Semen cryopreservation for men banking for oligospermia, cancers, and other pathologies: prediction of post-thaw outcome

Semen cryopreservation for men banking for oligospermia, cancers, and other pathologies: prediction of post-thaw outcome using basal semen quality

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Objective: To evaluate post-thawing sperm parameters in a large series of men cryopreserving for different cancers and oligospermia.
Design: Retrospective observational study.
Setting: Semen cryopreservation laboratory.
Patient(s): Six hundred twenty-three patients undergoing semen cryopreservation for cancer or oligospermia who discontinued banking.
Intervention(s): None.
Main Outcome Measure(s): Postcryopreservation sperm motility and viability.
Result(s): In oligospermic men, recovery of motile sperm after cryopreservation was possible in only a few out of the 219 samples cryopreserved for this problem. Similarly, independent of the reason for which cryopreservation was required, if one basal semen parameter fell below the 5th percentile of the World Health Organization reference values, recovery of motile and viable spermatozoa after thawing was low. Among samples cryopreserved for cancer, those with testicular cancer showed the lowest basal semen quality and recovery after thawing. In cases of hematological cancers or other types of cancers, motility recovery was similar to that of non-cancer-related samples. Receiver operating characteristic analyses demonstrate that basal progressive and total motility predict the recovery rate of motile sperm after thawing with high accuracy, sensibility and specificity.
Conclusion(s): Our study demonstrates the ability of prefreeze semen parameters to predict cryosurvival in terms of sensitivity and precision. Using this information, the clinician could perform appropriate counseling about the future possibilities of fertility for the patient. (Fertil Steril® 2013;100:1555–63. ©2013 by American Society for Reproductive Medicine.)
Key Words: Sperm cryopreservation, sperm motility, oligospermia, cancer, sperm viability

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Cryopreservation of spermatozoa is, at present, the most valuable and used way to preserve reproductive function in men undergoing gonadotoxic treatments such as chemo- or radiotherapies. In addition, sperm cryopreservation is increasingly used in case of other disorders, such as autoimmune diseases and myelodysplastic syndromes requiring treatments that may affect reproductive functions. Moreover, sperm cryopreservation is offered to patients with severe oligospermia (or even cryptozoospermia) or ejaculatory disorders with the intent of using cryopreserved sperm in case no sperm are found in the ejaculates on the day of intracytoplasmic sperm

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injection (ICSI) (1) to have sperm available in the event of a decline in sperm count, which may occur in these patients (2).

Cryopreservation of spermatozoa was introduced in the early 1960s and is performed with different procedures. In the last version of the World Health Organization (WHO) manual for semen analysis, a protocol is indicated, but the procedure is not standardized (3). The consequences of cryopreservation on sperm functions are well-known. Spermatozoa may be heavily damaged by freezing-thawing procedures and total motility, viability, and morphology are severely affected in most samples (4–7). However, whether the different types of pathology requiring sperm cryopreservation may result in a different outcome of sperm quality at thawing has been studied only for cancer patients, and results are often controversial (8–14). For instance, when results of semen cryopreservation from testicular cancer patients were compared with those of donors, Said et al. (11) and Hotaling et al. (13) found lower cryosurvival rates, whereas Hallak et al. (9) did not find relevant differences. Similarly, whereas Hallak et al. (10) found lower post-thaw motility recovery in Hodgkin's lymphoma patients with respect to healthy donors, motility reduction in the former category of patients was similar to that of healthy donors in the study by Hotaling et al. (13). Outcome of cryopreservation in cases of severe oligospermia has not been evaluated so far. In addition, whether the expected decrease in sperm functions is related, or can be predicted, on the basis of semen quality on the day of cryopreservation has also been poorly documented. One study (15) reported that Kruger strict morphology assessment, among the conventional semen parameters, was the best predictor of progressive motility recovery after thawing in a small number of normozoospermic samples. Other studies (16, 17) evaluating the relationship between prefreezing and post-thawing semen characteristics demonstrated that higher concentration and prefreeze motility and fewer abstinence days are associated with an increased recovery rate in donors for a sperm bank. In general, it appears that, for normospermic samples, postcryopreservation recovery is related to basal semen quality; however, sensitivity and specificity of basal semen parameters in predicting cryosurvival rates have not been established.

