ORIGINAL ARTICLE

Relationship between Sperm Hyaluronan-binding Assay (HBA) Scores on Embryo Development, Fertilisation, and Pregnancy Rate in Patients Undergoing Intra-cytoplasmic Sperm Injection (ICSI)

Carine To Chiou Fen, *BSc,* Shaw Ni Lee, Mui Nee Lim, Su Ling Yu, *MBBS, MMED (O & G)*

Centre for Assisted Reproduction, Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore

ABSTRACT

Introduction: The novel development of the sperm hyaluronan-binding assay (HBA) has now been routinely used in some laboratories worldwide to predict sperm maturity and functionality. Hence, the purpose of this study is to evaluate if embryo development, quality, fertilisation and pregnancy rates are affected by low HBA values.

Methods: A total of192 female patients who underwent intra-cytoplasmic sperm injection (ICSI) were compared retrospectively in terms of embryo development, fertilisation, and pregnancy rates with their husbands' HBA score. Patients' husbands were required to undergo a HBA test before the start of their stimulation cycle to determine if their semen was suitable to undergo ICSI or in-vitro fertilisation (IVF). *P*-value < 0.05 was considered significant.

Results: Patients were divided into four groups, group A (HBA ≤ 15%), group B (HBA > 15% < 35%), group C (HBA ≥ 35% < 60%), and group D (HBA ≥ 60%). The fertilisation rate for groups A, B, C, and D were 67.9%, 73.1%, 72.5%, and 77.1% respectively. Group D had a fertilisation rate significantly higher than the rest of the groups ($p = 0.016$). The pregnancy rate for group D was also significantly higher amongst the four groups ($p = 0.041$), whereas the pregnancy rate for groups B and C was similar (42.4% versus 41.1% respectively). Day three cleavage rate (the ability to reach six cells and beyond) was highest for group D compared to the other groups ($p = 0.002$).

Conclusion: The higher the HBA score, the better the fertilisation, pregnancy, and cleavage rates. This shows that HBA does have the ability to select mature sperms with normal chromosome development and oocyte-binding capability.

Keywords: Chromosomal abnormalities, DNA integrity, Sperm plasma membrane remodelling

INTRODUCTION

Routine semen analysis is widely performed as a major test for male fertility potential by assessing sperm concentration, motility, and morphology of the spermatozoa. However, these results do not provide accurate diagnostic or prognostic information about human fertility either *in vivo* or *in vitro*[1–8]. Sperm function may not be predicted by semen analysis, as the fertilisation process involves a large number of biochemical events not measured by these parameters. Nearly a third of male infertility etiologies remain idiopathic. Clinically, these patients with unexplained infertility have difficulty deciding which of the assisted reproductive technologies (ART) would be the best option to assist them to achieve pregnancy with lower cost and a less invasive procedure.

Poor predictive values of routine semen analysis for sperm-fertilising ability is not because of a large variation of semen parameters between ejaculates[8–11]. It is mostly because routine semen analysis only determines sperm concentration, motility, and morphology but cannot detect many other aspects of sperm function such as nuclear maturity, DNA normality, and the ability of sperm to interact with oocytes[12–14]. Hence, many tests such as sperm penetration assay (SPA) have been developed. However, most of these tests are time consuming and costly. Therefore, there is a need for a more economical and technically easier alternative for assessing sperm function.

The sperm HBA that has been developed as a commercial diagnostic kit for assessing sperm

maturity and function[13,14], is a simple, short, and less costly test. This test is based on previous studies that hyaluronic acid (HA) can selectively bind to mature sperm with intact acrosome with better morphology. As we know, HA has a natural sperm-selective function. HA is normally present in the extracellular matrix (ECM) of the cumulus oophorus surrounding the oocyte in the natural human fertilisation process. The ECM is a formidable barrier that only mature spermatozoa that have extruded their specific receptors to bind to and digest HA, can overcome to reach and penetrate the zona pellucida and fertilise the oocyte[15]. HBA is a simple technique to predict sperm performance and fertilisation potential.

The aim of this study was to determine the relationship between HBA score with the embryo development, fertilisation and pregnancy rate in patients undergoing the ICSI procedure.

METHODS

Patient Population

The study population included 192 female patients and their husbands undergoing ICSI cycles in the Singapore General Hospital, Centre for Assisted Reproduction (CARE), Department of Obstetrics and Gynaecology from January 2009 to June 2010. The age of female patients ranged from 21- to 44-years-old. The age group for group A was between 21- to 44-years-old, group B was 27- to 43-years-old, group C was 27- to 42-years-old, and group D was 29- to 40-years-old. The main cause of infertility in this population of patients was male infertility. These patients' husbands were required to produce a fresh sample on the day of oocyte retrieval for the processing of semen for the ICSI procedure. HBA test was done prior to the day of oocyte retrieval, before the start of the patients' stimulation cycle to determine if their husbands' semen is suitable to undergo ICSI or IVF.

