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Research article

Glycans in spent embryo culture medium are related to the implantation ability of blastocysts

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ABSTRACT

Research question: Does glycan profile in spent blastocyst culture medium have the potential to be used as a biomarker to predict implantation outcome.

Design: A nested case-control study was conducted in Northwest women's and children's Hospital, Xi'an, China. The patients underwent fresh IVF/ICSI cycles with single blastocyst transfer were included. Total 78 cases were included and separated into groups according to success (n = 39) and failure (n = 39) implantation outcomes. The glycosylation patterns in spent blastocyst culture medium were detected by lectin microarray containing 37 lectins using pooled samples and confirmed by reversed lectin microarray using individual sample.

Results: Binding signals of 10 lectins were found to be different between samples from successful and failed implantation. And 8 of them were confirmed that glycans binding to lectin NPA, UEA-I, MAL-I, LCA and GNA were significantly increased while DBA and BPL were decreased in the successful implantation compared to failed implantation. The glycan binding to lectin PHA-E + L had no difference between two groups. No significant differences in the glycan profile were found in spent culture medium of embryos with different morphological grades except the glycan binding to UEA-I between blastocysts of Poor and blastocysts of Medium.

Conclusion: Detection of glycan profile in spent culture medium may lead to a novel non-invasive assessment assay of embryo viability. In addition, these results may be helpful to further understanding molecular mechanisms in embryo implantation.

1. Introduction

Assisted reproductive technology (ART) has been widely applied for treatment of infertility worldwide in recent years. Embryo culture and transfer are important steps in the procedure of ART. In spite of the dramatically improvement in embryo culture technology, the implantation rate of transferred embryos is far from optimal [1]. In order to increase the efficiency, multiple embryo transfers have long been applied in clinical practice. However, it often results in multiple pregnancies as well as other adverse outcomes [2]. A significant trend has emerged in recent years that the numbers of embryos being transferred are reduced to single, which has been shown to be with improved ART outcomes [3]. Besides, it has been proved that the clinical pregnancy rate of blastocyst

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transfer is higher than that of cleavage embryo transfer [4,5]. Therefore, single blastocyst transfer has been used more widely. Selection of blastocysts with high quality is essential for the success of single blastocyst transfer.

Various embryo screening methods have been utilized in clinical practice. At present, the main method of screening of the blastocyst quality is still mainly based on morphological observation. The blastocyst quality is scored by observing the degree of blastocyst expansion, the number and the looseness of inner cell mass and trophoblast cells, which is inevitably subjective [6]. A variety of technologies have been developed in recent years to improve the accuracy of blastocyst quality screening. Firstly, several models of embryo division dynamics have been established by combining Time-Lapse technology with morphological observation to evaluate the quality of blastocysts, which is still controversial whether it can improve the accuracy of blastocyst quality screening [6,7]. Secondly, preimplantation genetic testing (PGT) is developed to detect the abnormal number or structure of embryo chromosomes and the pathogenic genes of genetic diseases, which is invasive inspection [8]. Finally, several methods of blastocyst quality screening, which are based on secreted proteins or metabolites in embryo culture fluid, have emerged [9]. However, there are still no indicators with high sensitivity and accuracy for improving the prediction of implantation potential in practice. Therefore, it is of great interest to identify novel biomarkers and develop an accurate, rapid and non-invasive method to quantify embryo viability.

Majority of human proteins are modified by glycosylation during protein synthesis. Glycan biosynthesis, which depends on coordination of multiple metabolic processes, is susceptible to physiological and pathological conditions. Therefore, the change of glycan structures may be good diagnostic markers for health conditions [10,11]. Glycan profile has been widely explored as diagnostic markers for various cancers, as well as in other pathological conditions such as inflammation, aging and age-related diseases [12–14]. In ART related studies, it has been studied in quality assessment of sperm [15]. The serum N-glycan has also been suggested to be correlated with pregnancy in animal assays [16]. However, no glycan profiles of embryo culture medium have been studied for assessment of embryos viability.

We hypothesized that glycan profile would be different in spent culture medium between embryos successfully implanted and those with implantation failure or between embryos with different morphological grades, which would be correlated with implantation ability of blastocysts. Therefore, in the current study, an exploratory analysis was performed to characterize the profile of glycans secreted in spent culture medium of blastocyst and to investigate whether the profile of glycans could be used as predictive markers of embryo viability in ART.

