Hyaluronic acid binding ability of human sperm reflects cellular maturity and fertilizing potential: selection of sperm for intracytoplasmic sperm injection

Gabor Huszar, Sinan Ozkavukcu, Attila Jakab, Ciler Celik-Ozenci, G. Leyla Sati and Sevil Cayli

Purpose of review

The current concepts of sperm biochemical markers and the central role of the HspA2 chaperone protein, a measure of sperm cellular maturity and fertilizing potential, are reviewed.

Recent findings

Because HspA2 is a component of the synaptonemal complex, low HspA2 levels and increased frequency of chromosomal aneuploidies are related in diminished maturity sperm. We also suggest a relationship between HspA2 expression in elongating spermatids and events of late spermiogenesis, such as cytoplasmic extrusion and plasma membrane remodeling that aid the formation of the zona pellucida binding and hyaluronic acid binding sites. The presence of hyaluronic acid receptor on the plasma membrane of mature sperm, coupled with hyaluronic acid coated glass or plastic surfaces, facilitates testing of sperm function and selection of single mature sperm for intracytoplasmic sperm injection. The frequencies of sperm with chromosomal disomy are reduced approximately fourfold to fivefold in hyaluronic acid selected sperm compared with semen sperm, comparable to the increase in such abnormalities in intracytoplasmic sperm injection offspring. Hyaluronic acid binding also excludes immature sperm with cytoplasmic extrusion, persistent histones, and DNA chain breaks.

Summary

Hyaluronic acid mediated sperm selection is a novel technique that is comparable to sperm zona pellucida binding. Hyaluronic acid selected sperm will also alleviate the risks related to intracytoplasmic sperm injection fertilization with sperm of diminished maturity that currently cause worldwide concern.

Keywords

chromosomal disomy and diploidy, intracytoplasmic sperm injection, sperm maturation, zona pellucida binding

Curr Opin Obstet Gynecol 18:260–267. © 2006 Lippincott Williams & Wilkins.

The Sperm Physiology Laboratory, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut, USA

Correspondence to Gabor Huszar MD, The Sperm Physiology Laboratory, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA
Tel: +1 203 785 4010; fax: +1 203 737 1200; e-mail: gabor.huszar@yale.edu

Sponsorship: This study was supported by the US National Institute of Health (HD-19505, HD-32902, OH-04061)

Current Opinion in Obstetrics and Gynecology 2006, 18:260–267

Abbreviations

FISH	fluorescence in-situ hybridization
HTF	human tubal fluid
ICSI	intracytoplasmic sperm injection

1040-872X

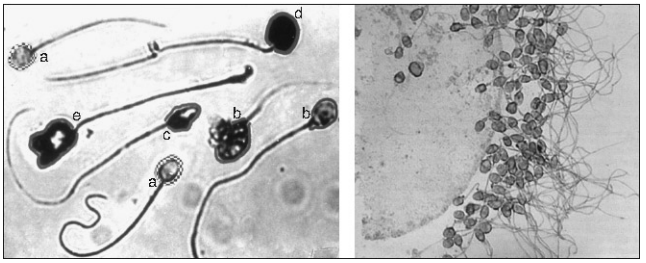
Introduction

The primary interest of our laboratory has been the development of objective biochemical markers of human sperm maturity and function that would predict male fertility, independently from the traditional semen criteria of sperm concentration and motility. In measurements of sperm creatine-*N*-phosphotransferase or creatine kinase, we found significantly higher sperm creatine kinase content in men with diminished fertility [1,2]. The sperm creatine kinase immunostaining patterns indicated (Fig. 1, left panel) that the high sperm creatine kinase activity was a direct consequence of increased cytoplasmic protein and creatine kinase concentrations in the spermatozoon [3]. This suggested to us that we had identified a sperm developmental defect in the last phase of spermiogenesis when the surplus cytoplasm (unnecessary for mature sperm) is normally extruded and left in the adluminal area as 'residual bodies' [4].

In addition to the creatine kinase-B isoform, we found another adenosinetriphosphate-containing protein, which was proportional to the incidence of mature sperm, characterized by lack of cytoplasmic retention [5]. We have identified this developmentally regulated protein as the 70-kDa testis expressed chaperone protein, which in man is called HspA2 [6].

We have further shown that mature and diminished maturity sperm are different with respect to HspA2 levels, as expressed by the concentrations of sperm creatine kinase and HspA2 [%HspA2/(HspA2 + creatine kinase-B)], morphological and morphometric attributes, zona pellucida binding properties, and fertility [7,8]. Furthermore, we have established that in spermiogenesis, simultaneously with cytoplasmic extrusion and the commencement of HspA2 synthesis, the sperm plasma

Figure 1 Creatine kinase-immunostained sperm patterns



Left panel: mature (a) and diminished maturity sperm with various degrees of cytoplasmic retention (b–e) after creatine kinase immunostaining. The interrupted and continuous contours represent the presence or lack of plasma membrane remodeling in the mature and immature sperm, respectively. Right panel: creatine kinase immunostained sperm–hemizona complex. Observe that only the clear-headed mature spermatozoa without cytoplasmic retention are able to bind. Reproduced from Reproduction BioMedicine Online by Cayli *et al.* [2] with permission from Reproductive Healthcare Ltd.

membrane also undergoes a maturation-related remodeling. This remodeling step facilitates the formation of the sites for zona pellucida binding and, very importantly from the point of view of andrology testing and intracytoplasmic sperm injection (ICSI) sperm selection, for hyaluronic acid binding in mature sperm [9]. All of these maturational events are completed by the time the sperm enter the caput epididymidis [10].

