Clinica Chimica Acta 491 (2019) 46–51

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Clinical application of immunomagnetic reduction for quantitative analysis of beta-subunit of human chorionic gonadotropin in blastocyst culture media to differentiate embryo quality

Chen-Yu Chen[a,b], Yuh-Ming Hwu[a,b,c,*], Yu-Wen Weng[b], Chung-Hao Lu[b], Ying-Jie Chen[b], Fang-Ju Sun[d]

[a] Department of Medicine, Mackay Medical College, Taipei, Taiwan
[b] Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan
[c] Mackay Junior College of Medicine, Nursing, and Management, Taipei, Taiwan
[d] Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan

ARTICLE INFO

Keywords:
Immunomagnetic reduction
Beta-subunit of human chorionic gonadotropin
Blastocyst
Embryo quality
In vitro fertilization

ABSTRACT

Background: The most important factor for a successful pregnancy after in vitro fertilization is embryo quality. The aim of this study was to explore the possibility that using the immunomagnetic reduction (IMR) assay to quantitatively measure β-subunit of human chorionic gonadotropin (β-hCG) in blastocyst culture media to differentiate embryo quality.

Methods: This was a prospective case-control study including 28 samples of blastocyst culture media. We used single-step blastocyst culture and IMR assay to analyze β-hCG concentrations in culture media. We also explored the relationship between IMR signals of β-hCG and morphological grading of blastocysts.

Results: β-hCG concentration-dependent IMR signals were highly correlated with blastocyst morphological quality (Spearman correlation coefficient: 0.731). Receiver-operating characteristic curve analysis showed a cut-off IMR value to differentiate embryo quality of 0.873%, with an area under the curve of 0.947, sensitivity of 0.882 and specificity of 0.818. Furthermore, subanalysis also revealed a positive correlation between β-hCG concentration-dependent IMR signals and trophectoderm grading, with a Spearman correlation coefficient of 0.576.

Conclusions: An IMR assay can quantitatively measure β-hCG in blastocyst culture media, and may be a potential clinical tool to assist in the assessment of good blastocyst quality before embryo transfer.

1. Introduction

Infertility, defined by the failure to achieve a successful pregnancy within 12 months of unprotected intercourse, occurs in 10–15% of couples of reproductive age [1,2]. Assisted reproductive technologies include infertility protocols to achieve conception, of which in vitro fertilization (IVF) is one of the most common. During the past decade, blastocyst transfers in IVF protocols have increased implantation and pregnancy rates and decreased high-order multiple gestations due to a reduction in the number of transferred embryos compared with embryo transfer during the cleavage stage [3,4].

The outcome of IVF depends on many factors, including the etiology of infertility, patient characteristics, and management protocols, however the most important factor for a successful pregnancy after IVF is embryo quality [5]. Various grading systems using morphological criteria have been developed to identify embryo quality; however inter- and intra-observer variations remain high due to differences in morphological interpretation by individual embryologists [6–8]. Thus, these grading systems are not always consistent with regards to embryo implantation potential. Selecting an ideal embryo with the highest embryo implantation potential is one of the chief challenges in IVF, and a quantitative method to evaluate embryo quality before transfer is crucial.

Human chorionic gonadotropin (hCG), a member of the glycoprotein hormone family, consists of α- and β-subunits. The α-subunit of hCG consists of 92 amino acids, and the β-subunit of hCG (β-hCG)

Abbreviations: IMR, immunomagnetic reduction; β-hCG, beta-subunit of human chorionic gonadotropin; IVF, in vitro fertilization

* Corresponding author at: Department of Obstetrics and Gynecology, Mackay Memorial Hospital, No. 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan.
E-mail address: d96548019@ntu.edu.tw (Y.-M. Hwu).

https://doi.org/10.1016/j.cca.2019.01.012
Received 21 November 2018; Received in revised form 7 January 2019; Accepted 15 January 2019
Available online 16 January 2019
0009-8981/

