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Original Article

Expression of heparin-binding epidermal growth factor in the endometrium is positively correlated with IVF-ET pregnancy outcome

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Abstract: Heparin-binding epidermal growth factor (HB-EGF) is regarded as a marker of endometrial receptivity. However, the significance of HB-EGF expression for in vitro fertilization and embryo transfer (IVF-ET) remains unclear. In this study, we aimed to investigate the relationship between HB-EGF expression in endometrium in the window of implantation and the pregnancy outcome after IVF-ET. Total 56 women with infertility who received IVF-ET from October 2009 to April 2010 were enrolled in this study, and divided into pregnancy group (23 cases) and non-pregnant group (33 cases) according to clinical pregnancy outcome. The expression of HB-EGF was detected by real-time RT-PCR. Pearson correlation analysis was used to analyze the correlation between HB-EGF expression and endometrial thickness. We found that HB-EGF expression in endometrium of pregnancy group in the window of implantation was significantly higher than that of non-pregnant group ($P < 0.05$). Endometrial HB-EGF expression was positively correlated with endometrial thickness ($r = 0.746$, $P < 0.01$). In addition, HB-EGF expression in endometrial thickness of 9-16 mm group was significantly higher than in endometrial thickness of 6-8 mm group ($P < 0.001$). In conclusion, HB-EGF expression in endometrium in window of implantation in patients undergoing IVF-ET was positively correlated with endometrial thickness and pregnancy outcome. HB-EGF in endometrium may be used as one predictor for implantation receptivity and pregnancy outcome of IVF-ET.

Keywords: Heparin-binding epidermal growth factor, endometrium, window of implantation, in vitro fertilization-embryo transfer (IVF-ET), pregnancy

Introduction

With the rapid development of human assisted reproductive technology, the pregnancy rates after in vitro fertilization and embryo transfer (IVF-ET) are increasing, but pregnancy outcomes are still not satisfactory. To achieve a successful pregnancy, the embryo must implant and build good connection to maternal circulation, while the endometrium must be receptive. Endometrium is the inner mucous membrane of the mammalian uterus. The "window of receptivity" of the endometrium has become the focus of pregnancy research in recent years. If the endometrium is not receptive for the implantation of the embryo, the implantation will fail and pregnancy is impossible. Studies have shown that 60% of embryo implantation failure was due to inadequate endometrial receptivity, leading to the decrease of pregnancy success rate [1].

Heparin-binding epidermal growth factor (HB-EGF) is a member of epidermal growth factor (EGF) family and is synthesized as a transmembrane precursor which undergoes proteolytic processing to produce a soluble form sHB-EGF, or it may remain bound to cell membrane. Increasing evidence has indicated that HB-EGF is involved in the regulation of implantation [2]. During mouse blastocyst implantation, the synthesis of HB-EGF is closely associated with the implantation site. In mouse endometrium HB-EGF is produced as a protein bound to cell membrane of endometrial epithelium and it could bind heparan sulfate on the surface of the blastocyst. Consequently, endometrial HB-EGF acts as the blastocyst growth

Table 1. Comparison of general data in pregnant group and non-pregnant group

Group	Pregnant group	Non-pregnant group	*t*	*P*
No. of cycles	23	33		
Age (years)	29.33 ± 4.10	30.62 ± 4.75	-0.92	0.35
Infertility duration (years)	5.62 ± 3.74	5.97 ± 4.21	-0.53	0.64
BMI (kg/m^2)	21.17 ± 2.11	22.62 ± 2.69	-0.45	0.68
bFSH (mIU/ml)	3.94 ± 2.86	4.79 ± 3.49	-0.96	0.34
bE_2 (pg/ml)	29.30 ± 4.01	31.02 ± 4.63	-0.90	0.37
Gn dosage	38.61 ± 8.13	40.76 ± 10.13	0.56	0.61
No. of oocytes	14.78 ± 11.78	11.93 ± 6.42	-1.52	0.13
No. of embryos implanted	2.17 ± 0.38	2.00 ± 0.53	-1.17	0.21
No. of high quality embryos	8.25 ± 4.56	8.21 ± 4.17	-1.04	0.23
No. of frozen embryos	5.58 ± 5.21	3.62 ± 2.37	-1.52	0.18
hCGday LH (mIU/ml)	2.02 ± 1.46	1.97 ± 1.86	0.104	0.92
hCGday E_2 (pg/ml)	2622 ± 1829	3408 ± 2387	-1.33	0.19
hCGday P (ng/ml)	3.92 ± 1.92	5.39 ± 3.72	-1.73	0.09
ETday E_2 (pg/ml)	1418 ± 1031	1427 ± 1186	-0.03	0.98
ETday P (ng/ml)	33.11 ± 22.26	32.73 ± 25.03	0.06	0.95

