DOI Number: 10.37506/v11/i2/2020/ijphrd/195040

New Approach of Hyaluronic Acid Bound Spermatozoa-ICSI in Iraqi Infertile Patients

Ezdehar N. Ali, Hayder A.L. Mossa

High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, IRAQ

Abstract

Background: Ability of spermatozoa to binding to hyaluronan is related to sperm membrane maturity and fertilizing potential, thus it has been suggested that sperm selection using Hyaluronic acid (HA) could increase the implantation rate in Intracytoplasmic Sperm Injection(ICSI). Basically Physiological -ICSI (PICSI) is a scientific technique used in ICSI, is a specialized form and more filtered way of selecting the best competent sperm for fertilization process. Despite the fact that conventionally spermatozoa are selected for ICSI based on their morphology and motility.

Objective: To evaluate whether (PICSI) has the potential to improve the fertilization rate and embryo grading.

Patients, Materials and Methods: Fifty-three infertile couples subjected to an *in vitro* fertilization stimulation program; Thirty-five underwent (ICSI) cycle and eighteen underwent (PICSI) cycle, assessed the fertilization rate and embryo grading at cleavage stage then the results were statistically analyzed.

Results: In spite of the fertilization rate of PICSI group was better than ICSI group but statistically no significant difference was noticed between both of them. In regarding to embryo grading at cleavage stage, there was a significant increase($P<0.05$) in Grade 1 and Grade 2 for PICSI group as compared to ICSI group.

Conclusions: The percentage of fertilization for PICSI group is better than for ICSI group. In regarding to statistical analysis, there was no significant differences between two groups. Regarding to embryo quality at cleavage stage, the PICSI cycle significantly improves embryo quality(Grade 1 and Grade 2) at day 2 and day 3 of development as compared to the ICSI cycle.

Keywords: *Hyaluronic Acid Bound Sperm, ICSI,PICSI, infertile men.*

Introduction

Infertility is a complex disorder and a unique medical condition,it involves a couple, rather than a single individual with significant medical, psychosocial, and economic problems[1].

Male infertility is usually caused by either sperm production or its transport disorders[2]. Semen analysis has two major quantifiable attributes: the total number of spermatozoa which reflects sperm production by the testes and the patency of the post-testicular duct system and the total fluid volume formed by the various accessory glands which reflect the secretory activity of these glands [3].

In Vitro Fertilization (IVF) is an assisted reproductive technology in which spermatozoa and oocytes are combined outside of the human body in a laboratory dish. The main steps in any IVF cycle are controlled ovarian hyperstimulation, retrieval of oocytes, fertilization, embryo culture and embryo transfer[4].

The conventional IVF could not help the couples with severe male factor infertility including very low sperm count, motility impairment and abnormal sperm morphology as they leading to failure of fertilization[5]. The ICSI proceduremean" entails the deposition of a single spermatozoon directly into the cytoplasm of the oocyte, thus bypassing the zona pellucida and the

oolemma"[6]. Up to 15% of all couples of reproductive age have been diagnosed with infertility and about one-third of them have male factor infertility as a contributing factor, ICSI has confirmed to be precious for couples with severely compromised semen parameters [7].

Another proposed indication for the use of ICSI includes: unexplained infertility, poor quality oocyte, advanced maternal age, low oocyte yield, prior fertilization failure with conventional IVF, Pre implantation genetic diagnosis, fertilization after *in vitro* maturation, and fertilization of cryopreserved oocyte [8].

Hyaluronic acid (HA) is the main component of the cumulus oophorus; it plays a role in the natural selection of mature spermatozoa during in vivo fertilization. Therefore, the sperm ability to bind to hyaluronic acid and subsequently to the zona pellucida can be used as the basis for *in vitro* sperm selection. Since HA is a physiological component of the cervix, cumulus cells and follicular fluid, it should pose no additional safety risks when used for sperm selection[9]. Huszar and colleagues discovered that sperm bound to hyaluronic acid *in vitro* have markers of cellular maturity, minimal DNA fragmentation, normal shape, and low frequency of chromosomal aneuploidies[10].

In the past few years, both HA-mediated devices, the sperm HA- binding assessment in the Andrology laboratory, and the ICSI sperm selection device, the PICSI dish (an IVF Petri dish that carries an HA spot), has been increasingly accepted and used worldwide[11].

The PICSI dish may take only a few minutes. Further, the PICSI dish provides a spacious area for sperm selection. It's equipped with lines of orientation, and all spermatozoa are within the same level of microscopic focus range. Thus, the embryologist has a good opportunity to compare the available spermatozoa with respect to shape (morphology) and also HA binding, as well as the important specific response of the fully developed sperm to HA contact [12,13].There are now several laboratories that have initiated HA mediated sperm selection. It's important that none of the groups practicing the HA sperm selection reported any adverse effects regarding fertilization or embryo development[14].

