NextGen® Home Sperm Banking Kit: Outcomes of Offsite vs Onsite Collection—Preliminary Findings

Ashok Agarwal, Reecha Sharma, Sajal Gupta, and Rakesh Sharma

OBJECTIVE
To compare cryosurvival rates between remote collections with NextGen kit (offsite) and onsite collection of semen samples from infertile men and those with cancer.

METHODS
Prefreeze and post-thaw sperm motility, total motile sperm, and percent cryosurvival rates were compared between samples collected from infertile men onsite at the Andrology Center (n = 10) and samples collected from infertile patients at home (offsite; n = 9), which were shipped by NextGen to our laboratory. A second group (n = 17) consisted of 10 semen samples from cancer patients collected onsite, which were compared with 7 semen samples from cancer patients shipped by the NextGen. All semen samples were assessed within 18 hours of collection.

RESULTS
In the infertile men, percent cryosurvival rates were similar with NextGen compared with those of onsite collection (53.14 ± 28.9% vs 61.90 ± 20.46%; P = .51). Similarly, in the cancer patients, all 4 parameters were comparable between the onsite and NextGen. Cryosurvival rates were also similar between NextGen compared with those of onsite collection (52.71 ± 20.37% vs 58.90 ± 22.68%; P = .46).

CONCLUSION
Cancer patients can bank sperm as effectively as men banking for infertility reasons using the NextGen kit. UROLOGY 85: 1339–1346, 2015. © 2015 Elsevier Inc.

Cryopreservation of human spermatozoa has emerged as an important area in assisted reproductive technology programs and oncology programs. Patients are often referred for semen banking, followed by assisted reproductive techniques, when pregnancy is desired.1,2 The American Society of Reproductive Medicine Ethics Committee and the American Society of Clinical Oncology both recommend that physicians counsel all cancer patients about options available for fertility preservation before treatment.4,5

Advances in diagnostic techniques and therapies have dramatically improved cancer survival rates,6 and the current cure rates for patients with testicular cancer and lymphoma are as high as 90%.7 Because it is difficult to predict the precise impact the cancer therapy will have on an individual’s fertility, patients should have the opportunity to preserve their fertility before treatment.

Approximately, 70,000 adolescents and young adults, aged 15-39 years, are diagnosed with cancer each year in the United States.3 Many of these young men desire future fertility, and more than three-quarters are without children at the time of their cancer diagnosis.9,10 Furthermore, there is a strong desire to be notified of options for fertility preservation among young men diagnosed with cancer, and fertility has been identified as an important issue for all cancer patients.10,11 However, sperm cryopreservation is underused, and referrals to fertility specialists are inconsistently offered across the United States.12

Cryopreservation of sperm also impacts fertilization potential by increasing the concentration of free radicals.13 After freezing and thawing, the sperm motility further decreases by 25%-75%.14 Loss of sperm function, oxidative stress, apoptosis, and deoxyribonucleic acid (DNA) fragmentation are commonly observed after thawing.14,15 Therefore, it becomes crucial to carefully review sperm banking protocols to ensure the highest level of sperm cryosurvival. Factors such as maintenance of proper temperature and the specific diluent used affect the viability of semen specimens during transport.16 Repeated freeze-thaw processes negatively affect sperm quality and may also worsen any underlying sperm defects.17,18 There are many challenges that these men experience. For many, sperm collection in a laboratory may be embarrassing or uncomfortable. Traveling to other cities or neighboring states from their homes for sperm banking creates an emotionally traumatic experience for the patients.19 Furthermore, the delay caused by waiting for infertility test results heightens patient
anxiety.20 Some of these problems can be alleviated by collecting a semen sample in a private setting and directly shipping it to a central laboratory for testing and storage. We have recently developed a specialized sperm collection and transport kit (NextGen®, Path-Tec, Columbus, GA). It is a first-of-its kind product evaluated in a clinical setting and specially designed primarily for men with cancer who are about to undergo treatment (surgery, chemotherapy, and radiation therapy), which can render them infertile.

