Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies

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Objective: To test a newly invented intracytoplasmic sperm injection (ICSI) sperm selection method based on sperm hyaluronic acid (HA) binding.
Design: Comparison of chromosomal disomy and diploidy frequencies in sperm arising from semen and in HA-bound sperm.
Setting: Academic andrology laboratory.
Patient(s): Men presenting for semen analysis.
Intervention(s): Washed sperm fractions of 32 semen samples were applied to Petri dishes or glass slides coated with immobilized HA. The unbound sperm were rinsed gently, and the HA-bound sperm were removed with an ICSI pipette. The control sperm population was the unselected sperm. Both HA-selected and unselected sperm were treated with fluorescence in situ hybridization with centromeric probes for the X, Y, and 17 chromosomes.
Main Outcome Measure(s): Chromosomal disomy and diploidy frequencies.
Result(s): In the HA-bound sperm (495–2,079 per man, 41,670 in all) compared with unselected sperm (4,770 per man, 162,210 in all), the chromosomal disomy frequencies were reduced to 0.16% from 0.52%, diploidy to 0.09% from 0.51%, and sex chromosome disomy to 0.05% from 0.27% (a 5.4-fold reduction vs. 4-fold respective increase in ICSI offspring).
Conclusion(s): The HA sperm selection method for ICSI, which is based on a relationship between sperm receptors for zona pellucida and HA, will likely reduce the potential genetic complications and adverse public health effects of ICSI. (Fertil Steril® 2005;84:1665–73. ©2005 by American Society for Reproductive Medicine.)

Key Words: Sperm maturity, chromosomal aneuploidies, ICSI sperm selection, genetic integrity, paternal contribution

The technological advance of intracytoplasmic sperm injection (ICSI) enabled a single sperm to be introduced into an oocyte (1). Although this approach has provided a unique opportunity for infertile men to father children, serious concerns remain. First, male offspring conceived by ICSI have been linked to increased rates of chromosomal aneuploidies and propagation of disorders related to Y-chromosome deletions (2–4). Second, for the first time in evolution the natural process of sperm–oocyte self-selection is superseded by a random choice of an embryologist. Thus, sperm that have never been part of the fertilizing pool might initiate zygote formation.

In the United States, >30,000 cycles of ICSI are conducted yearly; some centers use ICSI for up to 70% of IVF cycles, to enhance fertilization rates. Proponents of ICSI argue that in the approximately 10,000 ICSI children (who are now up to 10 years old), the rate of congenital malformations does not differ from that of the normal population, although ICSI has been linked to a threefold to fourfold increase in de novo chromosomal aberrations in offspring compared with conventional fertilization (2–6). However, other investigators suggest that ICSI might cause an increase in malformations and adverse developmental effects and pregnancy outcome (7–9). Furthermore, the future public health consequences of ICSI with sperm containing fragmented DNA are as yet unknown with respect to individual physical and mental development, as is the life span or cancer rates of the ICSI offspring. Another concern related to ICSI with diminished-maturity sperm is the possible presence of an abnormal apoptotic process in such sperm (10). However, we have recently shown that hyaluronic acid (HA)-selected sperm are devoid of DNA degradation and of active caspase-3, which is a central component of the apo-

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ptotic process (11). In general, it seems desirable to keep 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. In this report, we describe a scientifically based, noninvasive ICSI selection strategy for mature spermatozoa that show a normal frequency of chromosomal aneuploidies and are devoid of apoptosis (11).

The research leading to this sperm selection method is related to our earlier search for objective biochemical markers of human sperm maturity and function. In measurements of the sperm enzyme, creatine-N-phosphokinase, we have found substantially elevated cytoplasmic content in men with diminished fertility. Using various experimental approaches, we confirmed that diminished sperm fertility is associated with the retention of the surplus cytoplasm that is extruded from elongating spermatids in the course of normal sperm development (12). Furthermore, we demonstrated that only mature sperm without cytoplasmic retention were able to bind to the zona pellucida of oocytes (13). This finding led us to hypothesize that the sperm plasma membrane undergoes a maturation-related remodeling. We established that this concept is correct on the basis of tight correlations between cytoplasmic and membrane-specific biochemical markers (14). This remodeling step facilitates the formation of the sperm binding sites for the zona pellucida of oocytes and for another membrane feature that is the fundamental element of our ICSI sperm selection method, the binding site(s) for HA (15–20). Indeed, immature sperm that fail to undergo membrane remodeling are unable to bind to immobilized HA, as is the case with immature sperm that fail to bind to the zona pellucida (13, 20).

