Factors associated with the quality before freezing and after thawing of sperm obtained by microsurgical epididymal aspiration

Rakesh K. Sharma, Ph.D.
Osvaldo F. Padron, M.D.
Anthony J. Thomas, Jr., M.D.
Ashok Agarwal, Ph.D.*

Andrology Research and Clinical Laboratories, Department of Urology, The Cleveland Clinic Foundation, Cleveland, Ohio.

Abstract

Objective: To identify whether the cause, site of ductal obstruction, and characteristics of fluid aspirates are associated with the cryosurvival and fertility after thawing of sperm obtained during reconstruction of the excurrent ducts with microsurgical epididymal sperm aspiration, vasal sperm aspiration, or both.

Design: Prospective study.

Setting: Andrology center at a tertiary care institution.

Patient(s): Men undergoing reconstruction of the excurrent duct and sperm aspiration (n = 42) or microsurgical epididymal sperm aspiration (n = 11).

Intervention(s): Sperm were tested for an association with the cause and site of obstruction. Fertilization and pregnancy rates after sperm aspiration and intracytoplasmic sperm injection (ICSI) were evaluated for fresh and frozen aspirates.

Main Outcome Measure(s): Motile sperm count and percentage motility after thawing.

Result(s): The motile sperm count before freezing was significantly higher in the caput epididymis than in the corpus. The motile sperm count before freezing was related inversely to the distance from the caput where the sperm were aspirated. Sperm from clear and opaque fluid aspirates had better motility than those from cloudy and creamy fluid aspirates. High fertilization and pregnancy rates were achieved using both fresh and frozen epididymal sperm.

Conclusion(s): None of the factors studied was associated with cryosurvival of aspirated epididymal or vasal spermatozoa. Because motility is low after thawing, these specimens are best used with ICSI. (Fertil Steril 1997;68:626-31 Copyright © 1997 American Society for Reproductive Medicine.)

Key Words: Microsurgery, epididymal spermatozoa, cryopreservation, MESA, ICSI

It is now common practice to cryopreserve sperm obtained at the time of excurrent duct reconstruction for possible later use with intracytoplasmic sperm injection (ICSI). The reasons for sperm cryopreservation at the time of reconstruction are the possibility of failure of the procedure or subsequent obstruction after initial success and patenty. After vasoepididymostomy, azoospermia persists in approx-
thawed epididymal sperm produce similar fertilization rates and PRs with microsurgical epididymal sperm aspiration and ICSI (7). Therefore, a cryopreserved sample can obviate the need for additional surgical procedures to obtain sperm.

The purposes of this study were as follows: (1) to assess the relations between the site, cause, and duration of vasal and epididymal obstruction and the character of sperm-containing epididymal fluid obtained with microsurgical epididymal sperm aspiration with the sperm characteristics before freezing and after thawing; (2) to determine whether a specific motility before freezing could predict a motility after thawing of greater than zero; and (3) to assess the outcome of microsurgical epididymal sperm aspiration/ICSI using fresh and frozen sperm aspirates.

MATERIALS AND METHODS

This study was approved by the institutional review board. Semen specimens were obtained from 53 patients undergoing excurrent duct reconstruction or microsurgical epididymal sperm aspiration alone. Forty-two patients had vas deferens or epididymal reconstruction (vasovasostomy, n = 13; epididymovasostomy, n = 26; both vasovasostomy and epididymovasostomy, n = 3). The results of semen specimens from 2 patients who had vasectomy and were treated for obstruction were excluded from the analysis because their sperm counts were so low that the percent motility values were elevated erroneously (semen was sampled from the vas deferens in one and from the cauda epididymis in the other).

Eleven men underwent microsurgical epididymal sperm aspiration and ICSI with fresh aspirates. Three of these men subsequently required the use of their cryopreserved aspirates. The remaining patients did not elect ICSI; they preferred to wait for the success or failure of their surgical intervention. When multiple specimens were obtained (from the same or contralateral side), the average percent motility values were used in the analysis.

Microsurgical Epididymal Sperm Aspiration Technique

Using the operating microscope, we explored the epididymis beginning at the cauda and moving toward the caput until finding long-tailed, normal-appearing sperm. If microsurgical epididymal sperm aspiration was performed without reconstruction (n = 10), exploration of the epididymis was continued until motile sperm were found. If reconstruction was to take place, it was done at the site where normal appearing sperm were found, either motile or nonmotile. A 0.5-cm long incision was made in the epididymal tunic, and a single convoluted tubule was isolated using blunt-tipped, curved microscissors (Figure 1). When a loop of the tubule was freed completely from the surrounding connective tissue, a 0.5-mm longitudinal incision was made in the isolated loop using a microsurgical knife.