2. Material and methods

2.1. Study design and subjects

The spent culture medium of embryos from patients underwent fresh IVF/ICSI cycles with single blastocyst transfer in Assisted Reproduction Center, Northwest women's and children's Hospital, Xi'an, China was collected. Clinical records were reviewed to exclude patients with following conditions: (1) uterine infertility/abnormal endometrium/abnormal uterus; (2) endometriosis/immune infertility/unexplained infertility; (3) patients with history of $\geq$2 embryo transfer without implantation; (4) cycles with PGD/PGS; (5) cycles with the risk of OHSS, fluid in the uterine cavity, pelvic inflammation, and other acute maternal problems; (6) one or both spouses have an abnormal karyotype (including polymorphism). Culture medium was collected on day 3–5 and the embryo was transferred on day 5. Total 78 samples were separated into groups according to success (defined as ultrasonographic visualization of gestational sac) (n = 39) or failure (n = 39) implantation outcomes. The analysis was performed in two phases: At first, half of the culture medium from each sample was pooled for lectin microarray analysis. According to the results of lectin microarray, glycan profiles in individual samples were further confirmed using reversed lectin microarray analysis. The results were compared between the group of successful implantation and the group of failed implantation.

The written consented form for the study was acquired from each patient. The study was approved by the Ethical Committee of Northwest women and children's Hospital.

2.2. IVF policy

The protocol of controlled ovarian hyperstimulation (COH) for most patients was the standard GnRH agonist long protocol (GnRH-a, Decapeptyl Germany) and recombinant FSH (GONAL-f, Merck Serono Italy; Puregon, Organon Netherlands). GnRH antagonist protocol (Cetrotide, Merck Serono France) was also adopted in COH. 10000 units of human chorionic gonadotrophin (hCG) was administered when more than 3 follicles were >18 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided aspiration. The OCCs were cultured in the media (IVF; Vitrolife, Sweden) after retrieval. The fertilization was completed in fertilization media (IVF; Vitrolife, Sweden) 39–40 h (39–42 h for ICSI) after hCG administration. Then the zygotes were transferred into the embryo culture media until day 5 for selective single blastocyst transfer. The blastocyst was transferred by the catheter (COOK IRELAND LTD, Ireland). All patients were given luteal support (Duphaston 20 mg/day and Progesterone injection 60 mg/day). The clinical pregnancy was confirmed by ultrasonographic visualization of one or more gestational sacs.

2.3. Embryo culture and sample collection

The embryos were cultured in the micro-drop (35 μl) individually with a sequential system (Vitrolife G5 Series, Sweden) and 5% O_2 culture system (Model c200; Labotect, Germany), which includes G1 medium for cleavage embryo and G2 medium for blastocyst. Both G1 and G2 medium were supplemented with HSA-solutions purchased from Vitrolife. And the HSA-solution used for this research was

consistent across the sampling period. The embryos were transferred to the blastocyst culture medium on day 3. Prior to this point, residual cumulus cells were cleaned by pipetting the embryos repeatedly through a plastic pipette until no visible granular cell under the microscope. Spent culture medium of blastocyst (from day 3 to day 5) were collected on day 5 after blastocyst transfer and stored at −80 °C.

2.4. Lectin microarray analysis

To normalize the differences between the subjects and to tolerate individual variation, the half of each spent culture medium sample was pooled and the other half maintained for further validation. The proteins in each pooled group were labeled with Cy3 fluorescent dye (GE Healthcare, Buckinghamshire, U.K.) and purified with Sephadex G-25 columns according to the manufacturer's instructions. Glycosylation patterns were screened using a lectin microarray loaded with 37 lectins (Supplementary Table 1). The lectin microarray was produced by 37 lectins (purchased from Vector Laboratories, Sigma-Aldrich, and Calbiochem) with different binding preferences covering N- and O-linked glycans. Table S1 showed the glycan-binding specificities of the lectins. The lectin microarray was incubated with the pooled and labeled samples. After incubation the microarray were scanned with the 70% photomultiplier tube and 100% laser power settings using a Genepix 4000B confocal scanner (Axon Instruments, USA). The acquired images were scanned at 532 nm for Cy3 detection using Laser scanning confocal microscope FV 1000 (Olympus, Tokyo, JPN) and data was analyzed using Genepix 3.0 software (Axon Instruments, Inc., USA). The generated original data were further analyzed by HCE (V3.0). Differences between the arbitrary two data sets were evaluated by Student's t-test to each lectin signal using SPSS statistics 19.