We have also identified another developmentally related protein in human sperm, the 70-kd testis-expressed chaperone, HspA2 (21). This chaperone protein is expressed in spermatocytes as HspA2, which is similar to the homologous chaperone protein hsp70-2 in mice, and is part of the synaptonemal complex that directs and supports the meiotic process (22, 23). In view of the key role of HspA2 in meiosis, we hypothesized that the frequency of chromosomal aneuploidies will be higher in immature sperm (characterized by low HspA2 levels and increased cytoplasmic retention) compared with mature sperm (24, 25). We have examined this by fluorescence in situ hybridization (FISH) in 10 men by evaluating approximately 14,000 sperm nuclei in each subject (142,086 sperm in all), using centromeric probes for the X, Y, and 17 chromosomes. The proportions of immature sperm and frequency of chromosomal disomies were closely related ($r = 0.7$, $P < .001$, or $r = 0.78$, $P < .001$ for Y disomy), indicating that disomies originate primarily in immature sperm. This finding also suggested that selection of mature sperm might alleviate the various genetic concerns and the ICSI-related increase of sex chromosome aberrations (24).

These experiments provided insight regarding the relationship between diminished sperm maturation (often associated with oligozoospermia), low levels of HspA2 expression, increased frequencies of chromosomal aneuploidies, presence of the apoptotic process, and fragmented DNA (10–13, 20, 21). Several other studies reported an overall relationship between oligozoospermia and increased frequencies of chromosomal aberrations, particularly those involving the sex chromosomes, that are propagated in the ICSI offspring (26–34).

At present, sperm selection by the embryologist depends on finding "the best-looking sperm" by eye or by nuclear features detected by specialized microscopy (35). In addressing the validity of this concept, we first demonstrated that sperm retain their original shape after the steps of decondensation and denaturation (36). Furthermore, we have shown that sperm shape does not predict the presence or absence of chromosomal aneuploidies. Thus, sperm shape is an invalid parameter for selection of mature sperm without chromosomal aberrations for ICSI (37).

Recognizing the association between the presence of plasma membrane HA receptors and the various upstream features of sperm maturity, we hypothesized that [1] mature sperm would selectively bind to solid-state HA; this assumption was recently proven by assay of the various cytoplasmic and nuclear biochemical markers in HA-bound spermatozoa (20), [2] diminished-maturity spermatozoa, which have low levels of HspA2, increased levels of chromosomal aberrations, and which have failed to undergo spermatogenetic membrane remodeling, will not bind to solid-state HA, and [3] HA binding would facilitate the selection of individual mature sperm with low levels of chromosomal aneuploidies.

MATERIALS AND METHODS

Patient Population and Experimental Design

The study subjects were men who presented for semen analysis at the Sperm Physiology Laboratory, Department of Obstetrics, Gynecology and Reproductive Sciences at Yale University School of Medicine. Semen was collected by masturbation after 2 days of abstinence.

The studies are composed of three HA sperm selection experiments. In the first and second experiments, we studied samples of 12 borderline oligospermic men and 12 samples in the high normospermic range that were further enhanced in mature sperm by gradient centrifugation. Collection of 20–60 HA-bound sperm occurred by a sweeping and suction motion of the ICSI micropipette. In the third experiment, using samples from 10 oligospermic men, we collected the HA-bound sperm one by one.

All studies were approved by the Yale Human Investigation Committee.