Cryopreserved semen is used in assisted reproductive techniques (ART) and, in case of low motility recovery, ICSI is mandatory. Although most studies comparing ICSI using fresh or thawed spermatozoa do not reveal differences in reproductive outcome (18, 19), it appears that ICSI performed with motile spermatozoa gives better results with respect to immotile ones (20, 21). As a matter of fact, ICSI performed using cryopreserved spermatozoa from patients with different types of cancer or pathologies gives rise to variable clinical pregnancy and live-birth rates (12, 22). Owing to the detrimental effects of cryopreservation, the chance of finding motile sperm after thawing to perform the ICSI procedure is greatly decreased. Such a chance may decrease even more in pathological conditions (like testicular cancer and oligospermia) associated with detrimental effects on semen quality (11, 23–25). In light of these considerations, prediction of cryopreservation outcome on the basis of basal semen quality and type of pathology for which cryopreservation is required may help in the management and counseling of these patients.

The present study evaluated sperm motility and viability recovery rates after thawing and the relationship with precryopreservation semen quality in 822 semen samples from men affected by different types of neoplasia, oligospermia, or other pathologies requiring cryopreservation who discontinued sperm banking. Receiver operating characteristic (ROC) curves were used to identify the accuracy of the different semen parameters in predicting motility recovery rates. Our results demonstrate that the recovery rate of sperm motility and viability varies among the different pathologies. In addition, we show that precryopreservation sperm motility predicts motility recovery with a high accuracy.

MATERIALS AND METHODS

Patients

The study was conducted in semen collected from 623 patients undergoing semen cryopreservation in the Laboratory of Andrology of the Azienda Ospedaliera-Universitaria of Florence from 1998 to 2010 who discontinued sperm banking. A total of 822 semen samples have been collected from these subjects, as some patients underwent more than one semen collection to increase the number of cryopreserved straws. Of the 822 samples, 183 were cryopreserved because of hematological malignancies (122 for Hodgkin's lymphoma, 31 for non–Hodgkin's lymphoma, 27 for leukemia), 158 for testicular cancer (78 seminoma, 16 nonseminomatous germ cell tumors, 64 unknown), 83 for mixed cancer pathologies (15 urinary tract, 26 skeletal-muscle, 17 cerebral, 7 gastrointestinal, and 18 other types of cancer), 239 for oligospermia, 56 for ejaculatory disorders, 42 for other pathologies (mostly multiple sclerosis and autoimmune pathologies), and 61 for spinal cord injury (37 using electroejaculation and 24 with vibratory stimulation). All cancer patients cryopreserved sperm before initiation of the antineoplastic treatment. In case of testicular cancer, the majority of patients underwent cryopreservation after orchiectomy. Semen samples cryopreserved with baseline 0% viability ($n = 34$), although cryobanked for ethical reasons, were not considered in the statistical analysis.

All the data provided were collected as part of the routine clinical procedure, and therefore, according to the Italian law, approval from the local Ethics Committee was not required. In addition, informed consent had been obtained from all patients to use discarded, cryopreserverd sperm for research purposes.

Semen Samples for Cryopreservation

Semen samples were collected the same day of cryopreservation by masturbation in the laboratory. In exceptional cases, semen collection was performed at home. With the exception of spinal cord injury patients, all subjects were asked to observe 2–7 days of sexual abstinence. After semen analysis (see below), semen samples were frozen in liquid nitrogen tanks by a manually controlled freezing procedure according to Gandini et al. (26) with minimal modifications. Briefly,

samples were diluted 1:1 (vol:vol) by drop-wise addition of test yolk buffer with glycerol and gentamycin (Irvine scientific). After equilibration at room temperature for 5–10 minutes, sperm were loaded in 500 μL high security sperm straws (Cryo Bio System). Straws were frozen by 8 minutes of exposure to liquid nitrogen vapors and a final plunge into liquid nitrogen. Thawing was carried out by transferring the straw at room temperature for 15 minutes followed by 15 minutes at 37°C before evaluations.

Pretreatment and Post-Treatment Semen Analysis

Semen analysis was performed according to WHO guidelines (3, 27). Pre- and postcryopreservation sperm motility was assessed by optical microscopy, according to WHO criteria (3, 27). Briefly, sperm motility was evaluated by a Leica DMLS microscope using a ×40 objective. The percentages of progressive, nonprogressive, and immotile spermatozoa were evaluated on 200 spermatozoa/sample. Sperm viability was evaluated by using an eosin test according to the WHO manual (3, 27). The Laboratory of Andrology of the Azienda Ospedaliera-Universitaria of Florence has been participating in the UK-NEQAS (United Kingdom National External Quality Assessment Service) external quality control program for semen analysis since 2005. The mean (±SD) percent biases of the laboratory for the years 2012–2013 were 29.3 (±17.5) and 15.6 (±12.1), respectively, for total and progressive motility and 5.7 (±1.5) for sperm concentration (n = 6, data from UK-NEQAS).