Spermiogenetic maturation and sperm fertilizing function

The predictive value of sperm HspA2 levels in the assessment of male fertility was tested in two blinded studies of couples undergoing in-vitro fertilization (IVF). First in 1992, we classified 84 husbands from two different IVF centers (with no information on their semen parameters or reproductive histories) based only on their sperm HspA2 ratios into 'high likelihood' (>10% HspA2 ratio) and 'low likelihood' (<10% HspA2 ratio) for fertility groups [8]. All pregnancies occurred in the 'high-likelihood' group. No pregnancy occurred in the 'low-likelihood' group. In the 'high-likelihood' group, if at least one oocyte was fertilized, the predictive rate of HspA2 ratio for pregnancy was 30.4% per cycle. An additional important utility of the HspA2 ratio became apparent: nine of the 22 'low-likelihood' men were normospermic but had diminished fertility. Thus, the HspA2 ratio provided, for the first time, a diagnostic tool for unexplained male infertility (infertile men with normal semen) [8]. In a recent second study [11], we reexamined the utility of HspA2 ratios in 194 couples treated at Yale. The receiver operating characteristic analysis indicated a 100% predictive value for failure to achieve pregnancy below the 10.4% HspA2 threshold.

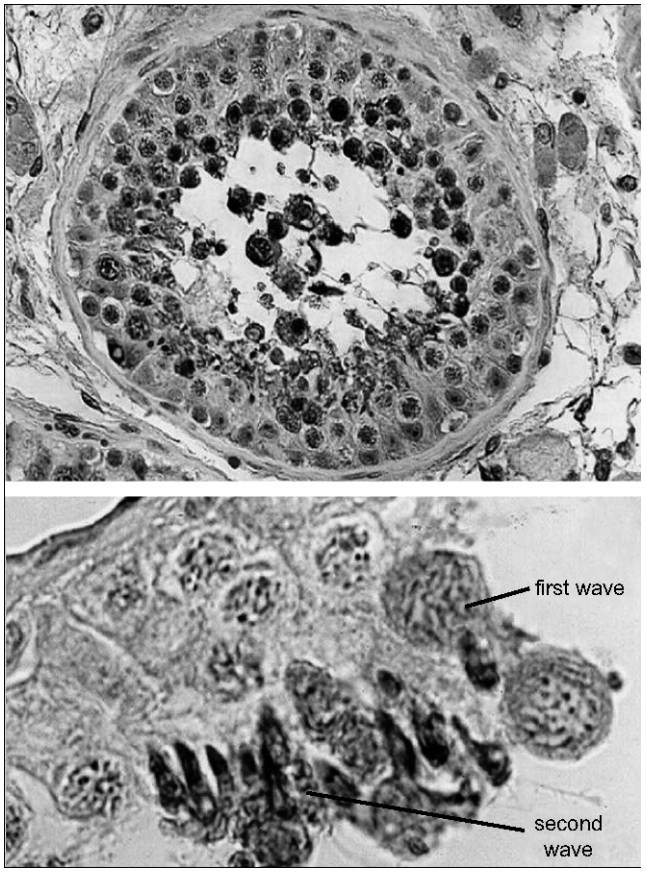
To identify the steps of the fertilization process at which the low HspA2 immature sperm are deficient, we explored human sperm–oocyte binding. With the study of sperm–hemizona complexes, we established that only the clear-headed (low creatine kinase) mature sperm were able to bind to the zona (Fig. 1, right panel) [7]. Sperm with retained cytoplasm were deficient in the oocyte binding site, the formation of which may occur with plasma membrane remodeling simultaneously with cytoplasmic extrusion [9].

From the perspective of male infertility, it is important that synthesis of the HspA2 and HSP70 family of proteins is developmentally regulated and that they first appear during the meiotic prophase as a component of the synaptonemal complexes [12]. An apparent function of HSP70-2 in mice is maintaining the synaptonemal complex and assisting chromosome cross-over during meiosis and spermatocyte development. Accordingly, the targeted disruption of the *hsp70-2* gene causes arrested sperm maturation and azoospermia [13]. Regarding human sperm, our laboratory was the first to demonstrate the expression pattern of the HspA2 protein in human testis and sperm and to correlate the expression level of HspA2 to sperm function [6]. Figure 2 clearly demonstrates the two-wave expression of HspA2, first in spermatocytes related to meiosis, and then at the time of terminal spermiogenesis in elongated spermatids [2,6].