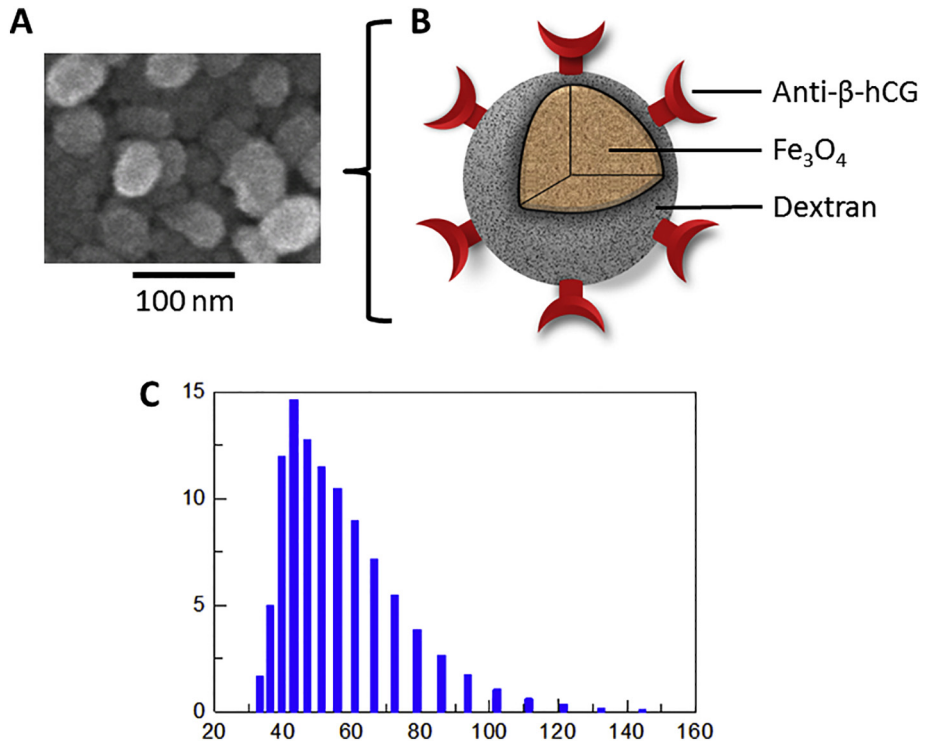

Fig. 1. Characterization of magnetic nanoparticles. (A) A typical image of nanoparticles under dehydrated condition by SEM (100,000×). (B) Illustration of a magnetic Fe_3O_4 nanoparticle coated with dextran and anti-β-hCG antibodies. (C) Size and distribution of the anti-β-hCG magnetic reagent determined by DLS.

consists of 145 amino acids. The α-subunit structures of glycoprotein hormones are basically similar, whereas the β-subunit structures differ in amino acid sequences which confer the biological and immunological specificity [9]. After conception, hCG is initially synthesized by trophoblastic cells and has various functions such as stimulating progesterone production by corpus luteal cells, promoting angiogenesis in uterine vasculature, and assisting in the growth of cytotrophoblast and syncytiotrophoblast cells [10]. hCG was firstly detected in embryo culture medium in 1984 [11]. However, few studies have assessed the relationship between hCG or β-hCG in culture media and embryo development using conventional assays such as enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA) [12,13]. This may be because there are several limitations to these methods such as the amount of time required for washing and incubation between the addition of reagents, decay of color signal intensity, requirement of two different antibodies, and extremely short luminescent decay time.

To overcome these limitations, magnetically-labeled diagnostic systems have been explored to identify biomolecules using bio-functionalized magnetic nanoparticles since the late 1990s [14,15]. Recently, the immunomagnetic reduction (IMR) assay has emerged as a novel quantitative method to detect biomarkers, and it has been shown to enhance the sensitivity and specificity of biomarker detection in body fluids including serum, plasma, urine, and even cervicovaginal secretions [16–19]. In this pilot study, we investigated whether the IMR assay can be used to detect β-hCG concentrations in blastocyst culture media. We also explored the relationship between IMR signals of β-hCG and morphological grading of blastocysts.

2. Materials and methods

2.1. Sample collection

We conducted this prospective case-control study in women undergoing IVF treatment at Mackay Memorial Hospital in Taiwan from June 2015 to March 2016. After insemination, individual embryos were transferred to a 30-μL micro-droplet of one-step embryo culture medium (global, Life Global, Guilford, CT, USA) for culture to day 5 (blastocyst stage). Each 5-μL sample volume was drawn from the culture medium and diluted 25 times with phosphate buffered saline (PBS) solution (pH = 7.4). We then sent the samples to our laboratory to measure the concentration of β-hCG using the IMR assay.

At the same time, embryo morphological grading was assessed by two experienced embryologists who were blinded to the IMR results. The blastocyst morphology was graded based on the criteria established by Gardner and Schoolcraft [20]. The blastocysts were first graded according to their size: (1) early blastocyst (EB), a blastocoel cavity less than half the embryo volume; (2) blastocyst, a blastocoel cavity greater than or equal to half the embryo volume; (3) full blastocyst, a blastocoel cavity completely filling the embryo; (4) expanded blastocyst, a blastocoel cavity larger than that of the early embryo with zona thinning; (5) hatching blastocyst, the trophectoderm had started to herniate through the zona; (6) hatched blastocyst, the blastocyst had completely escaped from the zona. The blastocysts were graded from 3 to 6, and the development of the inner cell mass (ICM) and trophectoderm was assessed further. The ICM was graded as follows: (A) many tightly packed cells; (B) several loosely grouped cells; (C) very few cells. The trophectoderm was graded as follows: (A) many cells forming a cohesive epithelium; (B) few cells forming a loose epithelium; (C) very few large cells.

This study was approved by the Mackay Memorial Hospital Institutional Review Board (IRB #15MMHIS066e), and the methods were carried out in accordance with the approved guidelines. Informed consent obtained from all of the enrolled women for the collection and examination of clinical samples.

