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Sperm Function and Choice of Preparation Media: Comparison of Percoll and Accudenz Discontinuous Density Gradients

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ABSTRACT: We compared the sperm populations prepared by Accudenz (35–65%) and Percoll (40–80%) density gradients in 21 normospermic specimens (concentration, 53.6 ± 3.8 × 10^6 sperm/ml; motility, 44.5 ± 3.5%). Accudenz facilitated a higher recovery of sperm and motile sperm (68.4 ± 6.6% vs. 49.3 ± 4.9%, $P < 0.001$, and 87.8 ± 4.1% vs. 77.8 ± 3.7%, $P < 0.01$, respectively). Sperm motility was lower in the Accudenz compared to the Percoll pellets; thus the values of total motile sperm recovered were not different (17.1 ± 2.4 vs. 15.1 ± 2.2 × 10^6 sperm/ml). The long-term retention of sperm motility was substantially improved in Accudenz (at 24 hours, 34.9 ± 2.8% vs. 26.3 ± 1.5%; 60% vs. 40% of the initial motility, $P < 0.001$), and the Accudenz vs. Percoll samples also exhibited a higher retention of total motile sperm (at 24 hours, 9.8 ± 1.2 vs. 6.1 ± 0.5 × 10^6 motile sperm/ml, $P < 0.05$). The sperm motility index, a multiple of velocity and motility in the sample that reflects the efficiency of the sperm population in sperm–oocyte interaction, was 75% higher in the Accudenz samples at 24 hours (3.6 ± 0.4 vs. 2.1 ± 0.2, μm/second, $P < 0.01$). Sperm cellular maturity by the creatine phosphokinase (CK) activity and CK-M to CK-B isoform ratio parameters (in the original samples 0.14 ± 0.02 IU CK/100 × 10^6 sperm and 57.9 ± 3.7%, respectively) were improved in both the Accudenz and Percoll pellets ($P < 0.001$), with no difference between the two sperm fractions. Sperm activation status monitored by chlortetracycline fluorescence indicated that after 4 hours of incubation the incidence of fully acrosome-reacted spermatozoa in the Accudenz versus Percoll pellets was 6.2 ± 0.3% versus 13.1 ± 1.0% ($P < 0.001$), a 100% increase in Percoll. We can conclude that Accudenz yields a higher concentration of motile spermatozoa, with improved retention of motility, velocity, and acrosomal integrity and without an increase of sperm with diminished cellular maturity. Thus, in sperm preparation for intrauterine insemination, in which the timing of ovulation and insemination frequently do not correspond, Accudenz-prepared sperm, with a better retention of motility/velocity and acrosomal integrity and with a consequential higher resistance to activation by the female reproductive tract, are expected to be more effective.

Key words: Sperm maturity and creatine kinase, motile sperm yield, sperm activation, chlortetracycline.

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Sperm preparation methods for assisted reproduction are directed to the separation of sperm from seminal fluid and from other cellular components of semen as well as to the concentration of the motile sperm population. Approaches most frequently used include sperm "washing" in various media, "swim-up" or "swim-down" preparations in which the motile sperm population migrates away from the nonmotile elements of semen, and centrifugation of sperm through various density gradients, exploiting the higher density of mature sperm that completed cytoplasmic extrusion during spermiogenesis (Pertoft and Laurent, 1977; Lessley and Garner, 1983; Makler et al, 1984; Berger et al, 1985; Dravland and Mortimer, 1985; Hyne et al, 1986; Velez de la Calle, 1991; Huszar and Vigue, 1993).

Concerns about the adverse "iatrogenic" effects of sperm preparation techniques have focused upon three issues: the choice of sperm preparation media, the mechanical damage due to centrifugation/resuspension, and the propagation of lipid peroxidation during sperm pelleting due to the close vicinity of leukocytes and spermatozoa that have already been damaged that may generate reactive oxygen species (Alvarez et al, 1987; Aitken and West, 1990; Mortimer, 1991; Aitken et al, 1994). Another concern is the effect of centrifugation/resuspension on the integrity of the sperm acrosomal membrane that, if diminished, would shorten the lifetime and fertilizing efficiency of spermatozoa. This is of particular significance for intrauterine insemination, the most frequently em-

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ployed assisted reproduction modality, in which an extended presence of motile and acrosome-intact sperm population is advantageous.