The aim of this study was to evaluate the quality of semen samples collected and shipped overnight from different states (offsite) and delivered to our Andrology and Reproductive Tissue Bank the next day via NextGen kit. We compared the baseline semen parameters in samples collected onsite (Andrology laboratory) vs those collected offsite and shipped by NextGen. We chose to study cancer patients and infertile men as these groups request cryobanking most frequently. Furthermore, many of these men are young, unmarried, and in their reproductive years and desire to father their biological children.

**METHODS**

**Patients and Semen Samples**

On study approval by the institutional review board, semen samples were collected from men with a history of infertility (group 1; n = 19) who presented for infertility treatment or were diagnosed with cancer. All infertile patients were seen by an andrologist or urologist and they had a diagnosis of male-factor only. Female-factor was excluded in these couples. Similarly, all cancer patients who banked onsite had testicular cancer as the main diagnosis. A consent form was provided to each subject, and the purpose of the study was clearly explained. Semen samples were collected in the Andrology laboratory (onsite) from 10 infertile patients, and 9 semen samples were collected from infertile patients and shipped by NextGen (offsite).

The inclusion criteria were as follows: (1) all subjects were attending the male infertility clinic for fertility issues; (2) all were evaluated for proven male-factor infertility as assessed by the male infertility specialist; (3) all underwent history, physical, and laboratory evaluation; and (4) the female partners of the infertile men had undergone gynecologic evaluation and female factor was ruled out on a fertility workup.

Participants were excluded if there was a history of smoking and/or illicit drug use. In addition, participants were not included if they presented with azoospermia, cryptorchidism, and/or incomplete semen analysis results.

A second group consisted of cancer patients (n = 17) who were in the process of initiating cancer treatments. Of these, 10 semen samples were obtained from cancer patients onsite, and 7 semen samples were collected from cancer patients (offsite) and shipped by NextGen. Patients were included if they had a strong desire to bank specimens before initiating cancer treatment and excluded if there was an absence of motile sperm in their ejaculate.

**Cryopreservation of Semen Samples**

After complete liquefaction, semen samples were mixed with an equal volume of 10% glycerol-based cryoprotectant (glycerol-egg yolk-citrate medium) in 4 equal supplements and plunged into liquid nitrogen.22 For thawing, the cryovials containing the semen samples were removed from the liquid nitrogen tank and allowed to thaw at room temperature for 5 minutes. The samples were then incubated for 20 minutes at 37°C. After thawing, sperm concentration and motility were evaluated and recorded.

**Statistical Analysis**

Offsite samples collected by NextGen and onsite collections between infertile patients and cancer patients were compared with respect to quantitative parameters using Wilcoxon tests. Quantitative variables are summarized as mean ± standard deviation and interquartile values. Associations with categorical variables such as collection sites were analyzed by the Fisher exact test or the chi-square test. Associations with quantitative and ordinal variables such as percent motility, total motile sperm (TMS), and percent survival were assessed with the
Wilcoxon rank sum test. All analyses were performed with use of R, version 2.3.1 (The R Foundation, www.R-project.org). *P* values <.05 were considered statistically significant.

**RESULTS**

**Difference between Semen Parameters—Offsite Collection vs Onsite Among Infertile Men**

The distribution of semen parameters for all infertile patients (group 1) is shown in Table 1. The overall sperm characteristics between the offsite collection via the NextGen and onsite collection for infertile patients were within normal limits. For offsite collections (NextGen), the average prefreeze motility was 56.26 ± 18.51%, and the average prefreeze TMS concentration (×10⁶ sperm) was 59.46 ± 42.76 for infertile patients. For onsite collections, the average prefreeze motility was 42.30 ± 15.88%, and the average prefreeze TMS concentration (×10⁶ sperm) was 32.41 ± 28.22 for infertile patients. For onsite vs those collecting offsite, there was no statistically significant difference in sperm motility or TMS concentration.