2.2. Preparation of magnetic reagents

Fig. 1A shows a typical image of nanoparticles (MagQu, New Taipei City, Taiwan) under dehydrated conditions using a scanning electron

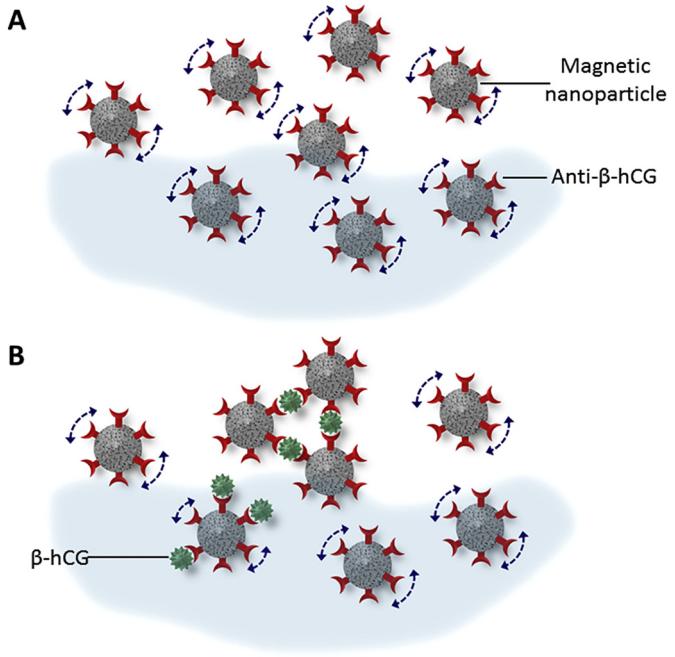

Fig. 2. Illustration of the relationship between β-hCG and magnetic nanoparticles coated with anti-β-hCG antibodies in an IMR assay. (A) With the applied external alternating current magnetic fields, all of the magnetic nanoparticles oscillated and spun individually. (B) The magnetic nanoparticles became larger and clustered after binding with β-hCG, and thus oscillated and spun much slower than the original individual magnetic nanoparticles.

microscope (SEM; JSM-6700F, JEOL, Tokyo, Japan). Magnetic Fe_3O_4 nanoparticles were covered with dextran as a hydrophilic surfactant and anti-β-hCG antibodies (ab764, Abcam, Cambridge, MA) (Fig. 1B). Aldehyde groups (–CHO) were initially formed on the dextran via an oxidation reaction to bio-functionalize the magnetic nanoparticles with antibodies against β-hCG, in which anti-β-hCG formed a covalent bond (−CH═N−) with the dextran aldehyde groups. This was then used to measure the concentration of β-hCG. The size distribution of the magnetic nanoparticles was analyzed using dynamic laser scattering (DLS; Nanotrac 150, Microtrac, PA, USA), and the mean diameter was found to be 52.8 nm (Fig. 1C). The concentration of magnetic reagent was 0.1 emu/g, and it was stored at 4 °C before measurement.

2.3. Mechanism of IMR

Fig. 2A shows that individual magnetic nanoparticles layered with hydrophilic surfactant and anti-β-hCG were well disseminated in PBS buffer because of the homogeneous size of the nanoparticles. Magnetic reagents have been shown to exhibit superparamagnetic characteristics in PBS buffer [21]. Because of the thermal motion of nanoparticles, the direction of magnetization of each nanoparticle is isotropic, which leads to zero magnetization. After the application of external multiple alternating current (AC) magnetic fields, individual magnetic nanoparticles oscillate and spin. The magnetic reagent then manifests a magnetic property, termed mixed-frequency AC magnetic susceptibility (χ_{ac}). In this study, the χ_{ac} was expressed as $\chi_{ac,o}$ before the magnetic nanoparticles connected with the targeted β-hCG via the anti-β-hCG antibody coating on the shell surface, with the immune complexes becoming larger and clustered (Fig. 2B). Subsequently, the magnetic nanoparticles spun slowly, resulting in attenuation of χ_{ac}, termed as $\chi_{ac,\phi}$, where ϕ refers to the concentration of β-hCG. A greater reduction in χ_{ac} was anticipated if the solution contained higher concentrations of β-hCG, which caused larger and greater clustering of the magnetic nanoparticles. The final IMR signal was obtained by measuring the attenuation in the percentage of AC magnetic susceptibility of the magnetic reagent, and calculated using the following equation:

$$IMR(\%) = (\chi_{ac,o} - \chi_{ac,\phi})/\chi_{ac,o} \times 100\% \quad (1)$$

The IMR assay was accomplished with homogeneous suspension of anti-β-hCG functionalized magnetic nanoparticles, so that β-hCG molecules in the sample volume could bind to three-dimensional magnetic nanoparticles and the total area of immuno-reaction with β-hCG molecules was greatly increased. An IMR analyzer (XacPro-E; MagQu, New Taipei City, Taiwan) was used to detect real-time IMR signals at room temperature (about 25 °C). IMR signals were measured in duplicate for each test.

2.4. Statistical analysis

SPSS version 21.0 (IBM, Armonk, New York) was used for all statistical analyses. The Spearman rank correlation coefficient was used to assess relationships between IMR signals and embryo morphological parameters. Receiver operating characteristic (ROC) curve analysis was used to identify the optimum cut-off value to predict high-quality embryos for the group with higher IMR signals. The optimum cut-off value was defined as the closest point on the ROC curve to point (0, 1) with a sensitivity of 100% and false positive rate of zero.