Preparation of HA-Coated Slides and Petri Dishes for Sperm Selection

The sperm selection platforms, Falcon Petri dishes or glass laboratory slides [sperm-hyaluronic acid binding test (HBA);

Biocoat Inc., Fort Washington, PA] were coated with immobilized bacterial HA (Biocoat), with proprietary coatings technology. The HBA slides are modified disposable glass sperm counting chambers (Cell-Vu; Millenium Sciences, New York, NY) that have been treated with a bilaminar hyaluronan coating. The coating consists of hyaluronan grafted to a base coat, cross-linked with a polyfunctional isocyanate. Total coating depth is <1 μm.

Sperm Preparation and HA Sperm Selection

Experiments 1 and 2. Washed sperm were prepared by dilution of semen with three to five volumes of human tubal fluid–0.5% bovine serum albumin (HTF; Irvine Scientific, Irvine CA.). The diluted semen was centrifuged at 1,200 $\times$ *g* for 15 minutes at room temperature. The sperm pellet was resuspended in 0.5 mL HTF to approximately 30 $\times$ 10^6 sperm per milliliter. In the second experiment, the sperm suspension was also subjected to centrifugation on a discontinuous 45%/90% Isolate gradient (Irvine Scientific, Santa Ana, CA).

In experiments 1 and 2, Falcon Petri dishes with an immobilized HA spot were used. A drop of sperm suspension was placed close to the edge of the HA spot, and the sperm were allowed to spontaneously migrate. The mature sperm that completed plasma membrane remodeling bound to the HA, whereas immature sperm with diminished HA receptor concentrations moved freely over the HA (Fig. 1). The HA-bound sperm also exhibited a vigorous beating, with increased tail cross-beat frequency (15, 16).

After 15 minutes (twice the maximum binding period) (20), the bound sperm were collected, fixed with methanol-acetic acid, and subjected to FISH. The control for the selection experiments was always the respective unselected sperm suspension, also treated with FISH.

Experiment 3. Semen was diluted with one volume of HTF, and the sperm was purified with a single phase 45% Isolate gradient at 1,500 $\times$ *g* for 15 minutes. The residual sperm pellet was washed with 2.0 mL HTF at 800 $\times$ *g* for 15 minutes, and the sperm were resuspended in HTF to a concentration of approximately 20 $\times 10^6$/mL, and 5–10-μL drops of sperm suspension were placed on HA-coated glass slides. After a 5-minute HA binding period, the slide was placed on a slight angle, and the unbound sperm was eliminated by slowly applying drops of HTF until no free sperm were visible. The HA-bound sperm were removed one by one and placed in a PAP-pen–circled area wetted with HTF, fixed, and the HA selected sperm were subjected to FISH.

FISH Procedures

Preparation of sperm nuclei, decondensation, denaturation of the nuclei, application of the centromeric DNA probes for chromosomes X, Y, and 17, in situ hybridization, scoring criteria, and data collection were all carried out as previously described (24, 36–37).

Statistical Analysis

Statistical analyses were performed with commercial software (SigmaStat 2.0; Jandel Corporation, San Rafael, CA). Differences in disomy and diploidy frequencies in the semen and HA-bound sperm fractions were analyzed with chi-square analysis of contingency tables. All *P* values are given in Table 1. All data are mean ± SEM.

FIGURE 1

(**A**) Sperm approach from the periphery and then bind to the HA-spot. (**B**) Sperm being picked up with the ICSI pipette.

