<Original Article>

The Evaluation of Sperm's Hyaluronic Acid Binding Ability as a Predictive Value for the Clinical Success of Intracytoplasmic Sperm Injection

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ABSTRACT

Selecting the best sperm for intracytoplasmic sperm injection (ICSI) has been performed over the years using various approaches. We compared the clinical results between ICSI using sperm hyaluronic acid binding (HA-ICSI) and ICSI using conventional sperm selection (C-ICSI). Fifteen patients who received ICSI with HA-selected sperm and 15 patients who received conventional ICSI as a control group from June 2016 to January 2017 were enrolled in the study. We examined the clinical outcome of HA -ICSI and compared it to the clinical outcome of C-ICSI. Although there was no statistical difference in the fertilization rate (72.2 % vs 77.8 %) and high-quality embryo rate (67.4 % vs 79.1 %) between the two groups, the blastocyst rate in the HA-ICSI group (55.9 %) was statistically higher than that in the C-ICSI group (34.2 %) ($p < 0.05$). The implantation rate and pregnancy rate revealed no statistical difference. However, HA-ICSI improved the high-quality blastocyst rate in patients who had previously undertaken C-ICSI. Although further studies are necessary, HA-binding may be better than conventional sperm selection for obtaining high-quality sperm for ICSI.

INTRODUCTION

Selecting the best sperm for intracytoplasmic sperm injection (ICSI) has been performed over the years using various approaches. Some anxiety over the use of poorer-quality sperm are warranted; however, it remains unclear as to what is present in a spermatozoon that may adversely affect fetal outcome. The strategy to select the best sperm, or to get rid of those that are abnormal from the population, can be performed using various methods. No available sperm test, how-

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ever, can directly isolate and evaluate the DNA damage in a single sperm cell.

ICSI using hyaluronic acid (HA) containing media has been suggested to have a higher specificity and lower biological risk than other selection methods, and a number of recent studies have indicated that HA-bound sperm selected for the ICSI procedure may lead to increased implantation rates. Parmegiani L et al. reported that all outcomes (fertilization, embryo quality, implantation and pregnancy rates) were the same or improved in the HA-bound sperm group in their study [1]. Worrilow KC et al. also stated that in couples in which less than 65 % of sperm were bound to hyaluronan, the selection of HA-bound sperm for ICSI significantly reduced pregnancy loss rates [2].

Tarozzi et al. have also mentioned the ability of hyaluronan to select spermatozoa with higher DNA integrity and morphology; however, they also add that the clinical value of HA is rather limited under Italian law. Moreover, Petersen CG et al. have described that HA has limited efficacy in selecting motile sperm with normal morphology at high magnification. Therefore, it appears that the effectiveness of HA in ICSI remains controversial [4].

The objective of this study was to investigate the effectiveness of HA by comparing the clinical results between ICSI using a hyaluronan binding assay (HA-ICSI) and ICSI using conventional sperm selection (C-ICSI). Furthermore, to scrutinize the utility of HA-ICSI we compared the clinical results between C-ICSI and HA-ICSI in those patients who had been previously unsuccessful with C-ICSI.

MATERIALS and METHODS

We investigated patients who underwent ICSI at Umeda Fertility Clinic from June in 2016 to January in 2017.

First, we retrospectively compared the clinical outcome between HA-ICSI (n = 27) and C-ICSI (n = 30). We then retrospectively compared the clinical outcome between HA-ICSI and C-ICSI in 12 patients who had not become pregnant using the latter method.

The Practice Committee of the American Society for Reproductive Medicine (ASRM) has published a set of guidelines for a standard infertility evaluation [5]. It includes a semen analysis, assessment of ovulation, a hysterosalpingogram and, if indicated, tests for ovarian reserve and laparoscopy. The inclusion criteria for this study were as follows: 1) 23–39 years of age with regular menstrual cycles and an anti-Mullerian hormone (AMH) level greater than 0.5 ng/ml and 2) the absence of any endocrine disease. The exclusion criteria were as follows: 1) evidence of postmenopausal FSH levels and 2) any suspicion of ovarian disease (ex. ovarian endometrioma). Our institutional review board approved this protocol (16–003) and consent form, and we received informed consent for this study from all participants.

