Journal of Andrology, Vol. 21, No. 3, May/June 2000

Evaluation of a Container for Collection and Shipment of Semen With Potential Uses in Population-Based, Clinical, and Occupational Settings

MICHAEL O. ROYSTER,* DANNELLE T. LOBDELL,† PAULINE MENDOLA,* SALLY D. PERREAULT,‡ SHERRY G. SELEVAN,§ SUSAN A. ROTHMANN,‖ AND WENDIE A. ROBBINS¶

*From the *HSD/NHEERL, US Environmental Protection Agency, Chapel Hill, North Carolina; the †Department of Social and Preventive Medicine, School of Medical and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York; ‡RTD/NHEERL, US Environmental Protection Agency, Research Triangle Park, North Carolina; §NCEA, US Environmental Protection Agency, Washington, DC; ‖Fertility Solutions Inc, Cleveland, Ohio; and the ¶Center for Occupational and Environmental Health, University of California, Los Angeles, California.*

ABSTRACT: Large, population-based studies of semen quality are encumbered by the logistics and expense of obtaining semen samples from men who live in a variety of locations. A prototype semen collection and transportation kit, the TRANSEM100™, can be distributed to study participants and then directly shipped to a central laboratory for analysis. This study was designed to evaluate the ability of male volunteers to correctly use the kit. Thirty volunteers aged 20 to 44 years with no history of diabetes, recent chemotherapy, fertility problems, or vasectomy were recruited through a newspaper advertisement, interviewed to obtain demographic information, and instructed on the use of the kit. Twenty-six of the initial subjects provided at least 1 semen specimen using the kit and returned the specimens by overnight delivery to the laboratory for analysis, 25 completed a follow-up interview on the use of the collection kit, and 20 submitted a second semen sample using the same method. The average volunteer was white, 27.8 years old, and held at least a college degree. Forty percent of the volunteers were married. In general, participants correctly followed the instructions for collecting, packaging, and shipping the semen samples. Volunteers were instructed to collect samples after at least 2, but no more than 7 days of abstinence. For the first and second samples submitted, participants collected semen samples after an average of 3.3 and 3.9 days of abstinence, respectively. Seventeen (65%) of the samples from the first sampling period and 16 (80%) of the samples from the second period were received in the laboratory the day after they had been collected. In summary, the TRANSEM100™ may prove to be useful for collecting human semen in field studies. Further testing of this method is warranted to evaluate preservation of sample quality and use of the kit by men among diverse socioeconomic groups.

Keywords: Male reproductive health, human sperm analysis, sperm sample.

J Androl 2000;21:478–484

Semen analysis is a key element in population-based studies of male reproductive health (Schrader et al, 1992). Effects of environmental and occupational toxicants on male fertility often are reflected in sperm count and morphology (Overstreet, 1994). Recent evidence that sperm counts may be declining and concerns about reproductive damage from endocrine-disrupting agents have prompted an increased interest in the surveillance of exposed populations (Auger et al, 1995; Lahdetie, 1995; Irvine, 1997; Rasmussen et al, 1997; Swan et al, 1997). Therefore, practical and reliable methods of semen collection that promote participation and reduce the likelihood of selection bias are important in broadening the understanding of male reproductive health.

Occupational and population-based studies are hampered by low participation rates (30–50%; Lahdetie, 1995) and by the need to sample individuals in diverse locations. Reasons for low participation rates include embarrassment about providing specimens and privacy concerns because, typically, samples are collected in a clinical setting (Lahdetie, 1995; Wyrobek et al, 1997). In addition, multiple semen samples may be required to evaluate changes over time, such as variations in exposure conditions; or to stabilize highly variable measures, such as sperm concentration (Opsahl et al, 1996), which requires that a study team return to collection sites multiple times. The ability to collect a sample in a private setting and directly ship it to a central laboratory may help alleviate some of these problems.

The purpose of this study was to assess the ease of use of a kit designed to collect semen in a private setting and to ship the specimen to a laboratory. The kit was evalu-

Supported by EPA Request for Application 7D1397TASX with Fertility Solutions, Inc.

Correspondence to: Dr Pauline Mendola, US Environmental Protection Agency, MD-58A, Human Studies Facility, Research Triangle Park, NC 27711.

Received for publication August 26, 1999; accepted for publication December 13, 1999.

ated for the ability of men to follow instructions for semen collection, packaging, and shipment. Semen analyses were performed for each sample submitted, with the objective of identifying problems associated with sample handling and preservation after shipping. Feedback was collected from participants to improve methodologies for future studies.

