A prospective randomized study to evaluate the effect of hyaluronic acid sperm selection on the intracytoplasmic sperm injection outcome of patients with unexplained infertility having normal semen parameters

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Abstract

Purpose Sperm quality plays an important role in determining embryo development and intracytoplasmic sperm injection (ICSI) outcome. Selection of competent sperm based on its ability to bind to hyaluronic acid (HA) has been suggested as one of the methods to assess sperm quality. The aim of the present study was to examine whether injection of HA bound sperm helps in improving outcome in patients undergoing ICSI with unexplained infertility having normal semen parameters.

Methods Patients with unexplained infertility having normal semen parameters in accordance with WHO 2010 criterion, undergoing their first IVF-ICSI cycle were enrolled during the course of the study. 156 patients were prospectively randomized after oocyte retrieval and were assigned to either the ICSI group, where sperm selection for injection was based on visual assessment, or the PICS group, where sperm were selected based on their ability to bind to HA. Only fresh embryo transfers were included in the analysis.

Results There was no difference in the fertilization rates, number of top quality embryos and clinical pregnancy rates between the ICSI and PICS groups (65.7 % vs 64.7 %; 45.8 % vs 43.6 % and 35 % vs 35.2 % respectively). However,

a higher pregnancy loss rate was observed in the ICSI group (25 % vs 12 %; $P=0.227$) as compared to the PICS group, but the difference was not statistically significant. Implantation rates were 22.03 % and 18.84 % in the PICS and ICSI groups respectively. There were 22 (31 %) live births in the PICS group and 21 (26.3 %) live births in the ICSI group.

Conclusions Patients with unexplained infertility having normal semen parameters may constitute a patient group which does not benefit from this sperm selection method. A larger study may be necessary to establish a relationship between PICS and pregnancy loss rate in patients undergoing IVF with unexplained infertility.

Keywords Hyaluronic acid binding · Sperm selection · PICS · Unexplained infertility · Pregnancy loss rate

Introduction

Sperm quality plays an important role in determining embryo development and intracytoplasmic sperm injection (ICSI) outcome. In spermiogenesis, during sperm plasma membrane remodeling, along with the formation of zona pellucida receptors, receptors for hyaluronic acid (HA) are also formed [1]. Therefore sperm that binds to HA should also bind to the zona pellucida. Selection of competent sperm based on its ability to bind to HA has been suggested as one of the methods to assess sperm quality. HA binding by human sperm indicates increased levels of cellular maturity, viability and un-reacted acrosomal status [2–4]. The proportion of Tygerberg normal morphology sperm has been found to be 3.04 fold higher in HA selected sperm [5]. It has also been reported that sperm

Capsule Injection of HA selected sperm (PICS) did not help in improving the clinical outcome in patients undergoing ICSI with unexplained infertility having normal semen parameters.

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that can bind to HA have a reduced risk of being aneuploid [6] or having fragmented DNA [7, 8]. Initially, a number of studies have suggested a beneficial effect of HA sperm selection on assisted reproductive technology (ART) outcomes [7–11]. However, recently a large study comparing ICSI using HA and conventional ICSI showed no statistical difference in embryo quality and pregnancy rates [12]. Moreover, improvement in fertilization rate and pregnancy rates has been observed only in semen samples exhibiting either poor morphology [13] or compromised HA binding [14, 15]. This suggests that while PICSi might prove to be a valuable tool for sperm selection in patients with male factor infertility, its role needs to be further investigated in patient populations other than male factor infertility.

The aim of the present study was to examine whether injection of HA bound sperm helps in improving outcome in patients undergoing ICSI with unexplained infertility having normal semen parameters.