factor through the receptors HER1 and HER4 [3, 4]. Moreover, HB-EGF expression exhibits periodical changes following the different stages of a menstrual cycle. In detail, HB-EGF expression is low during proliferation, then gradually increases after ovulation, and eventually increases to the peak at the time of implantation. Therefore, HB-EGF has been accepted as one important marker of endometrial receptivity [5].

Up to now, the significance of HB-EGF expression for IVF-ET pregnancy rate remains unclear. In this study, we aimed to investigate the relationship between HB-EGF expression in endometrium in the window of implantation and the pregnancy outcome after IVF-ET.

Materials and methods

Study subjects

The subjects were 56 women with infertility who received IVF-ET for the first time at Center for Reproductive Medicine, Ningxia Medical University General Hospital from October 2009 to April 2010. They included 40 cases of primary infertility and 16 cases of secondary infertility, aged 22 to 42 years old (average 30.00 ± 4.20 years), duration of infertility 1 to 18 years (average 5.62 ± 3. 01 years). Inclusion criteria: (1) having normal levels of basic reproductive hormones; (2) having a normal menstrual cycle (26 to 32 days); (3) No history of hormone use or uterine operations within three months; (4) no history of IUD placement; (5) there are at least two Class I or II stage during IVF-ET cycles suitable for embryo implantation. This study was approved by Ethics Committee of Ningxia Medical University General Hospital, and all subjects signed informed consent.

Grouping

At 35 days after embryo implantation, abdominal ultrasound was carried out. If intrauterine gestational sac, fetal bud and fetal heart beat were detected, clinical pregnancy was confirmed. According to clinical pregnancy outcome after IVF-ET, 56 patients were divided into pregnancy group (23 cases) and non-pregnant group (33 cases). The age, duration of infertility, body mass index (BMI), endocrine basis, Gn dosage, the number of oocytes, the number of embryos implanted, the number of high quality embryos, the number of frozen embryos of pregnant group and non-pregnant group were compared.

Specimen collection

On the 10th day before a cycle of IVF-ET, vaginal ultrasound and urinary ovulation test were carried out to monitor ovulation. At 5-7 days after ovulation the simulation of implantation was carried out, and some endometrial tissues were taken and placed in diethylpyrocarbonate (DEPC) water treated sealed tubes for storage at -80°C. If endometrial biopsy showed the spiral arteries hyperplasia, endometrium was in the window of implantation and specimens collected were good.

Real-time PCR

Total RNA was extracted from endometrial tissues using AxyPrep total RNA Miniprep Kit (Axygen Biological, USA). cDNA synthesis and

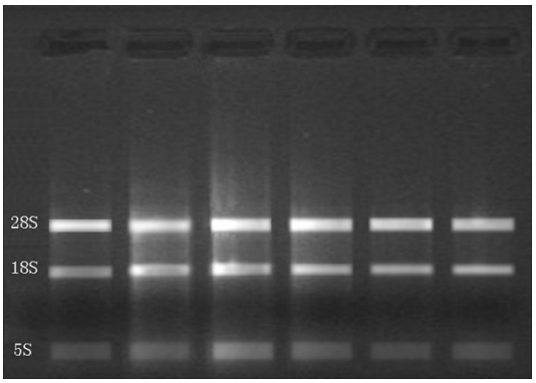

Figure 1. Agarose electrophoresis of total RNA extracted from endometrium. Shown were 6 samples.