Fluid exuding from this tubule was aspirated with a 24-gauge angiocatheter attached to a tuberculin syringe. An aliquot of the aspirate was mixed with human tubal fluid (HTF) medium (Enhance-W; Conception Technologies, La Jolla, CA), placed on a sterile glass slide, and examined by light microscopy for the presence of normal-appearing sperm (Figure 1B). If sperm were not seen, another incision was made 0.5-1 cm more proximal, and the procedure was repeated until sperm were found. Using a flexible ruler, we
measured the distance in millimeters from the tip of the caput to the point where motile sperm were found. The site from which the sperm were obtained (vas deferens, caput, corpus, or cauda epididymis) and the fluid characteristics of the aspirates were determined by one observer (A.J.T.). Fluid aspirates were categorized as follows: clear, water-like clarity; opaque, slightly turbid appearance but can be seen through if placed on a glass slide; cloudy, nontranslucent white to yellowish fluid; or creamy, thick, paste-like substance, generally yellow.

Motile sperm were aspirated and mixed with HTF. If no reconstruction was planned, the epididymal tubule was closed using 10-0 nylon suture and the epididymal tunic was closed using 9-0 nylon suture. Otherwise, either a two-layer vasovasostomy or end-to-side vasoepididymostomy was performed as indicated.

**Semen Analysis**

Five microliters of the fresh aspirate was loaded on a 20-micro L sperm-counting chamber (Microcell; Conception Technologies, La Jolla, CA) and analyzed on a computer-assisted semen analyzer (Motion Analysis Corp., Palo Alto, CA). Each specimen was analyzed before and after cryopreservation. In addition, 8-10 fields with a minimum of 200 sperm each were assessed manually by light microscopy to validate the semen analyzer results. The motile sperm count and percentage of motile sperm were determined.

**Cryopreservation**

The freezing medium used was TES and Tris-yolk buffer with glycerol (TEST; Irvine Scientific, Santa Ana, CA). A 5-mL vial of TEST was thawed by incubation at 37 (degree sign) C. An aliquot of frozen medium equal to 25% of the original specimen volume was added to the specimen. The specimen was mixed gently for 5 minutes using a Hematrek aliquot mixer (Miles, Elkhart, IN). This procedure was repeated until an equal volume of freezing medium was added. The specimen was then divided equally and placed in polypropylene cryovials (1.2-mL capacity, Corning; Fisher Scientific Products, Pittsburgh, PA). An additional vial was cryopreserved to assess the survival after 24 hours. Cryovials were placed in the freezer at -20 (degree sign) C for 8 minutes, immersed in liquid nitrogen vapor for 2 hours, and finally submerged in liquid nitrogen at -196 (degree sign) C for long-term storage. For evaluation after thawing, a vial was removed from the liquid nitrogen, thawed at room temperature for 5 minutes, and incubated at 37 (degree sign) C for 20 minutes. A 5-micro L aliquot was analyzed as described earlier.

**Statistical Analysis**

Correlations between the duration of obstruction, epididymal measurement, and sperm characteristics were assessed with Spearman's rank-order correlation coefficient. The Kruskal-Wallis test was used to determine whether sperm characteristics changed with the site of obstruction, fluid character, or cause of obstruction. For any significant Kruskal-Wallis test, a Wilcoxon rank-sum test was used for all pair-wise comparisons with Bonferroni corrections. A P value of <= 0.05 was considered statistically significant; all tests were two tailed. All statistical analyses were performed using the SAS statistical package (Cary, NC).

**RESULTS**

A total of 107 patients consented to sperm cryopreservation at the time of excurrent duct reconstruction. The study was done between June 1995 and June 1996, when the first clinical trials with ICSI started in our institution. The causes of obstruction in the patients who had sperm present during reconstruction were congenital absence of the vas deferens (n = 5), congenital obstruction (n = 10), inflammation (n = 8), vasectomy (n = 25), and other (n = 3). Included in the "other" category were anejaculation (n = 1), irreparable inguinal vas obstruction (n = 1), and severe scarring and epididymal obstruction after hydrocele surgery (n = 1). The aspirates from 56 patients were not cryopreserved because of the absence of motile sperm.