Sperm morphology data were not analyzed. Our methods of assessing sperm morphology varied during the study years [according to the fourth edition of the WHO manual (27) until January 2008, and after that, using strict criteria as indicated in the fifth edition (3)]. Thus, our sperm morphology data did not lend themselves to the large-scale data analysis needed for this study.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 20.0 (SPSS) for Windows. Recovery rates of total and progressive motility were evaluated, excluding those samples (n = 52) showing 0% basal motility. The Kolmogorov-Smirnov test was used to test the parameter distribution. Owing to the abnormal distribution of the parameters, statistical significance differences were evaluated by nonparametric Kruskal-Wallis test. The Mann-Whitney test was used for comparisons among groups. Correlations between pre- and postcryopreservation parameters and recovery rates were assessed by Spearman's correlation test. $P<.05$ was considered statistically significant. ROC was used as a binary classifier system to identify the accuracy of precryopreservation semen parameters in predicting post-thawing recovery of at least 1% motility.

Data are shown as median values and 95% confidence limits in the tables and text. The figures have been generated by SPSS software and show median values of the different parameters and the 10th, 25th, 75th, and 90th percentiles.

RESULTS

Effect of Cryopreservation on Sperm Motility and Viability in the Different Patient Categories

Precryopreservation sperm parameters in the different subgroups of patients are shown in Table 1. The median age of the entire population was 34 years (34–35 years) and did not significantly differ among the groups. The median time of cryostorage was 3.01 years (2.9–3.2 years) and did not differ among the groups (not shown). The overall poorest semen quality was found in oligospermic patients. Among patients undergoing cryopreservation for cancer, the lowest semen quality was observed in testicular cancer (Table 1). No significant differences were observed between the two types of germ cell tumors of the testis (seminoma and nonseminoma; Supplemental Table 1) and among Hodgkin's, non-Hodgkin's lymphoma, and leukemia patients (Supplemental Table 2). Although both total and progressive motility are low, patients with spinal cord lesions show, on average, an elevated number of spermatozoa (Table 1).

When the analyzed samples were considered as a whole (n = 788), cryopreservation determined an average median recovery of total and progressive sperm motility of 12.4% (10.67%–15.52%) and 5.8% (4%–7.69%), respectively, and of sperm viability of 32.8% (30%–35.56%). Figure 1 shows the average percentage recovery of sperm motility (panel A, total; and panel B, progressive) and viability (panel C) in the different subgroups of patients. Nonparametric analysis (Kruskal-Wallis test) revealed a significant difference among subgroups for both total and progressive motility and for

TABLE 1

Median (95% confidence interval) of basal semen parameters in the different groups of patients undergoing sperm cryobanking.

	Volume, mL	Total sperm count/ejaculate, ×10^6	Vitality, % vital sperm	Progressive motility, %	TMN, ×10^6
Total, n = 788	2.80 (2.5–3)	37.50 (30.75–46.5)	65.00 (64–68)	31.00 (28–34)	18.15 (12.69–22.78)
Hematological cancer, n = 180	2.40 (2.1–2.5)	96.60 (67.2–129.6)	71.00 (70–75)	50.00 (43–52)	57.87 (41.44–82.16)
Testicular cancer, n = 150	3.00 (2.5–3.3)	46.00 (31.5–59.4)	74.00 (70–76)	46.00 (34–54)	26.46 (15.9–37.54)
Other cancer, n = 83	3.00 (2.6–3.1)	100.80 (60–143)	73.00 (70–76)	44.00 (37–55)	67.58 (29.52–106.26)
Other pathologies, n = 42	2.40 (2–3.2)	113.00 (64–183.6)	75.00 (70–80)	56.00 (48–61)	74.72 (40.32–138.62)
Spinal cord injury, n = 60	1.70 (1.2–2.5)	149.40 (80.6–372)	25.00 (21–32)	11.00 (2–22)	35.23 (11.55–88.8)
Oligospermia, n = 219	3.20 (3–3.6)	5.40 (4.5–7.14)	50.00 (45–54)	15.00 (10–17)	1.20 (0.63–1.72)
Ejaculatory disorder, n = 54	2.55 (1.8–3.4)	44.48 (19.8–100)	65.00 (52–70)	28.00 (17–44)	23.74 (11.6–41.44)