2.5. Verification of glycan profile by reversed lectin microarray with individual sample

Individual samples were spotted on the surface of array-chips with Stealth micro-spotting pins (SMP-10B) (TeleChem USA) by a Capital smart microarrayer (CapitalBio, Beijing, China). And then array-chips were incubated with different Cy5-labeled lectins. And the microarrays were scanned and the data was analyzed as above.

Table 1
Baseline characteristics of patients with successful implantation and implantation failure.

	Successful implantation	Implantation failure	P value
No. of cases	39	39	
Age (y)	29.31 ± 3.58	30.28 ± 3.87	0.252
BMI ($kg.m^{-2}$)	21.53 ± 3.02	22.84 ± 3.47	0.080
Basal serum FSH level (mIU/ml)	6.09 ± 1.34	7.29 ± 6.02	0.228
Fertilization procedure, n			
IVF	31	31	1.000
ICSI	8	8	
Main etiology of infertility, n			
Tubal factor	23	21	0.736[a]
Ovarian factor	2	4	
Male factor	9	11	
Other reasons	5	3	
Type of infertility, n			
Primary infertility	19	19	1.000
Secondary infertility	20	20	
Ovarian stimulation protocol, n			
GnRH agonist long protocol	35	27	0.025
GnRH antagonist protocol	4	12	
Endometrial thickness (mm)	11.47 ± 1.77	11.43 ± 2.38	0.937
Morphological characteristics of blastocyst transferred[b]			
Stage of development			
2	3	10	0.112[a]
3	5	4	
4	31	25	
ICM			
A	1	2	0.507[a]
B	25	20	
C	13	17	
TE			
A	1	1	0.363[a]
B	26	20	
C	12	18	

[a] Fisher's exact test.
[b] Gardner scale (Gardner and Schoolcraft, 1999). Stage of development: 2 = blastocyst, the blastocoel greater than half the volume of the embryo; 3 = full blastocyst, the blastocoel completely filling the embryo; 4 = Expanded blastocyst. Inner cell mass (ICM) and trophectoderm (TE) were assessed as follows: A = Good; B=Fair; C=Poor.

2.6. Statistical analysis

SPSS 19 (IBM, Armonk, NY, USA) software was used for statistical analysis. For baseline characteristic analysis, continuous quantitative data was presented as mean ± standard deviation. Differences among groups were compared using student *t*-test. Categorical data was presented as rate (percentage) with differences among groups analyzed using Chi-square test. Fluorescent intensities data were compared using Mann–Whitney *U* test. Statistical analysis was tested on two-sided settings, with $P < 0.05$ considered as statistically significant.

3. Results

3.1. The glycan profile in spent culture medium of blastocyst is different between the group of successful implantation and the group of failed implantation

The baseline characteristics of patients were compared between the success and the failure group of implantation of blastocysts in Table 1. There was no significant difference detected in groups for all parameters except for ovarian stimulation protocol.

The lectin microarray including 37 different lectins (shown in Supplementary Table 1) was used to detect glycans in spent culture medium of blastocysts, which was obtained from successful or failed implantation group separately.

The glycan profile results showed significant differences between the spent culture medium of blastocyst with successful and with failed implantation (Fig. 1). Compared to the medium with implantation failure, glycans respectively binding to NPA, UEA-I, MAL-I, LCA, PHA-E + L, GNA were increased while glycans respectively binding to AAL, LTL, DBA, BPL were decreased in the blastocyst culture medium with successful implantation. The changes of glycan profiles between the spent culture medium with the implantation success group and with the implantation failure group was confirmed with individual medium samples (Fig. 2). As shown in the microarray analysis, 7 candidate lectins (NPA, UEA-I, MAL-I, LCA, GNA, DBA and BPL) showed significant differences between successful implantation and failed implantation (Fig. 2B-G and I) which was consistent with the initial microarray analysis, while the lectin PHA-E + L showed no significant difference (Fig. 2H).

