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Correlation analysis of human embryo Le^Y glycan antigen expression and embryo quality

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Abstract. This study assessed the feasibility of using Le^Y glycan secretion level in human embryos as a method of judging embryo quality. Embryo culture media from patients receiving *in vitro* fertilization-embryo transfer was collected, and quality scores of embryos were recorded. Secretions of Le^Y in the culture media in different development stages (from 4-cell to 10-cell), embryos in the same development stage of the same patients (8-cell/I) and embryos in the same development stage of different patients (8-cell/I) were examined by dot-blot. Embryos were divided into a hypersecretion group and hyposecretion group, based on their Le^Y secretion level. The embryo quality was evaluated by clinical observations, the number which developed to D3 cell stage and the number of successful embryo transplantations. Le^Y secretion increased as embryos developed from 4-cell to 10-cell ($P<0.05$); secretion of Le^Y of 8/I is not identical; development speed of embryos with different secretion level of Le^Y was also different. The number of embryos which developed to 6-cell or higher was 82.2% in the Le^Y hypersecretion group but only 60% in the hyposecretion group. The rate of successful transplantation was significantly higher in the hypersecretion group (71.1 vs. 40%). In conclusion, Le^Y glycan secretion level in human embryos is closely related to embryo quality. Le^Y may become a useful measure to evaluate embryo quality in the future.

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Introduction

With the development of assisted reproductive techniques, the fertilization rate of *in vitro* fertilization-embryo transfer (IVF-ET) has reached 90%, cleavage rate is 70%, while clinical pregnancy rate after embryo transplantation is only 40-50%. The main factors influencing pregnancy rate are embryo quality, endometrial receptivity and effective embryo transplantation. Embryo quality is measures by grading cleavage embryos after embryo transplantation from the aspect of morphology (including symmetry of blastomere, cell granulations, cytoplasmic distribution, and transparency of blastocyst and containing debris). Grade I to III embryos are transferable, but grade I embryos have different implantation rates after embryo transplantation; therefore, several embryos have to be selected to improve the success rate. However, this increases the risk of multiple pregnancy for patients receiving IVF-ET. Because multiple pregnancy may influence health levels of newborn babies, and their incidence rate of congenital disease is high, selecting high quality embryos becomes the key point of ensuring successful pregnancies.

Glycan on the surface of cell membrane engages in transmission of biological information among cells, and fucoidan antigen Le^Y expresses highly continuously in endometrium epithelial cells and blastocyst of mammals during implantation (1,2); Le^Y of closed blastocyst and endometrium surface not only can block identification and adhesion between the fetus and the mother, but also can inhibit secretions of blastocyst and other implantation factors of endometrium surface (3-7), acting as informational molecules to regulate embryo implantation (8-10). In animal experiments, embryos of grade I have different Le^Y glycan secretion levels, and the Le^Y glycan hypersecretion embryos have good development speed and implantation capacity (11). Measuring glycan secretion in human embryo culture media does not damage embryos, and can be used to assist in monitoring embryo quality besides morphological evaluation and improve prediction of embryo implantation capability.

In order to assess the feasibility of using Le^Y glycan secretion in human single embryos as an adjunct method of judging