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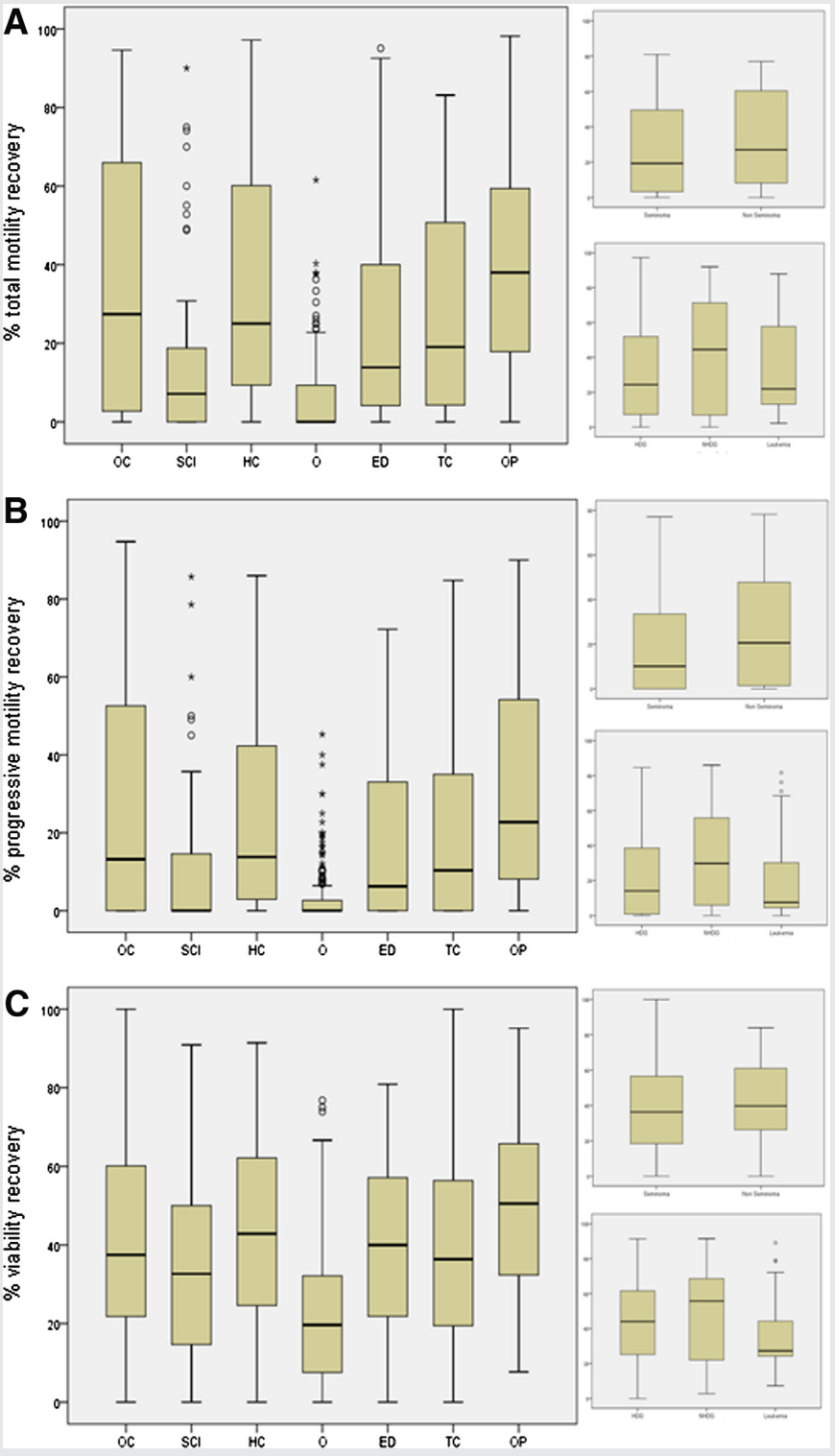
FIGURE 1



Sperm motility and viability recovery in the different groups of subjects undergoing cryopreservation for different reasons. (A) Total motility; (B) progressive motility; (C) viability. The insets in the three panels show progressive (panel A) and total (panel B) motility and viability (panel C) percent recovery in patients with seminoma (n = 76) or nonseminoma (n = 14) testicular cancers (upper insets) and Hodgkin's (n = 120), non-Hodgkin's (n = 30), and leukemia (n = 27) patients (lower insets). OC: other cancers; SCI: spinal cord injury; HC: hematological cancer; O: oligospermia; ED: ejaculatory disorders; TC: testicular cancer; OP: other pathologies. Data are presented as box plots, showing the median values as well as the 10th, 25th, 75th, and 90th percentiles. The single points represent the outlier values.

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viability ($P<.0001$). The highest detrimental effects were observed in oligozoospermic subjects, where the median value of recovery approximates 0% for motility and 20% for viability, with few outliers showing good recovery (Fig. 1). Interestingly, when patients were divided according to the 5th percentile of the new WHO reference values (3), the overall recovery of total (Fig. 2 upper panels) and progressive (Fig. 2 middle panels) motility and viability (Fig. 2 lower panels) after cryopreservation was the lowest in subjects showing basal number, total motility, and viability below the 5th percentile, independent of the pathology for which cryopreservation was required.