3.2. No significant differences in the glycan profile are found in spent culture medium of embryos with different morphological grades except for UEA-I

Both blastocysts with successful implantation and with failed implantation were scored according to the system of Gardner and divided into three group: Good (Both inner cell mass and trophoblast were scored A and B, n = 38), Poor (Both inner cell mass and trophoblast were scored C, n = 20) and Medium (All others, n = 20). The expression of glycans respectively binding to BPL, DBA, GNA, LCA, MAL-I, NPA, PHA-E + L and UEA-I in the spent culture medium of blastocysts of above three groups were detected (Fig. 3). There were no significant differences in all above 8 lectins (Fig. 3A-H) except that a significant difference was found in glycans binding to UEA-I between blastocysts of Poor and blastocysts of Medium (Fig. 3H).

4. Discussion

Results of the current study showed that there were significant differences in glycan profiles in spent culture medium between embryos with successful and with failed implantation. These findings suggested the possibility of using glycans from spent culture medium, which was easy to collect, as non-invasive biomarkers of embryo implantation during IVF cycles.

Glycosylation is a post-translational modification of proteins that glycans attach to amino acid residues, mainly to Asn (N-linked) or Ser/Thr (O-linked) residues. Subjected to various physiological and pathological stimulations, proteins will have different glycosylation forms, varying in glycosylation properties such as glycan structure and composition. Glycans participate in a broad range of biological activities, such as intermolecular recognition, cell-cell recognition and regulation of protein function. They affect the cell

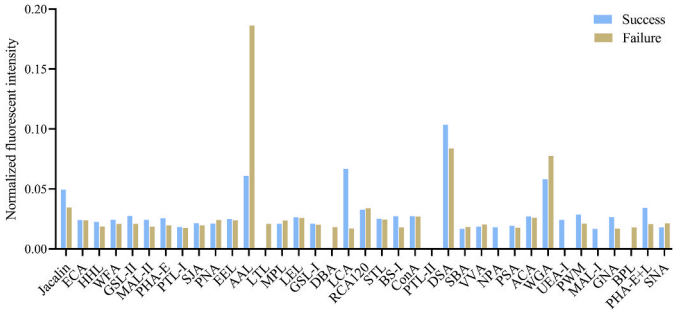

Fig. 1. Analysis of glycan profiles in spent culture medium using lectin microarray. Thirty-nine samples of blastocyst culture medium from cases with successful implantation or failed implantation were pooled separately and detected by a microarray containing 37 lectins. The normalized fluorescent intensities (NFLs) of each lectin were compared between two groups.