Relationship between diminished sperm maturity and chromosomal aneuploidies

Assuming that HspA2 is a component of the synaptonemal complex in man as well as in rodents, we hypothesized that the frequency of chromosomal aneuploidies will be higher in immature compared with mature sperm [14]. We have examined this question in mature and diminished maturity sperm fractionated from the same ejaculate in 10 oligozoospermic men. Immature sperm with retained cytoplasm, which signifies spermiogenetic arrest, were identified by immunocytochemistry. We have evaluated with fluorescence in-situ hybridization (FISH) approximately 7000 sperm nuclei in each of the 20 fractions (142 086 sperm in all) using centromeric probes for the X, Y, and 17 chromosomes. Indeed, there was a close correlation between the incidences of immature sperm with cytoplasmic retention and disomies ($r = 0.7$ with all three chromosomes, and $r = 0.76$ in the case of the Y disomy, $P < 0.001$ in both), indicating that disomies originate primarily in immature sperm. Thus, the idea that the common factor underlying sperm immaturity and aneuploidies is the diminished expression of the HspA2 is valid [14]. There was no relationship with diploidies,

Figure 2 Human testicular biopsy tissues immunostained with HspA2 antiserum



The upper and lower sections represent low and high magnifications to illustrate the tubular structure of testis and the staining pattern of the adluminal area. The first wave of HspA2 expression occurs in meiotic spermatocytes. The second wave is predominant during terminal spermiogenesis in the elongated spermatids and spermatozoa at the edge of the seminiferous tubuli. Reproduced from Reproduction BioMedicine Online by Cayli *et al.* [2] with permission from Reproductive Healthcare Ltd.

however ($r < 0.1$). Thus, chromosomal diploidy is likely to arise by diverse cellular mechanisms [15].

Sperm-head shape or dimensions do not aid the selection of mature sperm for intracytoplasmic sperm injection

The relationship between abnormal sperm morphology and chromosomal aberrations has been of long-term interest. In earlier studies, however, the proportion of abnormal sperm and the frequency of aneuploidies were determined in different sperm cells in the same ejaculate [16,17]. The study of the direct relationship between sperm shape and numerical chromosomal aneuploidies in the same sperm was made possible because we determined that sperm preserve their shape after undergoing the decondensation and denaturation steps that are a prerequisite for performing FISH [18].

We examined the relationship between numerical chromosomal aberrations and sperm shape, as well as the applicability of such data to sperm selection for ICSI [19]. In order to accomplish this goal, we studied the post-FISH status of sperm whether haploid, disomic, or diploid, and also evaluated the shape and dimensions of images in a selected sperm population that had much higher proportions of disomic and diploid spermatozoa than occur physiologically.

First, using computer-assisted morphometry, we evaluated 1286 individual sperm from 15 men: 900 haploid, 256 disomic and 130 diploid sperm, using centromeric FISH probes for the X, Y, 10, 11, and 17 chromosomes. We studied normal, disomic, and diploid genotypes in sperm images utilizing three-color FISH (17, X, and Y) and two-color FISH for the 10 and 11 chromosomes (60 sperm from each men; 30 sperm with X, Y, 17 chromosomes, and 30 sperm with 10, 11 chromosomes).

In another approach, we sorted the 900 haploid sperm and classified them into 'small-head', 'intermediate-head', and 'large-head' groups. Furthermore, we sorted the 256 disomic and 130 diploid sperm according to the head-size parameter ranges established in the three non-aneuploid sperm dimension ranges, and determined the frequencies of disomies and diploidies within the three head-size groups.

Disomies and diploidies were present within all three groups. The frequency of chromosomal aberrations positively correlated with sperm-head size, as size reflects cytoplasmic retention and immaturity. The mean percentages of disomies in small, intermediate, and large sperm-head categories were 27 ± 2%, 23 ± 1%, and 50 ± 2%, respectively. Moreover, the mean percentages of diploidies in the three sperm-head categories were 3 ± 1%, 8 ± 1%, and 89 ± 2%, respectively.

When we asked the question, 'How many of the disomic or diploidic sperm will fall within the smallest third of the 900 nonaneuploid sperm', the 'most normal' sperm category, we have shown that sperm of any head size or shape may have chromosomal aberrations. Furthermore, about 27% of sperm with disomy and 3% with diploidy of the 386 sperm selected for this analysis were among the 300 sperm with smallest dimensions.

In another analysis, we classified the same 1286 sperm according to their shape characteristics of normal ($n = 367$), intermediate ($n = 368$), abnormal ($n = 504$), and amorphous ($n = 47$). Disomic and diploid sperm

were present in all four groups with an increasing frequency of 18%, 18%, 41%, and 98%, respectively, in line with the severity of the sperm-shape abnormality, which was most apparent in the abnormal and amorphous sperm-shape categories [19].

Finally, we classified the 1286 spermatozoa according to the Kruger strict morphology method as normal and abnormal. The normal strict morphology scores of the haploid ($n = 900$), disomic ($n = 256$), and diploid ($n = 130$) sperm were 24%, 10%, and 1%, respectively. These values are also in accordance with the morphometric results, which indicate that the haploid, disomic, and diploid sperm are different from each other, not only in genetic or morphometric aspects but also in morphology.