3. Results

3.1. Time-dependent χ_{ac} signal

Fig. 3A shows the real-time magnetic response χ_{ac} of the mixture of magnetic reagent and β-hCG at various concentrations from 0.01 to 100 ng/mL. The data from 0 to 1.5 h indicated the magnetic response $\chi_{ac,o}$ of the mixture of magnetic reagent and β-hCG before incubation. The decrease in the data from 1.5 to 4 h indicated that the magnetic nanoparticles were reacting with β-hCG, and corresponded to the incubation period. After the reaction at room temperature, the magnetic response $\chi_{ac,\phi}$ of the mixture of magnetic reagent and β-hCG reached another stable status, as shown by the data from 4 to 4.5 h. The IMR signals were calculated using Eq. (1). The significant reduction in the magnetic response verified the conjugation between the magnetic nanoparticles and β-hCG. The β-hCG concentration of each sample was obtained from duplicate measurements of IMR signals (Fig. 3B). According to these real-time magnetic responses, it took about 4 h for each sample.

3.2. β-hCG concentration-dependent IMR signal

In our previous study, we have investigated IMR signals as a function of β-hCG concentration and established a calibration curve [17]. The β-hCG concentration in this study ranged from 0.01 ng/mL to 10,000 ng/mL, and the mean value and standard deviation of each β-hCG concentration were obtained through duplicate IMR measurements. The data fit the following four parameter logistic equation:

$$IMR(\%) = (A - B)/[1 + (\phi_{\beta-hCG}/\phi_o)^r] + B \quad (2)$$

Where A is the minimal signal, B is the maximal signal, $\phi_{\beta\text{-hCG}}$ is the β-hCG concentration, ϕ_o is the concentration of the inflection point, and r is the slope at the inflection point of the calibration curve. By fitting the measured β-hCG concentration-dependent IMR signals to Eq. (2), the values of A, B, ϕ_o, and γ were 0.75, 1.92, 9.80, and 0.51, respectively.

In Eq. (2), parameter A (= 0.75) denoted the noise level of the IMR signal, where $\phi_{\beta\text{-hCG}}$ was equal to zero. The noise of IMR measurements was primarily generated from electronic interactions with the IMR analyzer. We used a 30-μL micro-droplet of the one-step embryo culture medium without embryos that had been cultured for 5 days as a blank medium, and the mean value and standard deviation of IMR signal were

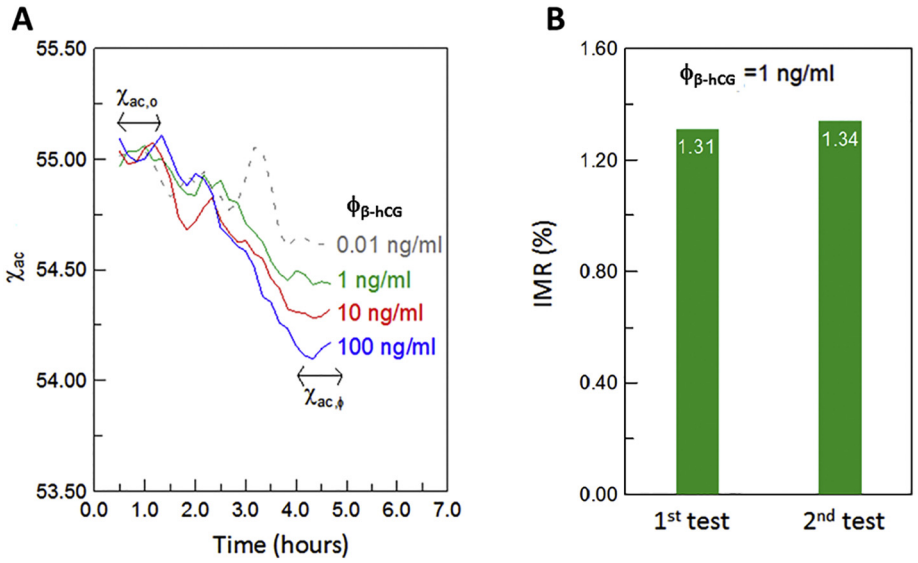

Fig. 3. (A) Real-time χ_{ac} signals of the mixture of magnetic reagent and β-hCG at various concentrations ranging from 0.01 to 100 ng/mL. (B) IMR signals for independent duplicate tests of 1 ng/mL β-hCG solution.

0.75 and 0.042, which were consistent with parameter A. The lower detection limit (LDL) was defined as a concentration higher than the noise level using two standard deviations for the IMR signals at the lowest detectable concentration, and our previous study has shown that the LDL of $\phi_{β\text{-hCG}}$ to assay β-hCG using IMR was as low as 0.03 ng/mL [17].

