Original Article
Growth hormone intrauterine perfusion combined with replacement cycle in the treatment of non-response thin endometrium: report of 5 cases

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Abstract: To investigate the effects of the frozen embryo transplantation for patients with poor outcome of endometrial growth by using growth hormone intrauterine perfusion combined with replacement cycle in the treatment of non-response thin endometrium. This is a prospective study and study participants were consecutively recruited between Jun 2014 and December 2014. A total of 5 non-response thin endometrium patients were selected, with treatment by frozen thawed embryo transfer from the Reproductive Center of Hunan Provincial Maternal and Child Health Hospital. The mean endometrial thickness of 5 patients on progesterone day was 7.96 ± 0.71 mm, more than the mean endometrial thickness of the first perfusion (5.78 ± 0.65) mm, the difference was statistically significant (t=10.46, P<0.05). All endometrial thickness on transplantation day was more than 7 mm. The average concentration of E2 was 9387.8 ± 2623.87 pmol/l on the progesterone day. Clinical pregnancy and implantation rate of all cases was 72.73% (8/11), including 2 patients with full-term delivery and 1 patient with continued pregnancy; Embryo development of 1 patient diapause due to incision pregnancy, abortion embryo genetic testing results indicated normality. GH uterine cavity perfusion was a useful method for treatment of non-reactivity thin endometrium, and was helpful for improvement of endometrial thickness and receptivity, improve embryo implantation environment by assistance for HRT under the high estrogen levels.

Keywords: Frozen embryo, transplantation, growth hormone intrauterine perfusion, non response thin endometrium

Introduction

In recent years, assisted reproductive technology (ART) developed rapidly due to a variety of advanced technology, but with high quality embryo conditions, the average of success rate of test tube baby in vitro fertilization (IVF) was around 50%, and the final outcome of pregnancy is still not optimistic [1]. Study indicated that 50%-70% of pregnancy failure is caused by abnormal endometrial implantation of embryo after transplantation; successful implantation depends on the blastocyst invasion and establishment of uterine receptivity [2]. Some studies [3-5] suggested that the thin endometrium refers to endometrial thickness below the threshold thickness which was needed for pregnant. Give the luteal support day or HCG day in ART, in the ultrasound image, endometrial thickness is less than 7 mm or 8 mm, which could met the standard that can achieve the best pregnancy rate of uterine endometrium (more than 7 mm), it was the best that endometrial thickness was more than 9 mm. Study [6] reported that the incidence rate of women was about 5% in subjects with the age of less than 40 in the natural cycle of thin endometrium, and the incidence rate was 25% in 41-45 year old females. It was a recognized problem to improve endometrial receptivity, transplantation opportunities, improve planting rate and live birth rate of this type of patients with endometrial thickness. The human uterus has a complete insulin-like growth factor (insulin-like growth, factors, IGFs), growth hormone (growth hormone, GH) and its receptors, play a
role by autocrine and paracrine, and associated with endometrial receptivity, periodic variation, embryo implantation and development. This is the first study on growth hormone intrauterine perfusion combined with replacement cycle in the treatment of non-response thin endometrium, to investigate the effects of the frozen embryo transplantation for patients with poor outcome of endometrial growth.

**Materials and methods**

**Study subjects**

This is a prospective study and study participants were consecutively recruited between Jun 2014 and December 2014.

A total of 5 non-response thin endometrium patients were selected, with treatment by frozen thawed embryo transfer from the Reproductive Center of Hunan Provincial Maternal and Child Health Hospital. We selected study subjects using the following methods: 1) Regular menstrual cycle, at least within the past 6 months, the use of artificial cycle, femoston vaginal medication, endometrial stimulation, sildenafil, ovulation induction and other methods to ovulation or progesterone with endometrial thickness increased to 7 mm or above; 2) hysteroscopy examination found no obvious abnormalities within the past 6 months; 3) age <40 years old; 4) infertility due to pelvic tubal or male factors.