When the overall duration of obstruction was used as the basis for the association between duration of obstruction and sperm motility, the motile sperm count and percent motility before freezing and after thawing were not correlated with the duration of obstruction (Table 1). The motile sperm count before freezing was related inversely to the distance from the caput where the sperm were aspirated (r = -0.50, P = 0.03). The motile sperm count (before freezing: r = -0.06, P <0.69; after thawing: r = -0.11, P <0.47) and sperm motility (before freezing: r = -0.12, P = 0.39; after thawing: r = -0.13, P = 0.37) showed no correlation with the duration of obstruction (Table 1). Sperm from the caput had a significantly higher motile count before freezing than those from the corpus (P = 0.002; Table 1). Regardless of the region of the epididymis from which those the sperm were obtained, motile sperm counts were low after thawing.
A motility of >0 after thawing was seen if sperm motility before freezing was >5%. This motility after thawing could be predicted from the motility values before freezing with an accuracy of 78.3%.

Table 1  Sperm Characteristics Before Freezing and After Thawing in 51 Men Undergoing Excurrent Duct Repair or Microsurgical Epididymal Sperm Aspiration

<table>
<thead>
<tr>
<th>Site of obstruction</th>
<th>Total sperm count (x10^6/mL) Before freezing</th>
<th>Motile sperm count (x10^6/mL)</th>
<th>Motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput (n = 18)</td>
<td>3.39 (0.82-11.88)</td>
<td>0.41 (0.06-1.33)</td>
<td>0.01 (0.00-0.16)</td>
</tr>
<tr>
<td>Corpus (n = 17)</td>
<td>14.1 (6.4-33.4)</td>
<td>1.5 (0.56-2.9)</td>
<td>0.035 (0.00-0.28)</td>
</tr>
<tr>
<td>Cauda (n = 4)*</td>
<td>16.7 (7.5-22.9)</td>
<td>0.09 (0.0-0.8)</td>
<td>0 (0.00-0.13)</td>
</tr>
<tr>
<td>P value †</td>
<td></td>
<td></td>
<td>0.005 †</td>
</tr>
<tr>
<td>Fluid character</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear (n = 11)</td>
<td>10.8 (0.95-19.9)</td>
<td>0.65 (0.03-2.4)</td>
<td>0.00 (0.00-0.07)</td>
</tr>
<tr>
<td>Inflammation (n = 8)</td>
<td>15.5 (8.9-21.3)</td>
<td>0.86 (0.16-6.1)</td>
<td>0.06 (0.00-0.31)</td>
</tr>
<tr>
<td>Vasectomy/others (n = 28)‡</td>
<td>11.1 (2.5-28.3)</td>
<td>0.26 (0.00-1.23)</td>
<td>0.00 (0.00-0.20)</td>
</tr>
<tr>
<td>P value †</td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>Cause of obstruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAVD/congenital (n = 15)</td>
<td>5.83 (0.85-14.2)</td>
<td>0.84 (0.1-2.0)</td>
<td>0.02 (0.00-0.26)</td>
</tr>
<tr>
<td>Inflammation (n = 8)</td>
<td>17.7 (9.0-42.9)</td>
<td>0.00 (0.00-1.5)</td>
<td>0.00 (0.00-0.09)</td>
</tr>
<tr>
<td>Opaque (n = 26)</td>
<td>11.1 (1.1-19.9)</td>
<td>0.43 (0.12-1.5)</td>
<td>0.00 (0.00-0.11)</td>
</tr>
<tr>
<td>Creamy (n = 3)*</td>
<td>19.4 (16.4-33.4)</td>
<td>0.33 (0.00-4.7)</td>
<td>0.17 (0.00-0.33)</td>
</tr>
<tr>
<td>P value †</td>
<td>0.12</td>
<td>0.1</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Note: Values are medians, with interquartile range in parentheses. CAVD = congenital absence of the vas deferens.

* Cauda and the creamy fluid groups were not included in the statistical analysis because of small sample size.
† Kruskal-Wallis test; P ≤ 0.05 was considered statistically significant.
‡ Wilcoxon rank-sum tests were used for all pairwise comparisons among the three sites, with Bonferroni correction. Caput and corpus groups were significantly different (P = 0.0003); cloudy group was significantly lower than both clear group (P = 0.001) and opaque group (P = 0.0003).
§ Included in others; an ejaculation (n = 1), irreparable inguinal vas obstruction (n = 1), and severe scarring and epididymal obstruction after hydrocele surgery (n = 1).