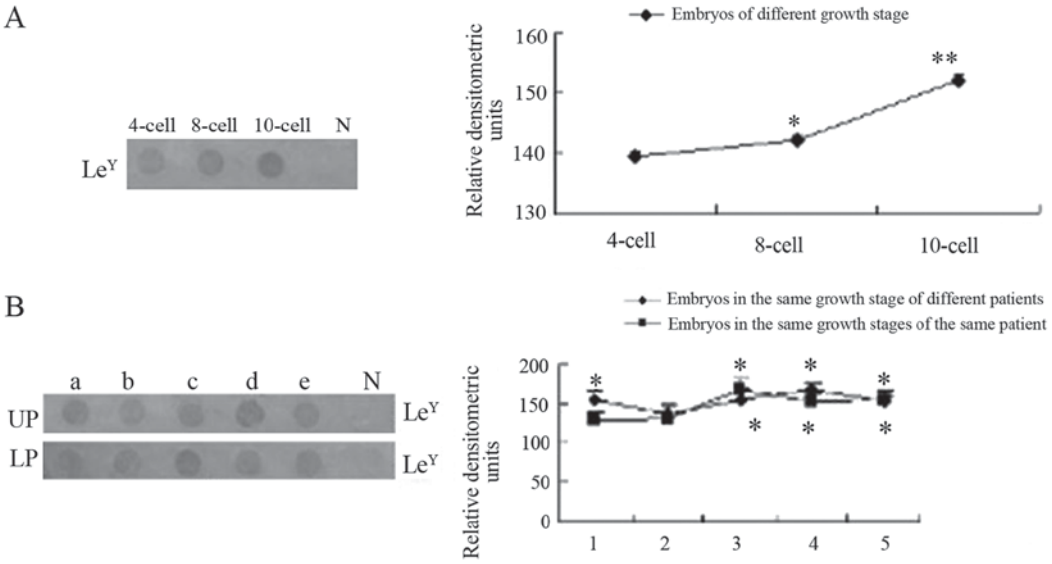

Figure 1. Dot-blot detection of Le^Y secretion level of human individual embryo. (A) Dot-blot detection of Le^Y secretion of embryo with different development stage and densitometric analysis of Le^Y secretion levels of embryo. (B) Dot-blot detection of Le^Y secretion levels of human individual embryos with same development stage and densitometric analysis of Le^Y secretion levels of embryo. UP, Le^Y secretion level of different patients' embryos; and LP, Le^Y secretion level of same patients' embryos. UP, upper panel; LP, lower panel; N, negative control. *P<0.05, **P<0.01. a, patient a; b, patient b; c, patient c; d, patient d; e, patient e; N, negative control.

embryo quality, we collected embryo culture media after *in vitro* fertilization. We used a dot-blot method to detect Le^Y glycan secretion in culture media, and analyzed correlations of Le^Y glycan secretion and embryo development, quality and transfer success observed in clinic. Results indicate that Le^Y glycan secretion level may be useful as an index for judging embryo quality and implantation potential.

Patients and methods

Embryo culture media was provided by Sino-American Shanghia Jiai Inheritance and Sterility Clinical and Center Reproductive Center Laboratory. Clinicians made ovary of infertility patients with multiple-follicle maturity at the same time by using ovulation stimulants before assisted reproduction, B-ultrasound was used to monitor follicular development, and ovum was picked up through guidance of trans-vaginal B-ultrasound at the appropriate time. After *in vitro* fertilization of ovum taken from laboratory and semen, embryos were put in 5% CO_2 incubator at 37˚C to carry out single embryo culture. Embryos were often transferred to patients' uterus 3 days after fertilization. Embryos were cultured *in vitro*, and embryo culture media was changed in 4-cell, 8-cell and different development stages after egg-sperm fusion; embryo culture media was collected and classified according to development stages of different embryos, different patients' embryos in different growth stages and different embryos of the same grade and of the same patients, time of embryos develop to different stages, and grade standard embryos were provided by the reproductive center, noting that they were used to transplanted embryos. The study was approved by the Reproductive Ethics Committee of Xuzhou Central hospital.

Standard of clinical embryo quality (embryo evaluation standard). Embryo quality was judged by morphology, and embryo quality was divided into four grades mainly on the basis of number and size of blastomere, symmetry of segmentation sphere, debris number of segmentation sphere and thickness of zonapellucida (12). Grade I: Segmentation spheres are of the same size, homogeneous and transparent, and no debris; grade II: Segmentation spheres are not of the same size, homogeneous, and no debris; grade III: Segmentation spheres are of the same size, homogeneous, and with little debris; grade IV: Segmentation spheres are not of the same size, blackening, and inhomogeneous particles. Embryos of grade I to III are suitable for transplantation.