Criteria for the diagnosis of endometrial thickness: natural ovulation cycle or the use of gonadotropin stimulated ovarian follicular development cycle, at least 1 follicle and larger than 18mm in diameter, endometrial thickness is less than 8mm; or an ovulatory patient, exogenous estrogen, the serum estradiol level more than 200 pg/ml for over 14 days, endometrial thickness is less than 8 mm.

**Methods on FET (frozen-thawed embryo transfer) endometrial preparation**

A total of 5 patients were treated by hormone replacement cycle FET. B ultrasound detection of bilateral ovaries on the third day of menstruation, and it showed no dominant follicle ovary, and then subjects were given estradiol vale rate (E2V, progynova, 1 mg/, Bayer, Germany), with a constant dose of 3 mg/time, 2 times/day, menstruation eighth days with 17-beta estradiol (17 P-Estradiol, 17 P-E2, the frontal 14 of 17 Femoston beta -E2 tablets, 2 mg/package, Abbott Company), vaginal drug. The first GH intrauterine perfusion was conducted at 8-12 day after menstruation every other day. The blood concentration was at least 1830 pmol/L, according to the serum estradiol level and endometrial thickness [7]. At the same time, estrogen was used for about 14-21 days, and when endometrial thickness increased significantly compared with the early cycle endometrial, or endometrial thickness was close to or more than 8 mm, ultrasound monitoring indicated that growth of endometrial was halt for 3 days, then given progesterone 60 mg/days for the further promotion for the proliferation of endometrial glands and blood vessels, make the secretory phase endometrium changes for embryo implantation. If the pregnancy existed, we will continue to use until the 8th weeks of pregnancy, and then reduce the amount of drugs, stop luteal support the tenth week after pregnancy.

**Endometrial monitoring**

Endometrial thickness was measured by ultrasound. In the uterine endometrium strong echo median sagittal plane and muscle layer at the junction of the measured maximum diameter, measurements were repeated for 3 times, the average of the value was obtained. According to the suggestion by Gonen [8], the methods for classification standard were as follows: the endometrium is divided into A, B and C. A: three line, the outer layer and the central line was the strong echo, low echo area or dark area was between the outer layer and the uterine cavity midline; type B: moderate intensity echo uniform, strong uterine cavity intermittent unclear midline echo type: C; homogeneous echo, no uterine midline echo.

**GH intrauterine perfusion combined with frozen thawed embryo transfer**

GH perfusion fluid: 6 IU GH (6 IU/, Anke biotechnology) diluted with 0.5 ml 0.9% saline. GH intrauterine perfusion method: routine vaginal disinfection, 0.5 ml GH solution was extracted by 1 ml syringe, connected to COOK one-off artificial insemination tube (AC18, Smiths, Australia), in the mouth of the cervix is guided by ultrasound, uterine cavity at the bottom of the 0.5 cm-1.0 cm at the distance of slow injection. Then let the patient hip elevation for 15-30 degrees after perfusion, the patient have free activity time after bed rest for 15 minutes.
### Table 1. Clinical situation of 5 GH intrauterine perfusion patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patient number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.6</td>
</tr>
<tr>
<td>Duration of Infertility (years)</td>
<td>4</td>
</tr>
<tr>
<td>Obstetrical history</td>
<td>G2P0</td>
</tr>
<tr>
<td>The past Hysteroscopy</td>
<td>Intrauterine</td>
</tr>
<tr>
<td>The number of intrauterine adhesions</td>
<td>2</td>
</tr>
<tr>
<td>History of fresh and frozen embryo transfer cycles</td>
<td>1</td>
</tr>
<tr>
<td>The previous situation of embryo transfer</td>
<td>M1.0, M1.0</td>
</tr>
<tr>
<td>Previous transplantation of endometrial thickness (mm)</td>
<td>5.8</td>
</tr>
<tr>
<td>The outcome of pregnancy</td>
<td>Not pregnant</td>
</tr>
</tbody>
</table>
Application of sequencing slow freezing method was used for embryo freezing. Freezing protective agent was 1.5 M propylene glycol and 0.1 M sucrose (Sigma, USA), by using the Freeze Control program freezing equipment (CL-8800i, Australia). The rapid recovery method was also used, and more than 50% blast meres were viable embryo after recovery. Embryo was grouped for four levels. Grade 1: blast mere size was relatively uniform, and shape was regular, the rate of fragments was less than 5%, and no bubble; grade 2: blast mere size was not uniform and shape was irregular slightly, the rate of fragments was between 5%-10%, with or without a little bubble; grade 3: blast mere size was not uniform and shape was irregular significantly, the rate of fragments was between 21%-50%, with bubbles; grade 4: blast mere size was not uniform significantly, the rate of fragments was more than 50%, with many bubbles.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software system for Windows version 18.0 (SPSS Inc. Chicago, USA). Continuous variables of participants, such as endometrial thickness, were calculated as mean with standard deviation and were compared by using t test. All reported p-values were two-tailed, and those less than 0.05 were considered as statistically significant.