Materials and Methods

The study was approved by the hospital ethics committee. All patients gave their informed consent prior to inclusion in the study. Patients with unexplained infertility having normal semen parameters in accordance with WHO 2010 criterion [16], undergoing their first IVF-ICSI cycle were enrolled during the period, Jan to Nov 2012. The exclusion criterion were as follows: 1) age >38, 2) presence of any uterine anomalies like adenomyosis and fibroids larger than 3 cm in size, 3) any demonstrable hydrosalpinx, 4) moderate and severe endometriosis and 5) 3 or less oocytes at retrieval. 156 patients were prospectively randomized with the help of a computer generated randomization table after oocyte retrieval and were assigned to either the ICSI group, where sperm selection for injection was based on visual assessment, or the PICSi group, where sperm were selected based on their ability to bind to HA. Outcome measures studied were fertilization rate, number of top quality embryos, implantation rate, clinical pregnancy rate, live birth rate and pregnancy loss rate. Only fresh embryo transfers were included in the analysis.

All patients underwent controlled ovarian stimulation using standard long protocol. After confirming pituitary down regulation on day 2 or 3 of the cycle, 150–225 IU of recombinant FSH (Gonal F; Merc Serono, Geneva) was administered daily depending on the patients anticipated response, with or without addition of 75 IU recombinant LH (Luvris; Merc Serono, Geneva) in the late follicular phase based on the patients response to stimulation. When at least two follicles reached 18 mm in diameter, recombinant hCG (250 µg, Ovitrelle, Merck Serono, Geneva) was used to trigger ovulation. Transvaginal ultrasound guided oocyte retrieval (OCR) was performed 35–36 h later. Following aspiration, the follicular

fluid was examined and oocyte cumulus complexes (OCC's) were retrieved and transferred to GIVF (Vitrolife, Sweden) for culture at 37 °C and 6 %CO₂ for 3 to 4 h. In the meanwhile, the semen sample obtained from the husband was subjected to 40:80 double density gradient centrifugation (Puresperm, Nidacon, Sweden) at 300 g for 10 min. The resulting pellet was washed twice (Sperm Rinse, Vitrolife, Sweden) and a subsequent swim-up was performed to obtain motile sperm for injection. OCC's were denuded mechanically using a 150 µ flexipet (Stripper Tips, Origio, USA) after brief exposure to 80 IU hyaluronidase (Hyase-10X, Vitrolife, Sweden) for 30 s.

Mature oocytes were injected with sperm according to the centers established ICSI protocol. In the ICSI group, an elongated 10 µl poly vinyl pyrrolidone drop (PVP, Medicult, Denmark) under oil, was used to select spermatozoa with normal morphology for subsequent injection. In the PICSi group, sterile PICSi dishes (Origio MidAtlantic Devices, USA) with three hyaluronan microdots attached to the interior bottom, were used. 10 µl droplets of culture medium (GMOPS, Vitrolife) were placed over the hyaluronan microdots and an elongated 10 µl drop of PVP was made below the drops, before covering the dish with oil. 1–2 µl of sperm suspension was then added to the hyaluronan microdot containing droplets. After 5 min of incubation at 37 °C, HA bound sperm with normal morphology were removed with an injecting micropipette (TPC, Australia) to the adjacent PVP droplet, immobilized and subsequently injected. Fertilization was checked the next morning at 16–18 h post insemination. 2pn embryos were then cultured sequentially in G1P from day 1 to 3 and G2P from day 3 to 5. Based on the number and quality of embryos available, up to 3 embryos were selected for transfer under ultrasound guidance on either day 2 or 3, and the remaining embryos were cultured till day5 and good quality blastocysts frozen. Embryos were graded in accordance with the latest guidelines for embryo assessment [17], taking in to account both morphology and cleavage rates. All patients received progesterone support daily, from the day of OCR in the form of vaginal tablets (400 mg BD) alternating with injectable natural micronized progesterone (100 g), until day 14 after embryo transfer when they were tested for βhCG.

Fertilization rate was calculated as a ratio of number of 2pn embryos to the total number of MII oocytes injected, expressed as a percentage. A clinical pregnancy was defined as presence of one or more gestational sacs on ultrasound 2 to 3 weeks after positive βhCG. Ectopic pregnancies were not counted as clinical pregnancies. Live birth rate was defined as a ratio of number of embryo transfers resulting in the live birth of one or more babies to the total number of embryo transfers performed. Pregnancy loss rate was defined as the number of clinical pregnancies lost before 20 weeks of gestation divided by the total number of clinical pregnancies.