Methods

Subject Recruitment

Thirty men aged 20 to 44 years were recruited from central North Carolina in September 1998 using advertisements placed in local newspapers. To encourage participation the men were paid $10 if they completed an initial interview, $25 for each semen sample they submitted (2 samples maximum), and a $25 study-completion bonus. Men were excluded from participating in the study if they had a history of fertility problems or vasectomy, chemotherapy in the past year, or insulin-dependent diabetes.

Initial Subject Interviews

Face-to-face interviews were conducted by trained interviewers at the U.S. Environmental Protection Agency, Human Studies Facility, in Chapel Hill, North Carolina. Interviewers collected demographic information and reviewed instructions for the use of the kit with each participant.

Semen Collection Kit

The TRANSEM100™ semen collection kit (Fertility Solutions Inc, Cleveland, Oh) contains a sterile, screw-cap polypropylene specimen jar, which was tested for sperm toxicity. A temperature indicator (ColdSnap™, Telatemp Corporation, Fullerton, Calif), designed to indicate whether the temperature during transportation fell below 2°C, was attached to the top of the specimen container. The kit and termperature indicator were sealed in a plastic biohazard specimen bag and placed in a tamper-resistant, insulated, plastic secondary container, which was placed in an insulated, cardboard shipping box. The instructions for use and shipment were placed in a sealed bag that was attached to the outside of the box.

Semen Collection Instructions

During the initial interview, the men were given oral instructions, shown a demonstration of how to assemble the collection kit components, and how to activate the Coldsnap™ gauge. They were instructed to abstain from ejaculation for at least 2, but no more than 7 days, before collecting a semen sample. The men were instructed to wash and dry their hands and penis before collecting the sample and to collect the sample by masturbation without the use of a lubricant. They were asked to record on the collection container label the time and date of collection, the amount of ejaculate collected, and date and time of their most recent previous ejaculation. They were also instructed how to package the samples inside the transportation tube and to arrange for the samples to be picked up by an overnight delivery service. The men were instructed to collect specimens so that they could be picked up Monday through Thursday before 2 PM.

Follow-up Telephone Interview

Follow-up interviews were performed with all but 1 subject after the first set of semen samples were received. Participants who had not submitted a sample were telephoned and encouraged to submit one. Interviews assessed the acceptability of the sample collection, packaging, labeling, and shipping procedures. Participants were also asked to submit a second sample using the same method.

Semen Analysis

When each TRANSEM100™ kit was received at Fertility Solutions Inc in Cleveland, Ohio, the packages were evaluated to see if the instructions had been followed. The outer and inner containers were examined for evidence of leakage. The ColdSnap™ gauge was examined to determine if it had been correctly activated and whether the indicator showed that the temperature had fallen below 2°C during transportation.

Semen analysis was performed using methods described in Kinzer and Rothmann (1998). Each specimen was macroscopically examined for consistency and completeness of liquefaction. Samples were mixed well and volume was determined using a 5-mL disposable polycarbonate serologic pipet. Sperm concentration was manually determined using a 12-μ-deep Microcell™ counting chamber and phase contrast microscopy. Percent motility was determined using an objective immobilization procedure. Bacterial contamination or sperm aggregation or clumping was also noted. Analyses were performed by counting the sperm in all of the squares within the reticle eyepiece grid. All steps of the procedure were performed at ambient room temperature, approximately 22°C.

Two semen smears of each sample were prepared, fixed with hairspray (AquaNet, Chesebrough-Ponds USA Co, Greenwich, Conn), and stained using a modified Papanicolaou method. The same andrologist assessed the morphology of 200 cells for each specimen using criteria established by both the World Health Organization (WHO) and Tygerberg Strict Criteria (Menkveld et al, 1990, 1991; World Health Organization, 1992; Rothmann, 1997; Rothmann et al, 1998). Two additional semen smears of each sample were prepared and stained with eosin Y and counterstained with nigrosin to evaluate sperm viability.

Laboratory Quality Control

On days that samples were analyzed, microscope illumination was calibrated, accuracy and precision of counting were assessed using 2 levels of AQC™ Stabilized Sperm Concentration (Fertility Solutions Inc, Cleveland, Oh) and precision of motility was assessed. At least once a week, the microscope phase contrast was calibrated, the accuracy of viability was assessed using 2 levels of AQC™ Viability slides (Fertility Solutions), and the accuracy of morphology (95% confidence limits) was assessed using 2 levels of AQC™ morphology slides (Fertility Solutions).