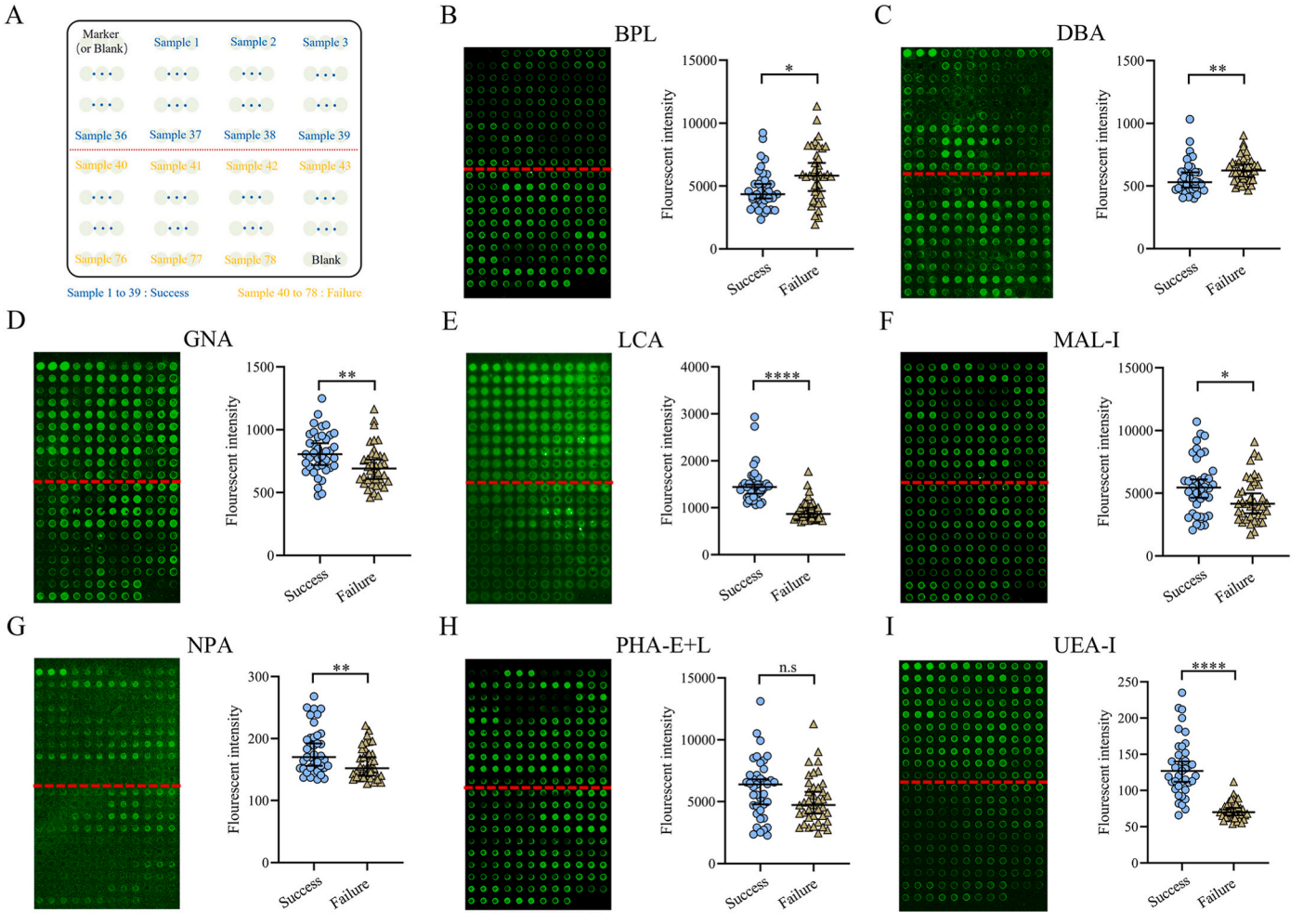

Fig. 2. Verification of glycans with differences in spent culture medium using individual sample. Individual spent culture medium from successful implantation and failed implantation were spotted in triplicate on the slide (A). Eight candidate lectins were further confirmed by reversed lectin microarray. The fluorescent intensities were compared between two groups in scatter plot (B–I) (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; n.s, no significance).