Controlled Ovarian Hyperstimulation (COH)

On day 3–5 of the menstrual cycle, all patients underwent blood sampling for the measurement of follicle stimulating hormone (FSH), estradiol (E2) and AMH serum levels. Under a gonadotropin releasing hormone antagonist (GnRHant) protocol, women enrolled started in vitro fertilization and embryo transfer (IVF-ET) cycles using GnRHant and, on day 3 of the treatment cycle, controlled ovarian stimulation was started by the daily injection of human menopausal gonadotropin (hMG) (Teizo HMG, Tokyo, Japan) at a dose of 150 to 300 IU per day. A daily dose of 0.25 mg of GnRH antagonist (Cetrotide) was initiated when the mean diameter of the leading follicle had reached 14–15 mm on transvaginal ultrasound.

Under a short protocol, 600 μg of GnRH agonist (GnRHa) was started on day 1, and controlled ovarian stimulation was started by the daily injection of hMG (Teizo HMG, Tokyo, Japan) at a dose of 150 IU per day up to 300 IU on day 3. In both protocols, 10,000 IU of human chorionic gonadotropin (hCG) was administered intramuscularly when leading follicles had reached a diameter greater than 18 mm, and transvaginal oocyte retrieval was carried out 35 hours thereafter.

Oocyte and sperm preparation

Oocytes were incubated at 37 ℃ under 5 % CO2 after retrieval. Cumulous-oocyte complexes were placed into a droplet of hyaluronidase solution for up to 40 seconds for denudation. Only those oocytes at metaphase II stage were selected for ICSI. Standard swim-up was carried out following single layer gradient centrifugation. For C-ICSI, mature sperm was captured based on morphologic characteristics

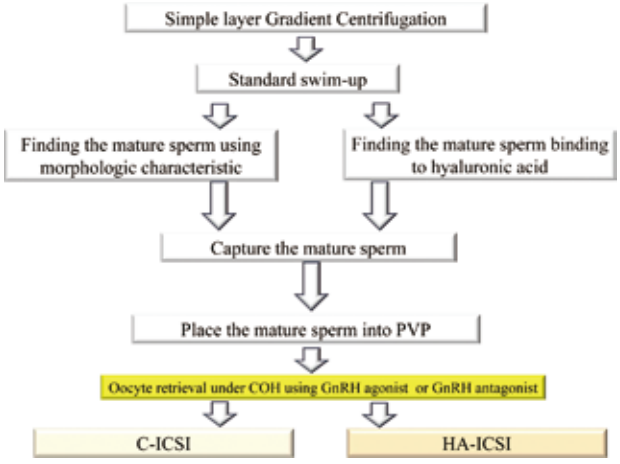

Fig. 1 The design of this study and the protocol of controlled ovarian hyper-stimulation.
For C-ICSI, mature sperm was captured based on morphologic characteristics and, for HA-ICSI, we captured mature sperm binding to hyaluronic acid. ICSI was performed following the standard protocol. In the both groups, oocyte retrieval was carried out under COH using GnRH agonist or GnRH antagonist.

and, for HA-ICSI, we captured mature sperm binding to hyaluronic acid. Afterward, the mature sperm was placed into Polyvinylpyrrolidone (PVP), and ICSI was performed following the standard protocol (**Fig. 1**).

Embryo transfer

Day 3 embryo quality was assessed by Veeck's criteria [6], and the blastocyst was evaluated by Gardner's criteria [7]. A high-quality embryo was defined as one having equal or higher than grade 2 on day 3 embryo or 3BB in blastocyst, respectively [8]. Pregnancy was confirmed during an ultrasound examination by the identification of an intrauterine gestational sac.

Statistical Analysis

Statistical analysis was conducted with StatMate IV (ATMS Co., Ltd. Tokyo, Japan). Comparisons between the two nonparametric groups were performed with the nonparametric student *t*-test or the Chi-square test. A p value of < 0.05 was considered as statistically significant.

RESULTS

Patients characteristic

Male age, female age, total dose of hMG/FSH in the HA-ICSI group showed no statistical difference in comparison with the C-ICSI group. Moreover, there was no statistical difference between the two groups in terms of endometrial thickness, serum estradiol level at the time of oocyte retrieval and the number of retrieved oocytes. Additionally, no significant difference in the pregnancy rate was identified (**Table 1**).

Comparing the clinical outcomes of C-ICSI with HA-ICSI

The 2 pronuclei (2PN) rate, fertilization rate and high-quality embryo rate showed no significant difference. However, the high-quality blastocyst rate in the HA-ICSI group was significantly higher than the rate in the C-ICSI group ($p = 0.034$) (**Fig. 2**).