3.3. Analysis of the clinical samples

A total of 28 blastocyst culture medium droplets from 16 patients were recruited. Characteristics of patients are listed in Table 1. In order to compare the β-hCG levels between different morphology of blastocysts, both poor and good quality of blastocyst culture media were collected. Blastocysts were analyzed and graded based on the criteria established by Gardner and Schoolcraft [20]. These blastocysts were divided into four groups from low to high embryo quality: Group 1: EB; Group 2: blastocysts with both the ICM and trophectoderm grade C; Group 3: blastocysts with the ICM or trophectoderm grade C, and the other A or B; Group 4: blastocysts with both the ICM and trophectoderm grade A or B. Table 2 shows the embryo grading, IMR signal, and β-hCG concentration of each sample. The low coefficients of variation with less than 5% in most of the samples indicated that the IMR assay was accurate and reproducible for measuring β-hCG.

A positive correlation was found between β-hCG concentration-dependent IMR signals and embryo morphological quality, with a Spearman correlation coefficient of 0.731 (Fig. 4). In ROC curve analysis regarding group 1 and 2 as negative subjects and group 3 and 4 as positive subjects, the cut-off value for IMR to differentiate embryo quality was 0.873%. The area under the ROC curve for the IMR assay was 0.947, and the corresponding sensitivity and specificity were 0.882 and 0.818, respectively (Fig. 5). Furthermore, subanalysis also revealed a positive correlation between β-hCG concentration-dependent IMR signals and trophectoderm grading, with a Spearman correlation

Table 1
Patient characteristics.

Characteristics	*N* = 16
Age (years)	33.75 ± 3.68
AMH level (ng/mL)	6.71 ± 4.34
Estradiol level at trigger (pg/mL)	1976.40 ± 956.44
Progesterone level at trigger (ng/mL)	0.77 ± 0.46
No. of oocyte retrieved	17.70 ± 5.94
No. of metaphase II oocyte retrieved	14.44 ± 4.66
No. of embryo transferred	1.38 ± 0.96
Main cause of infertility	
Male factor	7 (43.75)
Tubal factor	2 (12.5)
Ovary dysfunction	5 (31.25)
Uterine factor	1 (6.25)
Endometriosis	1 (6.25)

Data are mean ± standard deviation or number (percentage).
AMH, anti-Mullerian hormone.

Table 2
Embryo grading, IMR signal and β-hCG concentration of clinical samples.

No.	Embryo grading	Group	IMR (%)			$\phi_{β\text{-hCG}}$ (ng/mL)	β-hCG concentration (ng/mL) ($\phi_{β\text{-hCG}}$ × 25)
			Mean	SD	CV		
1	4BC	3	0.975	0.035	3.59	0.60	14.97
2	4AA	4	1.45	0.030	2.07	21.12	527.96
3	4AB	4	1.44	0.028	1.94	19.91	497.81
4	3CC	2	0.735	0.021	2.86	ND	ND
5	3CC	2	0.895	0.035	3.91	0.22	5.46
6	4BB	4	1.44	0.028	1.94	19.71	492.69
7	4AA	4	1.07	0.042	3.93	1.47	36.79
8	4BB	4	1.28	0.042	3.28	6.85	171.17
9	4AA	4	0.94	0.028	2.98	0.40	9.93
10	4BC	3	1.2	0.014	1.17	3.88	97.09
11	3BB	4	0.85	0.014	1.65	0.09	2.32
12	3BB	4	0.875	0.021	2.40	0.15	3.82
13	3BC	3	0.83	0.014	1.69	0.06	1.44
14	3BC	3	1.285	0.035	2.72	7.04	176.00
15	4BC	3	0.995	0.021	2.11	0.73	18.15
16	4CC	2	0.865	0.064	7.40	0.15	3.79
17	4BC	3	1.245	0.021	1.69	5.32	133.05
18	4BC	3	1.03	0.014	1.36	1.01	25.33
19	3CC	2	0.765	0.021	2.75	ND	ND
20	EB	1	0.635	0.021	3.31	ND	ND
21	EB	1	0.68	0.014	2.06	ND	ND
22	EB	1	0.6	0.014	2.33	ND	ND
23	3CC	2	0.87	0.014	1.61	0.14	3.45
24	3CC	2	0.89	0.014	1.57	0.19	4.86
25	3CC	2	0.81	0.014	1.73	0.03	0.78
26	3CC	2	0.775	0.021	2.71	0.01	0.16
27	4BC	3	1.395	0.007	0.50	14.51	362.87
28	4BC	3	1.11	0.014	1.26	1.99	49.84

IMR, immunomagnetic reduction; β-hCG, β-subunit of human chorionic gonadotropin; SD, standard deviation; CV, coefficient of variation; EB, early blastocyst; ND, not detectable.