Patients' husbands with a HBA score of less than 60% would definitely be channelled for ICSI. Husbands with HBA score greater or equal to 60%, would have to come back another day to provide another semen sample for sperm survival test. If the overnight sperm survival at 37^0C is less than 85% of motile sperm, the patient would also be channelled for ICSI. The sperm selection method during ICSI is based on the embryologist's subjective selection by choosing good nuclear morphology sperm for injection.

Husbands who were not able to produce a fresh semen sample on the oocyte retrieval day and frozen-thawed semen samples used for fertilisation were excluded from this study. Fertilisation check of the injected oocytes was assessed 16–18 hours after ICSI. Embryo development and embryo grading were both assessed on day two and day three. Embryos were graded in terms of degree of fragmentation, presence of vacuoles, symmetrical size of blastomeres, and morphology of embryos.

Sperm Preparation for ICSI Procedure

Semen samples were obtained by masturbation after two to five days abstinence. The ejaculated sperm was used for ICSI procedure. All sperm tests were performed after liquefaction of the semen within one hour. Sperm concentration and motility in semen were determined using standard methods (World Health Organization, 1999). Semen was processed for ICSI using 95%, 70%, and 50% Sil-Select gradient (from FertiPro N.V.), and only the 95% fraction was kept for ICSI after two washes with Quinn's Sperm Washing medium modified HTF with 5.0 mg/mL human albumin (from SAGE, In-Vitro Fertilization, Inc.), and a final swim-up process to select better quality sperm.

Sperm Hyaluronan-Binding Assay (HBA)

Commercial HBA kits were purchased from Biocoat (Fort Washington, PA, USA), and the HBA test was performed following the manufacturer's instructions. A total of 10 µl of semen (well-mixed) was added to the centre of the HBA chamber and the Cell-Vu grid cover slip was put on without entrapping air bubbles, this can be accomplished by slowly lowering the cover slip at an angle. The cover slip provided a grid of 100 squares (each 0.1 mm x 0.1 mm) within a viewing circle. After incubating the slide for 15 minutes at room temperature, the unbound motile sperm and bound motile sperm were counted in the same grid squares. For the HBA test, 200 motile sperms were counted. The percentage of hyaluronan-binding sperm was calculated using the bound motile sperm divided by the sum of bound and unbound motile sperm counted in the same squares and then multiplied by 100.

Sperm Survival Test

Husbands with HBA score of 60% and above were required to do a sperm survival test to determine if ICSI or IVF was suitable for them. Semen samples were obtained by masturbation after two to five

days abstinence. The ejaculated sperm was used for sperm survival test. All the sperm tests were performed after liquefaction of the semen within one hour. Sperm concentration and motility in semen were determined using standard methods (World Health Organization, 1999). Semen was washed once with Quinn's Sperm Washing medium modified HTF with 5.0 mg/mL human albumin (from SAGE, In-Vitro Fertilization, Inc.), and a final swim-up process to select better quality sperm. The swim-up sperm was further incubated overnight at 37^0C, and assessed for sperm motility the following day. If motility of sperm fell below 85%, the patient would be channelled for ICSI.

Statistical Test

The relation between HBA with fertilisation rate, pregnancy rate, day three embryo cleavage rate, and the embryo quality were analysed by Chi-square method using SPSS software. Statistical significance was set at $p < 0.05$.

RESULTS

Patients were divided into four groups, group A (HBA score ≤ 15%), group B (HBA score > 15% < 35%), group C (HBA score ≥ 35% < 60%), and group D (HBA score ≥ 60%). The fertilisation rate for group A, B, C, and D were 67.9%, 73.1%, 72.5%, and 77.1% respectively. Group D had a fertilisation rate significantly higher than groups A, B, and C ($p = 0.016$), as shown in Table 1. The pregnancy rate for group D was also the highest amongst the four groups ($p = 0.041$), as shown in the table, whereas pregnancy rate for groups B and C was similar (42.4% versus 41.1% respectively). Day three cleavage rate (the ability to reach six cells and beyond) was significantly higher for group D as compared to the other groups ($p = 0.002$), as shown in the table. Day three cleavage rate for group B and group C was 66.0% and 59.2% respectively. Group A had the lowest fertilisation, pregnancy, and day three cleavage rate amongst the four groups. HBA score did not seem to affect the embryo grading as the percentage of good quality embryos remained similar throughout the four groups: 79.3% (group A), 76.4% (group B), 78.7% (group C), and 73.6% (group D), $p = 0.324$, as shown in the table.