Data Analysis

Shipping information and semen analysis data were entered into a custom-made database that was derived from Lotus Approach.

Table 1. *Demographic characteristics by level of participation in a pilot study of home semen collection, North Carolina 1998**

Variable	Number of Semen Samples Submitted: 0 (n = 4)	1 (n = 6)	2 (n = 20)	Total Participants (n = 30)
Age, y				
Mean	25.0	26.5	28.8	27.8
Standard deviation	4.4	5.4	6.7	6.2
Minimum	20.0	22.0	21.0	20.0
Maximum	30.0	37.0	44.0	44.0
Ethnicity, n (%)				
White, non-Hispanic	3 (75)	6 (100)	17 (85)	26 (87)
Hispanic	. . .	. . .	1 (5)	1 (3)
Black, non-Hispanic	1 (25)	. . .	. . .	1 (3)
Asian or Pacific Islander	. . .	. . .	2 (10)	2 (7)
Education, n (%)				
High school	. . .	. . .	1 (5)	1 (3)
Some college	3 (75)	2 (33)	4 (20)	9 (30)
College	1 (25)	4 (67)	10 (50)	15 (50)
Graduate/professional school	. . .	. . .	5 (25)	5 (17)
Current student, n (%)				
Yes	3 (75)	2 (33)	9 (45)	14 (47)
No	1 (25)	3 (50)	10 (50)	14 (47)
Unknown	. . .	1 (17)	1 (5)	2 (6)
Marital status, n (%)				
Married	. . .	2 (33)	10 (50)	12 (40)
Living together	. . .	1 (17)	2 (10)	3 (10)
Never married	3 (75)	3 (50)	6 (30)	12 (40)
Unknown	1 (25)	. . .	2 (10)	3 (10)
Total pregnancies fathered, n (%)				
None	4 (100)	5 (83)	12 (60)	21 (70)
One or more	. . .	1 (17)	8 (40)	9 (30)

* There was no statistically significant difference in level of participation based on demographic characteristics.

Each entry was validated by 2 observers who compared original worksheet data with the database. Validation was performed twice. The data were imported into SPSS (SPSS Inc, version 7.5, Chicago, Ill) to statistically analyze frequencies, means, standard deviations, and ranges. In addition, we used analysis of variance and chi-square tests to observe the association between demographic factors and level of participation.

Results

Subject Characteristics

Recruitment was stopped after 30 men volunteered for the study. Of these, 1 volunteer withdrew after completing the initial interview and 3 who had agreed to submit a sample did not do so. Thus, 26 men (87% of volunteers) submitted at least 1 sample, and 20 of the 26 (77%) who submitted 1 sample also submitted a second sample.

Of the 6 men who submitted only 1 sample, 1 did not pick up the second kit; 4 expressed an interest in submitting a second sample but by the time they were interviewed no more collection kits were available; and 1 man submitted a sample after solicitation for second samples had ended.

Table 1 depicts the demographic characteristics of volunteers. They were predominantly white, mean age 27.8 years. All volunteers except 1 had some college education and two-thirds held college degrees. Less than 50% of subjects were students when they participated in the study. Fifty percent were married or lived with a partner, 40% were single, and 10% had an unknown marital status. No volunteer reported a history of fertility problems, and 30% of men had fathered at least 1 child.

Compliance Characteristics

Most men complied with the semen collection and shipping instructions (Table 2). Roughly three-fourths of the

Table 2. *Compliance with instructions for collecting and shipping semen samples in a pilot study of home semen collection, North Carolina 1998*

Variable	Semen Sample 1 (n = 26)	Semen Sample 2 (n = 20)
Days elapsed between receipt of collection kit and collection of semen sample, n (%)		
0–7	19 (73)	16 (80)
8–14	2 (8)	2 (10)
15+	5 (19)	1 (5)
Unknown	. . .	1 (5)
Mean (standard) deviation), range	7.73 (8.60), 0–31	4.95 (7.99), 0–35
Days of abstinence prior to collection of semen sample, n (%)		
<2	1 (4)	1 (5)
2–3	12 (46)	10 (50)
4–7	11 (42)	8 (40)
8+	1 (4)	. . .
Unknown	1 (4)	1 (5)
Mean (standard deviation), range	3.92 (2.27), 1–13	3.26 (1.10), 1–6
Days after collection of semen sample that it was received in the laboratory, n (%)		
1	17 (65)	16 (80)
2	6 (23)	1 (5)
3+	3 (12)	3 (15)
Mean (standard deviation), range	1.65 (1.32), 1–7	1.85 (2.21), 1–10

subjects who collected samples did so within a week of receiving the kit. The average amount of time subjects waited to collect a first sample was almost 8 days, compared with 5 days for the second samples.