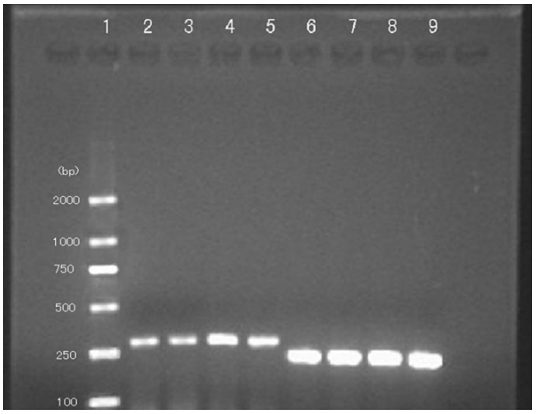

Figure 2. Agarose electrophoresis of PCR product. Lane 1: Marker; Lane 2-5: Product of HB-EGF; Lane 6-9: Product of GAPDH.

real-time fluorescent RT-PCR kit were from TransGen Biotech (Beijing, China). The primers were designed by using Primer5.0 software, and synthesized by Sangon Biotech (Shanghai, China). The primer sequences were as follows: HB-EGF upstream 5-ACAAGGAGGAGCACGGGAAAAG-3, downstream 5-CGATGACCAGCAGACAGACAGATG-3, product 276 bp; GAPDH upstream 5-GAAGGTGAAGGTCGGAGTC-3, downstream 5-GAAGATGGTGATGGGATTTC-3, product 226 bp. The real-time fluorescent RT-PCR amplification was performed using SYBR Green Kit on ABI Prism 7500 machine. The relative mRNA levels of HB-EGF were normalized to GAPDH and calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed by using SPSS13.0 software package. Independent sample t-test was used to compare the differences between groups, and Pearson correlation analysis was used to analyze the correlation between different factors.

Results

General clinical data of pregnant group and non-pregnant group

The clinical data on the age, duration of infertility, BMI, endocrine basis, Gn dosage, the number of oocytes, the number of embryos implanted, the number of high quality embryos, the number of frozen embryos in pregnant group and non-pregnant group were shown in **Table 1**. We found no significant differences in these parameters between the two groups.

HB-EGF mRNA levels in the endometrium of pregnant group and non-pregnant group

To compare HB-EGF mRNA expression levels in the endometrium in window of implantation of pregnancy group and non-pregnant group, we performed PCR analysis. First we isolated total RNA from the endometrium and examined the integrity of total RNA by 1.5% agarose gel electrophoresis. We observed three bands of 28S, 18S, and 5S, and the ratio of 28S:18S was approximately 2:1, while 5S band was weak (**Figure 1**). These data indicate that we obtained intact and high quality total RNA.

Based on 1.5% agarose gel electrophoresis, we detected PCR products of HB-EGF and GAPDH (**Figure 2**), confirming the specialty of PCR. Furthermore, by real-time PCR we calculated relative mRNA levels of HB-EGF based on melting curves (**Figure 3A**). HB-EGF relative mRNA level was of significantly higher in the endometrium in window of implantation of pregnancy group (2.39 ± 0.60) than in that of non-pregnant group (1.14 ± 0.61) ($P < 0.05$, **Figure 3B**).

Correlation analysis of HB-EGF expression and endometrial thickness

Linear correlation analysis of HB-EGF mRNA expression in the endometrium and endometrial thickness showed a significant positive correlation between them ($r = 0.746$, $P < 0.01$, **Figure 4A**).

Moreover, HB-EGF mRNA expression in mid-luteal phase in endometrium with the thickness