Patients, Materials and Methods

A prospective study conducted in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/AL-Nahrain University. Fifty-three infertile couples subjected to IVFstimulation program; Thirty -five infertile couples underwent (ICSI) cycle, and eighteen underwent (PICSI) cycle.Fifty-three infertile couples subjected to:

- Full history taking (age of women, type of infertility, duration & causes of male and female infertility, and number of previous IVF trials).
- Measurement of the body mass index (BMI).
- Baseline hormonal assay was performed at day 2 of the menstrual cycle include serum Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Estradiol (E2), Prolactin and Thyroid stimulating hormone (TSH)) for each woman.

The study included women that underwent to controlled ovarian stimulation (antagonist protocols) their age ranged between (20-40) years. The cases of infertile male with spermatozoa retrieval from testicular biopsy and frozen spermatozoa were excluded.

Oocytes retrieval was performed using a transvaginal probe 34-36 hours after the hCG injection just prior to the rupture of follicles. The procedure usually took 20-30 minutes. After that, the patients were given antibiotics, analgesics, and luteal phase support. All follicles within both ovaries are aspirated by ovum aspiration needle and follicular fluid given directly to the embryologist to identify the quality of the retrieved cumulus-oocytes complex[15].

After recovery, the oocytes were washed free of the follicular fluid, the hyaluronidase concentration and exposure must be kept to a minimum, mechanical dissection of cumulus oophorus and corona radiate was done. After denudation, to remove traces of hyaluronidase, oocytes should be thoroughly washed. Grading of the oocytes into germinal vesicle, metaphase I (MI), and metaphase II (MII), also classified into normal or abnormal oocyte. Later on transferred into drops of IVF media overlaid by paraffin/mineral oil in an incubator at temperature 37°C with 5% CO_2, and at 95% humidity. Finally, those ova which have been extruded the first polar body (metaphase II) and morphologically intact were suitable for microinjection[16]. The oocytes inseminated for 4–6 hours after aspiration and the spermatozoa must be prepared during this time. [17]. After insemination, zygotes observed for 18-20 hours to check for the presence of 2 pronuclei. At day 1, the presence of 2 pronuclei considered as a good prognostic sign. After that, evaluation of embryos at day 2 and day

3. The embryos with(4 cells at day 2) or with (7-9 cells at day 3) and containing <10% of cytoplasmic fragments are considered as good quality embryos [18]. Embryo transfer generally done at day 2,or at day 3 or at day 5 post ICSI procedure depending on patient's age, embryo quality, and the number of embryos available [6].

Luteal phase was supported since day of oocyte retrieval by vaginal progesterone (Cyclogest®400mg twice: or Crinone,® 8% progesterone gel) and continued daily. Serum ß-hCG assay was done on day 14 after the embryo transfer[19].

The Sperm Selection Device (PICSI) provides a means to select mature sperm based on their ability to bind to hyaluronan hydrogel. It's a polystyrene culture dish with three microdots of hyaluronan attached to the interior bottom. The device is sterile, free of endotoxin and non-toxic to embryos, the spermatozoa will be added to the pre-hydrated microdot in a volume equal to or greater than that used to pre-hydrate the dot (approximately 10 µl). Then the tip of the micropipette containing the sperm will be touched to the edge of the hydrating drop at the dish under the oil and expel the sperm. Once bound, hyaluronan -bound sperm are easily identified, they exhibit no progressive migration despite vigorous tail beating. Sperm binding begin normally in 5 minutes or less. However, some microdots may require 30 minutes or more to reach full binding capability. The captured sperm will be expelled into a Polyvinylpyrrolidone (PVP) drop to process them for ICSI, from the PVP droplet, select and load single, processed sperm for injection into the oocytes according to your standard injection protocol[10,20].

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20.For analysis, basic characteristics and hormones profile were analyzed using independent sample t-test. Chi square test was used to reveal the significant comparison among percentages of the fertilization rate, and embryo grading in this study.

Results

The current study illustrated that the percentage of infertile couples with primary infertility was (62.264%),while infertile couples with secondary infertility was (37.735%). All infertile couples who were enrolled in this study had different causes that led them to be infertile. The most common cases of males was oilgoasthenoteratozoospermia and for females was polycystic ovaries syndrome. The statistical analysis in table (1) showed no significant differences for the female age, infertility duration, BMI and basal hormonal profile level at cycle day 2 between infertile couples underwent ICSI cycle and infertile couples underwent PICSI cycle.

Table (1): Basic characteristic and hormonal profile at cycle day 2 for both groups.