Similarly, the cause of the obstruction did not affect sperm quality before freezing and after thawing. Sperm quality was associated with the characteristics of the fluid from the aspirate (P < 0.0004). Clear fluid had the highest motility before freezing, followed by opaque, cloudy, and creamy colored fluid. Both clear (P = 0.001) and opaque fluid (P = 0.0003) had a significantly higher motility before freezing compared with creamy fluid (Table 1). Although these differences were not statistically significant, clear fluid demonstrated better motility after thawing. When the samples were examined after thawing, 0% sperm motility was seen in clear fluid (n = 4), opaque fluid (n = 14), cloudy fluid (n = 7), and creamy fluid (n = 1).

To predict a motility of >0 after thawing, we analyzed the motility results before freezing to find a cutoff value that could be used in prediction. Predictive power was calculated solely on the basis of sperm motility before freezing, regardless of the anatomic site of aspiration or fluid characteristics. In this analysis, cutoff points ranging from 30% before freezing were used to predict a motility after thawing of >0 or = to 0. A motility of >0 after thawing was seen in 41.3% of the patients when the motility before freezing was >5%. This motility after thawing could be predicted from the motility values before freezing with an accuracy of 78.3%.

Microsurgical epididymal sperm retrieval and ICSI were performed for congenital absence of the vas deferens (n = 6) and failed vasoepididymostomy (n = 5). Of the nine patients who underwent microsurgical epididymal sperm aspiration and ICSI, 91% (9/11) had motile sperm in the fresh aspirates, whereas 64% (7/11) had motile sperm in the frozen aspirates. In the fresh aspirates, the average percent motility was 23% +/- 17.4%; total sperm count was 24.7 +/- 20.4 x 10^6/mL, and total motile sperm count was 6.5 +/- 10.1 x 10^6/mL. In the frozen aspirates, the average percent motility was 1.2% +/- 1.8%, and the total motile sperm count was 0.32 +/- 0.54 x 10^6/mL. Because the number of patients who opted to undergo assisted reproductive technology in our study was very small, we did not evaluate the relation to undergo assisted reproductive technology in our study.

A fertilization rate of 52% (52 of 101 oocytes) and a PR of 22% per cycle (2/9) were seen (Table 2). Among the cryopreserved aspirates, the fertilization rate was 67% (20 of 30 oocytes) and the PR was 67% (2/3) in the cryopreserved specimens used for ICSI.

**DISCUSSION**

Irreparable excurrent duct obstruction is an uncommon problem in the azoospermic male with normal spermatogenesis. Causes include congenital absence of the vas deferens, multiple obstructions along the excurrent duct system, or extensive...
Table 2 Fertilization and Pregnancy Outcomes With Fresh and Frozen Microsurgically Aspirated Sperm

<table>
<thead>
<tr>
<th>Sperm aspirate</th>
<th>Etiology</th>
<th>No. of oocytes</th>
<th>No. of embryos*</th>
<th>Percent fertilization rate (no. of embryos/no. of oocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (n = 11) †</td>
<td>Failed vasoproididymostomy (5)</td>
<td>44</td>
<td>15</td>
<td>34.1 (15/44)</td>
</tr>
<tr>
<td></td>
<td>CAVD (6/8)</td>
<td>57</td>
<td>37</td>
<td>64.9 (37/57)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>11</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Frozen (n = 3)</td>
<td>Failed vasoproididymostomy (1)</td>
<td>9</td>
<td>8</td>
<td>88.9 (8/9) ‡</td>
</tr>
<tr>
<td></td>
<td>CAVD (2)</td>
<td>21</td>
<td>12</td>
<td>57.1 (12/21)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3</td>
<td>20</td>
<td>66.7 (20/30)</td>
</tr>
</tbody>
</table>

* All embryos were frozen secondary to ovarian hyperstimulation and implanted in the next regular cycle.
† Congenital absence of the vas deferens.
‡ Microsurgical epididymal sperm aspiration and ICSI was not done in 2 of 11 patients because of the lack of mature oocytes at the time of harvesting.

scarring of the vas or epididymis, precluding the possibility of a good result with reconstructive surgery. Attempts to achieve pregnancies with the sperm from these men by creating an alloplastic spermatocele have had limited success (8,9). The potential strategy for epididymovasostomy is to perform the anastomosis at the most distal site of the epididymis where normal-appearing sperm are found. Microsurgical sperm aspiration allows spermatozoa to be retrieved from the epididymis and vas deferens in men with obstructive azoospermia, whether or not it is surgically correctable (10,11).