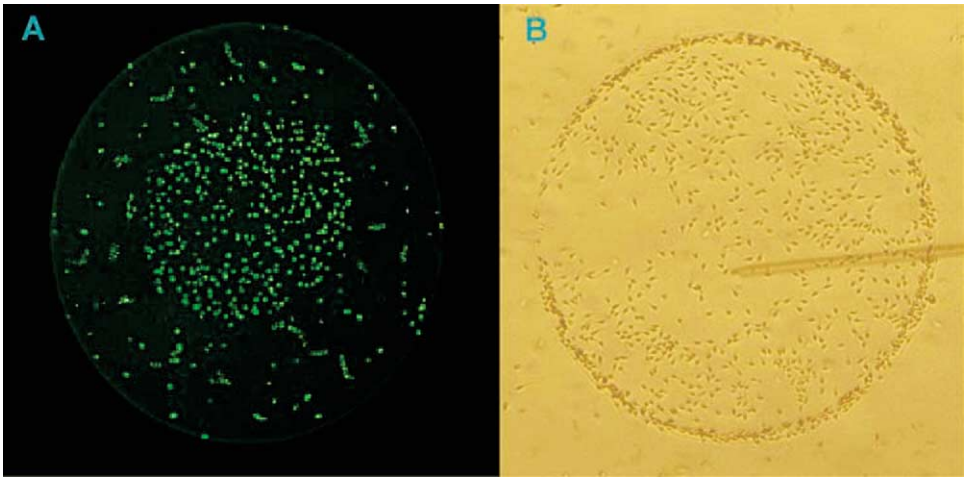

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TABLE 1

Disomy and diploidy frequencies in sperm populations arising from semen and HA-selected sperm fractions.

		17, X, Y Disomies						
		X/Y ratio	Disomy X	Disomy Y	Disomy X/Y	Disomy 17	Sex disomy	Total disomy
Experiment 1	Initial	1.07	0.16%	0.09%	0.11%	0.23%	0.35%	0.59%
(n = 12 men)	HA-bound	1.06	0.04%	0.03%	0.01%	0.04%	0.09%	0.13%
Reduction (×)			4.0	3.0	11	3.9	5.7	4.5
P (χ^2)			.01	NS	.01	<.001	<.001	<.001
Experiment 2	Initial	1.13	0.10%	0.10%	0.06%	0.12%	0.26%	0.38%
(n = 12 men)	HA-bound	1.05	0.02%	0.03%	0.02%	0.08%	0.06%	0.14%
Reduction (×)			5.0	3.3	3.0	1.5	4.3	2.7
P (χ^2)			.01	.02	NS	NS	<.001	<.001
Experiment 3	Initial	1.17	0.12%	0.08%	0.11%	0.27%	0.31%	0.58%
(n = 10 men)	HA-bound	1.10	0.01%	0.02%	0.02%	0.06%	0.05%	0.11%
Reduction (×)			12.0	4.9	6.0	4.5	6.2	5.1
P (χ^2)			<.001	.002	<.001	<.001	<.001	<.001

Note: NS = not significant.

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RESULTS

FISH Analysis of Sperm in the Initial Samples and in the HA-Selected Sperm Fractions

In the semen fraction of each man we analyzed a mean of 4,770 sperm, or 162,210 sperm in the 34 men. In the HA-bound and micropipette-collected sperm fractions, owing to the burdens of the task, we studied fewer sperm. In the first experiment we evaluated 7,530 sperm (range, 224–1,142 per man) and in the second experiment 9,720 sperm (range, 373–1,955 sperm per man). In the third experiment on individually selected sperm, we evaluated 24,420 sperm (range, 1,086–3,973 per man).

Experiment 1

Aliquots of the initial sperm suspension and HA-bound sperm from 12 moderately oligospermic men (sperm concentration, 20.6 ± 1.7 × 10^6/mL; motility, 52.1% ± 2.5%) were examined after FISH treatment. As Table 1 indicates, in the HA-selected sperm compared with initial semen, with the exception of Y disomy, the frequencies of all other aneuploidies and diploidies declined: 4-fold for 17 disomy, 5.7-fold for sex chromosome disomies, and 6.2-fold for diploidies. Indeed, no matter how high the frequencies were in the initial semen, the HA-selected sperm were within the range for normospermic men (Fig. 2).

Experiment 2

Semen samples from 12 normospermic patients (sperm concentration, 121.3 ± 21.4 × 10^6/mL; motility, 59.5% ± 4.9%) were studied. In this experiment we addressed the question of whether HA selection would cause a decline of chromosomal aberrations in "super sperm" fractions of high normospermic samples that were further enhanced in mature sperm with Isolate gradient centrifugation, which is used for ICSI sperm preparation in IVF laboratories (immature sperm with cytoplasmic retention have lower density and a diminished rate of sedimentation).