Parameter	ICSI group	PICSI group	P-value
Age (Year)	30.685 ± 0.853	28.555 ± 1.207	0.922
Duration of infertility (Year)	6.514 ± 0.600	5.944 ± 0.697	0.261
BMI (kg/m^2)	29.153 ± 0.592	28.762 ± 0.586	0.052
FSH (mIU/L)	6.278 ± 0.449	6.647 ± 0.720	0.552
LH (mIU/L)	7.139 ± 0.985	6.901 ± 1.391	0.984
Prolactin (mIU/L)	13.195 ± 1.401	14.380 ± 1.782	0.513
E_2 (pg/ml)	45.136 ± 1.755	43.477 ± 2.621	0.997
TSH (mIU/L)	1.477 ± 0.081	1.555 ± 0.117	0.936

The fertilization rate for PICSI group higher than for ICSI group. In regarding to statistical analysis, there was no significant differences between both groups, p-value = 0.207.

Assessment of embryo quality at cleavage period, according to number and size of cells and the percentage of fragmentation .Therefore, the grading of embryo divided into three grades (Grade 1, Grade 2 and Grade 3). The embryo with grade 1 represent the best embryo,while the worst embryo with grade 3. The statistical analysis showed significant increase in embryo grading (Grade 1 and Grade 2) for PICSI group, while no significant difference for Grade 3 for both groups, as shown in table (2).

Table 2: Comparison between ICSI group and PICSI group in main clinical embryological variables.

Chi square	P-value	PICSI group	ICSI group	Parameter	
1.596	0.207	78.378	63.385	Fertilization rate	
5.444	0.020	86.206	58.024	Grade 1	
13.889	0.000	10.344	35.802	Grade 2	Day 2
1.000	0.317	3.448	6.172	Grade 3	
5.321	0.021	82.758	55.555	Grade 1	
11.520	0.001	13.793	37.037	Grade 2	Day 3
1.600	0.206	3.448	7.407	Grade 3	

Discussion

In the current study there was no significant difference in basic characteristic and hormonal level at cycle day 2 for both groups to eliminate any variations that may affect the reproductive results.

Even though in this study the patients were treated with the same controlled ovarian stimulation regimen and ICSI was performed by the same embryologist, using the same instruments and media for gamete handling and culture. This study revealed that injection of HA-bound spermatozoa (HA-ICSI) determines a statistically significant improvement in embryo quality in day 2 and day 3 when performing HA- ICSI on a limited number of oocytes (between 1 and 3). It didn't observe a statistical significant difference in fertilization rate, in spite of the percentage of fertilization to selection of HA-bound spermatozoa group was higher than that for ICSI group .However,it's difficult to analyze the insignificant difference when HA-ICSI was performed on a limited number of oocytes and low number of patients which warrants further study to be carried out as a clinical trial.A statistically significant improvement in reproductive terms like fertilization rate, embryo quality and a reduction in the number of miscarriages were observed by Worrilow *et al*. Performing PICSI ® (MidAtlantic Diagnostic) versus conventional ICSI, in a study of 240 patients[21]. Recently, Nasr-Esfahani *et al*. have published a study (performed on 50 couples) observing a higher fertilization rate when injecting oocytes with HA-selected spermatozoa[22]. Against this, in two studies with a small number of patients involved [44 patients Van Den Berg *et al*, and 18 patients Sanchez *et al*.], no differences in fertilization[23,14].Use of hyaluronan-facilitated sperm selection did not exert any observed harmful effects to the recipient oocytes or resulting embryos. Consequently, the use of hyaluronan binding sperm in ICSI may directly influence the genetic integrity of the paternal contribution to the conceptus, minimizing the potential risks inherent to ICSI [20].

Conclusions

The percentage of fertilization for PICSI group is better than for ICSI group. In regarding to statistical analysis, there was no significant differences between two groups. Regarding to embryo quality at cleavage stage, the PICSI cycle significantly improves embryo quality(Grade 1 and Grade 2) at day 2 and day 3 of development as compared to the ICSI cycle.

Authors Contribution Statement: This research was done by Dr.Ezdehar N. Ali as a part of her Ph.D. thesis under the supervision of Assist. Professor Dr. Hayder A.L. Mossa (corresponding author).

Acknowledgement: The researchers are thankful for the coworkers.

Ethical Clearance: The study was approved by Ethical Approval Committee.

Conflict of Interest: Conflict of Interest declared none.

Source of Funding: By the researchers themselves.

Application of ICSI by (PICSI) was used for the first time in High Institute for Infertility Diagnosis and ART's/Al-Nahrain University.

References:

1. Cousineau TM and Domar AD. Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol. 2007; 21(2):293-308
2. McLachlan R, and Cook R. Male infertility: a child of our own, 4th ed. Andrology Australia. 2004.

3. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th edition. Geneva: WHO . 2010.
4. Perkins, KM. Boulet, SL. Kissin, DM. Jamieson, DJ. and the National ART Surveillance (NASS) Group, 'Risk of Ectopic Pregnancy Associated With Assisted Reproductive Technology in the United States, 2001–2011', ObstetGynecol, Jan 2015; 125(1): 70–8.
5. Devroey, Paul, and Andrè Van Steirteghem. A review of ten years, experience of ICSI. Human Reproduction Update, 2004; 10(1):19–28.
6- Neri QV, Monahan D, Rosenwaks Z, Palermo GD, and Rosenwaks Z. ICSI: technical aspects. In :Gradner DK, Weissman A, Howles CM, References 92, Shoham Z, editors. Textbook of Assisted Reproductive Technologies,Volume One: Laboratory Perspectives, 4th ed. London: Informa Health care. 2012. Pp172-185.
7. Babayev SN., Park C W, and Bukulmez O. ICSI indications: how rigorous? Seminars in reproductive medicine 2014; 32(4): 283-290.
8. American Society for Reproductive Medicine (ASRM). A Practice Committee Report. ICSI for non-male factor infertility. Ame Society Reprod Medic. FertilSteril 2012; 98: 1395-1399.
9. David K, Ariel W, Colin M, and Zeev S.: Textbook of Assisted Reproductive Techniques; 5th edition vol. 1: Laboratory Perspectives, 2018: Pp119.
10. Huszar G, Ozenci CC, Cayli S,Zavaczki Z, HanschE, andVigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability and unreacted acrosomal status. Fertil Steril,2003; 79(Suppl 3): 1616–24.
11. Worrilow KC, Eid S, Woodhouse D,Witmyer J, Khoury C, and Liebermann J, . Prospective, multi-center, double-blind, randomized clinical trial evaluating the use of hyaluronan-bound sperm in ICSI: statistically significant improvement in clinical outcomes. Abstract, ASRM annual meeting. Florida: Orlando, 2011.
12. Aoki VW, and Carrell DT. Human protamines and the developing spermatid: their structure, function, expression and relationship with male infertility. Asian J Androl 2003; 5: 315–24.
13. Carrell DT, Emery BR, and Hammoud S,. Altered protamine expression and diminished spermatogenesis: what is the link? Hum Reprod Update 2007; 13: 313–27.
14. Sanchez M, Aran B, Blanco J, Vidal f, Veiga A, BarriPN . Preliminary clinical and FISH results on hyaluronic acid sperm selection to improve ICSI. Hum Reprod 2005; 20 Supp 1: i200.
15. Orvieto R. Triggering final follicular maturation-hCG, GnRH-agonist or both, when and to whom?. J Ov Res .2015 Aug 21; 8(1):60.
16. Varras M, Polonifi K, Mantzourani M, Stefanidis K, Papadopoulos Z, AkrivisC, andAntsaklis A. Expression of antiapoptosis gene survivin in luteinized ovarian granulosa cells of women undergoing IVF or ICSI and embryo transfer: clinical correlations. Reproductive Biology and Endocrinology. 2012 Sep 7; 10(1):74.
17. Popal W, and Nagy ZP. Laboratory processing and ICSI using epididymal and testicular spermatozoa: what can be done to improve outcomes?. Clinics. 2013;68: 125-30.
18. Marinakis, G. and Nikolaou, D. 'What is the role of assisted reproduction technology in the management of age-related infertility?',Human Fertility, Feb 2011;14(1):8-15.
19. Satterfield, MC. Dunlap, KA. Hayashi, K. Burghardt, RC. Spencer, TE. Bazer, FW. 'Tight and Adherens Junctions in the Ovine Uterus: Differential Regulation by Pregnancy and Progesterone', Endocrinology, 01 August 2007; 148 (8): 3922-31.
20. Yagci,A,W. Mark.J.Stronk and G.Huszar. Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity :an acridine orange fluorescence study. JAndrol2010; 31(6):566-72.
21. Worrilow KC, Huynh T, Bower JB, Anderson AR, Schillings W, Crain JL. PICSI VS ICSI: statistically significant improvement in clinical outcomes in 240 IVF patients. FertilSteril 2007; 88 Supp 1: s37.
22. Nasr-Esfahani MH, Razavi S, Vahdati AA, Fathi F, Tavalaee M. Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. J Assist Reprod Genet 2008; 25: 197-203.
23. Van Den Bergh MJ, Fahy-Deshe M, Hohl MK. Pronuclear zygote score following ICSI of hyaluronan-bound spermatozoa: a prospective randomized study. Reprod Biomed Online 2009.