Results

The general clinical data

The general clinical situation was shown in Table 1. The mean age was 32.2 ± 5.5 years old. 4 patients with secondary infertility, 1 patient with primary infertility, and ovarian reserve function of all subjects were normal. All patients received hysteroscopy investigation before frozen embryo transplantation, 4 patients received intrauterine adhesion separation operation for 6 times due to intrauterine adhesions. The number 3 case was not pregnant after fresh embryo transplantation, the growth of endometrium was slow, mature follicle on endometrial thickness is less than 8 mm, hysteroscopy suggested thin endometrium.

Fresh and frozen embryo transfer cycles was conducted for 5 times for 4 patients, transplantation of endometrium with 5.8 mm-9.2 mm thickness was transplanted, a total of 10 embryos were transplanted, and the embryo transplantation rate was 90% (9/10), including 1 clinical pregnancy cases (number 2 case), pregnancy abortion at the 54th day, and the embryo implantation rate was 10% (1/10); the transplantation of 1 patients was cancelled because endometrium was 6.5 mm on the day of oocyte retrieval. Intrauterine adhesions was found for number 2 case after the operation, endometrial repair for 5 cycles, endometrial thickness was less than 7mm nearly a year.

GH intrauterine perfusion combined with replacement cycle frozen embryo transplantation

A total of 5 patients received the first time menstuation GH uterine infusion based on the replacement cycle on the ninth to twelfth day, each patient would receive 4-5 times GH intrauterine perfusion therapy. Some information of this study, including the information on the first time for the perfusion, and endometrium conditions for the first and terminal perfusion, endometrial progesterone, corpus luteum ketone on...
blood E2 and LH levels and endometrial conditions on transplantation day was shown in Table 2. The mean endometrial thickness of 5 patients on progesterone day was 7.96 ± 0.71 mm, more than the mean endometrial thickness of the first perfusion (5.78 ± 0.65) mm, the difference was statistically significant (t=10.46, P<0.05). All endometrial thickness on transplantation day was more than 7 mm. The average of E2 was 9387.8 + 2623.87 pmol/l on the progesterone day.

The outcome of pregnancy

Embryos, the blood HCG level for 14 days after transplantation, ultrasound results for 4 weeks after transplantation of 5 cases and the outcome of pregnancy were shown in Table 3. A total of 11 embryos were transplanted for five patients with GH intrauterine perfusion, and good embryo rate was 72.73% (8/11) in transplantation. All cases obtained clinical pregnancy and implantation rate was 72.73% (8/11); There were 2 patients with full-term delivery and 1 patient with continued pregnancy; Embryo development of 1 patient diapause due to incision pregnancy, abortion embryonic genetic testing results indicated normality (Multiplex ligation dependent probe amplification, MLPA).