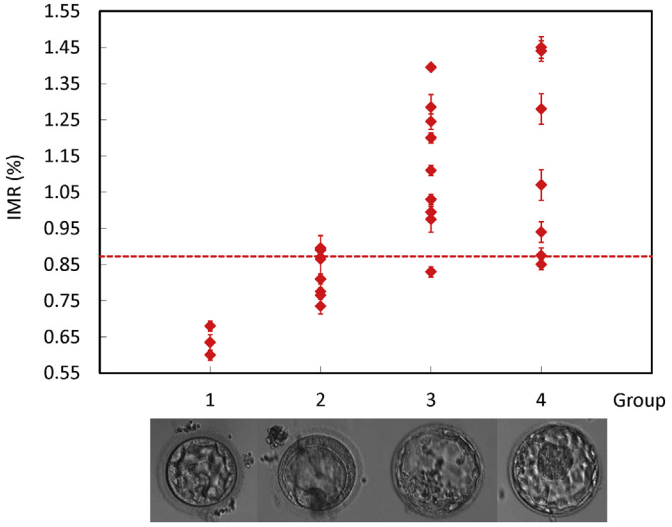

Fig. 4. Relationship between β-hCG concentration-dependent IMR signals and embryo morphological quality with a Spearman correlation coefficient of 0.731, and examples of four grouped blastocysts from low to high embryo quality (left to right). The calculated cut-off value of 0.873 is shown as the dashed line.

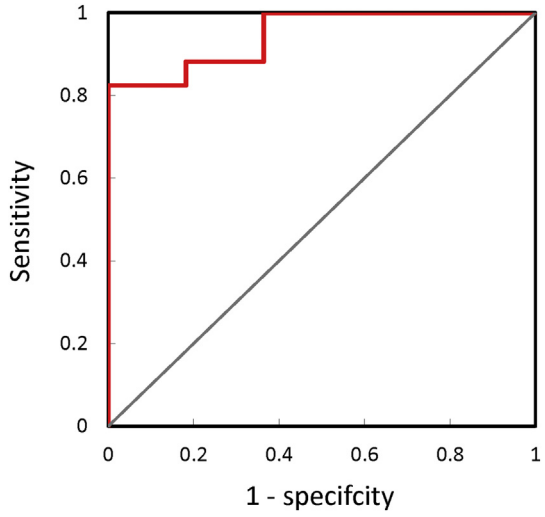

Fig. 5. ROC curve to differentiate blastocyst quality by the IMR assay. The calculated cut-off value was 0.873%, and the area under the ROC curve was 0.947.

coefficient of 0.576.

4. Discussion

Identifying good embryo quality is essential to improve the success rate of pregnancy in IVF procedures, and morphological grading systems are the most common methods used to assess embryo quality. Although these grading systems are thorough and use various morphological parameters, inter- and intra-observer variations among embryologists remain high due to the different interpretations [6–8]. Recently, single blastocyst transfer has been reported to be an effective method of reducing the number of multiple gestations while maintaining high implantation and pregnancy rates [22]. From this point of view, since the morphological assessment of embryo quality is subjective, a quantitative method with high sensitivity and specificity to assess the embryo quality of the blastocyst pre-implantation is critical.

hCG is the first reported biomarker in embryonic media, and is initially produced by the embryo and later by the syncytiotrophoblast [11]. Few studies have investigated the relationship between hCG or β-hCG in spent culture media and embryo development [12,13]. Ramu et al. assessed hCG concentrations using ELISA in embryo culture media from day 2 after intracytoplasmic sperm injection, and found that hCG may be a useful biomarker for embryo competence [12]. Xiao-Yan et al. investigated β-hCG concentrations using ECLIA in embryo culture media from day 1 to day 5, and concluded that β-hCG is a valuable biomarker for embryo selection in IVF procedures [13]. However, there were limitations to previous studies, such as different durations between measuring hCG and embryo transfer, using two-step (interrupted) culture media systems, and requiring a larger sample volume (20 to 50 μL) in the spent culture media. In the present study we measured β-hCG concentrations just before blastocyst transfer, and used single-step (uninterrupted) culture media rather than two-step culture media. Thus, β-hCG was allowed to accumulate in the culture medium from day 1 to day 5, and a sample volume of only 5 μL was needed to detect IMR.

The bioactivity and concentration of hCG secreted by blastocysts are time-dependent [23,24]. Since single blastocyst transfer is a valid method of decreasing multiple gestations while retaining a high pregnancy rate, the present study was designed to measure β-hCG concentrations just before the blastocyst transfer, thus allowing for an accurate comparison of the correlation between β-hCG levels and blastocyst morphological grade. We found a highly positive correlation between β-hCG concentration-dependent IMR signals and embryo morphological quality (Spearman correlation coefficient 0.731), and also a positive correlation between IMR signals and trophectoderm grade (Spearman correlation coefficient 0.576), which is consistent with previous studies that have demonstrated that β-hCG is secreted by trophectoderm [25,26].

Although a recent systematic review study found insufficient evidence as to whether a single-step versus two-step media blastocyst culture was more beneficial, a single-step medium was shown to have higher blastocyst formation rates and some practical advantages [27]. Single-step media have also been reported to have important benefits over two-step media, such as accumulation of endogenous growth factors, decreased embryo disturbance, lower labor and cost, simplified laboratory procedures, and allowing embryos to select the nutrients needed [28]. The stability of the reagent is crucial for an accurate result during the application of nanotechnology to detect biomarkers. A single-step blastocyst culture does not involve medium replacement on day 3, and thus avoids abrupt changes in the culture setting, which effectively minimizes environmental stress on the embryo.