Secretion of Le^Y in human single embryo was examined by dot-blot method. Culture media of 86 embryos from 12 patients was selected from embryo culture media provided by clinic, 30 μl of culture media point samples was collected and adsorbed to nitrocellulose filter through dot-blot machine (MBI Fermentas, Vilnius, Lithuania), and the filter was put in 5% BSA (Sigma-Aldrich, St. Louis, MO, USA) at 37˚C and closed for 2 h; after that, it was incubated at 37˚C with mouse monoclonal LeY antibody (dilution, 1:500; cat. no. ab15095; Abcam, Cambridge, MA, USA) and specific monoclonal antibody AH6 (presented by Professor Sen-itiroh Hakomori from Washington University; 1:400, diluted by pH 7.4 TBS) containing Le^Y, IgM (1:1,500, diluted by pH 7.4 TBS; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used to label membrane and alkaline phosphatase, incubated with NBT/BCIP (Gibco-BRL, Carlsbad, CA, USA) and used as alkaline phosphatase chromogenic substrate to avoid light and develop color, and secretion of human single embryo Le^Y was examined.

Analysis of correlation of Le^Y secretion level of embryo and growth quality of embryo. The Le^Y secretion results of embryo examined by dot-blot was scanned and analyzed

Table I. Effects of different Le^Y secretion levels on embryo development.

Groups	D3 embryo growth period		
	<6-cell	6-8-cell	>8-cell
Hypersecretion (45 cells)	8	27	10
Hyposecretion (30 cells)	12	16	2

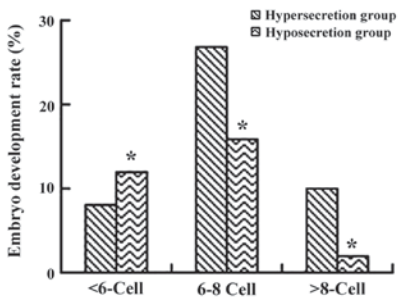

Figure 2. Effects of different Le^Y secretion levels on embryo development. *P<0.05.

Table II. Effects of different Le^Y secretion levels on embryo transplantation.

Groups	Embryo number	Number-embryo transplantation	Transplant rate (%)
Hypersecretion	45	32	71.1
Hyposecretion	30	12	40.0

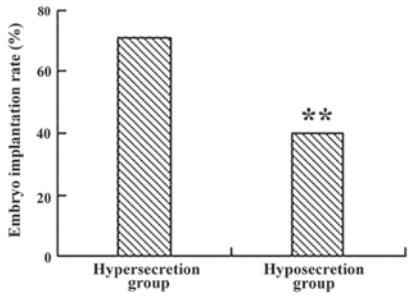

Figure 3. Effects of different Le^Y secretion levels on embryo transplantation rate. Embryo total no., 75; high secretion group, 45; low secretion group, 30. **P<0.01.

by Labworks 4.60 software (Taiwan Taida Co., Guangzhou, China), taking gray level >150 as hypersecretion group and gray level <140 as hyposecretion group, and medial secretion level was not used in the analysis. Relations of secretion level of Le^Y embryo and growth speed of different embryos and embryo transplantation provided by clinic were analyzed. According to Le^Y secretion level of embryo, embryo quality was evaluated through observations and clinical records of cell stages that embryos develop to D3 and the embryo number of transplantation.

Results

Le^Y secretion of a single embryo was examined. Results of western blotting (Fig. 1) show that: In human embryo culture media, secretion of Le^Y glycan of embryo was examined from 4-cell stage, and Le^Y expression increased with the development of the embryos. For embryos in the same growth stage of different patients (8-cells), secretion levels of Le^Y were different (Fig. 1B, upper panel), and for different embryos in the same growth stages of the same patient, their secretion levels of Le^Y were also different (Fig. 1B, lower panel), and the differences were significant while using single factor analysis of variance.

Influence of Le^Y secretion level of single embryo on embryo development speed in vitro. The time of forming fertilized egg after *in vitro* fertilization was recorded as the first day (D1), and cell stages from embryo development to D3 were recorded. The relationship of Le^Y secretion level of different embryos was examined (Table I and Fig. 2). Results indicate that embryos with different Le^Y secretion levels had different growth speed, and number of embryos with hypersecretion of Le^Y developed to 6-cell and higher than 6-cell (37/45) was more than the hyposecretion group (18/30); the number of embryos that were lower than 6-cell in hypersecretion group (8/45) was less than in hyposecretion group (12/30).