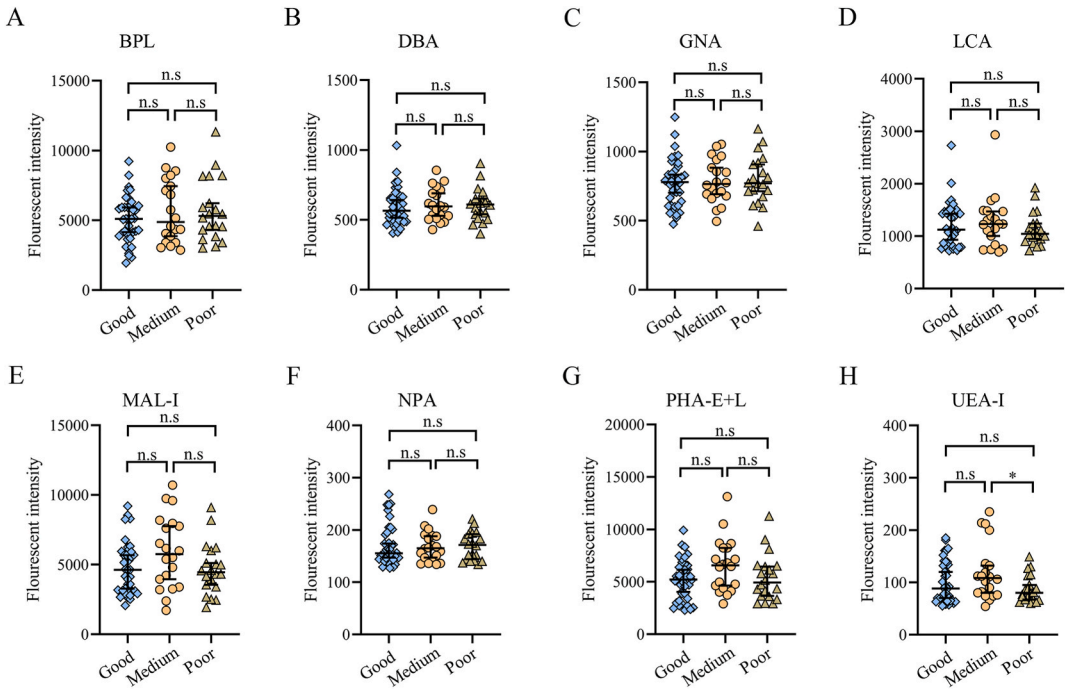

Fig. 3. Analysis of glycan profiles in spent culture medium of embryos with different morphological grades. Glycans binding to eight candidate lectins were detected in individual spent culture medium of blastocysts with different morphological grades. The fluorescent intensities were compared between three groups in scatter plot (*P < 0.05; n.s, no significance).

cycle, cell differentiation, apoptosis, host–pathogen interactions, inflammation and tumorigenesis [17]. As aberrant glycosylation can be the result of a disease, monitoring changes in the glycosylation of proteins is becoming a popular diagnostic tool, which already led to several potentially promising markers for several types of cancer and diseases [18,19]. Hence, we studied the glycan profile in spent culture medium of blastocysts with successful implantation or failed implantation, which could help in identifying implantation-competent embryos.

Lectin is a class of proteins that can recognize specific glycan structures. Lectin microarray contains different lectins that each lectin can bind to multiple types of glycans [20,21]. Although the numbers of available lectin probes are far less than the glycan epitopes [22], lectin microarray still provide a useful tool to identify various biomarkers in disease diagnosis [23]. There have been reports that lectin microarray was used to identify potential biomarkers for diabetes [24], cancers [25,26] as well as other clinical diseases. Successful implantation is a complex process involving reciprocal interactions between blastocyst and uterus. Glycosylated proteins are often found on cell surface and in extracellular matrix making it the first point of contact in cellular interactions, which is involved in blastocyst-endometrial recognition for implantation. Therefore, the glycans in culture medium, which may have dissociated from the surface glycoproteins, will reflect the blastocyst surface glycoproteins status. Besides, glycosylation can alter the activity of proteins including proteins secreted from blastocysts or endometrium which may play a key role in implantation. Thus, glycan profiles in spent culture medium may indicate the implantation of blastocyst. In current study, we have found that glycans in spent culture medium of embryo with successful implantation outcomes had significantly increased binding to lectins including NPA, UEA-I, MAL-I, LCA and GNA but decreased binding to DBA and BPL, which suggested that there might be a signature glycan profile in culture medium for embryo with better implantation potential. Among the above differential lectins, several lectins have been found in human embryonic stem cells (ESCs) and pluripotent stem cells (PSCs) and to undergo alteration during differentiation. During adipogenic differentiation of ESCs, the binding signal of UEA- increased while the binding signal of NPA, LCA and GNA decreased [27]. Besides, the lectin UEA-I have been found to be a potential tool for isolating viable hPSCs [28] and the signal intensities of the lectin MAL-I, for which the medullary thymocytes was positive [29], was higher in fertilized eggs compared with 2-cell embryos [30].

The implantation of embryo is closely related to both the embryo quality and the endometrium receptivity. The implantation rate of transferred embryos is directly correlated with the morphological scoring [31]. Therefore, the glycan profile was analyzed by groups of blastocysts with different morphological grade in this study. Interestingly, no significant difference in the glycan profile was found in spent culture medium of embryos with different morphological grades except UEA-I. But this result does not mean that the current morphological score is not an indicator of pregnancy outcomes of blastocyst. We believe that the blastocyst with better grade from the same patient in the same oocyte retrieval cycle will have a higher pregnancy rate compared to other blastocysts, while the relationship between blastocyst grade and implantation potential from different patients is complicated. On one hand, the number of samples in our study might be not sufficient to clarify the relationship between the expression levels of glycosides and morphological grade of blastocyst from different patients. On the other hand, the above different glycans in spent culture medium might affect the

implantation of embryos through not affecting the embryo viability but affecting the cross-talk between the embryo and the endometrium or affecting the endometrium receptivity. And we believe that our research results can help patients choose blastocysts to be transplanted when there was no significant difference in blastocyst morphology, and achieve better clinical pregnancy outcomes.