In this study, we tried to measure the concentration of β-hCG before selecting the blastocyst with the best quality, and thus we could not use the whole volume of culture media in order to keep cultivating the blastocyst in residual media. Therefore, both a small sample volume and minimal use of reagent are prerequisites when measuring β-hCG. In the present study, only a 5-μL sample volume was required to detect IMR signals, which implies that the IMR assay is a potential candidate to clinically assist in the screening of good blastocyst quality before embryo transfer.

Compared with using ELISA or ECLIA to measure β-hCG, there are several advantages of the IMR assay. First, magnetic signals rather than optical signals are used in the IMR assay, which excludes the limitation of color intensity decay. Moreover, optical signals can be easily affected by sample colors caused by other biomarkers. In contrast, magnetic signals are transparent, and therefore the IMR assay has a much lower background signal level. Second, no bound or free particle separation procedures are necessary in the IMR assay, which reduces quality control processes. Third, only one monoclonal β-hCG antibody is necessary, which increases the cost-effectiveness of IMR assays. Fourth, as mentioned above, only a very small sample volume is required to measure β-hCG, which makes the IMR assay a potential clinical tool in IVF procedures. Furthermore, it is thus possible to keep sufficient

sample volume to analyze other biomarkers simultaneously in the embryo culture media.

There are several limitations to this study. First, we did not perform an interference test because of the relatively small sample volume of embryo culture media compared with blood or urine. However, previous studies have reported no obvious interfering factors when measuring serum biomarkers using an IMR assay [29]. Interference in ELISA is mainly due to color change of the substrates, which may then affect fluorescence detection. In addition, the emission intensity of ECLIA varies with time and may be influenced by various environmental factors. Magnetic signals are transparent and are thus theoretically not affected by the color of the sample. Second, the complete magnetic reaction required nearly 4 h. Through appropriate adjustments in laboratory parameters such as incubation temperature, reagent concentration, and sample volume, it may be possible to reduce the reaction time to within 30 min [30]. Third, although a positive correlation was found between β-hCG concentration-dependent IMR signals and blastocyst quality, the IVF outcome depends on a range of factors including infertility etiology and patient characteristics. Thus, a high β-hCG level does not guarantee a successful implantation. Fourth, the number of clinical samples in this study was limited, and further prospective randomized trials are necessary to verify our preliminary results.

5. Conclusions

One of the most important factors for a successful pregnancy after IVF is embryo quality. Our results revealed that the IMR assay demonstrated an ideal LDL and low coefficients of variation in β-hCG measurements in day 5 blastocyst culture media, with high sensitivity and specificity. Furthermore, a positive relationship was shown between β-hCG concentration and blastocyst morphological grading. In this pilot study, we verified that the IMR assay may be a potential clinical tool to assist in the assessment of good blastocyst quality before embryo transfer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by Mackay Memorial Hospital (MMH-104-85) and Ministry of Science and Technology of Taiwan (MOST-106-2314-B-195-013-MY3).