Compared with the normal motility pattern in a nonobstructed epididymis, where motility increases from caput to cauda, an inverse motility pattern is present in patients with epididymal obstruction. Essentially, in an obstructed system, the oldest (i.e., “dead” or “senescent”) sperm are seen in the most distal region (cauda), and the fresh and most motile sperm are found in the more proximal region (caput). Sperm in the cauda portion of an obstructed epididymis have poor motility, whereas more motile sperm are present in the caput region.

Patency rates and PRs are higher with corpus and caudal anastomosis compared with surgery at the caput area (Thomas AJ, American Urological Association meeting, San Antonio, TX, 1993 abstract). The patency rate depends on both the surgical technique and the epididymal characteristics. Sperm harvested from the proximal caput epididymis and efferent ductules of patients with bilateral absence of the vas deferens have the best quality in terms of motility, viability, and fertility, whereas sperm obtained from the more distal aspects of the remnant epididymis rarely are viable (12,13).

Immotile sperm adversely influence ICSI fertilization rates and PRs, and poor results have been ascribed to the use of dead sperm for ICSI (7,9). In our study, we attempted to predict motility after thawing on the basis of sperm motility values before freezing. The fluid characteristics have been well recognized and written about in other studies concerning vasectomy reversal. In general, clear fluid may or may not contain sperm. When present, sperm are almost always intact. Opaque fluid often contains sperm without much degeneration. Cloudy and creamy fluids are more likely to have sperm debris and macrophages and are generally of poor sperm quality.

Our study demonstrated that after thawing, 55% (22/40) of the specimens in the epididymal duct reconstruction group contained no motile sperm, compared with 36% (4/11) in the microsurgical epididymal sperm aspiration group alone. This difference in motility after thawing can be explained by our approach to reconstructive surgery. Our primary aim was to maintain intact as much of the excurrent duct as possible; sperm quality at aspiration was secondary. On the other hand, in microsurgical epididymal sperm aspiration the reverse was true, and retrieval of the best quality sperm was our main concern. Pregnancy rates with cryopreserved sperm are not significantly different from those with fresh epididymal sperm when ICSI is performed (7,14,15). Cryopreserving epididymal sperm can be a valuable adjunct to vasectomy reversal and may eliminate the need for repeated surgeries.

Intracytoplasmic sperm injection has altered radically beliefs about the quality and quantity of spermatozoa required for successful fertilization with fresh and frozen-thawed epididymal spermatozoa, and ICSI is far superior to conventional IVF (6,16-19). This study confirms previous findings that microsurgically aspirated epididymal sperm can be cryopreserved successfully (14,17). As part of our discussion with couples before surgical reconstruction and cryopreservation, we emphasize the significance and need for ICSI if frozen sperm specimens are to be used. If the couple is not interested in the ICSI procedure, cryopreservation is discouraged. Fertilization rates and PRs with microsurgical epididymal sperm aspiration and ICSI were high in
our study using fresh and frozen-thawed epididymal sperm. In the three specimens in which cryopreserved sperm were used, the fertilization rate was 67% and the PR also was 67%. A fertilization rate of 45% and a cleavage rate of 82% were reported using thawed epididymal spermatozoa with microsurgical epididymal sperm aspiration (17).

The protocol for cryopreserving the microsurgically aspirated epididymal sperm affords the couple much more flexibility in future planning and offers them the opportunity to have spermatozoa aspirated in one facility and ICSI performed in another. Because only a few sperm are required for ICSI, the unused spermatozoa obtained during a fresh microsurgical epididymal sperm aspiration procedure can be cryopreserved and used for a subsequent treatment cycle if needed.

On the basis of our results, we conclude the following: (1) Motile sperm are more likely to be acquired from the caput epididymis; (2) clear or opaque fluid is more likely to be associated with motile spermatozoa than is cloudy or creamy fluid; and (3) about 55% of patients undergoing epididymal duct reconstruction show sperm motility in their aspirates after thawing, compared with 64% of patients having microsurgical epididymal sperm aspiration. These specimens can be used for ICSI only as a result of low motile sperm count after thawing.

Acknowledgments. The authors thank Jar-Chi Lee, M.S., Department of Biostatistics and Epidemiology, for her help in the statistical analysis of the results; and Cheryl Fitzugh, M.T., Clinical Andrology Laboratory, Department of Urology, for technical assistance.

REFERENCES