The implantation outcomes are influenced by numerous risk factors including ovarian stimulation protocol. The long-acting GnRH agonist follicular protocol was beneficial in improving implantation rate compared with the antagonist protocol [32]. In this study, more patients adopted GnRH agonist long protocol in the group of blastocysts with successful implantation. Therefore, the data for each group of the two different protocols was analyzed separately (Supplementary Fig. S1). And in the group of patients using the long-acting GnRH agonist follicular protocol, a similar result was obtained with the previous analysis of mixed data except for MAL-I (Supplementary Figure S1A). But in the group of patients using the GnRH antagonist protocol, no significant differences were found in the glycan profile except for GNA and UEA-I, which might be due to the small sample size ($n = 4$) in the successful implantation group (Supplementary Figure S1.B).

In conclusion, the result in this study have indicated that glycan profiles of spent culture medium may provide a novel non-invasive assessment assay of embryo viability. The change of glycan profile may provide hints about embryo development as well as interaction between blastocyst and uterus. However, there were several limitations in this study, including the small sample size and lack of prospective evaluations prior to transfers. Therefore, the result of the current study should be further verified in future study with larger sample size and prospective follow-up. In addition, the exact structure features of glycans binding to the microarray have not been determined. The characteristics of specific candidate glycan as well as their potential functions in embryo implantation need to be further addressed.

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Author contribution statement

Dongyang Wang, Ph.D: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zhenghao Zhao; Xia Xue: Performed the experiments.

Juanzi Shi: Contributed reagents, materials, analysis tools or data.

Wenhao Shi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supplementary data

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References

[1] European IVF-Monitoring Consortium (EIM), Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE, European Society of Human Reproduction and Embryology (ESHRE), M. S. Kupka, et al. Hum. Reprod. 31 (2016) 233–248.

[2] ACOG Committee on Obstetric Practice, ACOG Committee on Gynecologic Practice, ACOG Committee on Genetics., ACOG Committee Opinion #324: perinatal risks associated with assisted reproductive technology, Obstet. Gynecol. 106 (2005) 1143–1146.

[3] R. Cutting, Single embryo transfer for all, Best Pract. Res. Clin. Obstet. Gynaecol. 53 (2018) 30–37.