### Table 1. Prefreeze and post-thaw sperm motility, total motile sperm, and cryosurvival rates in infertile men using NextGen

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NextGen</th>
<th>Onsite</th>
<th><em>P</em> Value</th>
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<tbody>
<tr>
<td><strong>Motility (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefreeze</td>
<td>56.26 ± 18.51</td>
<td>42.30 ± 15.88</td>
<td>.09</td>
</tr>
<tr>
<td>Post-thaw</td>
<td>27.6 ± 13.6</td>
<td>27.90 ± 17.07</td>
<td>.90</td>
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<tr>
<td><strong>TMS (×10⁶ sperm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefreeze</td>
<td>59.46 ± 42.76</td>
<td>32.41 ± 28.22</td>
<td>.12</td>
</tr>
<tr>
<td>Post-thaw</td>
<td>26.97 ± 22.73</td>
<td>19.34 ± 15.53</td>
<td>.57</td>
</tr>
<tr>
<td><strong>Cryosurvival (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefreeze</td>
<td>53.14 ± 28.9</td>
<td>61.90 ± 20.46</td>
<td>.51</td>
</tr>
<tr>
<td>Post-thaw</td>
<td>46.5 (32.9-66.0)*</td>
<td>60.5 (47.8-77.2)*</td>
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</table>

TMS, total motile sperm.
Values are mean ± standard deviation or *median (25th-75th percentile). *P* <.05 was considered significant by the Wilcoxon rank sum test.

**Comparative Study of Semen Parameters—Onsite vs Offsite Collection Among Cancer Patients**

The distributions of semen parameters for all cancer patients (group 2) are shown in Table 2. In the cancer patients, all 3 semen parameters were comparable between the offsite samples shipped by NextGen and those collected onsite. There were no significant differences observed in the prefreeze motility and post-thaw motility in samples collected from cancer patient offsite vs onsite. For onsite collections, the average prefreeze motility for cancer patients was 39.60 ± 16.67%, and the average prefreeze TMS (×10⁶ sperm) was 94.72 ± 206.42 (median [25th-75th percentile], 13.2 [5.1-51.3]). TMS concentration, both prefreeze and post-thaw, was comparable in cancer patients collecting offsite or onsite (P = .20 and P = .77; Fig. 2). Similarly, percent cryosurvival rates in cancer patients were comparable in both groups collecting offsite vs those collecting onsite (P = .46; Fig. 2).

**COMMENT**

More than 50% of cancer patients desire future fertility, and of those, more than three-quarters are without children at the time of their diagnosis. At present, semen banking is the gold standard for fertility preservation in men diagnosed with cancer. Less than a quarter of cancer patients bank sperm, and the most common reasons for not doing so is lack of information, time, high costs, and lack of convenient facilities. Hence, there is an urgent need to evaluate alternate methods of cryopreservation available to young male cancer survivors. Young et al and Zavos et al have successfully used remote semen collection for therapeutic purposes. Young et al used the TRANSEM semen transport container (Fertility Solutions Inc.) to hold semen specimens at 22 ± 1°C for 24 hours to simulate overnight shipping; the samples were then snap frozen.