Discussion

Currently, more and more endometrial dysplasia patients appeared, due to endometrial congenital dysplasia or by infection, inflammation or mechanical damage, including endometrial thin, poor shape, endometrial and sub-endometrial blood flow signal difference and function abnormal basal layer of the endometrium, leading to decreased embryo implantation rate. The pathological and physiological features of thin endometrium including glandular epithelium of slow growth, increased resistance of uterine artery blood flow, angiogenesis and vascular endothelial growth factor with low expression of vascular endothelial growth factor (VEGF) [12]. Estrogen can stimulate endometrial basal layer of adult stem cells [13]. The proliferation and differentiation could maintain endometrial regeneration ability, endometrial hyperplasia degree is positively associated with the level of estrogen, when the basal layer damage, endometrial stem cells may be reduced and lead to abnormal function, cause decreased endometrial proliferation ability, resulting in non-implantation thin because of thin endometrium. Side population (SP) cells may be a source of endometrial stem cells, estrogen can stimulus SP cells proliferation and differentiation significant, and this effect was dose dependent, it can promote recurrent value-added in the SP cells and promote endometrial thickening when physiological dose more than about 10 times higher than that of large dose of estrogen.

However the peak serum estradiol (E2) level in the natural ovulation cycle is 200-300 pg/ml, so over physiological dose of estrogen level was needed in the treatment of thin endometrium. Estradiol vale rate (e2v) is a plant derived estrogen, which could convert to 17 beta-E2 by liver metabolism, and renovation of Valerie acid base, about 3% of the estradiol was utilized biologically after oral e2v or 17 beta-E2, and the serum concentration could increase by 30-40 pg/ml per milligrams. We investigated and compared the impact of oral and vaginal use for 17 beta-E2, the result indicated that vaginal medication can significantly increase thickness of endometrium and uterine blood flow by using the same dose of estradiol (2 mg/day), the vaginal medication can make estrogen play a role by more direct and efficient transport to the uterus. In addition, vaginal medication could avoid hepatic first pass effect, and concentrations of serum estrogen were significantly higher (about 10 times) than that by oral administration, vaginal use for 17 beta-E2 2 mg daily could make serum reach to about 1000-1500 pg/ml [14] Therefore e2v was conducted during the menstrual period in this study, combination with 17-beta E2 used for preparation of artificial membrane after menstrual for 3 days.
### Table 3. The pregnancy outcome of 5 GH patients with uterine cavity perfusion

<table>
<thead>
<tr>
<th>Cases</th>
<th>Embryo transplantation Dn days</th>
<th>Number of transplantation embryo</th>
<th>Information on transplantation embryo</th>
<th>HCG level for 14 days after transplantation (IU/L)</th>
<th>Number of pregnancy bag/fetal heart for 4 weeks after transplantation</th>
<th>The outcome of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D4</td>
<td>2</td>
<td>M^1,0, M^2,0</td>
<td>624.70</td>
<td>2/2</td>
<td>33+3 weeks of pregnancy, one male live birth by Cesarean section, birth weight was 1900 g; one female live birth, birth weight was 1100 g.</td>
</tr>
<tr>
<td>2</td>
<td>D3</td>
<td>2</td>
<td>5^1,0; 6^1,0</td>
<td>955.00</td>
<td>2/1</td>
<td>37+6 weeks of pregnancy, one male live births by delivery, birth weight was 2700 g</td>
</tr>
<tr>
<td>3</td>
<td>D5</td>
<td>3</td>
<td>B^1,0; eb^2,0; eb^2,0</td>
<td>1037.00</td>
<td>3/3</td>
<td>Sistens for one of the three fetal embryo; 38+2 weeks of pregnancy, two female live births by cesarean, birth weight were 3150 g and 2800 g.</td>
</tr>
<tr>
<td>4</td>
<td>D4</td>
<td>2</td>
<td>12^1,0; 11^1,0</td>
<td>923.00</td>
<td>2/1</td>
<td>Singleton pregnancy duration</td>
</tr>
<tr>
<td>5</td>
<td>D4</td>
<td>2</td>
<td>M^1,0; M^2,0</td>
<td>297.5</td>
<td>1/1</td>
<td>Lower segment uterine incision pregnancy, pregnancy embryonic diapaused for 66 days</td>
</tr>
</tbody>
</table>

*p<0.05, compared to endometrium conditions for the first perfusion.*
Serum E2 concentration can reach to a high level (9387.8 ± 2623.8 pmol/l) on the progesterone day, which could provide an adequate level of estrogen for thin endometrium treatment.

