PICSI® Dish and SpermSlow™ Media

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Introduction

The pathway to normal fertilization *in vivo* and *in vitro*, is closely guarded by the oocyte and the follicular cells surrounding it, the *corona radiata* and *cumulus oophorus*, collectively termed the cumulus-oocyte complex (COC). To successfully navigate this pathway, competent spermatozoa require sufficient motility to migrate to the COC, and appropriate morphology and physiology to bind to the *zona pellucida* (ZP), penetrate it and, subsequently, bind to the oolemma to fuse with it and activate the oocyte. Once activated, the oocyte responds by forming the male and female pronuclei, which contain the genetic contributions of each gamete to the zygote and begins cleaving to form an embryo. Meanwhile, the oocyte is obliged to unravel the compacted chromatin within the male pronucleus and repair any damaged DNA in time for embryonic genome activation to be initiated by the 8-cell cleavage stage, which allows further development to the blastocyst stage. Essentially, these mechanisms have evolved to ensure that a normal oocyte is fertilized by a normal spermatozoon and, thereby, increase the likelihood that a blastocyst will have sufficient viability to implant and maintain an ongoing pregnancy, with a resulting healthy live birth.

Intracytoplasmic sperm injection (ICSI) was introduced to enable incompetent spermatozoa to circumvent their limitations in fertilizing oocytes but, to do so, it bypasses the oocyte's gatekeepers which would otherwise help to police sperm quality. ICSI operators must assume the oocyte's role of sperm quality control but only have sperm motility and morphology to assist them, which provides little indication of the sperm's genomic integrity. Indeed, even morphologically normal spermatozoa in men with oligoasthenoteratozoospermia have higher rates of aneuploidy.[1] Therefore, various techniques have been developed to better identify genetically healthy spermatozoa. One of these methods, originally termed physiologic ICSI,[2] relies upon the differential binding of spermatozoa to the naturally occurring glycosaminoglycan, Hyaluronic Acid (HA), also known as Hyaluronate or Hyaluronan.

Biological rationale for sperm selection using Hyaluronan

Endogenous HA is a major constituent of follicular fluid and the COC, and is found throughout the female reproductive tract, from the fallopian tubes to the cervix.[3] Unlike other glycosaminoglycans, HA is not covalently linked to a core protein but is synthesized at the plasmalemma and exists as a free polysaccharide within the extracellular matrix of the COC.

During late spermiogenesis, loss of the residual body via cytoplasmic extrusion is accompanied by maturation-related remodelling of the sperm plasmalemma, which facilitates specific cell membrane ZP- and HA-binding site formation in mature spermatozoa.[4] Indeed, Hyaluronan-Binding Protein 1 (HABP1) is present on the spermatozoa of many species, where it participates in sperm-oocyte interaction via its mannose residues, and has been correlated with sperm motility.[5] In fact, this relationship with sperm motility led to the development of the sperm HA-binding assay (HBA).[6] In this respect, immature spermatozoa are unable to bind to HA, their diminished maturity being correlated with meiotic defects, abnormal sperm head morphology, decreased binding to the ZP, increased lipid peroxidation, and sperm DNA fragmentation (SDF).[7] On the contrary, the ability of spermatozoa to bind to HA is correlated with sperm maturity, decreased aneuploidy, increased chromatin integrity, and decreased SDF. Therefore, inability to bind HA may be pertinent to impaired reproductive success, including early pregnancy loss, which has been associated with SDF.[8] Hence, HA based sperm selection should benefit clinical outcomes.

Hyaluronan binding sperm selection

Two different but related approaches have been developed for sperm selection via their ability to bind to HA: the PICSI® dish (Figure 1) and SpermSlow™ medium (Figure 2).

The PICSI® dish is manufactured from polystyrene with three microdots of dehydrated HA placed on the bottom of the dish, their location indicated by three arrowheads embossed on the exterior surface of the dish. Preparation of the PICSI® dish is performed at room temperature just prior to use by rehydrating the microdots of HA using an appropriate medium such as HEPES/MOPS buffered holding medium containing 5mg/ml^{-1} Human Serum Albumin (HSA), immediately overlaid with mineral oil. Rehydration and swelling of the HA microdots usually commences after five minutes, at which time different concentrations of the sperm prep may be added to the edge of each microdot such that spermatozoa are obliged to migrate towards the microdot as a secondary selection parameter to optimize sperm selection. Sperm binding to HA may be observed immediately, though maximal binding occurs after 30 minutes. Since the PICSI® dish may also be used for ICSI, it is important to ensure the PICSI® dish has reached 37°C before commencing ICSI, though less sperm binding may be observed due to increased sperm velocity at higher temperature. Spermatozoa that have bound to HA are clearly identified by their head spinning around at a fixed location due to their tail motility, whereupon they may be easily aspirated into an ICSI micropipette. Once transferred to a more viscous medium, sperm morphology may be assessed prior to rupture of the sperm plasmalemma and microinjection into an oocyte.