Statistical Methods

Statistical analysis was performed by the SPSS program for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean $\pm$ SEM, and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the student's *t*-test, whereas the Mann–Whitney *U* test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the chi square test or Fisher's exact test. For all statistical tests, a *P* value less than 0.05 were considered statistically significant.

Results

Out of 156 patients that were enrolled and randomized for the study, 5 were excluded from the analysis since they did not have a fresh transfer. A total of 71 cases were analyzed in the PICS group and 80 cases were analyzed in the ICSI group. There were no significant differences in male age, female age, BMI, duration of infertility, total dose of gonadotropin used, endometrial thickness on day of hCG, peak serum E2 on day of hCG, number of oocytes and MII's retrieved, and number of embryos transferred between the two groups (Table 1). There were 22 (31 %) live births in the PICS group and 21(26.3 %) live births in the ICSI group. There was no difference in the fertilization rates, number of top quality embryos and clinical pregnancy rates between the ICSI and PICS groups (65.7 % vs 64.7 %; 45.8 % vs 43.6 % and 35 % vs 35.2 % respectively). However, a higher pregnancy loss rate was observed in the ICSI group (25 % vs 12 %; *P*=0.227) as compared to the PICS group, but the difference could not reach statistical significance. Implantation rates were 22.03 % and 18.84 % in the PICS and ICSI groups respectively. Clinical outcome has been tabulated in Table 2.

Discussion

Primary indication for ICSI is male factor infertility constituting patients with subnormal semen parameters. However, ICSI has also been used to treat couples with unexplained infertility, characterized by normal seminal sperm concentration and motility [22]. Since sperm quality plays an important role in determining ICSI outcome, our study sought to investigate if better sperm selection through PICS would help improve outcome in patients with unexplained infertility. Based on our results, PICS was not found to be an effective method in improving outcome in patients undergoing IVF cycles with unexplained infertility. Our results demonstrated that there were no differences in fertilization rates, number of top quality embryos or clinical pregnancy rates between PICS and conventional ICSI in this patient group. The implantation rates were also similar between the two groups. The only benefit that seemed to emanate from injecting HA selected sperm was visibly a lower pregnancy loss rate which consequently translated to a higher live birth rate, both of which however were not statistically significant.

The contribution of sperm towards embryogenesis can be understood to be spread over two periods. First is the early period that extends from the point of fertilization to the period of early embryo development, generally considered to be up to the eight cell stage. Second is the late period that extends from the eight cell stage (point of embryonic genome activation) to birth and even beyond. In the early period, the sperm exerts its influence by affecting fertilization, syngamy and the first mitotic division. This is achieved by oocyte activating factor, which brings about second meiotic division in the oocyte, and by proximal centriole, that is necessary for spindle assembly during syngamy and mitotic division.

In the late period (8 cell stage and beyond), after the embryonic gene switches on, the sperm can influence embryogenesis by way of the genome that it contributes. If the genome is altered by DNA breakages, it may manifest into a poor development to blastocyst stage, poor implantation or

Table 1 Comparison of patient and cycle characteristics

	ICSI group (n=80)	PICS group (n=71)	P value
Male age (years)	34.79 $\pm$ 0.526	34.30 $\pm$ 0.410	0.466
Female age (years)	31.54 $\pm$ 0.372	31.76 $\pm$ 0.358	0.666
BMI	25.27 $\pm$ 0.739	24.98 $\pm$ 0.446	0.742
Duration of infertility (years)	6.43 $\pm$ 0.378	6.95 $\pm$ 0.375	0.182
Total dose of gonadotropin used (IU)	2095.71 $\pm$ 109.46	1973.30 $\pm$ 84.91	0.774
Endometrial thickness on day of hCG (mm)	9.60 $\pm$ 0.167	9.25 $\pm$ 0.179	0.158
Peak serum E2 on day of hCG (pg/ml)	1677.16 $\pm$ 85.81	1578.69 $\pm$ 83.30	0.451
No. of oocytes retrieved	10.08 $\pm$ 0.52	10.34 $\pm$ 0.51	0.541
No. of MII oocytes	7.23 $\pm$ 0.448	7.82 $\pm$ 0.384	0.156
No. of embryos transferred	2.39 $\pm$ 0.092	2.49 $\pm$ 0.087	0.350