The cautions about media selection were well justified because Ham's F-10, which was for years the medium of choice for assisted reproduction, increases the rate of destructive lipid peroxidation due to its iron content (Huszar and Vigue, 1994). The major disadvantage of the swim-up/swim-down techniques is that they preferentially select the most motile sperm fractions, whereas normal sperm, which are not as active at the time of preparation, are discarded. The discontinuous density gradient preparation method now used by most laboratories is the most advantageous because the more dense, mature, and motile sperm of the pellet are separated from immature sperm and other cellular elements of semen (Huszar and Vigue, 1993). Motile sperm yields of up to 60% of the original semen have been reported (Lessley and Garner, 1983; Berger et al, 1985; Huszar and Vigue, 1994).

In the past few years, the use of Percoll density gradients has become widely accepted. However, it is of concern that Percoll is not approved by the Food and Drug Administration (FDA) (it is actually not marketed for sperm preparation by the manufacturer), because it may contain endotoxins and its constituent silica particles may cause sperm membrane damage. We wished to systematically examine an alternative density gradient medium, Accudenz (formerly called Nycodenz, as used in Gellert-Mortimer et al [1988] and Serafini et al [1990]), which is also approved by the FDA as a contrast medium for internal use. It was reported that Accudenz produces sperm fractions with a more extended motility than Percoll (Gellert-Mortimer et al, 1988).

In order to ascertain the benefits of Percoll versus Accudenz, we compared sperm populations prepared from the same semen samples by discontinuous density gradients of the two media. We examined by computer-assisted semen analysis (CASA) the yield of recovered sperm, sperm motility, and long-term retention of sperm motility. Because of recent advances in understanding the importance of sperm capacitation and acrosomal status (Byrd and Wolf, 1986; Tesarik, 1989; Fenichel et al, 1991; DasGupta et al, 1993; Calvo et al, 1994; De Jonge, 1994), we followed these parameters using the chlortetracycline (CTC) binding fluorescence pattern method (Lee et al, 1987; DasGupta et al, 1993; Perry et al, 1995). In addition, we have also monitored the sperm cellular maturity in the initial semen and in the sperm pellet fractions by creatine phosphokinase (CK) activity and CK-M to CK-B isoform ratio parameters, which in previous studies were predictive for sperm cellular maturity, occurrence of pregnancies in a blinded study of *in vitro* fertilization (IVF) couples, sperm–oocyte binding, and for the rate of sperm lipid peroxidation (Huszar and Vigue, 1990, 1994; Huszar et al, 1992, 1994).

Materials and Methods

Experimental Design

We studied 21 normospermic semen samples processed in our Sperm Physiology Laboratory without preselection. After the CASA parameters were determined, an aliquot of 5–10 × 10^6 sperm was reserved for the CK determinations, the semen samples were diluted to 1:1 with human tubal fluid (HTF) (Irvine Scientific Company, Santa Ana, California) supplemented with 0.5% bovine serum albumin (BSA), and the samples were divided into four aliquots. Centrifugations were carried out in discontinuous gradients of 2.0 ml each 40% and 80% Percoll or in 2.0 ml each 35% and 65% Accudenz at 400 × *g* for 20 minutes. The two Percoll and two Accudenz sperm pellets were washed with 1.0 ml of HTF-BSA (500 × *g*, 5 minutes) and resuspended in 0.4 ml HTF-BSA. The CASA parameters were determined at 0 time and after 4 hours and overnight incubations at 37°C. The CTC assays were carried out at 0 time and after 4 hours.

Analysis of Sperm Motility—Using a 10-μm-deep Makler chamber (Zygotek Systems, Inc., Springfield, Massachusetts) and an Olympus microscope (Olympus, Lake Success, New York) equipped with a phase-contrast objective, two drops of semen (three fields in each) per sample at each time point were studied. The CASA parameters (Cryo Research, New York, New York) were as follows: 15 frames at 30 frames/second, threshold velocity 8 μm/second, and maximum velocity 150 μm/second. We monitored sperm concentrations, motility (%), curvilinear velocity (VCL, μm/second), linearity, mean amplitude of lateral head displacement (ALH, μm), and cross-beat frequency.