As expected, the FISH data showed that, in the initial fractions of "super sperm," the frequency of disomies and diploidies were lower compared with that of oligospermic men (Fig. 2). However, HA-binding showed a substantial selection effect even in this "super sperm" group: there was a 4.3-fold decline in sex chromosome disomies and a 5.8-fold reduction in diploidies. As in experiment 1, whether the HA-selected sperm originated in the oligospermic or the normospermic groups, the frequency of aneuploidies or diploidies was in the very low <0.02%–0.1% range (Fig. 2).

Experiment 3

In this experiment we studied 10 oligospermic men (sperm concentration, 12.6 ± 1.2 × 10^6/mL; motility, 49.3% ± 4.0%). We set out to investigate the efficacy of HA-sperm selection, as it would occur in a clinical setting when the HA-bound sperm are removed individually. In this approach, which required marathon collection sessions, we studied a mean of 2,442 HA-selected sperm in each of the 10 men (range, 1,086–3,973). As Table 1 indicates, neither the frequencies of disomies nor the reduction of disomy frequen-

TABLE 1

Continued.

		Diploidies			
		XX diploidy	XY diploidy	YY diploidy	Total diploidy
Experiment 1 (n = 12 men)	Initial	0.35%	0.29%	0.17%	0.81%
	HA-bound	0.08%	0.02%	0.03%	0.13%
Reduction (×)		4.4	14.5	5.7	6.2
P (χ^2)		<.001	<.001	.003	<.001
Experiment 2 (n = 12 men)	Initial	0.19%	0.25%	0.14%	0.58%
	HA-bound	0.03%	0.06%	0.01%	0.10%
Reduction (×)		6.3	4.2	14	5.8
P (χ^2)		<.001	<.001	<.001	<.001
Experiment 3 (n = 10 men)	Initial	0.06%	0.05%	0.03%	0.14%
	HA-bound	0.01%	0.03%	0.01%	0.05%
Reduction (×)		7.8	1.5	3.9	2.8
P (χ^2)		<.001	NS	NS	.002

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cies in the HA-selected sperm differed from those in experiments 1 and 2. The lower rate of reduction in diploidies is related to the lower frequency of diploidies in the initial semen samples.

The frequency of disomic nuclei, or the aggregate frequency of disomic and diploid nuclei in the HA-selected sperm fractions, was similar in all three experiments (Fig. 2). Thus, the higher number of HA-selected selected and individually collected sperm in experiment 3 further confirmed the validity of the HA method.

DISCUSSION

The development of this novel sperm selection method with HA binding is based on the recognition that during spermatogenesis and plasma membrane remodeling the formation of the zona pellucida–binding and HA-binding sites are commonly regulated. Indeed, we have found a close correlation ($r = 0.73$, $P < .001$, n = 54) of sperm binding scores either to HA or to the zona of bisected human oocytes (unpublished observation). Thus, the HA-selected mature sperm have a frequency of chromosomal aberrations comparable to that of sperm selected by the zona pellucida in conventional fertilization.