SpermSlow is a semi-viscous medium containing a high concentration of HA in solution. Therefore, it may be used for both sperm selection and manipulation prior to microinjection. Set-up of the ICSI dish containing SpermSlow is the same as with standard ICSI dish preparation, the only difference being that a micro-drop of SpermSlow and a micro-drop of the sperm prep are bridged together using the same medium as that used for ICSI, and immediately overlaid with mineral oil.

Spermatozoa are then obliged to migrate towards the SpermSlow via the bridging medium, those able to bind to HA becoming caught in the three-dimensional net of SpermSlow. Hence, those spermatozoa that progress freely through the SpermSlow are ignored whereas those whose progress is arrested at the interface between the SpermSlow and bridging medium are selected and assessed for normal morphology before being manipulated for microinjection.

Figure 1: The PICSI® Dish: arrowheads indicate the location of the three microdots of HA

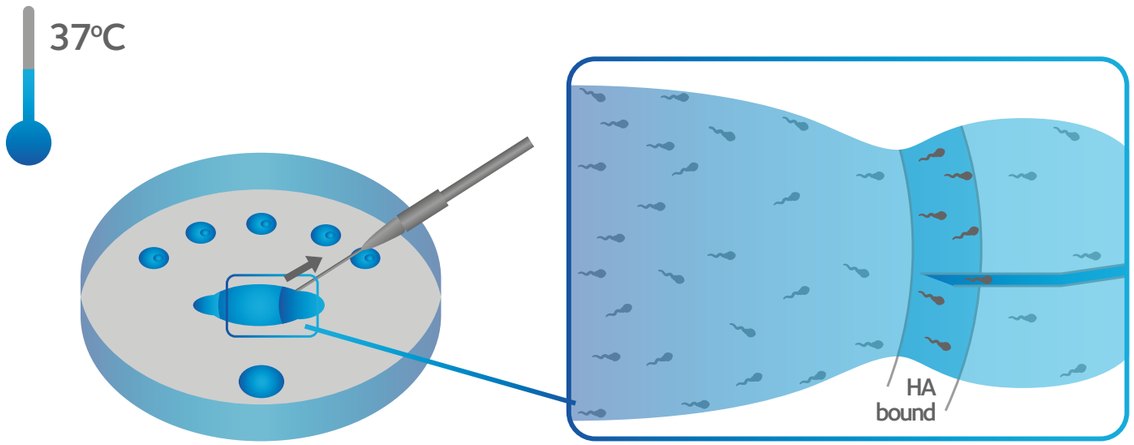

Figure 2: SpermSlow™ medium set-up: sperm selection at interface with bridging drop in ICSI dish

Clinical application

The application of physiologic ICSI and SpermSlow was compared in a prospective, randomized trial, yielding comparable clinical results, as might be expected, though physiologic ICSI did take three minutes longer than the SpermSlow procedure.[9] Naturally, ICSI results vary between different peer-reviewed published studies, though the overall trend suggests a benefit of HA-based sperm selection (Table 1).

So far, the largest and most definitive prospective, randomized study is the HABSelect trial which was conducted at 16 assisted reproduction units within the UK.[17] Physiologic ICSI was demonstrated to have a highly significant impact on one of the secondary endpoints of that study, in markedly reducing the miscarriage rate (MR) in patients with advanced age. Considering the strong correlation between HA binding and the genomic integrity of spermatozoa, along with the known relationship between SDF and miscarriage, a reduction in MR by physiologic ICSI is exactly what would be expected. Interestingly, a sub-group analysis of the HABSelect trial indicated that the MR was associated more with female age than with the hyaluronan binding score (HBS).[23] Indeed, a parallel mechanistic analysis of the data from that study showed that physiologic ICSI can mitigate the typical age-related decline in live birth rate observed with ICSI (Figure 3).[24] Furthermore, the mechanistic analysis demonstrated that the effect of physiologic ICSI was connected to sperm DNA quality.