It has also become clear that the strict morphology evaluation is not discriminatory with respect to the identification of haploid spermatozoa.

Test of sperm maturity and function by hyaluronic acid binding in the andrology and in-vitro fertilization laboratories

We recognized the fact that plasma membrane remodeling facilitates the formation of the zona pellucida binding and hyaluronic acid binding sites [9]. Concurrently with the sperm maturation studies, we investigated the effects of hyaluronic acid or hyaluronan, which is a linear repeating polymer of disaccharides, on human sperm function. Hyaluronic acid in the medium increased the velocity, retention of motility, and viability of freshly ejaculated, as well as cryopreserved/thawed, mature human spermatozoa [20,21]. The enhancement of sperm motility and velocity occurred as a direct response to hyaluronic acid, as indicated by two observations:

(1) There was an instantaneous increase in sperm tail cross-beat frequency and sperm velocity upon hyaluronic acid exposure.
(2) When we transferred the hyaluronic acid exposed sperm, after density gradient centrifugation, to a regular medium, the motility and velocity properties returned to those of the control sperm. We concluded that hyaluronic acid effects on sperm are receptor-mediated. Indeed, the presence of the hyaluronic acid receptor in human sperm has been established by three laboratories [22–24].

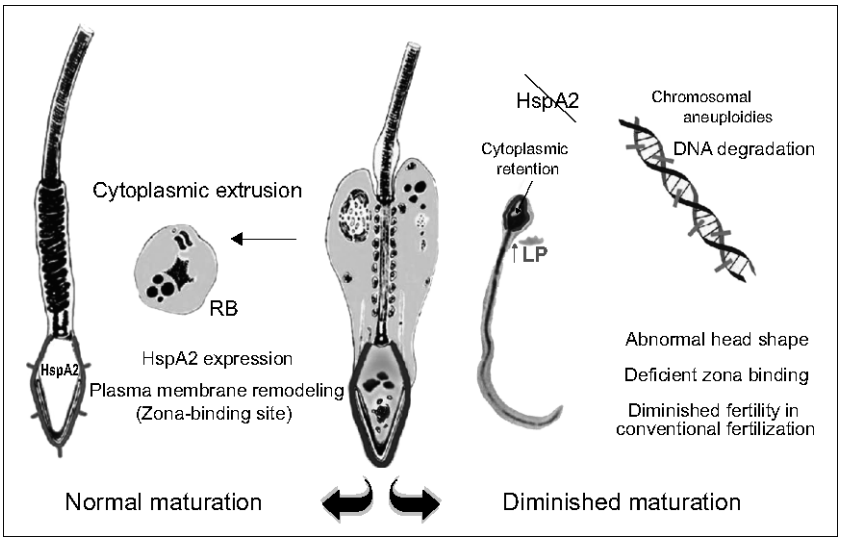
In immature sperm with cytoplasmic retention, there are low densities of zona binding sites and also of hyaluronic acid receptors [6,7,20,25].

Our current ideas on sperm maturation in men are summarized in Fig. 3.

As a result of the association between the presence of plasma membrane hyaluronic acid receptors and the various upstream features of sperm maturation, we were interested in developing the sperm hyaluronic acid binding assay to a clinical andrology test, as well a device for selection of mature sperm for ICSI [25]. We hypothesized firstly that mature sperm would selectively bind to solid-state hyaluronic acid. This assumption has recently been proven by studies using the various cyto-

Figure 3 A model of normal and diminished maturation of human sperm

In normal sperm maturation (events toward the left direction), HspA2 is expressed in the synaptonemal complex of spermatocytes, supporting meiosis. HspA2 is probably also involved in the processes of late spermiogenesis, such as cytoplasmic extrusion (represented by the loss of residual body, RB), plasma membrane remodeling, and the formation of the zona pellucida binding and hyaluronic acid binding (the binding sites are represented by the stubs). Diminished maturity sperm lack HspA2 expression (right direction), which causes meiotic defects and a higher rate of retention of creatine kinase and other cytoplasmic enzymes, increased levels of lipid peroxidation (LP) and consequent DNA fragmentation, abnormal sperm morphology and deficiency in zona binding and hyaluronic acid binding. Reproduced with permission from [6].



plasmic and nuclear biochemical markers: hyaluronic acid bound sperm are devoid of cytoplasmic retention, persistent histones and caspase-3, which signifies an ongoing apoptotic process [26]. All sperm bound to hyaluronic acid are viable; nonviable sperm lose their hyaluronic acid binding ability. Secondly, diminished maturity spermatozoa, having low HspA2 ratios, chromosomal aberrations, and lack of spermatogenetic membrane remodeling will not bind to solid-state hyaluronic acid; thus hyaluronic acid binding would facilitate the selection of individual mature sperm with low levels of chromosomal aneuploidies.