[4] Y. Li, S. Liu, Q. Lv, Single blastocyst stage versus single cleavage stage embryo transfer following fresh transfer: a systematic review and meta-analysis, Eur. J. Obstet. Gynecol. Reprod. Biol. 267 (2021) 11–17.
[5] M. Zeng, S. Su, L. Li, Comparison of pregnancy outcomes after vitrification at the cleavage and blastocyst stage: a meta-analysis, J. Assist. Reprod. Genet. 35 (2018) 127–134.
[6] S. Fishel, et al., Evolution of embryo selection for IVF from subjective morphology assessment to objective time-lapse algorithms improves chance of live birth, Reprod. Biomed. Online 40 (2020) 61–70.
[7] K. Lundin, H. Park, Time-lapse technology for embryo culture and selection, Ups. J. Med. Sci. 125 (2020) 77–84.
[8] R. Sciorio, L. Tramontano, J. Catt, Preimplantation genetic diagnosis (PGD) and genetic testing for aneuploidy (PGT-A): status and future challenges, Gynecol. Endocrinol. 36 (2020) 6–11.
[9] P. Hernández-Vargas, M. Muñoz, F. Domínguez, Identifying biomarkers for predicting successful embryo implantation: applying single to multi-OMICs to improve reproductive outcomes, Hum. Reprod. Update 26 (2020) 264–301.
[10] X. Wei, L. Li, Comparative glycoproteomics: approaches and applications, Briefings Funct. Genomics Proteomics 8 (2009) 104–113.
[11] S. Hua, H.J. An, Glycoscience aids in biomarker discovery, BMB Rep 45 (2012) 323–330.
[12] Y. Miura, T. Endo, Glycomics and glycoproteomics focused on aging and age-related diseases–Glycans as a potential biomarker for physiological alterations, Biochim. Biophys. Acta 1860 (2016) 1608–1614.
[13] M.J. Kailemia, D. Park, C.B. Lebrilla, Glycans and glycoproteins as specific biomarkers for cancer, Anal. Bioanal. Chem. 409 (2017) 395–410.
[14] H. Hwang, et al., Glycoproteomics in neurodegenerative diseases, Mass Spectrom. Rev. 29 (2010) 79–125.
[15] A. Xin, et al., Lectin binding of human sperm associates with DEFB126 mutation and serves as a potential biomarker for subfertility, Sci. Rep. 6 (2016), 20249.
[16] B. Adamczyk, et al., Pregnancy-Associated changes of IgG and serum N-glycosylation in camel (Camelus dromedarius), J. Proteome Res. 15 (2016) 3255–3265.
[17] H.J. Gabius, Cell surface glycans: the why and how of their functionality as biochemical signals in lectin-mediated information transfer, Crit. Rev. Immunol. 26 (2006) 43–79.
[18] R.L. Schnaar, Glycans and glycan-binding proteins in immune regulation: a concise introduction to glycobiology for the allergist, J. Allergy Clin. Immunol. 135 (2015) 609–615.
[19] R.R. Drake, Glycosylation and cancer: moving glycomics to the forefront, Adv. Cancer Res. 126 (2015) 1–10.
[20] J.J. Lundquist, E.J. Toone, The cluster glycoside effect, Chem. Rev. 102 (2002) 555–578.
[21] T.K. Dam, C.F. Brewer, Effects of clustered epitopes in multivalent ligand-receptor interactions, Biochemistry 47 (2008) 8470–8476.
[22] R.D. Cummings, The repertoire of glycan determinants in the human glycome, Mol. Biosyst. 5 (2009) 1087–1104.
[23] J.P. Ribeiro, L.K. Mahal, Dot by dot: analyzing the glycome using lectin microarrays, Curr. Opin. Chem. Biol. 17 (2013) 827–831.
[24] K. Inoue, et al., Urinary fetuin-A is a novel marker for diabetic nephropathy in type 2 diabetes identified by lectin microarray, PLoS One 8 (2013), e77118.
[25] K. Nakajima, et al., Establishment of new predictive markers for distant recurrence of colorectal cancer using lectin microarray analysis, Cancer Med. 4 (2015) 293–302.
[26] W.L. Huang, Y.G. Li, Y.C. Lv, X.H. Guan, H.F. Ji, B.R. Chi, Use of lectin microarray to differentiate gastric cancer from gastric ulcer, World J. Gastroenterol. 20 (2014) 5474–5482.
[27] W. Liu, et al., Alterations of protein glycosylation in embryonic stem cells during adipogenesis, Int. J. Mol. Med. 41 (2018) 293–301.
[28] Y.C. Wang, et al., Specific lectin biomarkers for isolation of human pluripotent stem cells identified through array-based glycomic analysis, Cell Res. 21 (2011) 1551–1563.
[29] E. Balcan, I. Tuğlu, M. Sahin, P. Toparlak, Cell surface glycosylation diversity of embryonic thymic tissues, Acta Histochem. 110 (2008) 14–25.
[30] J. Wang, et al., Integrated glycosylation patterns of glycoproteins and DNA methylation landscapes in mammalian oogenesis and preimplantation embryo development, Front. Cell Dev. Biol. 8 (2020) 555.
[31] A. Shulman, I. Ben-Nun, Y. Ghetler, H. Kaneti, M. Shilon, Y. Beyth, Relationship between embryo morphology and implantation rate after in vitro fertilization treatment in conception cycles, Fertil. Steril. 60 (1993) 123–126.
[32] R. Yang, Y. Guan, V. Perrot, J. Ma, R. Li, Comparison of the long-acting GnRH agonist follicular protocol with the GnRH antagonist protocol in women undergoing in vitro fertilization: a systematic review and meta-analysis, Adv. Ther. 38 (2021) 2027–2037.