The desired abstinence interval was 2 to 7 days. The average abstinence time was well within this range, with 88% to 90% of participants collecting their first and second samples after 2 to 7 days of abstinence.

Subjects were instructed to ship the samples the day that they were collected in order to arrive at the laboratory the next day. Seventeen (65%) of the first samples were received by the laboratory the day after collection. Sixteen (80%) of the second samples were received the day after collection. In each set of specimens, 3 specimens were not received in the laboratory until 3 or more days after collection.

Summing the average number of days to collect the specimen and the average number of days for the specimens to be received by the laboratory after collection yields an approximation of the turnaround time between distribution of kits and receipt of specimens in the laboratory. For the first samples, this time was roughly 9.4 days; for the second group it was approximately 7 days.

More than 90% of participants correctly assembled the collection kits. Incorrect assembly included failure to completely assemble the components of the collection kit as instructed. These minor errors did not cause any containers to leak or prevent sample analysis (Table 3). The ColdSnap™ temperature gauge was activated on all kits, but on 6 kits (13%), the pull-tab had been damaged. The damage did not prevent the ColdSnap™ from working and none of the indicators showed evidence of exposure to temperatures below freezing.

Table 3. *Compliance with instructions for packaging of semen specimens in a pilot study of home semen collection, North Carolina 1998*

Variable	Semen Sample 1 (n = 26), n (%)	Semen Sample 2 (n = 20), n (%)
Adequate component assembly	24 (92)	19 (95)
Container did not leak	26 (100)	20 (100)
ColdSnap activated	26 (100)	20 (100)

Twenty-four of 25 (96%) participants who were interviewed reported that they were able to follow the directions for collecting semen specimens and that the collection container was easy to use (Table 4). One of the 26 participants who submitted at least 1 sample was not interviewed because he submitted the first sample after the follow-up interviews had been completed.

All participants reported that they were able to correctly replace the lid on the collection container and to follow directions for sealing and labeling the container, and no collection containers leaked. However, 3 subjects

Table 4. *Responses from follow-up telephone interviews of participants in a pilot study of home semen collection, North Carolina 1998*

Variable	Affirmative Response (n = 25), n (%)
Sample collection	
• Able to follow directions	24 (96)
• Collection container easy to use	23 (92)
Sample packaging	
• Placed lid on correctly	25 (100)
• Able to follow directions for sealing and labeling	25 (100)
• Rating of packing method (1–5)	
5 (easy)	16 (64)
4 (above average)	9 (36)
Sample shipping	
• Able to follow directions for packing and shipping	24 (96)
• Rating of shipping method (1–5)	
5 (easy)	14 (56)
4 (above average)	8 (32)
3 (average)	2 (8)
2 (below average)	1 (4)
Willing to collect another sample	25 (100)

Table 5. *Semen volume, sperm concentration, and sperm morphology in the first and second samples submitted in a pilot study of home semen collection, North Carolina 1998*

Parameter	Semen Sample 1 (n = 26), n (%)	Semen Sample 2 (n = 20), n (%)
Volume (mL)		
<2	6 (23)	7 (35)
2–5	17 (65)	12 (60)
>5	3 (12)	1 (5)
Mean (standard deviation), range	3.0 (1.6), 0.5–7.4	2.8 (1.7), 0.5–7.7
Concentration (million sperm/mL)		
<10	2 (7)	1 (5)
10–20	3 (12)	3 (15)
20–250	21 (81)	16 (80)
Mean (standard deviation), range	48.9 (27.1), 8.5–105.0	60.3 (41.4), 6.7–169.6
Morphology		
Samples normal by World Health Organization criteria	18 (69)	14 (70)
Samples normal by strict criteria	19 (73)	13 (65)

did not label their collection containers with the date of collection or the date of their most recent ejaculation, as they had been instructed. The missing variables were inferred by laboratory personnel from shipping and activity data submitted by the participants.

On a scale of 1 to 5 for ease of use, all subjects rated the packing method as easy (5) or above average (4). The primary concern (mentioned by 13 subjects) was confusion over the correct manner in which to activate the ColdSnap™ temperature gauge.