Further details that may modulate hyaluronic acid binding of human sperm are related to the fact that sperm, similar to zona pellucida binding, attach with the head plasma membrane aspects. Thus, sperm that have proceeded to advanced stages of capacitation or acrosome reaction do not bind to hyaluronic acid [26]. Another theoretical issue is the presence of hyaluronidase in sperm. This does not appear likely to affect hyaluronic acid binding because hyaluronidase is located mostly in the post-acrosomal membrane (and acrosome-reacted sperm that are devoid of plasma membrane are not the subject of the binding assay), and the hyaluronic acid is immobilized to the glass or plastic surfaces as a linear polymer, thus cleavage of the polysaccharide backbone would not affect the binding template properties of hyaluronic acid.

A chamber device for the sperm hyaluronic acid binding assay

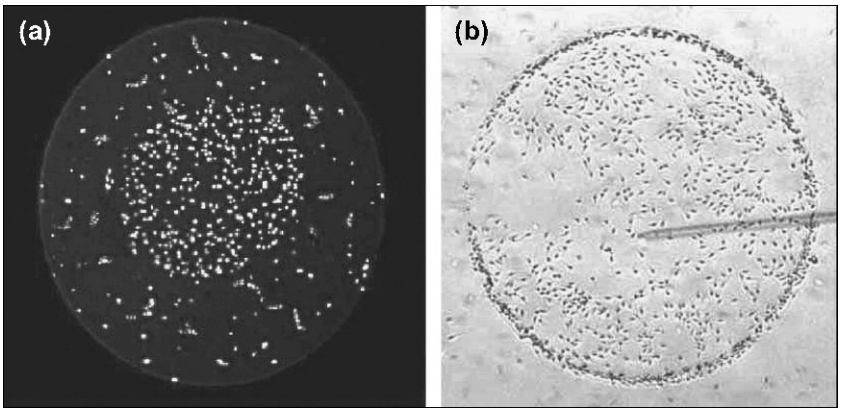
Based on the concepts of Fig. 3, we have examined whether, via the hyaluronic acid receptors, sperm would permanently bind to solid-state hyaluronic acid using a chamber device that is coated with hyaluronic acid in order to test the proportion of mature sperm exhibiting hyaluronic acid binding (Fig. 4). Indeed, sperm bind to hyaluronic acid and three sperm populations exist:

(1) sperm that bind permanently to hyaluronic acid;
(2) sperm that exhibit no binding;
(3) a small proportion of sperm (<5%) that initially bind to hyaluronic acid but are soon released, and then rebind.

We interpreted these three patterns as mature sperm with a high density of hyaluronic acid receptors, immature sperm with deficient maturity and plasma membrane remodeling, and sperm of intermediate maturity with a low density of hyaluronic acid receptors. It is of note that the hyaluronic acid binding assay has been approved by the US Food and Drug Administration for andrology testing.

We have shown that sperm hyaluronic acid binding has diagnostic utility. In a study, we evaluated the percentage binding of sperm to hyaluronic acid coated slides in 56 men. With respect to binding, we classified the sperm populations as follows: greater than 85% ($n = 32$), excellent binding, these men do not require intervention; binding between 65% and 85% ($n = 14$), intermediate, these couples may benefit from intrauterine insemination; diminished sperm binding properties, less than 60–65% ($n = 10$), these men should be retested. If the low binding score is confirmed, they should be treated with IVF or ICSI. In line with our previous findings, binding scores were largely independent from sperm concentrations. Among men within the less than 20 million sperm/ml concentration range ($n = 18$ of 56 men), we identified three excellent, seven moderate, and eight diminished hyaluronic acid binders [27].

Figure 4 Sperm movement patterns on the hyaluronic acid coated spots used for sperm selection



(a) Time-elapsed photography of the sperm movement patterns. Mature sperm that underwent plasma membrane remodeling bind to hyaluronic acid and are immotile, whereas diminished maturity sperm remain motile. (b) Sperm harvested by a micropipette. Reproduced with permission from [32••].

Selection of sperm with low aneuploidy frequencies for intracytoplasmic sperm injection

The third question, whether, due to the relationship between sperm maturity and the meiotic process, sperm with low levels of chromosomal aberrations would preferentially bind to hyaluronic acid, is addressed in the experiments below.

The development of this novel sperm selection method using hyaluronic acid binding, is based on the recognition that, during spermatogenesis, the formation of the zona pellucida binding and hyaluronic acid binding sites are commonly regulated. Indeed, we have found a close correlation ($r = 0.73$, $P < 0.001$, $n = 54$) between sperm binding scores either to hyaluronic acid or to the zona of bisected human oocytes [28]. Thus, hyaluronic acid selected mature sperm have frequencies of chromosomal aberrations comparable to those of sperm selected by the zona pellucida in conventional fertilization. This relationship is based on the dual role of the HspA2 chaperone, which supports meiosis as a component of the synaptonemal complex, and facilitates plasma membrane remodeling as well as the formation of the zona pellucida binding and hyaluronic acid binding sites during spermiogenesis [6].

Based on the association between sperm maturation and plasma membrane remodeling, we made the hypothesis that the presence of the hyaluronic acid receptor in mature, but not in immature, sperm, and a respective device with a hyaluronic acid coated surface will facilitate the selection of single mature sperm with high DNA integrity and low frequencies of chromosomal aneuploidies for ICSI [9,25,29–31]. As hyaluronic acid is a normally occurring component of the female reproductive tract, there should be no ethical concerns.