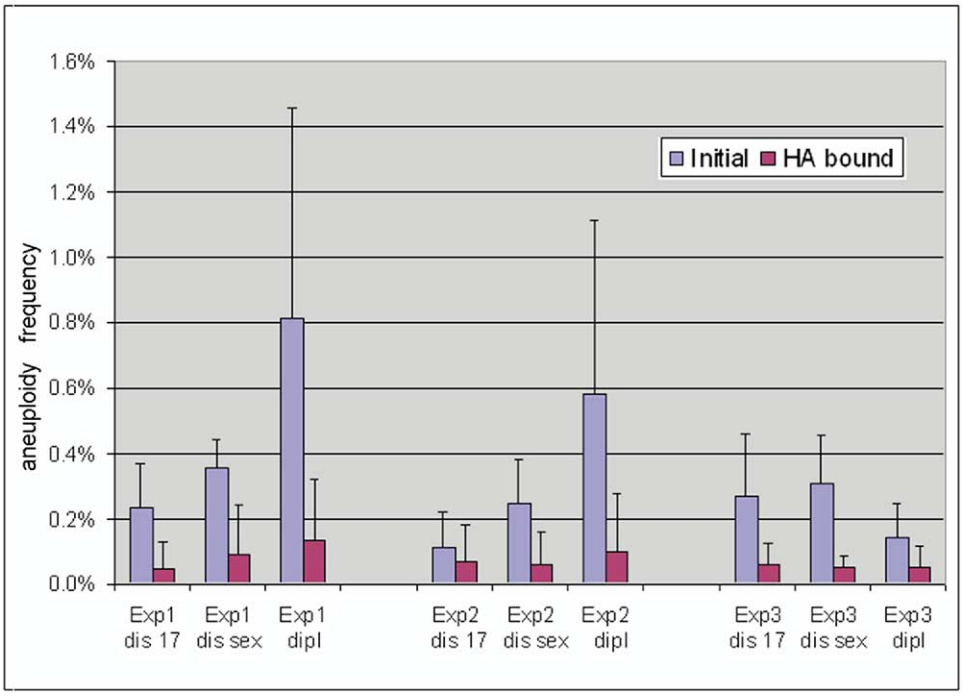
As hypothesized, HA selection reduced the frequency of sperm with disomy and diploidy. In spite of the sample-to-sample differences in the 34 men studied, the aneuploidy and diploidy rates in the HA-bound fraction declined to a narrow low 0.04%–0.10% range, which is comparable to that for normal fertile men. The HA-selected sperm showed low normal levels of numerical chromosomal aberrations, independently of the higher frequencies in the initial semen (Fig. 2). As Table 1 indicates, the clearance factor in the HA-bound sperm fraction depends on the initial frequency of sperm with disomies or diploidies, but the frequency of chromosomal numerical aberrations of HA-selected sperm is uniform. The five-fold decline of sex chromosome disomies is consistent with the increase of chromosomal aberrations in ICSI children conceived with visually selected sperm (2–6).

In addition to their role in meiosis, the chaperone proteins of the HspA2 family facilitate the assembly and intracellular transport of proteins, such as those needed for membrane remodeling, the process of histone–protamine replacement, or the delivery of the DNA repair enzymes. Thus, the HA-selected mature sperm, in addition to having low levels of meiotic errors, are devoid of cytoplasmic retention, persistent histones, the apoptotic process, and DNA fragmentation; these are factors that would adversely affect the paternal contribution of sperm to the zygote (10–12, 20 and unpublished observation). However, arrested plasma membrane remodeling with a consequential low density of the HA receptor causes diminished HA binding ability in immature spermatozoa (13, 14, 20). Thus, HA-mediated sperm selection is a novel and efficient technique that will alleviate the potential problems related to chromosomal aneuploidies and DNA chain fragmentation, that presently cause worldwide concern regarding ICSI fertilization with sperm of diminished maturity.

Although in men with severe oligospermia the frequencies of disomy and diploidy are much higher compared with those in the present study population, it was necessary to use

FIGURE 2

Bar graph of all three experiments. Exp = experiment; dis 17 = chromosome 17 disomy; dis sex = sex chromosome disomy; dipl = diploidy.



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these oligospermic, borderline oligospermic, and normospermic samples to collect sufficient numbers of spermatozoa to statistically validate the experimental results. The clinical utility of the method is well demonstrated by the selection of HA-bound sperm, particularly those selected one by one in experiment 3, and by the substantial reduction of sperm with aneuploid nuclei in the sperm fractions, even in sperm fractions of highly normospermic men that had been purified further by Isolate gradient centrifugation before the HA binding step. At the time of these studies we faced a technical limitation, in that we were able to detect only three FISH colors. Having established the validity of the HA selection method with adequate statistical power, we will now initiate studies in severely oligospermic men who are ICSI candidates, using a greater number of FISH probes (targeting X, Y, and several autosomal chromosomes) to further establish the improved genetic integrity of HA-bound sperm from men who are actually treated with ICSI.