Among the other noncancer pathologies, recovery rates of sperm motility were quite low in patients with spinal cord injury (Fig. 1A and B). However, in these patients, the low recovery rates were compensated for by a high number of spermatozoa in the ejaculates (Table 1). The highest recovery rates were observed in the subgroup of non-cancer-related pathologies (Fig. 1).

Among cryopreserved samples for cancer pathologies, the highest detrimental effects were, not surprisingly, observed in testicular cancer (Fig. 1), which also showed poor basal semen quality at cryopreservation (Table 1). When patients with testicular cancer were categorized according to subtypes (seminoma vs. nonseminoma), no significant differences were observed in recovery of motility and viability (Fig. 1, upper insets). In the subgroups of hematological and other (mixed forms) cancers, the overall recovery of motility and viability was similar to that of mixed, noncancer, pathologies or ejaculatory disorders subgroups (Fig. 1). When patients with hematological cancers were divided according to the subtype (Hodgkin's, non-Hodgkin's, and leukemia), no significant differences were observed for recovery parameters (Fig. 1A, lower insets).

Next we calculated the proportion of cryopreserved samples that recover a sufficient number of progressive motile spermatozoa to undergo IUI or IVF. We considered 1.5×10^6 progressive motile spermatozoa as the minimum requirement to undergo IUI and 0.3×10^6 as the minimum to undergo IVF. Such parameters were chosen in consideration of the fact that below 1×10^6 progressive spermatozoa, the IUI prognosis is unfavorable (28) and that, according to the current Italian law, no more than three oocytes can be used for IVF (with about 0.1×10^6 progressive motile spermatozoa/oocyte). Among our cryopreserved samples, 310 (39.3%) recover a number of progressive motile sperm over 1.5×10^6 and 375 (47.6%) over 0.3×10^6. Among the different patient categories, the higher percentages of samples recovering a number of progressive motile sperm over 1.5×10^6 were observed for hematological (61.1%) and other cancers (85.5%) and the lowest for oligospermia (5.5%). A similar pattern was observed when we considered the minimum requirement of progressive motile spermatozoa for IVF (not shown).

Correlations between Pre- and Postcryopreservation Sperm Parameters

We next evaluated the correlations between post-thaw sperm motility and viability and precryopreservation sperm parameters (concentration, total and progressive motility, and viability). As shown in Supplemental Table 3, all post-thawing parameters as well as the percentage recovery of viability and total and progressive motility were highly correlated to precryopreservation sperm number, motility, and total number of motile sperm. The strongest correlations were observed for precryopreservation progressive motility.