Preparation of Percoll and Accudenz Gradients—The 100% Percoll (Sigma, St. Louis, Missouri) stock solution was diluted to 90% with the 9:1 v/v addition of 1.5 M NaCl and 100 mM HEPES, pH 7.0 (Huszar and Vigue, 1993). This isotonic and isoionic 90% Percoll solution was considered "100% Percoll" and was diluted to 40% and 80% with HTF-BSA. The liquefied semen samples were layered upon the discontinuous 40–80% Percoll gradients. Accudenz (Accurate Chemical and Scientific Corporation, Westbury, New York) is a nonionic medium that is based on a tri-iodonated molecule. The density of the medium is 2.1 g/ml, and this high density derives from the presence of a substituted ring, which is linked to hydrophilic groups in order to facilitate the high water solubility of Accudenz. An isotonic solution is 27.6% (w/v), and in our application it was diluted with HTF-BSA to 35% and 70% final concentrations.

The Chlortetracycline (CTC) Assay—The CTC method utilized (DasGupta et al, 1993) is a modification of the original procedure described by Lee et al (1987). The CTC stock solution contains CTC-HCl (Sigma) in a 500 μM final concentration, 130 mM NaCl, 5 mM cysteine, and 20 mM Tris buffer (pH 7.8), and it is prepared fresh and kept wrapped in foil to exclude light. The sperm suspension (45 μl) is mixed with 45 μl CTC solution and 8 μl of 12.5% (w/v) paraformaldehyde in 20 mM Tris-HCl (pH 7.4) is added. Slides are prepared by placing 10 μl of sperm mixture on a clean slide along with 1 μl of 0.22 M 1,4-diazobicyclo (2.2.2) octane (DABCO, Sigma) in glycerol to retard fading of the fluorescence. Sperm are assessed using an Olympus microscope equipped with phase contrast and epifluorescent optics. One hundred sperm on each slide are classified according to one of three CTC staining patterns: uniform bright fluores-