References

[1] Practice Committee of the American Society for Reproductive Medicine, Definitions of infertility and recurrent pregnancy loss: a committee opinion, Fertil. Steril. 99 (2013) 63.
[2] A. Chandra, C.E. Copen, E.H. Stephen, Infertility service use in the United States: data from the National Survey of Family Growth, 1982-2010, Nat. Health Stat. Rep. 73 (2014) 1–21.
[3] D.K. Gardner, B. Balaban, Choosing between day 3 and day 5 embryo transfers, Clin. Obstet. Gynecol. 49 (2006) 85–92.
[4] E.G. Papanikolaou, E.M. Kolibianakis, H. Tournaye, et al., Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis, Hum. Reprod. 23 (2008) 91–99.
[5] A. Ahlstrom, C. Westin, E. Reismer, M. Wikland, T. Hardarson, Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer, Hum. Reprod. 26 (2011) 3289–3296.
[6] J.C. Arce, S. Ziebe, K. Lundin, R. Janssens, L. Helmgaard, P. Sorensen, Interobserver agreement and intraobserver reproducibility of embryo quality assessments, Hum. Reprod. 21 (2006) 2141–2148.
[7] A.E.B. Bendus, J.F. Mayer, S.K. Shipley, W.H. Catherino, Interobserver and intraobserver variation in day 3 embryo grading, Fertil. Steril. 86 (2006) 1608–1615.
[8] A. Storr, C.A. Venetis, S. Cooke, S. Kilani, W. Ledger, Inter-observer and intra-observer agreement between embryologists during selection of a single Day 5 for transfer: a multicenter study, Hum. Reprod. 32 (2017) 307–314.
[9] L.A. Cole, New discoveries on the biology and detection of human chorionic gonadotropin, Reprod. Biol. Endocrinol. 7 (2009) (8-1-37).
[10] L.A. Cole, Biological functions of hCG and hCG-related molecules, Reprod. Biol. Endocrinol. 8 (2010) (102-1-14).
[11] S.B. Fishel, R.G. Edwards, C.J. Evans, Human chorionic gonadotropin secreted by preimplantation embryos cultured in vitro, Science 223 (1984) 816–818.
[12] S. Ramu, B. Acacio, M. Adamowicz, S. Parrett, R.S. Jeyendran, Human chorionic gonadotropin from day 2 spent embryo culture media and its relationship to embryo development, Fertil. Steril. 96 (2011) 615–617.
[13] C. Xiao-Yan, L. Jie, J. Dang, L. Tao, L. Xin-Ru, Z. Guang-Lun, A highly sensitive electrochemiluminescence immunoassay for detecting human embryonic human chorionic gonadotropin in spent embryo culture media during IVF-ET cycle, J. Assist. Reprod. Genet. 30 (2013) 377–382.
[14] K. Enpuku, T. Minotani, T. Gima, et al., Detection of magnetic nanoparticles with superconducting quantum interference device (SQUID) magnetometer and application to immunoassays, Jpn. J. Appl. Phys. 38 (1999) L1102–L1105.
[15] S. Lee, W.R. Myers, H.L. Grossman, H.M. Cho, Y.R. Chemla, J. Clarke, Magnetic gradiometer based on a high-transition temperature superconducting quantum interference device for improved sensitivity of a biosensor, Appl. Phys. Lett. 81 (2002) 3094–3096.
[16] C.H. Chang, Z.X. Lai, H.L. Lin, et al., Use of immunomagnetic reduction for C-reactive protein assay in clinical samples, Int. J. Nanomedicine 7 (2012) 4335–4340.
[17] C.Y. Chen, Y.M. Hwu, C.P. Chen, C.C. Chang, Quantitative analysis of total β-subunit of human chorionic gonadotropin concentration in urine by immunomagnetic reduction to assist in the diagnosis of ectopic pregnancy, Int. J. Nanomedicine 10 (2015) 2475–2483.
[18] C.Y. Chen, C.C. Chang, C.W. Lin, Clinical application of immunomagnetic reduction for quantitative measurement of insulin-like growth factor binding protein-1 in the prediction of pregnant women with preterm premature rupture of membranes, Clin. Chim. Acta 438 (2015) 337–341.
[19] C.H. Wong, C.P. Chen, C.C. Chang, C.Y. Chen, Bio-functionalized magnetic nanoparticles for the immunoassay of fetal fibronectin: a feasibility study for the prediction of preterm birth, Sci. Rep. 7 (2017) (42461-1-9).
[20] D.K. Gardner, W.B. Schoolcraft, In vitro culture of human blastocyst, in: R. Janson, D. Mortimer (Eds.), Towards Reproductive Certainty: Fertility and Genetics beyond 1999, Parthenon Press, Carnforth, 1999, pp. 378–388.
[21] W.Q. Jiang, H.C. Yang, S.Y. Yang, et al., Preparation and properties of superparamagnetic nanoparticles with narrow size distribution and biocompatible, J. Magn. Magn. Mater. 283 (2004) 210–214.
[22] D.K. Gardner, E. Surrey, D. Minjarez, A. Leitz, J. Stevens, W.B. Schoolcraft, Single blastocyst transfer: a prospective randomized trial, Fertil. Steril. 81 (2004) 551–555.
[23] B.J. Woodward, E.A. Lenton, K. Turner, Human chorionic gonadotrophin: embryonic secretion is a time-dependent phenomenon, Hum. Reprod. 8 (1993) 1463–1468.
[24] A. Lopata, K. Oliva, P.G. Stanton, D.M. Robertson, Analysis of chorionic gonadotrophin secreted by cultured human blastocysts, Mol. Hum. Reprod. 3 (1997) 517–521.
[25] P. Licht, V. Russu, L. Wildt, On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation, Semin. Reprod. Med. 19 (2001) 37–47.
[26] M. Tsampalas, V. Gridelet, S. Berndt, J.M. Foidart, V. Geenen, S. Perrier d'Hauterive, Human chorionic gonadotropin: a hormone with immunological and angiogenic properties, J. Reprod. Immunol. 85 (2010) 93–98.
[27] I.A. Sfontouris, W.P. Martins, C.O. Nastri, et al., Blastocyst culture using single versus sequential media in clinical IVF: systematic review and meta-analysis of randomized trials, J. Assist. Reprod. Genet. 33 (2016) 1261–1272.
[28] J. Biggers, M. Summers, Choosing a culture medium: making informed choices, Fertil. Steril. 90 (2008) 473–483.
[29] K.W. Huang, S.Y. Yang, Y.W. Hong, et al., Feasibility studies for assaying alpha-fetoprotein using antibody-activated magnetic nanoparticles, Int. J. Nanomedicine 7 (2012) 1991–1996.
[30] S.Y. Yang, J.F. Chang, T.C. Chen, C.C. Yang, C.S. Ho, Study of the temperature dependent immuno-reaction kinetics for the bio-functionalized magnetic nanoparticle assay of bio-markers of colorectal cancer, Appl. Phys. Lett. 104 (2014) 013702.