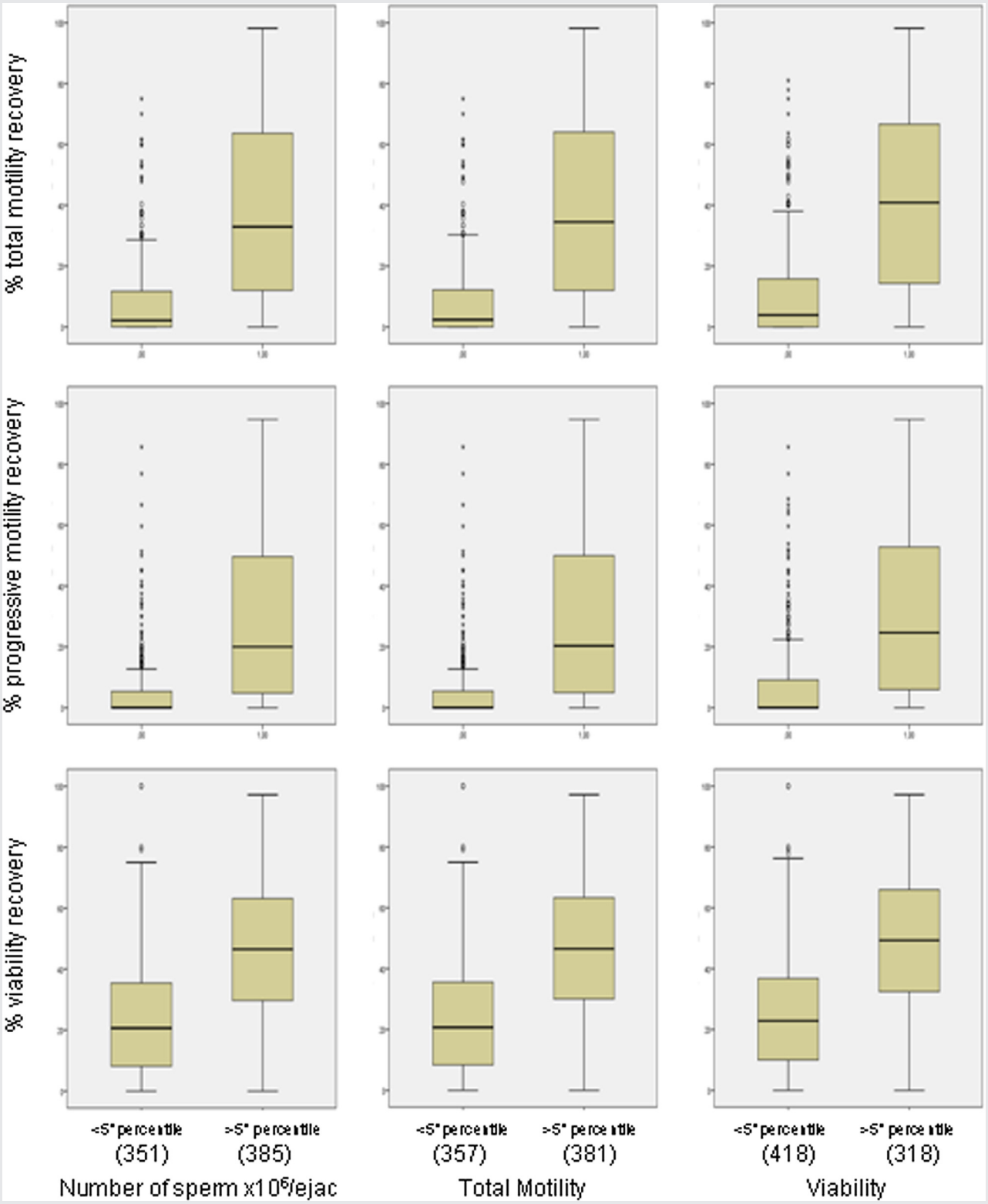
ROC analysis was used to identify the accuracy, sensitivity, and specificity of precryopreservation progressive (Fig. 3A) and total (Fig. 3B) motility, number of spermatozoa/ejaculate (Fig. 3C), and total number of motile sperm (TMN; Fig. 3D) in predicting total motility recovery of at least 1% after thawing. As can be observed in Figure 3, the accuracy was very high for all the parameters (89.0% $\pm$ 0.01%, $P = .000$ for progressive motility; 90.5% $\pm$ 0.01%, $P = .000$ for total motility; 84.0% $\pm$ 0.01%, $P = .000$ for number of sperm/ejaculate; and 90.0% $\pm$ 0.005%, $P = .000$ for TMN). The figure also shows the sensitivity and the specificity at the thresholds chosen for the three parameters.

DISCUSSION

Semen cryopreservation is widely used to preserve fertility in case of treatments that may be toxic for the testis. In addition, the process of cryopreservation is currently used in cases where a progressive decline of testicular function over time is suspected, such as in cases of severe oligospermia (1, 2). Cryopreserved semen is used for several ART procedures, including ICSI. Whereas procedures such as IUI or IVF-ET encompass some of the natural barriers of fertilization, the same cannot be said for ICSI, where the operator selects, when possible, a motile spermatozoon to inject into the oocyte, as this is the only way to assure viability and increase the probability of success (20, 21). We show in the present study that finding a motile spermatozoon after cryopreservation may be a hard task in some thawed semen samples. In particular, motile spermatozoa after thawing are rarely found in semen samples cryopreserved for severe oligospermia. Indeed, we show that only a few samples out of the 239 cryopreserved for this problem recovered motility and viability in a small percentage of spermatozoa (Fig. 1), and only a small percentage of them recover enough progressive motile spermatozoa to perform procedures such as IUI or IVF. It should be considered that, in addition to loss of motility and viability, there might be other detrimental effects after cryopreservation, such as an increase of sperm DNA fragmentation (11, 29, 30) and decondensation (31) as well as alterations of other sperm parameters that may be relevant for the fertilization process (5, 6). All the above data should be taken into account during patient management, and clinicians should inform the patients about the negative impact of cryopreservation on an already altered semen quality and that ICSI may be required in case of an ART procedure with cryopreserved semen.

We also show here that cryopreserved semen samples with basal number, motility, and viability falling below the 5th percentile of the WHO (3) reference value displayed the lowest recovery rates of motility and viability, independent of the reason for which cryopreservation was required. In

FIGURE 2



Sperm parameters recovery after cryopreservation in subjects with basal semen quality below the 5th percentile of WHO reference limits. Total (upper panels) and progressive (medium panels) motility and viability (lower panels) recovery (%) in subjects showing number of sperm/ejaculate (left panels), % total motility (medium panels), and viability (right panels) below the 5th percentile of reference values according to WHO 5th edition manual (27). Data are presented as box plots, showing the median values as well as the 10th, 25th, 75th, and 90th percentiles. The single points represent the outlier values. The number of samples for each group is in parentheses.