In a previous study, remote semen collection kits have also displayed significant potential to maintain semen samples from cancer patients for 24 hours before cryopreservation. The kits included a media solution containing Tyrode solution, human serum albumin, and penicillin-streptomycin. The kits maintained the semen specimens at 4°C-5°C in a temperature-regulated box containing chiller packs for 24 hours during overnight shipping. Although sperm motility was affected in the samples collected remotely, the ability of spermatozoa to withstand freezing and thawing was usually maintained. This is attributed to prolonged exposure of spermatozoa to exogenous protein supplement. We have earlier standardized the NextGen kit components (unpublished study). This included the transport media, the cooling components, and the effect of overnight shipping on sperm function. We tested 2 transport media—refrigeration media and human tubal fluid. The refrigeration media were selected. For the cooling conditions, 5
different combinations of ice packs were tested to study which combination best maintain the desired temperature during the overnight shipment of the NextGen kit. The cooling sleeve and 2 Tech Pack (Polar Tech Industries, Inc.) ice bricks were selected. To test the overnight shipping condition on the semen quality, sperm motility, total sperm motility, and sperm membrane integrity were tested in simulated shipped condition (37°C for 24 hours) and overnight shipped samples. Although there was a significant decline in the 3 parameters, the cryosurvival rates were comparable (50%) under the 2 test conditions. Thus, we have validated conditions that facilitate the overnight shipment of semen samples with the preservation of various sperm parameters.

Our study included both cancer and infertility patients. These were actual patients who resided out of state. Therefore, it was not feasible for them to travel and provide semen sample onsite. Hence, they could not serve as their own controls. Although there was a wide range in the number of TMS count before freezing in cancer patients, however, it was not statistically significant. The wide range in the TMS can be explained by the etiology of the disease, that is, testicular cancer patients, which is different than the general infertile population. Majority of these patients were oligospermic at the time of diagnosis. Furthermore, the dysfunctional germ cells or the precursors to malignancy present within the testicular tissue and the disease itself can also affect sperm quality and fertility. Our results showed no differences in change from prethaw to post-thaw TMS or motility between the offsite and onsite groups in infertile men and cancer patients. We observed no significant differences in the cryosurvival rates of semen samples collected offsite and transported via the NextGen kit to our Andrology Laboratory and Reproductive Tissue Bank. When compared between infertile and cancer patients—the 2 types of patients who are most likely to use sperm banking services—the NextGen kit showed promising results in preservation of sperm parameters. Sperm banking is a highly valued service that should be offered to both infertile and cancer patients. Cancer patients bank for longer time periods than infertile patients. Although onsite semen collection in cancer patients is important, the remote collection kits could be offered as an adequate alternative to onsite collection.

Our results demonstrate that cancer patients can bank sperm as effectively as men banking for infertility reasons. Our goal was not to merely establish the absence of a difference in the study groups but to establish the following: (1) if the NextGen kit is suited for shipment of semen samples from long distances using a commercial courier, and (2) if we can recover reasonable number of motile sperm from these samples after their shipment in frozen condition. We have successfully demonstrated that the overnight shipment of semen sample using the NextGen kit is feasible and a viable option for patients in remote locations who do not have ready access to sperm banking facilities. We have further shown that under standardized shipping conditions using the overnight shipping, all samples retained the motility after removal of the cryopreservative at the time of post-thaw analysis. It is important to mention here that sperm motility was retained in each and every sample that was shipped via NextGen kit before and after freezing.
To our knowledge, this is the first report comparing offsite and onsite sperm parameters in both fertile and cancer patients. Men diagnosed with cancer are suspected to have gonadotoxic exposure such as chemotherapy or radiotherapy, often reside at remote locations, or are unable to travel to a sperm bank laboratory. Thus, we suggest that a better option for cancer patients would be semen collection in the privacy and comfort of their homes, followed by analysis at a centralized andrology laboratory. We also demonstrated that sperm motility is preserved during shipping. In men with normal semen parameters, sperm freezing on average decreases motility by 25%-75%, which is attributed to oxidative stress.

Although usually not a significant problem for fertile men without illness, spermatozoa from men with cancer have been shown to be particularly sensitive to oxidative stress and damage from cryopreservation. Our results show that the NextGen kit preserved sperm parameters, thus providing strong support for its efficacy.

Despite a significant decline in semen quality, the sperm parameters were adequate for cryopreservation. As long as there are motile sperm in the sample before freezing, we can advise the patients to bank. This is similar to the requirements of spermatozoa by the embryologists and the gynecologists in patients banking fresh semen. The frozen specimens can be used for frozen specimens. The frozen specimens can be used for further fertility treatment.