High quality embryos were selected to transplant preferentially, thus transplantation rate can be used to judge the influence of Le^Y secretion level on embryo quality. Results indicate that the rate of embryonic transplant rate in hypersecretion group (71.1%) was higher than hyposecretion group (40.0%) (Table II and Fig. 3).

Discussion

The quality of cultured embryos *in vitro* should be judged from the aspects of cleavage stages and cleavage speed of embryos with normal morphology and embryos with no cellular debris, have the highest rate of implantation. Animal embryos are often developed to blastocyst stage via *in vitro* culture before being used to transplant to improve implantation rate. However, long-term *in vitro* culture of human embryos requires extraordinary conditions, since the number of embryos which develop to blastula is small, and embryo screening and transplantation to maternal uterus often take place in 8-12 cell stages in IVF-ET. Currently, embryo quality is mainly evaluated by morphological index (13), although more than 90% of patients can fertilize successfully in the treatment of IVF, implantation rate and fertility rate are still low, which suggests that morphological observations cannot judge embryo quality comprehensively and completely (14). Multi-embryo transplantation can improve implantation rate, but it brings the risk of multifetation; therefore, selecting high quality embryos to improve embryo implantation and development are very important.

Analysis of embryonic gene expression plays an important role in tracking embryo development and diagnosis of genetic defects, but collecting embryonic samples causes inevitable harm. Expression of some cytokines peak in accordance with

an 'implantation window' of the blastocyst, which regulates growth and development of the embryo. Research suggests that the level of implantation-related factors secreted into culture media is in proportion to embryo quality (14). These factors can regulate viability of embryos, and may be used to judge embryo quality and predict implantation potential (15).

Animal experiments indicate that expression of Le^Y glycan antigen has stage-specific variations; implantation rate of Le^Y glycan antigen that expresses highly in blastocyst stage decreases after being closed by specific antibody, and expressions of implantation-related factors epidermal growth factors (EGFs) and matrix metalloproteinases (MMPs) also decreases (3-7). In single embryo culture media, secretion levels of Le^Y glycan antigen in pre-implantation embryos judged as grade I by morphology is high and the embryos with hypersecretion of Le^Y glycan grow fast, which suggests that Le^Y glycan plays an important role in embryo implantation (3-5,16,17).

Here, a dot-blot method was used for semi-quantitative detection of protein factors secreted by a single embryo, and Le^Y glycan secretion level in culture media of embryos in different growth stages, of different embryos in the same growth stage and of different embryos of the same patient in the same grade before transplantation was examined. The results demonstrate that: i) Human embryos begin to secret Le^Y glycan from 4-cell stage and section of Le^Y glycan increases with the growth of embryos; and ii) Le^Y secretion levels of embryos of different patients in the same growth stage are still different, which suggest that embryo quality can be judged by secretion level of Le^Y. Faster growth was observed for embryos with higher secretion levels of Le^Y compared to those with low secretion levels of Le^Y. Among embryos that were used to transplant, embryo transplantation rate in Le^Y hypersecretion group (71.1%) was much higher than the hyposecretion group (40.0%), which suggests that Le^Y is related to the growth rate and quality of embryos. It is possible that adding Le^Y glycan may promote improved embryo culture conditions while culturing embryos *in vitro*.

Growth and implantation of embryos need coordination of a variety of factors, and leukemia inhibitory factor, EGF, insulin-like growth factor, MMPs and interleukin-1 all regulate implantation progress. Just using one of these indexes cannot reflect the overall situation. Examination of the secretion level of single embryo implantation factors in culture media may reflect their growth potential, and may be used for appraisal of embryo quality in addition to standard morphology. Using embryo culture media to examine expression of specific immunizing antigen of embryos will not damage examined embryos while appraising embryo quality, ensuring survival and transfusion of embryos, and is important in evaluating embryo quality before embryo transplantation of assisted reproduction.

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