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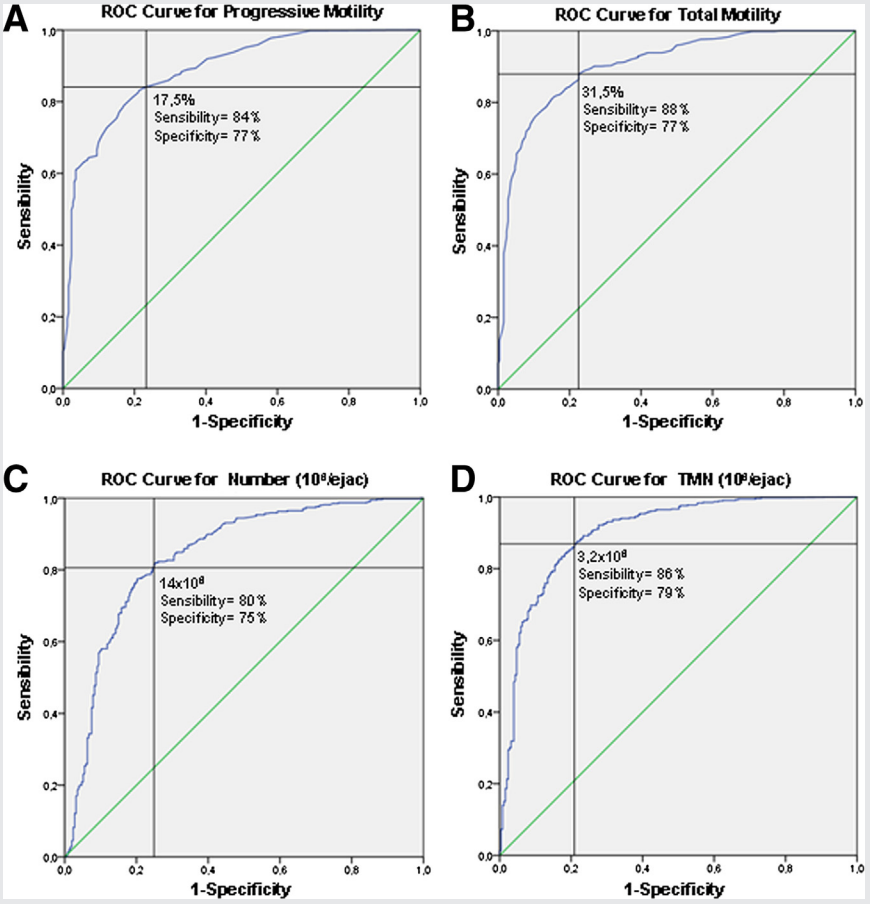
FIGURE 3



ROC curves for prediction of total motility recovery after cryopreservation. ROC curves for progressive (panel A) and total (panel B) sperm motility, number of sperm/ejaculate (panel C), and TNM (panel D) for probability of motility recovery after thawing. Sensibility and specificity at the specified threshold are indicated.

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such a situation, even if basal motility and viability are high, they cannot always guarantee a good recovery of motile sperm after thawing if the concentration falls below the 5th percentile of reference values. This result suggests that intrinsic characteristics of spermatozoa are the major determinant of recovery after thawing. In support of this hypothesis, we show that basal parameters (excluding morphology, which was not considered in our study for the reasons outlined in Materials and Methods) strictly correlate with motility recovery after cryopreservation, with basal total and progressive motility showing the stricter correlations. All these parameters were predictive of motility recovery after thawing with high values of accuracy, sensitivity, and specificity, demonstrating that they were of great help in patient counseling. This should be viewed in light of the fact that cryopreserved semen use for ART is very low among cancer patients (22, 23).

An open question concerns sperm cryopreservation for cancer patients. In fact, the probability of recovering motile sperm after thawing depends on the type of cancer for which cryopreservation is required, because the impact of a tumor on spermatogenesis is quite variable (11, 23, 25). Not surprisingly, several studies, including ours, report that, among men undergoing cryopreservation for cancer, those with testicular cancer show the lowest semen quality and low recovery rates after cryopreservation (8, 11–13, 22). On the other hand, there are several factors that may contribute to lower semen quality in testicular cancer, such as local production of toxic and inflammatory factors by the tumor, disruption of the blood-testis barrier, and, not last, the fact that some risk factors associated with testicular neoplasia are also associated to infertility, such as cryptorchidism (32). It should be considered that in our study azoospermic subjects were not included, and, thus, basal semen parameters may even be overestimated not only for testicular cancer patients but also for all other pathologies.