Hyaluronan-mediated sperm selection for intracytoplasmic sperm injection

In these experiments, we used as sperm selection platforms Falcon Petri dishes that have spots of immobilized bacterial hyaluronic acid that were prepared using proprietary coating technology (Biocoat Inc., Fort Washington, Pennsylvania, USA). The sperm–hyaluronic acid binding assay slides were based on Cell-Vu (Millennium Sciences Inc., New York, USA) disposable glass sperm counting chambers that were treated with a bilaminar hyaluronan coating.

We have tested the efficiency of sperm selection with respect to elimination of sperm with chromosomal aneuploidies and diploidies [32••]. Washed sperm from 34 moderately oligospermic men were studied. After incubation for 15 min, the hyaluronic acid attached sperm were collected using an ICSI micropipette. Both hyaluronic acid selected and unselected sperm were treated with FISH, using centrometric probes for the X, Y, and 17 chromosomes. Data were analyzed by chi-squared analysis.

Experiments 1 and 2

Washed sperm were prepared by dilution of semen with 3–5 volumes of human tubal fluid (HTF, Irvine Scientific Co., Irvine, California, USA)–0.5% bovine serum albumin. The diluted semen was centrifuged at 1200*g* for 15 min at room temperature. The sperm pellet was resuspended in 0.5 ml HTF to approximately 30 million sperm/ml. In the second experiment, the sperm suspension was also subjected to centrifugation on a discontinuous 45%/90% isolate gradient.

Using the Falcon Petri dishes with an immobilized hyaluronic acid spot, a drop of sperm suspension was placed close to the edge of the hyaluronic acid spot, and the sperm were allowed to spontaneously migrate. The mature sperm that had completed plasma membrane remodeling bound to the hyaluronic acid, while immature sperm with diminished hyaluronic acid receptor concentrations moved freely over the hyaluronic acid (Fig. 4). The hyaluronic acid bound sperm also exhibited vigorous beating with increased tail cross-beat frequency [20,21]. After 15 min (twice the maximum binding period) [25], the bound sperm were collected with the ICSI micropipette, fixed with methanol–ethanoic acid and subjected to FISH. The control for the selection experiments was always the respective unselected sperm suspension also treated with FISH (Fig. 5).

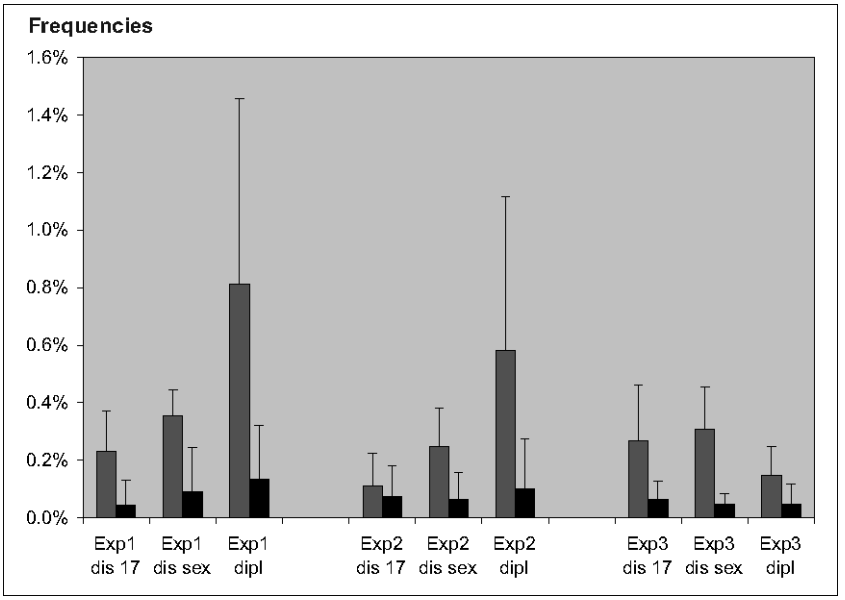
Experiment 3

Drops (5–10 µl) of sperm suspension were placed on hyaluronic acid coated glass slides. After a 5-min hyaluronic acid binding period, the slide was placed at a slight angle and the unbound sperm were eliminated by slowly applying and removing drops of HTF until no free sperm were visible. The hyaluronic acid bound sperm were removed one-by-one by micropipette and placed in a marker circled area wetted with HTF, fixed and subjected to FISH.

From the semen fraction of each man, we analyzed a mean of 4770 sperm, or 162 210 sperm in the 34 men. In the hyaluronic acid bound and micropipette-collected sperm fractions, owing to the burdens of the task, we studied fewer sperm. In the first experiment, we evaluated 7530 sperm (range 224–1142 sperm per man) and in the second experiment 9720 sperm (range 373–1955 sperm per man). In the third experiment of individually selected sperm, we evaluated 24 420 sperm (range 1086–3973 sperm per man).

