

Evaluation of transfer media containing different concentrations of hyaluronan for human in vitro fertilization

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Abstract

Purpose: The aim of the present prospective study was to investigate the efficacy of hyaluronan (HA) using two different concentrations as embryo transfer media for in vitro fertilization embryo transfer cycles.

Methods: A total of 169 cycles undergoing fresh embryo transfer on day 2 or 3, 561 cycles undergoing frozen-thawed embryo transfer on day 2 or 3, and 484 cycles of frozen-thawed blastocyst transfer were included in this study. Patients were randomly divided into two groups: transferred with low (l-HA) or high (h-HA) concentrations of HA in transfer media.

Results: In the case of fresh embryo transfer cycles, no significant differences were observed in the pregnancy (l-HA 27.2%, h-HA 31.2%), implantation (l-HA 22.1%, h-HA 24.2%), or abortion (l-HA 24.0%, h-HA 20.8%) rates between the two groups. In the case of frozen-thawed embryo transfer cycles, no significant differences were noted in the pregnancy (l-HA 20.9%, h-HA 22.9%), implantation (l-HA 13.4%, h-HA 15.8%), or abortion (l-HA 17.2%, h-HA 21.5%) rates. In the case of frozen-thawed blastocyst transfer cycles, no significant differences were observed in the pregnancy (l-HA 46.0%, h-HA 41.8%), implantation (l-HA 45.8%, h-HA 41.3%), or abortion (l-HA 17.6%, h-HA 26.9%) rates.

Conclusion: The present results showed that pregnancy, implantation, and abortion rates with transfer media containing different HA concentrations were similar.

KEYWORDS

hyaluronan, hyaluronic acid, in vitro fertilization and embryo transfer, transfer medium

1 | INTRODUCTION

Assisted reproductive technology (ART) has been widely accepted for the treatment of infertility.¹ However, the increased use of this technology has contributed to a higher rate of multiple births. The Japan Society of Obstetrics and Gynecology issued a recommendation for single embryo transfer. Selection of the best embryo for transfer is a clear requirement for successful in vitro fertilization and embryo transfer treatments. In order to improve the outcomes of in vitro fertilization and embryo transfer, many culture media for fertilization,

blastocyst cultures, and embryo transfer are commercially available.² The creation of a better environment for embryo cultures depends not only on culture media, but several other parameters. However, culture media are the most important factor for the acquirement of good-quality embryos.

Previous studies reported that culture media influence the quality of embryos, which, in turn, affect implantation and pregnancy rates.³⁻⁶ Recent studies investigated the efficacy of hyaluronan (hyaluronic acid [HA]) to support embryo implantation for successful pregnancies. Valojerdi et al showed that the addition of HA to transfer

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media contributed to better implantation rates.⁷ HA is one of the most abundant glycosaminoglycans in female reproductive organs.⁸ This glycosaminoglycan, which is naturally present in the in vivo follicular, oviduct, and uterine fluids, could be affected in the implantation process through binding with CD44 glycoprotein receptors on the surface of embryos, thereby allowing HA to act in the cell.^{9–11} Furthermore, HA increases the viscosity of transfer media and might improve the ability of embryos to interact with uterine fluid due to similarities in their consistencies.¹² Several commercial media are currently available for embryo transfer with HA. EmbryoGlue[®] contains a high concentration of HA (0.5 mg/mL). Although UTM[™] contains a lower HA concentration than EmbryoGlue[®], the exact concentration has not been disclosed. The addition of HA to culture media has been proposed in order to increase the efficiency of in vitro blastocyst production from in vitro matured bovine oocytes.¹³ Previous studies showed the highest rates of implantation and fetal development after blastocyst transfer when HA was added to culture media.¹⁴ We recently showed that media containing HA resulted in a significantly larger number of normal embryos and blastocysts.¹⁵

However, limited information is currently available on the optimal concentration of HA and the cumulative effects of transfer medium products supplemented with HA. The aim of the present study was to assess the effects of transfer media using two different concentrations of HA; that is, high HA and low HA.

2 | MATERIALS AND METHODS

2.1 | Study duration

The present study was carried out over a period of 13 months from June 2013 through June 2014.

2.2 | Study population

A total of 169 cycles undergoing fresh embryo transfer on day 2 or 3, 561 cycles undergoing frozen-thawed embryo transfer on day 2 or 3, and 484 cycles of frozen-thawed blastocyst transfer were included in this study. Patients were randomly divided into two groups: (1) the low HA group, where embryos were transferred with low HA concentration transfer media (UTM[™]; Origio, Måløv, Denmark), and (2) the high HA group, where embryos were transferred with HA-enriched transfer media (EmbryoGlue[®]; Vitrolife, Gothenburg, Sweden).