Publication	Study Design	Cycles	Primary Endpoint	Main Findings
Elraouf *et al.*, 2023[10]	Comparative, non-randomized	200	FR, EQ, IR, CPR	Significantly better EQ in teratozoospermic patients with SpermSlow (p=0.030)
Emirdar *et al.*, 2023[11]	Comparative, non-randomized	2815	FR, EQ, MK, BPR, CPR, MR	No significant difference in all endpoints
Erberelli *et al.*, 2017[12]	Comparative, non-randomized	56	FR, CR, CPR, MR	Significantly higher CPR with PICSI (p=0.009)
Hasanen *et al.*, 2020[13]	Prospective randomized (MACS/ICSI vs PICSI)	413	OPR	Trend towards higher IR (p=0.051), CPR (p=0.078) and OPR (p=0.097) in patients 30-35 years of age with PICSI
Kim *et al.*, 2020[14]	Longitudinal, non-randomized	152	FR, EQ	Significantly higher (p<0.001) FR and better EQ with PICSI
Liu *et al.*, 2019[15]	Sibling oocyte split cohort	21	FR, Time-lapse parameters	Significantly lower abnormal FR (p=0.017) with SpermSlow
Majumdar & Majumdar. 2013[16]	Prospective randomized	156	FR, EQ, IR, CPR, LBR, MR	Trend towards a higher MR with ICSI vs PICSI (25% vs 12%, p=0.227)
Miller *et al.*, 2019[17]	Multicenter, parallel, two-group, randomized trial	2752	Full-term LBR	Significantly lower MR with PICSI (p=0.003)
Mokánszki *et al.*, 2014[18]	Comparative, non-randomized to ICSI (HBS >60%) or PICSI (HBS ≤60%)	250	FR, IR, CPR, LBR, MR	Significantly (p<0.05) higher FR (HBS >60%), IR (HBS ≤60%), CPR (HBS >/≤60%), LBR (HBS ≤60%) and lower MR (HBS >/≤60%) with PICSI
Nasr-Esfahani *et al.*, 2008[19]	Sibling oocyte split cohort	50	FR, EQ	Significantly higher FR with HA-selected ICSI (p<0.05)
Novoselsky Persky *et al.*, 2021[20]	Sibling oocyte split cohort	45	FR, EQ	Significantly higher FR (p=0.008) and better EQ (p<0.01) with PICSI
Parmegiani *et al.*, 2010[2]	Prospective randomized (Under Italian law, only three oocytes injected)	232	FR, EQ, IR, CPR	Significantly better EQ with SpermSlow (p=0.046)
Scaruffi *et al.*, 2022[21]	Longitudinal, prospective, non-randomized	205	FR,CR,EQ,IR, CPR, LBR, MR	Significantly higher CR (p=0.026), better EQ (p=0.034), higher CPR (p<0.001) and IR (p<0.0001) with SpermSlow
Worrilow et al., 2013[22]	Multicenter, double-blinded randomized controlled trial	318 (HBS ≤65%)	CPR	Significantly lower MR with PICSI (p=0.016)

BPR: Biochemical Pregnancy Rate; CPR: Clinical Pregnancy Rate; CR: Cleavage Rate; EQ: Embryo Quality; FR: Fertilization Rate; HBS: Hyaluronan Binding Score; IR: Implantation Rate; LBR: Live Birth Rate; MK: Morpho-kinetics; MR: Miscarriage Rate; OPR: Ongoing Pregnancy Rate

Table 1: Studies comparing clinical outcomes between ICSI and HA-selected ICSI

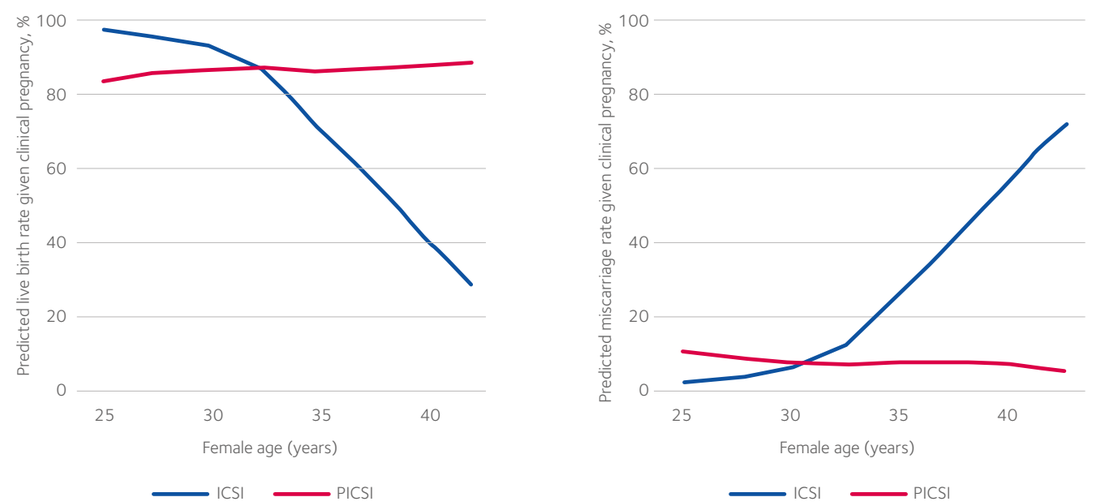

Figure 3: Data aggregated model for predicting ICSI and physiologic ICSI live birth rates in relation to age

Summary

Normal sperm maturation is associated with genomic integrity and formation of binding sites to HA. Methods such as physiologic ICSI and SpermSlow™ medium exploit this association to select sperm with reduced levels of SDF. The incidence of SDF increases with age and the ability of the oocyte to repair damaged sperm DNA following fertilization declines with age. Sperm selection with physiologic ICSI significantly reduces the MR and improves live birth outcomes among older couples.