Timely cryopreservation is critical because in some cases, >1 visit will be required to cryopreserve sufficient numbers of sperm, or there may be urgent need to start therapy. We have optimized our protocol for the best recovery of healthy sperm and investigated several strategies to further enhance post-thaw recovery of spermatozoa. The limitations of the present study include the following: (1) small sample size, (2) heterogeneity of the population, (3) lack of semen analysis before the overnight shipping of the sample using the NextGen kit, (4) inadequate matched control group, and (5) finally, the patients were counseled over the phone and then asked to review and mail the “Semen Collection and Storage Agreement” by mail. The most significant strength of our study is the comparison of onsite and offsite sperm banking in both cancer patients and infertile patients who banked at our institution.

**CONCLUSION**

A specialized sperm collection and transport kit (NextGen) has been recently developed at the reproductive tissue bank at the clinic. This is an innovative, first-of-its-kind product evaluated in a clinical setting and specially designed for men with cancer who are about to undergo treatment (surgery, chemotherapy, and radiation therapy), which can render them infertile. Patients can collect semen sample in the privacy of their own home and ship the samples overnight to the Andrology Laboratory for further processing and storage. Collecting semen at home and transporting the same overnight reduces emotional anxiety, need to travel from geographically distant places (different cities or states), and is cost effective. Semen parameters are comparable between the NextGen offsite collection and the control group of onsite collection in cancer patients. The NextGen kit provides a convenient method for men to ship their samples to the clinic from any part of the United States, especially in cancer patients with a need to preserve their fertility and in infertile men.

**Table 2. Prefreeze and post-thaw sperm motility, total motile sperm, and cryosurvival rates in cancer patients using NextGen**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NextGen</th>
<th>Onsite</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Cancer patients</td>
<td></td>
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<tr>
<td>Motility (%)</td>
<td></td>
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<td></td>
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<tr>
<td>Prefreeze</td>
<td>35.29 ± 11.97</td>
<td>39.60 ± 16.67</td>
<td>.33</td>
</tr>
<tr>
<td>Post-thaw</td>
<td>19.14 ± 11.91</td>
<td>22.10 ± 12.4</td>
<td>.66</td>
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<tr>
<td>TMS (×10⁶ sperm)</td>
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<td></td>
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</tr>
<tr>
<td>Prefreeze</td>
<td>9.94 ± 11.34</td>
<td>94.72 ± 206.42</td>
<td>.20</td>
</tr>
<tr>
<td>Post-thaw</td>
<td>13.04 ± 17.92</td>
<td>25.26 ± 46.05</td>
<td>.77</td>
</tr>
<tr>
<td>Cryosurvival (%)</td>
<td>52.71 ± 20.37</td>
<td>58.90 ± 22.68</td>
<td>.46</td>
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</table>

Abbreviation as in Table 1. Values are mean ± standard deviation or median (25th-75th percentile). P < .05 was considered significant by the Wilcoxon rank sum test.
Acknowledgment. The authors are grateful to the Andrology Center technologists Debbie Garlak, Carmen Caraballo, and Larry Harmych for scheduling the study subjects and Jeff Hammel, senior biostatistician, for his contribution to data analysis.

Figure 2. Differences in semen parameters between offsite and onsite groups in cancer patients. (A) Difference between prefreeze and post-thaw percent motility. (B) Difference between prefreeze and post-thaw total motile sperm. (C) Percent cryosurvival (n = 7 offsite; n = 10 onsite collections). (Color version available online.)