Table 1 The clinical results between ICSI using HA-ICSI ($n = 30$) and C-ICSI ($n = 27$)

Parameter	C-ICSI ($n = 30$)	HA-ICSI ($n = 27$)	p value
Male age (y)	38.7 ± 5.4	39.1 ± 6.99	0.815
Female age (y)	37.7 ± 3.7	36.9 ± 3.64	0.432
Total dose of FSH/hMG (IU)	1893.8 ± 860.0	1982.2 ± 1172.7	0.750
Endometrial thickness (mm)	10.6 ± 1.9	10.8 ± 2.4	0.697
Peak serum E2 on day of hCG (pg/ml)	2374.6 ± 1707.8	2248.0 ± 1134.9	0.742
No. of retrieved oocyte (n)	5.33 ± 1.42	7.00 ± 3.1	0.155
Pregnancy rate (%)	56.7 (17/30)	44.4 (12/27)	0.463

Note: Data are presented as mean ± SD or *n* (%).

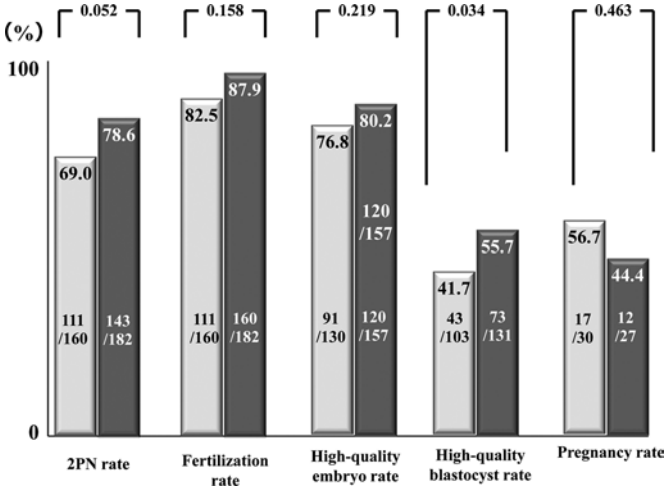

Fig. 2 Clinical outcomes of C-ICSI group and HA-ICSI group
The high-quality blastocyst rate alone in the HA-ICSI group was significantly higher than the rate in the C-ICSI group ($p = 0.034$). A number on top of each column represents p value.

Table 2 The clinical results between C-ICSI and HA-ICSI in the patients who had been previously unsuccessful with C-ICSI.

Parameters	C-ICSI *n* = 12	HA-ICSI *n* = 12	*p* value
Female age (y)	36.3 ± 5.1	36.7 ± 5.0	0.853
Male age (y)	38.4 ± 6.7	38.7 ± 6.2	0.911
Total dose of hMG/FSH (IU)	1629.1 ± 854.8	2445.6 ± 1377.5	0.106
Peak serum E2 on day of hCG (pg/ml)	2533.6 ± 1767.5	2224.0 ± 1074.3	0.613
Endometrial thickness (mm)	10.2 ± 2.4	11.0 ± 2.2	0.412
No. of retrieved oocyte (*n*)	5.8 ± 4.6	6.6 ± 2.8	0.597
Native sperm concentration ($\times 10^6$ /ml)	48.9 ± 41.7	61.7 ± 32.2	0.401
Pregnancy rate (%)	0 (0/12)	25.0 (3/12)	

Note: Data are presented as mean ± SD or *n* (%).

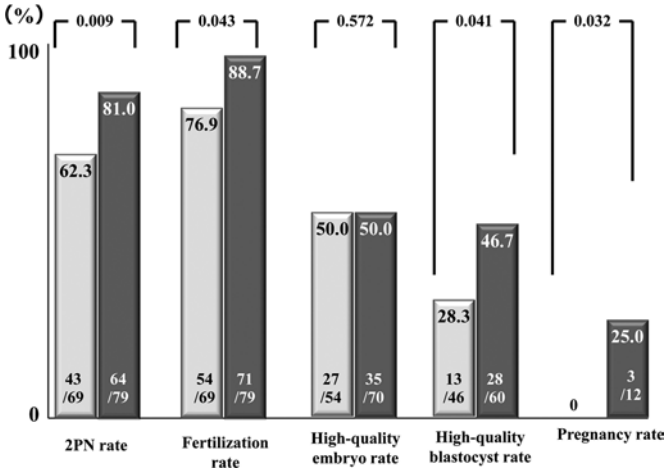

Fig. 3 The clinical outcome between HA-ICSI and C-ICSI in 12 patients who had not become pregnant using C-ICSI.
2PN rate, fertilization rate, high-quality blastocyst rate and pregnancy rate of HA-ICSI group were statistically higher than those of C-ICSI group. A number on top of each column represents *p* value.