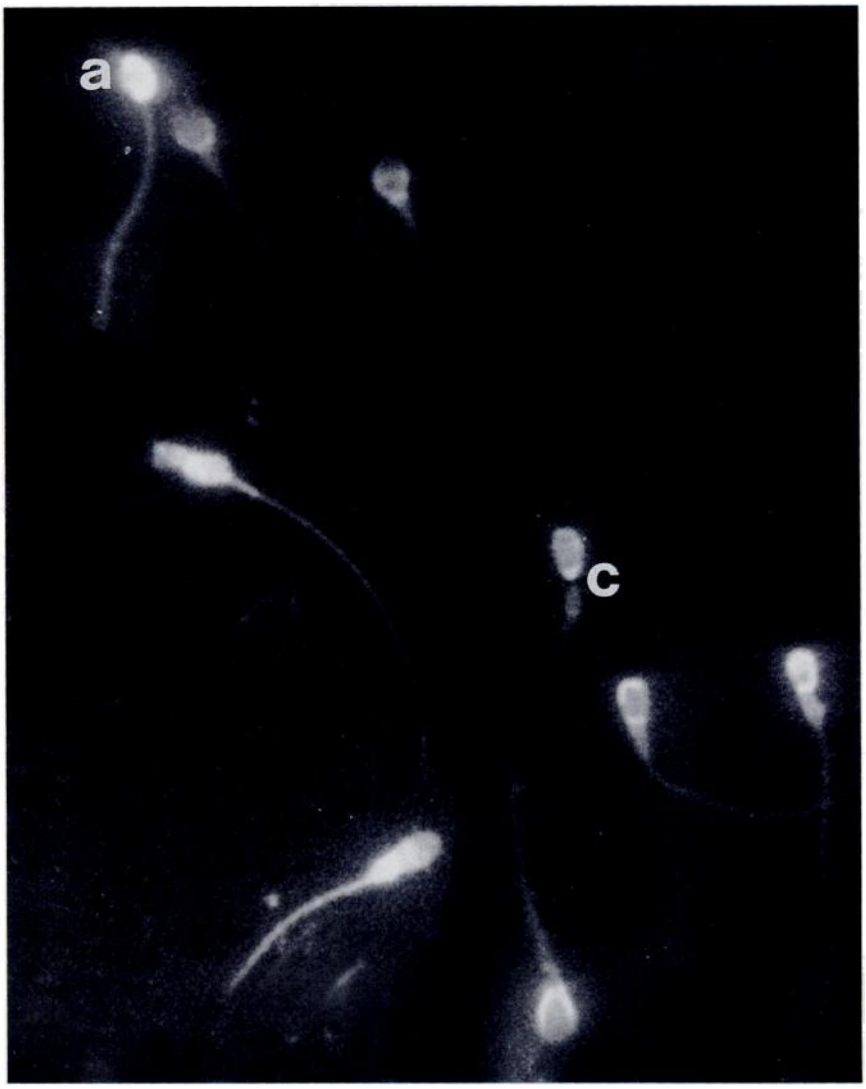

FIG. 1. CTC patterns of human spermatozoa in various stages of activation as described in the Materials and Methods section. A, Unreacted spermatozoa; C, acrosome-reacted spermatozoa. The other unlabeled sperm exhibit the reacted/capacitated pattern between the A and C endpoints.

cence of the whole area of the sperm head and the postracrosomal region, characteristic of "unreacted" cells; fluorescence-free band in the postacrosomal region or various degrees of absence of fluorescence in the center of the sperm head, characteristic of "reacted/capacitated" cells, and the patchy or total absence of head fluorescence, characteristic of "acrosome-reacted" cells. This classification system appears to provide easily discernible parameters for the three activation states that are objective and consistently recognizable by various investigators (Fig. 1).

Sperm CK Activity Measurements—Aliquots of the initial semen and of the Percoll and Accudenz pellet fractions were washed with 10–15 volumes of ice-cold 0.15 M NaCl and 0.30 mM imidazole, pH 7.0, at 5,000 × *g*, to remove seminal fluid, and the sperm pellets were disrupted by vortexing in 0.1% Triton X-100, 30 mM imidazole, pH 7.0, 10% glycerol, and 5 mM dithiothreitol (DTT). The homogenate was clarified by centrifugation at 5,000 × *g*, and an aliquot was subjected to CK activity determinations by a spectrophotometric kit (Sigma) as described previously (Huszar et al, 1990).

Sperm CK Isoform Determinations—The CK-M and CK-B isoforms were separated by electrophoresis on Agarose films (Helena Laboratories, Inc., Beaumont, Texas). The CK isoforms were detected by overlaying and incubating the gel with a fluorescent CK substrate for 20 minutes at 37°C, and they were integrated under long-wave ultraviolet light with a scanning fluorometer (Helena). The CK-M ratio (% CK-M/CK-M + CK-B) is calculated according to the relative areas under the respective CK isoform peaks (Huszar et al, 1992).

Statistical Evaluation—Statistical analysis was carried out using the YALE CLINFO statistical package, including parametric and nonparametric comparisons, and the Duncan *T*-test. Significance ($P < 0.05$) was calculated using the Student *t*-test and analysis of variance (ANOVA). All data are expressed as the mean ± standard error of the mean (SEM).

Results

Development of Accudenz Gradients

In the original Accudenz formulations, the manufacturer recommends a four-phase density gradient. We decided to develop a two-phase gradient system similar to that of the Percoll gradients used. In preliminary experiments we tested upper and lower Accudenz phases between 30 and 45% and 60 and 75%, and we also tried centrifugation speeds between 700 and 1,200 × *g* and 15- to 30-minute centrifugation times. In addition to the recovered sperm and recovered motile sperm, we also monitored the sperm CK activity and CK-M isoform ratios. These parameters allowed us to evaluate the sperm cellular maturity in the fractions recovered under the various gradient conditions. We found that the 35% and 65% Accudenz gradients, 500 × *g* speed, and 20-minute centrifugation time yielded sperm recovery and sperm quality within the optimal range. Also, these conditions are advantageous because the centrifugation speed and time are identical to that used with Percoll, thus allowing the sperm preparation to be carried out simultaneously in the two media.