DISCUSSION

The development of the sperm HBA is based on the fact that HA bound sperm had enhanced levels of developmental sperm maturity, including fewer chromosomal aberrations and higher sperm DNA integrity[14,16]. HBA has been marketed as an addition to semen analysis for predicting sperm fertilising ability. Therefore, the aim of this study was to predict how HBA score would affect the fertilisation and pregnancy rates as well as the development of the embryos in our ICSI patients.

The female patients in this retrospective study were divided into four groups according to their husband's HBA score, namely group A, B, C, and D. From this study, it showed that group D, those with high HBA score of more or equals to 60%, had the highest pregnancy, fertilisation rates, and the best developing embryos that were able to reach six cells and beyond on day three. Hence, this shows that HBA is positively correlated with embryo development, fertilisation, and pregnancy rates. The higher the HBA score, the better the fertilisation, pregnancy, and cleavage rate of the embryos.

Table 1. Correlation of fertilisation, pregnancy, cleavage rate and embryo quality.

	Group A HBA ≤ 15%	Group B HBA > 15% < 35%	Group C HBA ≥ 35% < 60%	Group D HBA ≥ 60%	*P*-value
Fertilisation rate (%)	319/470 (67.9)	445/609 (73.1)	380/524 (72.5)	182/236 (77.1)	0.016*
Pregnancy rate (%)	14/52 (26.9)	25/59 (42.4)	23/56 (41.4)	13/25 (52.0)	0.041*
Cleavage rate (%) ≥ 6 cells on D3	112/226 (49.6)	200/303 (66.0)	167/282 (59.2)	90/128 (70.3)	0.002*
Embryo quality (%)	252/319 (79.3)	340/445 (76.4)	299/380 (78.7)	134/182 (73.6)	0.324

*Difference was considered significant when *p*-value < 0.05

It should be highlighted that in this present study, the sperm selection process is based on the embryologist's subjective selection by visual observation of good nuclear sperm morphology under the microscope; hence the selected sperm for ICSI could either be a hyaluronan-binding or non-binding. However, the probability of selecting a hyaluronan-binding sperm would be increased for those patients with a high HBA score. It has been suggested that motile sperm with good nuclear morphology have superior binding to HA and in some aspects HA has some similarities to the human zona pellucida[12,17,18]. Huszar et al. (2003) showed that HA-bound human spermatozoa had intact acrosomes, mature nuclei, and better morphology with enhanced levels of developmental sperm maturity and higher sperm DNA integrity. It has also been shown that sperms that are able to bind to HA are mature and have completed the spermiogenetic processes of sperm plasma membrane remodelling, cytoplasmic extrusion, and nuclear histone-protamine replacement[14]. HBA has also been reported to select for mature sperm with low frequency of chromosomal abnormalities[19].

In the present study, patients' husbands with low HBA score of less than or equals to 15% had the lowest pregnancy and poorest cleavage rates. This may be due to the production of potentially defective embryos with chromosomal abnormalities as reported by Huszar et al (2006), which can affect the ability of embryos to develop further and thereby compromising the pregnancy outcome. There was no statistically significant difference between groups B and C in terms of fertilisation and pregnancy rates. This was probably because the lowest cut-off point to determine pregnancy and fertilisation rates might be 15%, meaning any HBA values above 15% would be able to generate a reasonable rate for fertilisation and pregnancy for ICSI patients.

However, HBA does not affect the number of good quality embryos generated as the percentage of good grading embryos remained similar in all four groups despite the HBA score. This probably suggests that sperm maturity and its DNA integrity does not play a major role in determining the quality of the embryos; the egg quality itself is more important in affecting the degree of fragmentation and vacuolation in embryos.

CONCLUSION

The higher the HBA score, the higher the fertilisation, pregnancy rate, and the better the cleavage rate. This shows that HBA does have the ability to select mature sperm with normal chromosome development and oocyte-binding capability. However, HBA score does not determine the embryo grading.

In conclusion, HBA is useful in routine semen analysis to improve the accuracy of male infertility diagnosis and would also help in identifying patients with poor reproductive prognosis in ICSI. The right approach for this group of patients with poor reproductive prognosis may be the use of two ready-to-use systems specially designed for sperm HA binding selection during ICSI, namely the PICSI^{R} Sperm Selection Device (MidAtlantic Diagnostic – FDA approved and CE-marked) or a viscous medium containing HA (Sperm Slow^{TM}, MediCult – CE marked). A study by Parmegiani L et al (2010) using Sperm Slow to select sperm prior to ICSI, reported a significant improvement in the embryo quality and implantation[20]. Our centre's preliminary data also showed promising results when using the PICSI Sperm Selection Device prior to ICSI for sperm selection.

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