References

1. Burrello, N. et al. Morphologically normal spermatozoa of patients with secretory oligo- astheno-teratozoospermia have an increased aneuploidy rate. Hum Reprod. 2004; 19: 2298- 2302.
2. Parmegiani, L. et al. "Physiologic ICSI": hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. Fertil Steril. 2010; 93: 598-604.
3. Salustri, A. et al. Hyaluronan and proteoglycans in ovarian follicles. Hum Reprod Update. 1999; 5: 293-301.
4. Cayli, S. et al. Biochemical markers of sperm function: male fertility and sperm selection for ICSI. Reprod Biomed Online. 2003; 7: 462-468.
5. Fouladi-Nashta, AA. et al. Regulation and roles of the hyaluronan system in mammalian reproduction. Reproduction. 2017; 153: R43-R58.
6. Huszar, G. et al. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril. 2003; 79(Suppl 3): 1616-1624.
7. Jakab, A. et al. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertil Steril. 2005; 84: 1665-1673.
8. Sakkas, D. & Alvarez, JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010; 93: 1027-1036.
9. Parmegiani, L. et al. Comparison of two ready-to-use systems designed for sperm-hyaluronic acid binding selection before intracytoplasmic sperm injection: PICSI vs. Sperm Slow: a prospective, randomized trial. Fertil Steril. 2012; 98: 632-637.
10. Elraouf, AA. et al. Comparative study between SpermSlow™ hyaluronan and traditional sperm selection in ICSI outcome. Zygote. 2023; 31: 180-187.
11. Emirdar, V. et al. Influence of a hyaluronan-binding system for sperm selection in intracytoplasmic sperm injection cycles on embryo morphokinetic parameters and in vitro fertilization cycle outcomes. Arch Gynecol Obstet. 2023 (in press).
12. Erberelli, RF. et al. Hyaluronan-binding system for sperm selection enhances pregnancy rates in ICSI cycles associated with male factor infertility. JBRA Assist Reprod. 2017; 21: 2-6.
13. Hasanen, E. et al. PICSI vs. MACS for abnormal sperm DNA fragmentation ICSI cases: a prospective randomized trial. J Assist Reprod Genet. 2020; 37: 2605-2613.
14. Kim, SJ. et al., Effect of sperm selection using hyaluronan on fertilization and quality of cleavage-stage embryos in intracytoplasmic sperm injection (ICSI) cycles of couples with severe teratozoospermia. Gynecol Endocrinol. 2020; 36: 456-459.
15. Liu, Y. et al. Intracytoplasmic sperm injection using hyaluronic acid or polyvinylpyrrolidone: a time-lapse sibling oocyte study. Hum Fertil. 2019; 22: 39-45.
16. Majumdar, G. & Majumdar, A. A prospective randomized study to evaluate the effect of hyaluronic acid sperm selection on the intracytoplasmic sperm injection outcome of patientswith unexplained infertility having normal semen parameters. J Assist Reprod Genet. 2013; 30: 1471-1475.
17. Miller, D. et al. Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. Lancet. 2019; 393: 416-422.
18. Mokánszki, A. et al. Is sperm hyaluronic acid binding ability predictive for clinical success of intracytoplasmic sperm injection: PICSI vs. ICSI? Syst Biol Reprod Med. 2014; 60: 348-354.
19. Nasr-Esfahani, MH. et al. Evaluation of sperm selection procedure based upon hyaluronic acid binding ability on ICSI outcome. J Assist Reprod Genet. 2008; 25: 197-203.
20. Novoselsky Persky, M. et al. Conventional ICSI vs. physiological selection of spermatozoa for ICSI (picsi) in sibling oocytes. Andrology. 2021; 9: 873-877.
21. Scaruffi, P. et al. Hyaluronic acid-sperm selection significantly improves the clinical outcome of couples with previous ICSI cycles failure. Andrology. 2022; 10: 677-685.
22. Worrilow, KC. et al. Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes—multicenter, double-blinded and randomized controlled trial. Hum Reprod. 2013; 28: 306-314.
23. Kirkman-Brown, J. et al. Sperm selection for assisted reproduction by prior hyaluronan binding: the HABSelect RCT. Efficacy and Mechanistic Evaluation. 2019; 6: 1-110.
24. West, R. et al. Sperm selection with hyaluronic acid improved live birth outcomes among older couples and was connected to sperm DNA quality, potentially affecting all treatment outcomes. Hum Reprod. 2022; 37: 1106-1125.

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