Sperm Motility and Velocity

The sperm concentration and motility in the initial semen samples were 53.6 ± 3.8 × 10^6 sperm/ml and 44.5 ± 3.5%, respectively. The analysis of the sperm fractions in the Accudenz versus the Percoll pellets at 0 time indicated that Accudenz facilitated a higher recovery of sperm from the original samples (29.9 ± 3.2 vs. 21.6 ± 2.4 × 10^6 sperm/ml or 68.4 ± 6.6% vs. 49.3 ± 4.9%, $P < 0.01$). The proportion of recovered motile sperm was also higher in the Accudenz versus the Percoll pellets (87.8 ± 4.1% vs. 77.8 ± 3.7%, $P < 0.01$). The sperm motility was lower vs. 66.6 ± 2.8%, $P = 0.02$). Thus, the total motile sperm initially recovered in the two media prior to the 4-hour and 24-hour incubations were not different (17.1 ± 2.4 vs. 15.1 ± 2.2 × 10^6 sperm/ml).

The retention of sperm motility was higher in the Accudenz compared to the Percoll pellets, both at 4 hours (55.7 ± 2.5% vs. 51.9 ± 3.8%) and at 24 hours (34.9 ± 2.8% vs. 26.3 ± 1.5%, or 60% vs. 40% of the 0 time values, $P < 0.01$). The Accudenz-prepared sperm also exhibited about 50% higher retention of total motile sperm concentration at both 4 hours (18.2 ± 2.5 vs. 12.3 ± 1.6 × 10^6 motile sperm/ml $P < 0.001$) and at 24 hours (9.8 ± 1.2 vs. 6.1 ± 0.5 × 10^6 motile sperm/ml, $P < 0.05$) compared with the Percoll samples. The CASA parameters in the motile sperm population prepared by Accudenz versus Percoll, including linearity, lateral head displacement, and sperm tail cross-beat frequency were not different. This was also true for velocity (VSL) at both 4 hours and 24 hours (47.4 ± 3.4 vs. 44.5 ± 2.4 μm/second and 36.5 ± 1.5 vs. 33.6 ± 2.3 μm/second). However, the sperm motility index, a multiple of velocity and motility in the sperm population that reflects the efficiency of the spermatozoa in that sperm–oocyte interaction, was about 58% and 75% higher in the Accudenz versus Percoll fractions at 4 hours and 24 hours, respectively (8.6 ± 0.6 vs. 5.5 ± 0.4 μm/second and 3.6 ± 0.2 vs. 2.1 ± 0.2 μm/second, $P < 0.01$ in both).

Sperm Cellular Maturity

As reported previously with Percoll gradients (Huszar and Vigue, 1993, 1994), sperm maturity measured by the CK activity and CK-M ratio parameters (initial sample 0.14 ± 0.02 IU CK/100 × 10^6 sperm and 59.7 ± 3.7%; normal values <0.25 IU CK/100 × 10^6 sperm and >12%) were improved in both the Accudenz and Percoll pellets ($P < 0.001$). However, there was no difference in sperm maturity or in the degree of improvement between the sperm populations recovered from the two media (CK activity 0.05 ± 0.005 and 0.06 ± 0.005 IU CK/100 × 10^6 sperm; CK-M ratio 89.7 ± 1.5% and 93.2 ± 1.8%).

Sperm CTC Binding Patterns in Percoll and Accudenz

In order to establish changes in sperm capacitation and acrosomal reaction status in response to the two gradient media, we followed the CTC fluorescent patterns by classifying sperm in the unreacted, reacted/capacitated, and acrosome-reacted states (Fig. 1). After the centrifugation step at 0 time, 69.2 ± 2.9% and 71.1 ± 2.1% of the sperm exhibited the unreacted/uncapacitated pattern in the sperm fractions prepared by Percoll and Accudenz respectively, whereas 29.7 ± 0.2% and 28.9 ± 0.4% were reacted/capacitated and 1.1 ± 0.4% and 1.0 ± 0.3% were acrosome-reacted. After the 4-hour incubation, the unreacted-type sperm were reduced in both media (13.0 ± 1.2% vs. 16.1 ± 2.0%). The incidence of reacted/capacitated sperm was 73.9 ± 2.7% versus 77.8 ± 3.1%, and the proportion of acrosome-reacted sperm increased to a 100% higher level in the Percoll compared to the Accudenz prepared fractions (13.1 ± 1% vs. 6.2 ± 0.4%, $P < 0.01$).