Figure 5 Disomy and diploidy frequencies in semen sperm and hyaluronic acid selected sperm fractions

In all three experiments, using samples with various sperm concentrations and sperm selection methods, the hyaluronic acid selected sperm fraction showed numerical chromosomal aberrations within the normal range, independent of the original frequency of disomies and diploidies in the various samples. HA, hyaluronic acid. Reproduced with permission from [32••].
■ : Initial; ■ : HA bound.



In the hyaluronic acid bound sperm compared with unselected sperm, with the three probes studied, the chromosomal disomy frequencies were reduced to 0.16% from 0.52%, the diploidy to 0.09% from 0.51%, and the sex chromosome disomy to 0.05% from 0.27% (a 5.4-fold reduction compared with a fourfold respective increase in ICSI offspring).

Our hyaluronic acid–sperm selection method provides a technique for reducing the genetic impact of ICSI fertilization at the traditional evolutionary level by introducing only mature spermatozoa that would have been part of the physiological fertilization pool.

Conclusion

The HspA2 chaperone protein is expressed in two waves. The first wave is in spermatocytes, as HspA2 is a component of the synaptonemal complex and supports the meiotic process. The second wave of HspA2 expression is simultaneous with major sperm protein movements underlying cytoplasmic extrusion and remodeling of the human sperm plasma membrane. We believe that retention of the cytoplasm, and the lack of zona binding and hyaluronic acid binding sites in immature sperm are directly related to the diminished expression of HspA2, and also to the consequential incomplete membrane remodeling.

There is an overall relationship between sperm-shape abnormalities and frequencies of chromosomal aneuploidies in spermatozoa. This relationship is probably based on the common upstream and spermiogenetic events of sperm development. Shape characteristics are, however, not predictive for ploidy in individual spermatozoa. Thus, visual shape assessment, that is, choosing the 'best looking' sperm, is an unreliable method for ICSI sperm selection.

The expected advantages of hyaluronic acid mediated sperm selection in improving ICSI outcome are as follows:

(1) In sperm selected by hyaluronic acid binding, the frequencies of chromosomal disomies and diploidies are in the normal range, independent of the aneuploidy frequencies of the initial semen. In this respect, the sperm selection properties of hyaluronic acid are similar to those of the zona pellucida. The fivefold decline of sex chromosome disomies is consistent with the increase in chromosomal aberrations in ICSI children conceived with visually selected sperm [33].

(2) Mature sperm selected by virtue of hyaluronic acid binding are also viable, devoid of persistent histones and apoptosis, and do not exhibit DNA fragmentation [25,26,33–35]. Thus, the paternal contribution of hyaluronic acid selected sperm will be improved. This should alleviate concerns related to the potential deterioration of individual development and increase in cancer rates following ICSI fertilization [33–35].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 345–346).

1 Huszar G, Corrales M, Vigue L. Correlation between sperm creatine phosphokinase activity and sperm concentrations in normospermic and oligospermic men. Gamete Res 1988; 19:67–75.

2 Cayli S, Jakab A, Ovari L, *et al.* Biochemical markers of sperm function: male fertility and sperm selection for ICSI. Reprod Biomed Online 2003; 7:462–468.

3 Huszar G, Vigue L. Incomplete development of human spermatozoa is associated with increased creatine phosphokinase concentration and abnormal head morphology. Mol Reprod Dev 1993; 34:292–298.

4 Clermont Y. The cycle of the seminiferous epithelium in man. Am J Anat 1963; 112:35–51.

5 Huszar G, Vigue L. Spermatogenesis-related change in the synthesis of the creatine kinase B-type and M-type isoforms in human spermatozoa. Mol Reprod Dev 1990; 25:258–262.

6 Huszar G, Stone K, Dix D, Vigue L. Putative creatine kinase M-isoform in human sperm is identified as the 70-kilodalton heat shock protein HspA2. Biol Reprod 2000; 63:925–932.

7 Huszar G, Vigue L, Oehninger S. Creatine kinase immunocytochemistry of human sperm-hemizona complexes: selective binding of sperm with mature creatine kinase-staining pattern. Fertil Steril 1994; 61:136–142.

8 Huszar G, Vigue L, Morshedi M. Sperm creatine phosphokinase M-isoform ratios and fertilizing potential of men: a blinded study of 84 couples treated with in-vitro fertilization. Fertil Steril 1992; 57:882–888.

9 Huszar G, Sbracia M, Vigue L, *et al.* Sperm plasma membrane remodeling during spermiogenetic maturation in men: relationship among plasma membrane beta 1,4-galactosyltransferase, cytoplasmic creatine phosphokinase, and creatine phosphokinase isoform ratios. Biol Reprod 1997; 56:1020–1024.

10 Huszar G, Patrizio P, Vigue L, *et al.* Cytoplasmic extrusion and the switch from creatine kinase B to M isoform are completed by the commencement of epididymal transport in human and stallion spermatozoa. J Androl 1998; 19:11–20.