Comparison of the clinical outcomes between HA-ICSI and C-ICSI in the same patients.

Male age, female age, total dose of hMG/FSH in the HA-ICSI group showed no statistical difference in comparison with the group that had previously undertaken C-ICSI (C-ICSI group). There was no statistical difference, as well, between the two groups in terms of endometrial thickness, serum estradiol level at the time of oocyte retrieval, the number of retrieved oocytes, and native sperm concentration. Three cases of pregnancy were observed in the HA-ICSI group (**Table 2**).

The 2 PN rate in the HA-ICSI group was significantly higher than that in the C-ICSI group. No statistical difference was identified in the high-quality embryo rate, however, high-quality blastocyst rate and pregnancy rate of HA-ICSI group were statistically higher than those of C-ICSI group (**Fig. 3**).

DISCUSSION

In this study, we found that HA-ICSI improved the 2PN, fertilization and high-quality blastocyst rate in patients who had not previously become pregnant via C-ICSI.

In ICSI, individual sperm are selected to inject into oocytes. Mostly, sperm are chosen based only on their morphology using the microscope. Until now, however, the ideal test to select healthy sperm accurately has not been available. In particular, the selection of the sperm without any DNA damage is critical in ICSI. Injecting spermatozoa with aneuploidy may produce chromosome aberrations in offspring [9,10], and

oocytes that have been fertilized with spermatozoa with damaged DNA may lead to an increased risk of pregnancy loss [11–15]. ICSI using HA-binding sperm results in a lower incidence of aneuploidies and DNA damage [16].

Regarding pregnancy outcomes, a recent study has revealed improved outcomes in patients with physiological intra-cytoplasmic sperm injection (PICSI) using commercial HA and PICSI assay kits [17]. Furthermore, it has been suggested that the selection of sperm with better developmental and nuclear competence influences preimplantation embryogenesis. An analysis of 4000 HA-bound spermatozoa from 20 patients by the sperm chromatin dispersion (SCD) method indicated a DNA fragmentation rate (5.3 %) reduction which was slightly lower than in 400 spermatozoa collected directly from PVP (11 %), as in C-ICSI [3]. Tarozzi et al. have also reported a very low DNA fragmentation level by the Tunnel assay in both HA-bound sperm and sperm prepared by density gradient separation (1.17 % and 1.59 %, respectively) [3]. However, regardless of these lower rates, they underlined the capacity of HA to select spermatozoa with higher DNA integrity. The different HA-binding methods (PICSI dish, sperm slow medium and HA-coated chamber) may have caused discrepancies among these three studies [1,3,18]. In addition, the low DNA fragmentation rate reported in the Tarozzi et al. study is different from the literature data, thus preventing the ability to draw any conclusions [19,20]. Because spermatozoa are transcriptionally silent cells, it is highly unlikely that new HA receptor proteins have been exhibited on the surface of the sperm membrane during culture. This indicates that HA binding properties could be typical for each spermatogenetic cycle and/or for each individual patient [21]. In our study, sperm retrieved by single layer gradient centrifugation followed by standard swim-up, might have improved the DNA fragmentation rate, thus leading to a better clinical outcome. Moreover, among the advanced sperm selection techniques, sperm selection by HA-binding is an easily available method and does not require expensive instrumentation [22]. Rougier et al. reported that it is especially important to shorten the time of semen preparation for ICSI in order to prevent the DNA damage of sperm [23].

In our study, no preimplantation genetical testing of aneuploidy was carried out, however, HA-ICSI improved the high-quality blastocyst rate and pregnancy rate in patients who had been previously unable to become pregnant with conventional ICSI.

In this respect, the incubation of sperm in HA has an advantage. Admittedly, there are some limitations to this study. First, we were unable to investigate cases of severe oligozoospermia. Second, the patients enrolled in this study were not selected at random.

In conclusion, although further studies are necessary to determine the effectiveness of HA-binding HA-ICSI may be an alternative for the retrieval of higher-quality blastocysts which lead to implantation.

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