Discussion

The goal of sperm preparation methods for assisted reproduction is the recovery of sperm from the seminal fluid with the highest possible yield and without introducing iatrogenic effects that may diminish sperm motility, viability, and, ultimately, fertilizing potential. Discontinuous density gradients appear to be the most efficient approach because they remove seminal fluid and other cellular elements of semen and concentrate in the pellet the mature and dense sperm with the least amount of

unextruded cytoplasm. In addition to the increased sperm motility (Pertoft and Laurent, 1977; Lessley and Garner, 1983; Berger et al, 1985; Velez de la Calle, 1991), the differences between the sperm populations of the initial semen and those recovered from the gradient pellets have been well demonstrated with sperm CK activity and CK-isoform ratios (Huszar and Vigue, 1993), by the rate of lipid peroxidation (Aitken et al, 1994; Huszar and Vigue, 1994), total lactate dehydrogenase (LDH) and LDH-X isoform activities (Orlando et al, 1988, 1994) and by strict sperm morphology (Hall et al, 1995).

Both Accudenz and Percoll gradients yielded spermatozoa with increased motility compared to the initial semen. However, the recoveries of the spermatozoa and motile sperm fraction were more efficient in Accudenz compared to Percoll ($P < 0.01$). The retention of sperm motility was higher in Accudenz (at 24 hours, 50% higher, $P < 0.01$), and thus the total motile sperm concentration in the Accudenz versus Percoll fractions was also increased at both 4 hours and 24 hours ($P < 0.01$ and $P = 0.03$). The sperm motility index, which reflects the rate of the potential sperm–oocyte encounters, was increased in Accudenz at 4 hours and 24 hours by 56% and 75% ($P < 0.001$), respectively. All of these data and the faster decline of long-term motility in the Percoll sperm fractions call attention to the potential membrane damage properties of Percoll (Arcidiacono et al, 1983; Gellert-Mortimer et al, 1988; Mortimer, 1991). The semen samples used in the present experiments were not oligospermic or asthenospermic due to the low motile sperm concentrations in the samples. This circumstance precluded a comprehensive investigation of all parameters. However, a previous study that focused upon the efficacy of Percoll and Accudenz in sperm samples with low motile sperm concentrations (Gellert-Mortimer et al, 1988) established that the sperm motility during a 21-hour incubation period was maintained with a better rate in Accudenz than in Percoll, whether the initial sperm concentrations were in the oligospermic or normospermic ranges.

The CK activity and CK–M ratio parameters indicated that sperm maturity was enhanced in the sperm fraction recovered from the gradients compared to that in the initial semen ($P < 0.001$), but there was no difference between Percoll and Accudenz. Thus, the higher recovery of motile sperm in Accudenz did not occur at the expense of sperm maturity. Other *in vitro* data also support that Accudenz does not adversely affect sperm function; there were similar penetration rates in the zona-free hamster oocyte penetration assay by sperm prepared with Percoll and Accudenz (Serafini et al, 1990). In addition to the benefits in recovery of motile sperm, retention of sperm motility, and maintenance of acrosomal integrity, Accudenz is a good choice of sperm preparation medium due to the lack of endotoxin formation, low rate of complications with internal human use (Assem et al, 1983; Dawson, 1983), and the fact that it is approved by the FDA for internal use. In the present work we also facilitated its convenience of use by developing conditions for a 35–65% gradient, which in work intensity is comparable to that of the 40–80% Percoll gradients. The recovery of the motile sperm was substantially higher (87.8% vs. 39%) in the two-phase versus the manufacterer-recommended four-phase Accudenz discontinous gradient (Gellert-Mortimer et al, 1988).