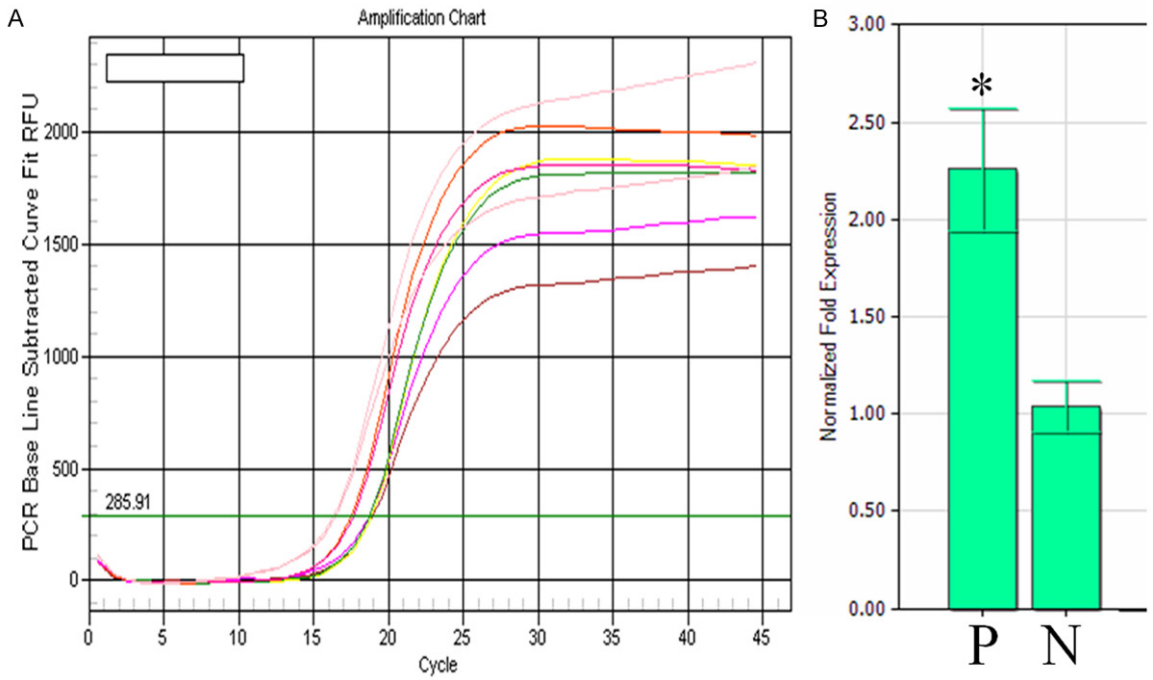

Figure 3. Real-time PCR for the detection of HB-EGF expression in endometrium of the window of implantation. A. Representative melting curves of real-time PCR. B. Relative mRNA level of HB-EGF in endometrium of the window of implantation. P: pregnancy group; N: non-pregancy group. *P < 0.05.

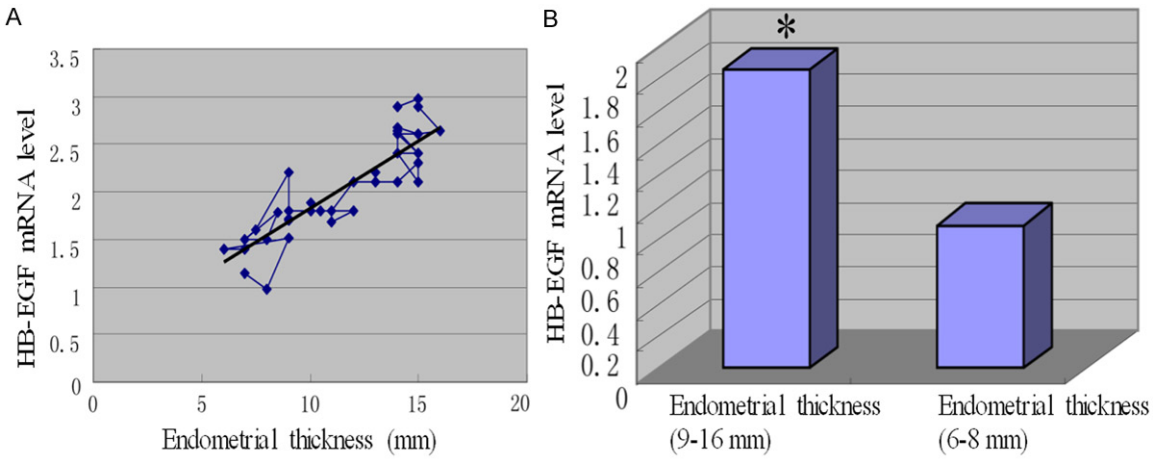

Figure 4. HB-EGF expression level is positively correlated with endometrial thickness. A. Linear correlation analysis of HB-EGF mRNA expression level and endometrial thickness. B. Comparison of HB-EGF mRNA expression level in group with endometrial thickness of 9-16 mm and with endometrial thickness of 6-8 mm. *P < 0.001.