References

The long-term impact of cancer treatment on reproductive function has become increasingly relevant in recent years given the number of successful treatment options. The American Society of Clinical Oncology and the American Society for Reproductive Medicine recommend that, when possible, at-risk patients bank sperm before gonadotoxic cancer therapy. Despite these guidelines, sperm cryopreservation before the initiation of cancer treatment remains undervalued. Unfortunately, some men do not have access to a sperm banking facility because they either live far away or are too sick to travel. Therefore, it is critical that we identify a reliable and an efficient method to cryopreserve sperm at a remote location. The authors’ pilot feasibility study of a sperm banking kit (NextGen; Path-Tec, Columbus, GA) that can be used at home or at a site away from the sperm banking facility. This study appears to be the first study to evaluate the outcomes after sperm cryopreservation attempted at a remote location. Several kits similar to NextGen have been marketed for sperm cryopreservation (OverNite Male (ReproTech, Ltd.), Priority Male (Cryogenic Laboratories, Inc. part of Fairfax Cryobank), and @Home). However, none of the providers appear to have published their outcomes.

Although there are benefits to sperm banking at a remote location, there exist some concerns. A semen analysis, which is performed routinely at a sperm banking facility before cryopreservation, cannot be performed if sperm is cryopreserved at a location without an andrology laboratory. It is well known that both cancer and cryopreservation can affect semen parameters, in particular, sperm motility. Therefore, the opportunity is missed to counsel men on results of semen analysis before cryopreservation. Nevertheless, the clinical message is important. Fertility preservation before undergoing cancer therapy is critical and should be evaluated and counseled by an expert in fertility preservation. If men have access to an andrology laboratory or a sperm banking facility, a semen analysis should be performed before cryopreservation. However, if accessibility is an issue, the sperm banking kit is an option that can be used as a last resort, provided the patient is appropriately counseled regarding the benefits and limitations of cryopreserving sperm at a remote location.

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References

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EDITORIAL COMMENT

We read with interest the thoughtful comments by the authors into the need for fertility options for cancer patients. NextGen® (Path-Tec, Columbus, GA) Remote Home Sperm Banking Kit is an innovative, product evaluated in a clinical setting and specially designed for men with cancer who are about to undergo treatment, which can render them infertile. Many of these men often reside at remote locations and do not have ready access to sperm banking facilities or often times are unable to travel to a sperm bank laboratory.

In a recent update of the fertility preservation options for cancer patients as recommended by the American Society of Clinical Oncology Guidelines, the unanimous decision was that cryopreservation is an effective but an underused option for fertility. The American Society of Clinical Oncology (ASCO) guidelines recommend that “all health care providers (including medical oncologists, radiation oncologists, gynecologic oncologists, urologists, hematologists, pediatric oncologists, and surgeons) should address the possibility of infertility with patients treated during their reproductive years (or with parents or guardians of children) and be prepared to discuss fertility preservation options and/or to refer all potential patients to appropriate reproductive specialists.” Although the initial concern of the patients is cancer diagnosis, they must be advised regarding the potential threat to their fertility as early as possible in the treatment process and of their options for fertility preservation.

The most significant strength of our study is the comparison of onsite and offsite sperm banking in both cancer patients and infertile patients who banked at our institution. We have successfully demonstrated that the sperm motility was retained in every sample shipped via NextGen kit, thus providing strong support for its efficacy. Although, the semen quality cannot be tested before freezing, however, as long as there are motile spermatozoa in the semen sample before freezing, we can advise the patients to bank. This is similar to the requirements of spermatozoa by the embryologists and the gynecologists in patients banking fresh “onsite” specimens. The frozen specimens can be used for procreation using the intratuberine insemination or assisted reproductive techniques (ART) techniques. In samples with extremely small number of motile sperm available after freezing, the patients will benefit with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Recent studies indicate no difference in pregnancy...
outcomes between ICSI success rates when using fresh vs cryopreserved sperm. Onsite banking should always be the first option and NextGen the last resort after the patient has been appropriately counseled. Educating both the patient and the health care providers of the availability of NextGen kit option is vital.