In current study, a total of 4 patients with intrauterine adhesions, and received intrauterine adhesions for 6 times, and there is a serious injury for endometrium of these patients. 1 patient with unexplained adverse endometrial growth was not sensitive for the conventional scheme. The endometrial thickness was always less than 7 mm in near half year, based on oral E2V combined with vaginal 17-beta E2 for intimal restoration. Currently, the mechanism for non-reactive thin endometrium occurrence mechanism is not very clear. It may be associated with endometrial stem cell damage, loss of function of tissue repair, the reasons for the low implantation rate was associated with endometrial epithelial cells of estrogen receptor defects and receptor dysfunction, and was also due to thinning of the functional layer of the endometrium, resulting in embryos exposed to basal layer spiral artery high concentration oxygen tension, which is detrimental to the embryo growth [5]. Endometrial could secrete a variety of cytokines and adhesion molecules with the effect of estrogen and progesterone, regulation by endocrine and secretory way, namely by steroid hormones, immune cells, cytokines, adhesion molecule network to achieve the endometrial receptivity in embryo implantation. Various molecules take part in the regulation of embryo implantation process, such as leukemia inhibitory factor (LIF), integrin family (integrin's), endocytosis process, calcitonin, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), IGFs, etc. [15].

GH is an important peptide hormone secreted from anterior pituitary, involved in cell growth and metabolism, and prompted the endometrial stromal loosen, vascular, glandular and glandular cavity increase in the endometrium by interacting with its receptors and IGFs, additionally, to promote endometrial inflammatory cytokine expression, including LIF, integrin, and mechanism of metalloproteinase 9 (MMP-9), at the same time, they could coordinate a variety of cytokines balance and promote endothelial cell mitosis, improve the womb GH mRNA levels, promote protein synthesis, so as to improve the endometrial receptivity [16]. Wolthers et al [17] indicated that protein synthesis was lower during oral estrogen both before and during GH administration. Oral estrogen antagonizes several of the metabolic actions of GH. It may aggravate body composition abnormalities already present in GHD women and attenuate the beneficial effects of GH therapy. Estrogen replacement in GHD women should be administered by a monorail route. Because the liver is the main organ of GH induced IGF-I, the impact of therapeutic doses estrogen on growth hormone activity was significantly associated with the route of administration, oral route of administration make the estrogen biological activity of reduced growth hormone estrogen associated with first pass effect. Wu et al [18] conducted a study on the treatment of FET in endometrial growth bad patients, found that endometrial thickness of 32 subcutaneous injection of GH transplantation group was 8.76 + 1.33 mm, was significantly higher than that of 30 non GH group (7.12 + 1.86) mm, the endometrial blood flow was improved significantly. The clinical pregnancy rate of GH group was 34.4%, was higher than that of non GH group, the clinical pregnancy rate of which was 23.3%, but the difference was not statistically significant, and was consistent with results of current study. It suggested [19] that IGF-I could promote endometrial hyperplasia, its content depends on the level of GH, IGF-I mRNA expression in uterine local gradually increased after embryo implantation, and is related to the embryo growth. IGF-III, IGF-II was associated with endometrial differentiation, and its expression in trophoblastic cells reached a peak, and may relate to trophoblastic invasion, differentiation and placenta formation.

The limitations of this study should be considered. Firstly, the number of the cases was limited, and large sample studies should be conducted in the future. Secondly, similar studies should be conducted in different population and races. Thirdly, the safety of GH intrauterine perfusion is still considered, although all patients in this study were safety after treatment.

In conclusion, GH uterine cavity perfusion was used for treatment of non-reactivity thin endometrium, pregnancies of all patients were obtained, indicating that the administration is
GH for endometrium

helpful to improvement of endometrial thickness and receptivity, improve embryo implantation environment by assistance for HRT under the high estrogen levels.

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Disclosure of conflict of interest

None.

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