9-16 mm group (44 cases) was significantly higher than in endometrium with the thickness 6-8 mm group (12 cases) (1.86 ± 0.85 vs. 0.89 ± 0.31, t = -3.85, P < 0.001, **Figure 4B**).

Discussion

Endometrium has a very short period of embryo implantation receptivity which is about 7-9 days after the ovulation in normal human menstrual cycle and called the window of implantation [2]. It is generally accepted that endometrial thickness and hormones and cytokines are improtant factors in regulating endometrial receptivity. These various factors coordinate and mediate cell-cell and cell-matrix interactions, thereby enhancing endometrial receptivity and

ensuring the success of embryo implantation [6].

HB-EGF is a new member of EGF family with a special heparin binding domain, and it has strong mitogenic activity. HB-EGF is encoded by the gene located on human chromosome 5, and its molecular weight is 19-23 kD. HB-EGF is synthesized as a transmembrane precursor protein proHB-EGF with 208 amino acids, including six domains, signal peptides, heparin-binding domain, epidermal growth factor-like domain, membrane proximal region, transmembrane region and cytoplasmic region. The membrane proximal region is hydrolyzed to release sHB-EGF, which could bind HER1 (EGFR) and HER4 with the mediation of heparan sulfate proteoglycan (HSPG) to initiate signal transduction between the embryo and the endometrium and regulate cell proliferation, migration and movement [5].

HB-EGF is an important factor to mediate the crosstalk between blastocyst and uterine during the window of implantation, and plays crucial role in blastocyst implantation [7]. Several studies showed that HB-EGF level in the proliferative phase of the menstrual cycle was very low, then gradually increased, and reached a high expression in the window of implantation [8, 9]. Ejskjaer et al. reported that the expression of HB-EGF in endometrium in window of implantation was higher than in follicular phase and late secretory phase, but there was no statistical significant [10]. In addition, women with consecutive miscarriages had significant lower expression of HB-EGF protein compared with control group [11]. These studies suggest that high expression of HB-EGF in endometrium in the window of implantation is necessary for the implantation of the blastocyst.

Currently, the relationship of HB-EGF expression in endometrium in the window of implantation and IVF-ET pregnancy outcome is rarely reported. In this study, we selected women who received IVE-ET and detected HB-EGF mRNA expression level in endometrium in the window of implantation. The results showed that the relative expression of HB-EGF mRNA in endometrium in window of implantation of pregnant group was significantly higher than in the non-pregnant group. These data indicate that a low level of HB-EGF in endometrium in window of implantation would not support the adhesion and implantation of blastocyst, resulting in the failure of IVF-ET implantation.

Al-Ghamdi et al. found that endometrial thickness was positively correlated with pregnancy rate, and endometrial thickness of at least 11 mm was most suitable for pregnancy [12]. Therefore, we further investigated the relationship between HB-EGF expression and endometrial thickness, and found that HB-EGF expression in endometrium in mid-luteal phase and endometrial thickness had a significant positive correlation, and HB-EGF expression in endometrial thickness of 9-16 mm group was significantly higher than in endometrial thickness of 6-8 mm group. These results provide strong evidence for the positive relationship between endometrial thickness and IVF-ET pregnancy outcome. Consistent with our findings, Sher et al. found that embryo implantation rate of IVF and clinical pregnancy rate had significant differences between endometrial thickness ≥ 9 mm group and endometrial thickness < 9 mm group [13]. Although there is currently no literature on the correlation between HB-EGF expression in endometrium and endometrial thickness, numerous studies suggest that integrin and VEGF expression is low and microvessel density is decreased in thin endometrium, leading to decreased endometrial receptivity [14]. In this study we found that HB-EGF expression in endometrium in mid-luteal phase and endometrial thickness was positively correlated, indicating that HB-EGF may mediate the growth of endometrium due to its mitogen activity. This may explain why HB-EGF expression in endometrium in mid-luteal phase is positively correlated to IVF pregnancy outcome.

In conclusion, in this study we provided the first evidence that HB-EGF expression in endometrium in window of implantation in patients undergoing IVF-ET was positively correlated with endometrial thickness and IVF pregnancy outcome. These results suggest that HB-EGF in endometrium may be used as one predictor for implantation receptivity and pregnancy outcome of IVF-ET.

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Disclosure of conflict of interest

None.

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