If there is an overall agreement both on basal and post-cryopreservation semen quality for testicular cancer patients, the same cannot be said for hematological cancers. Indeed, while some studies fail to demonstrate alterations of semen quality in Hodgkin's, non-Hodgkin's, and leukemia patients (24, 25, 29), other studies have shown alterations of semen

quality in leukemia (9, 13) and/or in Hodgkin's and non-Hodgkin's diseases (10). In our large study, we did not show significant alterations of basal semen quality in hematological or other (mixed types) cancers, and motility recovery after thawing for both groups was similar to that of noncancer pathologies and/or ejaculatory disorders. In the recent paper by Hotaling et al. (13), the lowest recovery rates of motility after thawing were observed for leukemia patients, whose performance was even worse than that of testicular cancer patients. However, although ours and Hotaling et al.'s (13) study were similar in terms of number of observations, a direct comparison between these two studies is not possible, as Hotaling et al. performed sperm selection or chose treatments that would be able to increase sperm motility before freezing. In general, a direct comparison among the different studies on this topic is not easy because several factors, such as the stage of the diseases, the copresence of other pathologies or complications, or the presence of risk factors for infertility are not systematically recorded. This is an important limitation of all the studies (including the present one) on sperm cryopreservation outcome in cancer patients and is mostly due to the urgency to collect semen for fertility preservation before gonadotoxic therapies.

The present study has strengths and limitations. One strength is represented by the fact that this is the first study showing the results of cryopreservation in a large number of oligospermic patients. In addition, we report for the first time ROC curves for the prediction of the outcome of cryopreserved semen based on prefreeze semen quality. One important limitation is that this is a single-center study and thus the derived information cannot be largely disseminated because of a lack of standardized protocols. Large multicenter studies are desirable in the future.

In conclusion, our large study on outcome of cryopreservation indicates that such a procedure does not always guarantee recovery of motile sperm for ART procedures in case of semen pathologies such as severe oligospermia and testicular cancers or when one basal semen parameter falls below the 5th percentile of the WHO reference value (3). In these cases, the clinician should perform appropriate counseling about the future possibilities of fertility for the patient.

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SUPPLEMENTAL TABLE 1

Median (95% confidence interval) of basal semen parameters in patients with testicular cancer according to the histological subtype.

Testicular cancer	Volume, mL	Total sperm count/ ejaculate, $\times 10^6$	Vitality, % vital sperm	Progressive motility, %	TMN, $\times 10^6$
Unknown, n = 60	3.20 (2.6–4)	50.95 (20.68–73.6)	75.00 (70–79)	45.50 (27–55)	32.93 (6.82–56.28)
Seminoma, n = 76	2.65 (2–3.2)	36.10 (18–50)	66.00 (62–75)	42.50 (24–55)	17.63 (6.72–27.77)
Mixed nonseminoma, n = 14	3.20 (2.5–4.2)	96.00 (54–135)	76.00 (70–84)	59.00 (33–66)	53.63 (25.58–96.77)

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SUPPLEMENTAL TABLE 2

Median (95% confidence interval) of basal semen parameters in patients with hematological cancers according to the cytological subtype.

Hematological cancer	Volume, mL	Total sperm count/ejaculate, $\times 10^6$	Vitality, % vital sperm	Progressive motility, %	TMN, $\times 10^6$
Hodgkin's disease, n = 120	2.20 (1.8–2.5)	79.00 (42–115.5)	73.00 (70–76)	47.00 (38–53)	43.94 (19–70.47)
Non-Hodgkin's disease, n = 30	2.80 (2.4–3)	122.10 (70.4–207.2)	70.00 (66–75)	49.00 (38–54)	75.67 (52.1–136.4)
Leukemia, n = 27	2.30 (1.6–3.2)	155.00 (54.4–212.5)	70.00 (64–78)	51.00 (40–57)	94.00 (34.15–137.7)

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SUPPLEMENTAL TABLE 3

Correlation coefficients between basal and post-thawing semen parameters.

		Basal values		
		Total sperm count/ejaculate, $\times 10^6$	**Progressive motility, %**	**Vitality, % vital sperm**
Post-thawing values	% Viability recovery	.229[a] n = 738	.539[a] n = 738	.325[a] n = 738
	% Total motility recovery	.254[a] n = 719	.537[a] n = 719	.396[a] n = 717

Note: n indicates the number of observations.
[a] $P < .0001$.

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