2.3 | Follicle stimulation protocol

All patients were stimulated with a standard IVF protocol. Patients were administered the gonadotropin-releasing hormone agonist, buserelin (Suprecur nasal solution 0.15%; Mochida Pharmaceutical, Tokyo, Japan), at a daily dose of 600 µg starting in the mid-luteal phase of the preceding cycle. All patients were treated with injections of 300 IU recombinant follicle-stimulating hormone on the third day of the cycle (Gonal-F; Merck Serono, Tokyo, Japan). The dose of the gonadotropin-releasing hormone agonist administered was continued

until the day of the human chorionic gonadotropin injection (human chorionic gonadotropin for injection; Fuji Pharma, Tokyo, Japan). Oocyte retrieval was carried out through vaginal ultrasound-guided follicle aspiration 36–38 hours after the administration of human chorionic gonadotropin.

2.4 | IVF procedure

All oocytes were inseminated in GM-HTF (Gynemed GmbH & Co. KG, Lensahn, Germany) or injected with sperm using the standard intracytoplasmic sperm injection technique. Fertilization was confirmed 14–16 hours after insemination or intracytoplasmic sperm injection by the presence of two pronuclei and extrusion of the second polar body. Fertilized oocytes were cultured in groups of a maximum of four oocytes in 1 mL of SAGE 1-Step[™] (Origio) until the day of transfer. The embryos were classified according to the criteria proposed by Veeck's criteria. A transferred embryo was defined as one that had reached the four-cell stage on day 2, and reached the seven-cell stage on day 3, and had <20% of its volume filled with fragments. A transferred blastocyst was defined as full blastocyst stage onward on day 5, and not including the inner cell mass and the trophectoderm with very few cells. Embryos were incubated for at least 2 hours in transfer medium before being transferred into the uterine cavity. All cultures were incubated at 37°C in 5% O₂ and 5% CO₂.

2.5 | Statistical analysis

Descriptive statistics and the Student's *t* test to compare groups were carried out using Microsoft Excel (Redmond, WA, USA).

3 | RESULTS

The characteristics of patients undergoing fresh embryo transfer on day 2 or 3 were shown in Table 1. No significant differences were observed in mean age, the number of oocytes collected, number of IVF trials, number of transferred embryos, or endometrial thickness between the two groups. There were also no significant differences in the rates of pregnancy (low-HA 27.2% [25/92], high-HA 31.2% [24/77]; *P* = .69), implantation (low-HA 22.1% [25/113], high-HA 24.2% [24/99]; *P* = .84), or abortion (low-HA 24.0% [6/25], high-HA 20.8% [5/24]; *P* = .94) between the two groups (Table 1).

The characteristics of patients undergoing frozen-thawed embryo transfer on day 2 or 3 are shown in Table 2. There were no significant differences in mean age, the number of IVF trials, number of transferred embryos, or endometrial thickness between the two groups. No significant differences were observed in the rates of pregnancy (low-HA 20.9% [58/277], high-HA 22.9% [65/284]; *P* = .65), implantation (low-HA 13.4% [58/432], high-HA 15.8% [66/418]; *P* = .42), or abortion (low-HA 17.2% [10/58], high-HA 21.5% [14/65]; *P* = .88) between the two groups (Table 2).

The characteristics of patients undergoing frozen-thawed blastocyst transfer are shown in Table 3. There were no significant

TABLE 1 Comparison of outcomes using two transfer media for fresh embryo transfer on day 2 or 3

	Low HA	High HA	P
No. cycles	92	77	
Mean age (years)	38.6 ± 12.1	38.7 ± 13.2	.85 ^a
Oocytes collected	9.3 ± 17.8	8.6 ± 14.7	.19 ^a
No. IVF trials	2.4 ± 3.6	2.5 ± 2.9	.55 ^a
No. transferred embryos	1.2 ± 0.2	1.3 ± 0.2	.34 ^a
Endometrial thickness (mm)	11.4 ± 5.0	10.9 ± 3.3	.10 ^a
Pregnancy/transfer rate (%)	27.2 (25/92)	31.2 (24/77)	.69 ^b
Implantation rate (%)	22.1 (25/113)	24.2 (24/99)	.84 ^b
Abortion rate (%)	24.0 (6/25)	20.8 (5/24)	.94 ^b

HA, hyaluronan; IVF, in vitro fertilization.

^aStudent's *t* test.

^b χ^2 -test.

TABLE 2 Comparison of outcomes using two transfer media for frozen-thawed embryo transfer on day 2 or 3

	Low HA	High HA	P
No. cycles	277	284	
Mean age (years)	40.9 ± 3.8	40.6 ± 3.5	.41 ^a
No. IVF trials	4.1 ± 3.5	3.9 ± 2.9	.45 ^a
No. of transferred embryos	1.6 ± 0.5	1.5 ± 0.5	.19 ^a
Endometrial thickness (mm)	10.6 ± 1.7	10.5 ± 1.9	.42 ^a
Pregnancy/transfer rate (%)	20.9 (58/277)	22.9 (65/284)	.65 ^b
Implantation rate (%)	13.4 (58/432)	15.8 (66/418)	.42 ^b
Abortion rate (%)	17.2 (10/58)	21.5 (14/65)	.88 ^b

HA, hyaluronan; IVF, in vitro fertilization.