The safety of HA-mediated sperm selection is supported by various lines of evidence. First, HA occurs normally in the female reproductive tract and in the cumulus oophorus. Thus, it is likely that HA is carried with the sperm into oocytes even with in vivo conception. In the case of ICSI, the removal of sperm from HA might cause a few HA molecules to attach to sperm, or a few square micrometers of sperm membrane could remain attached to the HA. In either case, fertilization with HA-selected sperm does not seem to differ from natural fertilization. Indeed, the U.S. Food and Drug Administration has already approved sperm preparation and embryo culture media [i.e., Sperm Select (Pharmacia AB, Uppsala, Sweden) or G 3.2 Vitrolife (Vitrolife AB, Kungsbacka, Sweden)] that contain HA.

In evaluating the benefits of this novel method of sperm selection, one should consider the potential adverse consequences of IVF-ICSI treatment. In the overall assessment, there is a reportedly higher incidence of spontaneous abortions, in the 18% range, compared with the 10% rate after normal conception (2). There were also somewhat increased rates of malformations reported, but not in infants born live in the United States or in Belgium. The majority of malformations were hypospadias in the male infants (2, 3, 5, 9). There were also reports from Scandinavia of elevated rates of neural tube defects; however, critical analyses indicated that the latter were due to multiple births after IVF and ICSI conception (4, 38). Another male-related adverse consequence of ICSI is the transmission of Y-chromosome deletions. It is estimated that approximately 10% of men with severe oligospermia or nonobstructive azoospermia, who require ICSI, have an azoospermia factor deletion, and these are transmitted to the male offspring (2). A further potential adverse consequence of ICSI, unrelated to the quality of

sperm, is the perturbation of the meiotic spindle in the oocyte to be fertilized (39).

Further reports indicated that, after ICSI fertilization, there were increased rates of de novo numerical chromosomal aberrations and cytogenetically detectable structural chromosomal aberrations (2–6). The numerical chromosomal aberrations are most likely due to the increased rate of chromosomal aneuploidies, primarily sex chromosome disomies, in sperm of oligozoospermic or severely oligozoospermic ICSI fathers. As discussed, these meiotic defects are likely to arise from ICSI fertilization with sperm of diminished maturity, which are deficient in the HspA2 chaperone protein expression that is associated with an increased incidence of aneuploidies and more extensive DNA fragmentation. The combined impact of chromosomal aberrations, apoptosis, and fragmented sperm DNA are likely to increase the chance for adverse paternal contribution of sperm to the zygote (10, 11, 20, 24).

Men participating in ICSI treatment also show a higher rate of chromosomal rearrangements, such as reciprocal and Robertsonian translocations. These rearrangements in some men are associated with oligozoospermia and infertility, as well as through interchromosomal effects, disomies, and diploidies. Thus, HA-mediated sperm selection for ICSI might reduce the risk of chromosomal aberrations in offspring if the common origin of chromosomal rearrangements and numerical aberrations in the sperm of their fathers is diminished sperm maturity (40–42).

The potentially increased risk of birth defects after IVF and ICSI has been addressed in two recent reviews (7, 8). Although differences in birth defect classifications in various countries warrant further interpretation, the reports suggested that there might be an elevated risk for birth defects in children treated with reproductive technologies. However, the potential risks seem to be comparable in ICSI and IVF, which suggests that as-yet-unknown compounding factors related to the extracorporal handling of the embryo might play a role (7, 8). These questions were further evaluated in 2,840 and 2,955 liveborn infants after ICSI and IVF, respectively. In these groups, the rates of multiple pregnancies, birth weights, neonatal complications, and occurrences of stillbirth or perinatal deaths were similar in the two groups. Major malformations (defined as those causing functional impairment or requiring surgical correction) were also similar: 3.4% in the ICSI and 3.8% in the IVF infants. The ICSI malformation rates were not related to sperm origin (whether ejaculated semen or testicular sperm extraction [TESE]), or quality of sperm evaluated by conventional semen parameters (43).