11 Ergur AR, Dokras A, Giraldo JR, *et al.* Sperm maturity and treatment choice of IVF or ICSI: Diminished sperm HspA2 chaperone levels predict IVF failure. Fertil Steril 2002; 77:910–918.

12 Allen JW, Dix DJ, Collins BW, *et al.* HSP70-2 is part of the synaptonemal complex in mouse and hamster spermatocytes. Chromosoma 1996; 104: 414–421.

13 Dix DJ, Allen JW, Collins BW, *et al.* Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci USA 1996; 93:3264–3268.

14 Kovanci E, Kovacs T, Moretti E, *et al.* FISH assessment of aneuploidy frequencies in mature and immature human spermatozoa classified by the absence or presence of cytoplasmic retention. Hum Reprod 2001; 16: 1209–1217.

15 Egozcue S, Blanco J, Vidal F, Egozcue J. Diploid sperm and the origin of triploidy. Hum Reprod 2002; 17:5–7.

16 Bernardini L, Borini A, Preti S, *et al.* Study of aneuploidy in normal and abnormal germ cells from semen of fertile and infertile men. Hum Reprod 1998; 13:3406–3413.

17 Templado C, Hoang T, Greene C, *et al.* Aneuploid spermatozoa in infertile men: teratozoospermia. Mol Reprod Dev 2002; 61:200–204.

18 Celik-Ozenci C, Catalanotti J, Jakab A, *et al.* Human sperm maintain their shape following decondensation and denaturation for fluorescent in situ hybridization: shape analysis and objective morphometry. Biol Reprod 2003; 69:1347–1355.

19 Celik-Ozenci C, Jakab A, Kovacs T, *et al.* Sperm selection for ICSI: shape properties do not predict the absence or presence of numerical chromosomal aberrations. Hum Reprod 2004; 19:2052–2059.

20 Huszar G, Willetts M, Corrales M. Hyaluronic acid (Sperm Select) improves retention of sperm motility and velocity in normospermic and oligospermic specimens. Fertil Steril 1990; 54:1127–1134.

21 Sbracia M, Grasso J, Sayme N, *et al.* Hyaluronic acid substantially increases the retention of motility in cryopreserved/thawed human spermatozoa. Hum Reprod 1997; 12:1949–1954.

22 Kornovski BS, McCoshen J, Kredentser J, Turley E. The regulation of sperm motility by a novel hyaluronan receptor. Fertil Steril 1994; 61:935–940.

23 Ranganathan S, Ganguly AK, Datta K. Evidence for presence of hyaluronan binding protein on spermatozoa and its possible involvement in sperm function. Mol Reprod Dev 1994; 38:69–76.

24 Cherr GN, Yudin AI, Li MW, *et al.* Hyaluronic acid and the cumulus extracellular matrix induce increases in intracellular calcium in macaque sperm via the plasma membrane protein PH-20. Zygote 1999; 7:211–222.

25 Huszar G, Ozenci CC, Cayli S, *et al.* Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril 2003; 79 (Suppl 3):1616–1624.

26 Cayli S, Sakkas D, Vigue L, *et al.* Cellular maturity and apoptosis in human sperm: creatine kinase, caspase-3 and Bclx expression in mature and diminished maturity spermatozoa. Mol Hum Reprod 2004; 10:365–372.

27 Huszar G, Celik-Ozenci C, Vigue L. Sperm maturity and fertility: testing by hyaluronic acid binding. ESHRE, Annual Meeting, Vienna, Austria, 2002. Oxford University Press, 2002.

28 Cayli S, Sakkas D, Celik-Ozenci C, *et al.* Hyaluronic acid binding is a test of human sperm maturity and function: correlation between the hyaluronic acid binding and hemizona binding tests. ESHRE, Annual Meeting, Madrid, Spain, 2003. Oxford University Press, 2003.

29 Seli E, Sakkas D. Spermatozoal nuclear determinants of reproductive outcome: implications for ART. Hum Reprod Update 2005; 11:337–349.

30 Sakkas D, Mariethoz E, St John JC. Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway. Exp Cell Res 1999; 251:350–355.

31 Sati L, Ovari L, Demir R, *et al.* Persistent histones in immature sperm are associated with DNA fragmentation and affect paternal contribution of sperm: a study of aniline blue staining, fluorescence in situ hybridization and DNA nick translation. Am Soc Reprod Med, Annual Meeting, Philadelphia, PA, 2004. Oxford University Press, 2004.

32 •• Jakab A, Sakkas D, Delpiano E, *et al.* Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertil Steril 2005; 81:1665–1673.
A demonstration of the benefits of the new ICSI sperm selection method, along with a discussion of the potential medical, genetic, and public health benefits.

33 Van Steirteghem A, Bonduelle M, Devroey P, Liebaers I. Follow-up of children born after ICSI. Hum Reprod Update 2002; 8:111–116.

34 Bonduelle M, Wennerholm UB, Loft A, *et al.* A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in-vitro fertilization and natural conception. Hum Reprod 2005; 20:413–419.

35 Simpson JL, Lamb DJ. Genetic effects of intracytoplasmic sperm injection. Semin Reprod Med 2001; 19:239–249.