Data are presented as
Mean $\pm$ SEM

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

Table 2 Comparison of results between ICSI versus PICS groups

Parameters	ICSI group	%	PICSI group	%	P value
Fertilization rate	371/565	65.7 %	353/546	64.7 %	0.724
No. of top quality embryos	170/371	45.8 %	154/353	43.6 %	0.460
Positive beta-hCG	35/80	43.8 %	31/71	43.7 %	0.991
Clinical pregnancy rate	28/80	35.0 %	25/71	35.2 %	0.978
Live birth rate	21/80	26.3 %	22/71	31.0 %	0.520
Pregnancy loss rate	7/28	25.0 %	3/25	12.0 %	0.227
Implantation rate	36/191	18.8 %	39/177	22.0 %	0.448

even a high risk of early pregnancy loss. DNA breakage is unlikely to affect embryogenesis in the period before the embryonic gene has been switched on [21]. Hence a sperm with DNA fragmentation is more likely to affect implantation rates and early pregnancy loss rate and less likely to affect fertilization rates, cleavage speeds or day2/3 embryo quality [20, 21]. Accordingly, our study was unable to establish any relationship between PICS and fertilization rates or embryo quality. On the other hand, injection of HA bound sperm, which has been demonstrated to have high DNA integrity [19] may have resulted in the reduced number of pregnancy losses, which however could not reach statistical significance probably due to lack of numbers.

Many authors have evaluated the effect of HA selection on both clinical outcome as well as association of HA binding with DNA fragmentation. While studies have found HA bound sperm to be associated with low DNA fragmentation [7, 8] there have been conflicting reports with regards to clinical outcome. While Nasr-Eshafani et al. [8] reported an increase in fertilization rate and Parmegiani et al. [9] showed an improvement in embryo quality and implantation rates after injection of HA bound sperm, Menezo et al. [12] found no difference in embryo quality or clinical pregnancy rates in HA-ICSI as compared to PVP-ICSI.

Recently in 2013, in a large multi-centric trial, Worilow et al. [15], reported a greater implantation rate and clinical pregnancy rate along with a statistically significant lower pregnancy loss rate when hyaluronan bound sperm was used for ICSI. However, this improvement was only attributed to patients demonstrating a compromised hyaluronan binding assay (HBA) score. A sperm binding score >65 % means that 7 out of 10 spermatozoa would qualify the selection criterion even if picked up only via visual assessment leading to a comparable chance of pregnancy either ways. HA selection becomes an important factor in cases with low binding scores of 50 % or 20–40 % where probability of finding the ‘right’ sperm is much lower. The HBA score could be a possible factor that could have explained the lack of improvement in ICSI outcome in our study. Alternately, our results may also suggest that probably it is the oocyte quality which exerts a greater influence on the outcome as compared to sperm

quality in patients of unexplained infertility with normal sperm parameters.

Since, the use of HA selected sperm has been found beneficial only in patients exhibiting specific binding characteristics, it was logical to assume that PICS would be useful only in selected patient populations. Our study was undertaken in the hope to identify patient populations where unnecessary PICS could be avoided. The basic premise of our study was similar to the study carried out by Choe et al. [18] who investigated the efficacy of HA sperm selection in a specific patient group with previous poor fertilizations or repeated implantation failures despite having normal semen parameters. The authors reported no benefit in terms of fertilization or embryo quality in such patients.

In summary, our results suggest that even though PICS may lead to selection of sperm of superior quality in terms of DNA integrity and a lower aneuploidy rate, no apparent clinical improvement was visible. A larger study may be necessary to establish a relationship between PICS and pregnancy loss rate in patients undergoing IVF with unexplained infertility. From our study it seems that clinical benefit may be derived from PICS only when applied to a selective patient population. Patients with unexplained infertility who have normal semen parameters may constitute a patient group which does not benefit from this sperm selection method.

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Conflict of interest The authors declare they have no conflict of interest.

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