^aStudent's *t* test.

^b χ^2 -test.

differences in mean age, the number of IVF trials, number of transferred embryos, or endometrial thickness between the two groups. No significant differences were noted in the rates of pregnancy (low-HA 46.0% [108/235], high-HA 41.8% [104/249]; *P* = .40), implantation (low-HA 45.8% [110/240], high-HA 41.3% [104/252]; *P* = .41), or abortion (low-HA 17.6% [19/108], high-HA 26.9% [28/104]; *P* = .14) between the two groups (Table 3).

4 | DISCUSSION

In the present study, no significant differences were noted in pregnancy, implantation, or abortion rates between low HA and HA-enriched transfer media. HA is a major glycosaminoglycan present

TABLE 3 Comparison of outcomes using two transfer media for frozen-thawed blastocyst transfer

	Low HA	High HA	P
No. cycles	235	249	
Mean age (years)	36.8 ± 4.1	37.1 ± 4.1	.72 ^a
No. IVF trials	3.6 ± 1.8	3.8 ± 2.0	.85 ^a
No. transferred embryos	1.01 ± 0.1	1.01 ± 0.1	.95 ^a
Endometrial thickness (mm)	10.5 ± 1.9	10.8 ± 1.9	.09 ^a
Pregnancy/transfer rate (%)	46.0 (108/235)	41.8 (104/249)	.40 ^b
Implantation rate (%)	45.8 (110/240)	41.3 (104/252)	.41 ^b
Abortion rate (%)	17.6 (19/108)	26.9 (28/104)	.14 ^b

HA, hyaluronan; IVF, in vitro fertilization.

^aStudent's *t* test.

^b χ^2 -test.

in several female reproductive organs, as well as cervical mucus, the cumulus, follicular fluid, and seminal plasma.¹⁶⁻¹⁸ This macromolecule is also present in the oviduct and uterine fluids. The synthesis of HA is induced at the time of implantation.¹⁰ HA is synthesized by integral plasma membrane glycosyltransferases, and is exported directly into the extracellular space.^{19,20} HA is chemically homogeneous, and is synthesized by the HA synthase enzymes Has1, Has2, and Has3 at the plasma membrane.²¹⁻²⁷ The loss of Has2 results in embryonic lethality, showing that Has2 is the major source of HA during this period of embryonic development.²⁸ The action of HA is known to be receptor-mediated through binding to its receptor CD44.²⁹⁻³¹ This process appears to be important for many physiological processes including embryonic development.³² In the present study, no significant differences were observed in the results obtained. However, the addition of HA might improve the developmental capacity of embryos under in vitro conditions. Bontekoe et al showed that clinical pregnancy and live birth rates were improved with the use of functional concentrations of HA in ART cycles.³³ However, the evidence obtained was of moderate quality, and also this study³³ did not show whether low concentrations of HA are sufficient or not. The increasing number of multiple births might be the result of the use of a combination of an adherence compound and a policy of transferring more than one embryo. Further studies are required in order to determine an appropriate concentration of HA.

We focused on differences with EmbryoGlue[®] containing a unique combination of a high concentration of HA and low concentration of recombinant human albumin. Recombinant human albumin might replace human serum albumin (HSA) as a protein source in culture media for IVF, and might reduce the risks of prion contamination and transmission of plasma-derived impurities. HSA contains fatty acids, whereas recombinant human albumin does not. Previous studies showed the important role of fatty acids in in vitro cultures of preimplantation embryos.³⁴ However, HSA added to IVF media contains numerous other proteins,

which, as a consequence, make it more difficult to analyze embryonic development. Some proteins, such as afamin, have been shown to contribute more to embryo development in culture media than a control. However, other proteins, such as chemokine ligand 13, had a negative impact on embryo development.³⁵ Further research is required in order to clarify the effects of the proteins in HSA and recombinant human albumin on embryonic development and fertility success.

Stern et al recently showed that high-molecular size HA is also anti-inflammatory and immunosuppressive.³⁶ The immunosuppressive effect is derived in part from the ability of high-molecular size HA to coat cell surfaces, thereby preventing ligand access to surface receptors. High concentrations of HA in the fetal circulation and amniotic fluid are largely responsible for immunosuppression in the developing fetus. In the present study, the abortion rate in the low HA group was lower than that in the high HA group for frozen-thawed blastocyst transfer cycles (17.6% vs 26.9%; $P = .14$). However, no significant differences were observed in the rates of abortion.

This is the first study to compare two embryo transfer media. The results obtained showed that pregnancy, implantation, and abortion rates with transfer media containing different HA concentrations were similar.

DISCLOSURES

Conflict of interest: Takuji Nishihara and Yoshiharu Morimoto declare that they have no conflict of interest. **Human and Animal Rights:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), and with the Helsinki Declaration of 1964 and its later amendments. Approval for this study was obtained from the local Ethics Committee of the IVF Japan group. This article does not contain any study with animal participants that has been performed by any of the authors.

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