Satisfaction with the shipping method was not as high as it was for the packing method (Table 4). Using the same scale of 1 to 5, 96% of participants said they were able to follow the directions and 88% rated the shipping method as easy (5) or above average (4). Seven subjects reported that it was inconvenient to collect samples only on Monday through Thursday before 2 PM. One participant expressed confusion about the acceptable amount of time between collection and kit pick-up by the overnight shipping service and whether temperature extremes would affect the specimen during this time. All of the volunteers who were interviewed (n = 25) expressed an interest in collecting a second sample.

Semen Characteristics

Table 5 summarizes the results of the semen analyses for semen volume, sperm concentration, and sperm morphology. The objective of collecting these data was to identify any problems with sample analysis after shipping, rather than to ascertain sample preservation because semen quality before shipment could not be determined. In general, results for the first and second series of samples were comparable. Two specimens each contained a volume of 0.5 mL, raising the possibility that they may have been incomplete samples. Eighty percent of the first samples and 81% of the second samples had sperm concentrations above the WHO reference value of 20 million sperm/mL of semen (WHO, 1992) and the range of sperm concentrations was wide, as is expected among the general population. Microscopic evaluation of semen revealed sperm clumping and aggregation in 10 (22%) samples. Of these, 7 were sent in late (an average of 3.5 days after collection). Four specimens contained bacteria; 3 of these had been received late (2, 4, and 10 days after collection).

Discussion

This pilot study demonstrates that the TRANSEM100™ collection kit has the potential for allowing men to collect semen in a private setting, and then to ship samples to a central laboratory for analysis. Such a kit could be useful for population-based studies in which it is desirable to obtain samples from men in diverse locations and to evaluate male reproductive health after adverse environmental or occupational exposures.

The intent of the study was to quickly obtain volunteers and no attempts were made to obtain samples from a diverse population. Volunteers were primarily white, college-educated men, ranging in age from 20 to 44 years, and lived in urban or suburban areas. Additional studies are needed to adequately evaluate the willingness of subjects with more varied socioeconomic backgrounds to participate in such a study and to use the kit. We hypothesize that semen collection in a setting of a subject's choice may enhance participation by increasing personal comfort and convenience, but this hypothesis remains to be tested in a larger, more diverse group of men.

Levels of compliance with instructions for semen collection, packaging, and shipping measured 2 factors: 1) the ability of participants to follow directions and 2) the clarity of the instructions that were provided. According to the follow-up questionnaires, participants expressed confidence in their overall ability to follow instructions for semen collection, packaging, and shipment. In addition, most men completed the study in a timely manner and as instructed. The few packaging errors encountered were not detrimental to the sample integrity or safety. Several men had difficulty activating the ColdSnap™ device but the device was successfully activated on all samples and showed that none of the samples were exposed to suboptimal temperatures, which indicates that the pack-

age insulation is adequate for protecting the samples during air shipment. The ColdSnap™, which is expensive, probably is not necessary and will be removed from future kits.

This pilot study provided information on the use of a home semen-collection kit that will be useful in designing follow-up studies. Most participants collected specimens within 1 week of receiving the kit; however, approximately 26% of the participants' samples were not received in the laboratory within 1 day of collection. This delay may be expected to result in excessive sample degradation before analysis, especially among samples that were not sent for several days after collection. Indeed, 3 of 4 samples that contained bacteria were shipped late, which suggests that compliance with the original shipping schedule is important to reduce bacterial contamination. The use of preservatives was tested in the initial development of the TRANSEM100™ but they were found to alter sperm morphology, and thus, were not used in this pilot study (Rothmann et al, 1998).

Some men reported that it was difficult to collect the specimen so that it could be picked up by the shipper between Monday and Thursday before 2 PM. Because most of the subjects were employed, compliance with the time restrictions may have been especially difficult to maintain; however, this inconvenience should have been much less than the inconvenience of participating in research that required semen collection at a study facility. Whatever the reason for noncompliance, the study demonstrated that some men did not comply with the collection and shipping schedule and this needs to be factored into sample-size calculation in future studies. Part of the shipping-compliance problem is probably related to misunderstandings about when to send the sample and suggests that instructions must be more explicit about the appropriate times and days of collection. The importance of sending the specimen on the day of collection must be emphasized. An instructional videotape showing how to use the TRANSEM100™ is in development for inclusion with the kit.

