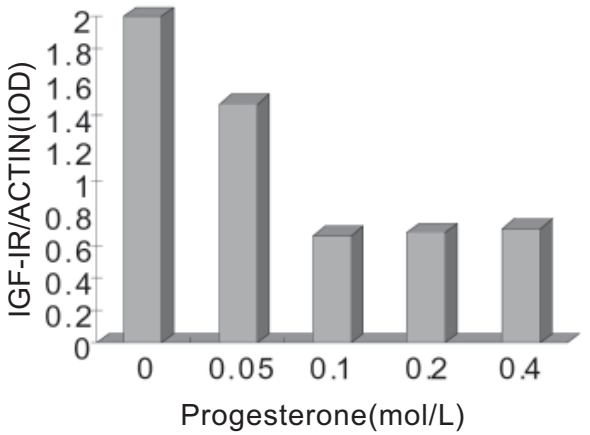


Fig 4. Effect of different concentrations of progesterone on the expression of IGF-IRmRNA in human decidual cells in vitro



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