A very recent multicenter study from five European countries focused on 5-year-old singleton children conceived either by ICSI (n = 540), IVF (n = 437), or natural conception (n = 538). The advantage of studying 5-year-old children is that malformations that might have been overlooked at the neonatal stage would become apparent. Compared with naturally conceived children (1.0), the odds for malformations were 2.77 for ICSI children and 1.80 for IVF children. In addition, ICSI and IVF children, compared with naturally conceived children, were more likely to have experienced significant childhood illnesses, surgery, or hospitalization. Among the malformations, there was a significant increase only in the rate of urogenital findings: 5%, 2%, and 1% after ICSI, IVF, and normal conception, respectively. The study does not distinguish whether the sperm originated in the ejaculate, in the epididymis, or in TESE tissue. However, the relevant question arises whether, in addition to chromosomal aberrations, these adverse effects are related to sperm apoptosis, DNA fragmentation, and perhaps incomplete DNA repair by the zygote because diminished-maturity sperm were selected for ICSI (6).

Regarding structural chromosomal abnormalities in ICSI-derived pregnancies, the incidence of abnormal karyotypes was examined by chorionic villus sampling and amniocenteses. In 1,586 subjects, there were 47 children (3%) with abnormal fetal karyotypes, and 25 of these (1.6%) were de novo. Regarding the role of sperm maturity (the proportion of immature sperm with various chromosomal aberrations is higher in oligozoospermic, and particularly in severely oligozoospermic men), in fathers with sperm concentrations of <20 vs. $>20 \times 10^6$/mL, the frequency of structural chromosomal abnormalities was approximately 10-fold higher (24/1,120 or 2.1% vs. 1/1,419 or 0.24%, $P = .006$). Thus, this is further indication that structural chromosomal aberrations might be related to injection of sperm with diminished maturity (3).

Because the diminished levels of the HspA2 chaperone affect the meiotic process and delivery of the DNA repair enzymes in immature sperm, one might anticipate that fertilization by ICSI vs. IVF would provide less progressive embryo development and higher rates of early miscarriages. Indeed, in a study on 59 products of conception from early miscarriages arising from IVF or ICSI, 32 (54%) showed abnormal cytogenetics. Furthermore, the level of aneuploidy was significantly elevated in ICSI vs. IVF concepti (76% vs. 41%, $P<.001$). In 27 of the 59 miscarriage material, there was no aneuploidy, but the potential impacts of diminished sperm DNA integrity are unknown. The relationship between pregnancy loss and sperm aneuploidy has been further confirmed in a study of 20 couples with three or more recurrent first-trimester abortions (44, 45).

Another question relevant to the adverse consequences of ICSI fertilization is the role of the oocyte. In a study of 46 ICSI offspring, umbilical cord blood samples were subjected to cytogenetic investigation. Overall, the incidence of aneuploidy was 2%, compared with 0.6% in the general population. The investigators suggest that the abnormalities found might be related to maternal factors, in addition to those of sperm. This is because some oocytes that would not be fertilized in natural conception are forced into zygote formation by ICSI (46). Similar conclusions were drawn in

another study of 263 female partners of IVF or ICSI couples. There was an increased frequency of chromosomal abnormalities in female partners of men with male factor infertility (47).

In summary, in light of our data and of the adverse ICSI consequences reviewed, it is of interest to define the expected advantages of HA-mediated sperm selection in improving ICSI outcome.

First, in sperm selected by HA binding, the frequencies of chromosomal disomies and diploidies are in the normal range, independently of the aneuploidy frequency of the initial semen. In this respect the sperm selection properties of HA are similar to those of the zona pellucida. Thus, we can expect an improvement by HA selection of the sperm comparable to that of the zona pellucida. It is not yet known whether this selection ability also extends to some of the structural chromosomal aberrations and chromosomal rearrangements in the offspring, but the increased rates of such aberrations in ICSI children would indicate a distinct possibility.

Second, mature sperm selected by virtue of HA binding are also viable and devoid of persistent histones and apoptosis, as evidenced by aniline blue staining and the absence of active caspase-3, respectively (11, 12). Thus, the paternal contribution of HA-selected sperm will be improved, and we would expect a lower rate of miscarriages after ICSI with HA-selected sperm.

Third, HA-selected mature sperm do not exhibit DNA fragmentation, as tested by DNA-nick translation and by the COMET assay. This should alleviate concerns related to the potential increase in cancer rates after ICSI fertilization.

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