In at least 4 cases, the shipping company apparently did not pick up the package at the arranged time or failed to recognize that the shipment was prepaid. This is likely to be a problem with any shipping company and will need to be considered for subsequent studies.

In this study, semen volume, sperm concentration, and sperm morphology values were consistent with expectations for an unselected group of young men. However, because semen analyses were performed only after the samples had been shipped, conclusions regarding specimen preservation cannot be made. Separate studies are under way to address this issue by examining all standard measures of semen quality (including sperm motility and viability) in samples assayed immediately after liquefaction and again 1 or more days after simulated shipping.

This pilot study demonstrated that the TRANSEM100™ has the potential for increasing participation in studies requiring semen analysis. It allows men to collect semen in a setting of their choosing and to ship samples to a central laboratory for analysis. The kit could be used for analysis of serial semen samples obtained before, during, and after voluntary exposure to potential reproductive toxicants such as chemotherapeutic agents, or after occupational or environmental exposures of interest. Such a kit could also be useful for population-based studies in which it is desirable to collect samples from men in diverse locations and to evaluate semen parameters after environmental or occupational exposures.

Acknowledgments

The authors acknowledge Rebecca L. Calderon, PhD, branch chief, Epidemiology and Biomarkers Branch, HSD/NHEERL, U.S. Environmental Protection Agency, for her contributions to the manuscript. The authors thank the recruitment and interviewing staff at Bionetics Inc, for their work in the pilot study. Their work was done under a ''Subject recruitment for clinical studies'' contract (68D30044).

This document has been reviewed and approved for publication in accordance with U.S. Environmental Protection Agency policy. Approval does not signify that the contents necessarily reflect EPA views and policies, nor does mention of trade names or commercial products constitute their endorsement or recommendation for use.

References

Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the last 20 years. *N Engl J Med.* 1995;332:281–285.

Irvine DS. Declining sperm quality: a review of facts and hypotheses. *Baillieres Clin Obstet Gynaecol.* 1997;11:655–671.

Kinzer DK, Rothmann SA. *The Andrology Trainer*. Cleveland, Oh: Fertillity Solutions Inc; 1998.

Lahdetie J. Occupation- and Exposure-related studies on human sperm. *J Occup Environ Med.* 1995;37:922–930.

Menkveld R, Stander FSH, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Hum Reprod.* 1990;5:586–592.

Menkveld R, Oettlé EE, Kruger TF, Swanson RJ, Acosta AA, Oehninger S. *Atlas of Human Sperm Morphology*. Baltimore, Md: Williams & Wilkins, 1991.

Opsahl MS, Dixon NG, Robins ER, Cunningham DS. Single vs Multiple semen specimens in screening for male infertility factors. A comparison. *J Reprod Med.* 1996;41:313–315.

Overstreet JW. Clinical approach to male reproductive problems. *Occup Med.* 1994;9:387–404.

Rasmussen PE, Erb K, Westergaard LG, Laursen SB. No evidence for decreasing semen quality in four birth cohorts of 1055 Danish men born between 1950 and 1970. *Fertil Steril.* 1997;68:1059–1064.

Rothmann SA, ed. *Sperm Confirm: A Photoslide Atlas of Semen Cytology and Sperm Morphology*. Cleveland, Oh: Fertility Solutions Inc; 1997.

Rothmann SA, Kinzer DK, Quigley J, Caruso C, Robbins WA, Perreault-Darney S. Evaluation of preservatives for semen collection and ship-

ping. In: *Proceedings of Hazardous Substances and Male Reproductive Health.* New York, NY: Academy of Medicine; 1998.

Schrader SM, Chapin RE, Clegg ED, Davis RO, Fourcroy JL, Katz DL, Rothmann SA, Toth G, Turner TW, Zinaman M. Laboratory methods for assessing human semen in epidemiologic studies: a consensus report. *Reprod Toxicol.* 1992;6:275–279.

Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect.* 1997;105: 1228–1232.

World Health Organization. *WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction.* 3rd ed. Cambridge, United Kingdom: The Press Syndicate of the University of Cambridge; 1992.

Wyrobek AJ, Schrader SM, Perreault SD, Fenster L, Huszar G, Katz DF, Osorio AM, Sublet V, Evenson D. Assessment of reproductive disorders and birth defects in communities near hazardous chemical sites. III. Guidelines for field studies of male reproductive disorders. *Reprod Toxicol.* 1997;11:243–259.