The comparable improvement of CK activity and CK-M ratios in the Percoll and Accudenz sperm fractions also reflects an enhancement in sperm with normal morphology and a decline in the rate of sperm lipid peroxidation, because CK activity, sperm morphology, and the rate of lipid peroxidation are related markers of the unextruded cytoplasm (Huszar and Vigue, 1993, 1994). We have shown, with a combination of CK immunocytochemistry and morphometry of individual spermatozoa, a close correlation among sperm CK content, the size and roundness of sperm heads, and the incidence of amorphous sperm (Huszar and Vigue, 1993). The improvement in CK parameters in both the Percoll and Accudenz sperm suggests a reduction in oxidative rates as well, because CK activity correlates with the rate of lipid peroxidation ($r = 0.43$, $P < 0.001$, $N = 142$; Huszar and Vigue, 1994). In the same publication, we also reported significant concomitant differences ($P < 0.01$) in CK activities and lipid peroxidation rates among the top, interface, and pellet fractions of 40–80% Percoll gradients ($P < 0.01$) and a correlation between CK activity and lipid peroxidation rates in the fractions ($r = 0.58$, $P < 0.001$, $N = 57$). Due to the various parameters that we followed in the present study, the amount of sperm available did not allow the lipid peroxidation measurements. However, based on the Percoll gradient data and on the decline of CK activity in the Accudenz fractions, we would expect a decline of sperm lipid peroxidation rates in the Accudenz pellets.

With respect to acrosomal status, at 0 time there were no differences between the Percoll and Accudenz sperm populations; about 70% of the sperm were unreacted in both pellets. At 4 hours following the centrifugation, 13.2 ± 1.2% and 16.1 ± 2.0%, respectively, of the sperm were still unreacted, and 72.3 ± 2.2% and 71.3 ± 2.9%, respectively, were in the reacted/capacitated state. Further, there was a 100% increase in the incidence of acrosome-reacted sperm in the Percoll versus the Accudenz pellets (13.1 vs. 6.2%, $P < 0.01$). All data are in line with earlier electron microscopic findings (Arcidiacono et al, 1983) that showed that Percoll centrifugation causes leakage of acrosomal enzymes and loss of sperm membrane integrity. The question arises whether the classification of sperm into the unreacted, reacted/capacitated, and acrosome-reacted patterns was affected by nonphysiological loss of

fluorescence due to diminishing sperm viability. This is not the case, because in monitoring the samples we followed the most specific measure of viability: sperm motility. Simultaneously with the major changes among the CTC activation patterns during the 4-hour incubation period, involving >70% of sperm equally in both the Accudenz and in Percoll populations, the decline of motility in the samples was only 4% and 22%, respectively. The preservation of motility indicates that the CTC patterns reflect genuine changes in the acrosomal membrane structure and not the demise of spermatozoa. In evaluating the reacted/capacitated sperm population, we could not detect (and for the purposes of this study it was not necessary) consistent and specific CTC fluorescence changes in the reacted/capacitated group that would suggest a sequential progression between the unreacted and acrosome-reacted endpoints. It is of interest that in a recent study of CTC binding patterns to human sperm, the authors were able to distinguish among eight different fluorescence patterns (Perry et al, 1995).

The 100% increase in acrosome reaction from 6.5 to 13% does not seem to be very high in absolute terms. However, it likely reflects an advanced state of sperm capacitation that in turn makes sperm susceptible to the stimuli of the female reproductive tract (for instance progesterone). Such stimuli will cause an increased rate of premature acrosome reaction and diminished lifetime in the Percoll prepared spermatozoa. Considering various modalities of assisted reproduction, the differences in motility and acrosomal integrity between the Accudenz and the Percoll prepared sperm are not likely to affect the outcome of IVF or gamete intrafallopian transfer (GIFT) due to the high abundance of sperm used and the short time frame between sperm preparation and the sperm–oocyte interaction. However, in intrauterine insemination, in which a delay of 12–24 hours may occur between the insemination and ovulation, the use of Accudenz and the consequential extended sperm viability may improve the chances for pregnancy. The best test of clinical efficacy will be trials in couples treated with intrauterine insemination, with the alternating use of Percoll and Accudenz in subsequent cycles.

The present study is a good example of how an improved understanding of sperm physiology, coupled with utilization of newer techniques, including CASA measurements, CK parameters, acrosomal and capacitation assessment with CTC, and consideration of lipid peroxidation rates, provides new insight into clinically relevant aspects of sperm function.

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