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References

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Standardisation of a novel sperm banking kit – NextGen® – to preserve sperm parameters during shipment

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Keywords
Home banking—human spermatozoa—hypoosmotic swelling—motility—sperm vitality

Summary
Many male patients diagnosed with cancer are within their reproductive years. These men are advised to freeze their spermatozoa prior to the start of cancer treatment. Very often, sperm banking facilities may not be readily available and patients may be required to travel to distant sperm bank centres. Our objective was to design and standardise a remote home shipping sperm kit that allows patients to collect a semen sample at home and ship it overnight to a sperm bank. A total of 21 semen samples and two transport media (refrigeration media and human tubal fluid) and five different combinations of ice packs were tested for maintaining desired shipping temperature. Ten semen samples were assessed for pre- and post-shipment changes in sperm motility, membrane integrity, total motile spermatozoa and recovery of motile spermatozoa. Even though motility, membrane integrity and total motile spermatozoa declined both in samples examined under simulated shipped conditions and in overnight-shipped samples, the observed motility and total motile spermatozoa were adequate for use with assisted reproductive techniques. Using refrigeration media, cooling sleeve and ice packs, adequate sperm motility can be maintained utilising NextGen® kit and these spermatozoa can be used for procreation utilising ART techniques such as intracytoplasmic sperm injection.

Introduction
In the United States alone in 2010, approximately 8400 cases of testicular cancer were diagnosed among men between the ages of 15 and 44 years old (Rosen et al., 2011). According to the Surveillance Epidemiology and End Results (SEER) cancer statistics, it is estimated that one of every two men will be diagnosed with cancer in his lifetime, of which 4% are under the age of 35 (Howlader et al., 2015). Over the past quarter century, incidence of cancer in adolescents and young adults has been on the rise and several countries have reported improvement in the survival rates of adolescents and young adults (Daudin et al., 2015). Patients with cancer comprise about 44% of all referrals in the United States alone (Tomlinson, 2010). The American Society of Clinical Oncology (ASCO) advocates sperm cryopreservation as an effective method of fertility preservation in young men with cancer (Hourvitz et al., 2008; Loren et al., 2013). In a recently published report, it was suggested that all males within the reproductive age group and even men undergoing radical prostatectomy for prostate cancer should cryopreserve their spermatozoa (Salonia et al., 2013; Niederberger, 2014).

Cryopreservation of human spermatozoa has evolved as an important area in assisted reproductive technology (ART), oncology, men undergoing vasectomy (Gangrade, 2013) and in men engaging in potentially life-threatening activities, such as those in the military and those faced with conditions that are likely to impair their fertility, such as occupational exposure (Williams, 2010). Fertility preservation enhances the quality of life in cancer survivors with gonadal dysfunction brought about by cytotoxic agents and radiation (Williams, 2013; Robinson & Knudtson, 2014).

Patients with cancer are generally instructed to bank multiple specimens within a short period of time, prior to the start of cancer treatment. This is to have adequate numbers of spermatozoa for procreation utilising ART techniques such as intracytoplasmic sperm injection or ICSI. In this technique, a single viable, morphologically normal spermatozoon is injected into the oocyte.
In order for any sperm collection kit to successfully transport semen specimens from a remote collection site to a laboratory for andrological evaluation and clinical use, it must be able to maintain sperm viability and fertilising potential. With shipping, three factors are important: the transport media, the cooling components and transport time (Zavos et al., 1998). Transport time should optimally take no more than 24 h because in vitro fertility of the motile subpopulation of spermatozoa declines over time due to a decrease in their functional quality (Maxwell & Stojanov, 1996; Krzyzosiak et al., 2001).

To meet this need, the Andrology Center of the Cleveland Clinic developed the NextGen Home Sperm Banking Service®, which uses an at-home collection kit and overnight shipping to expand fertility preservation options for men throughout the United States. This service uses a special collection kit that comes with instructions and packaging for overnight mail delivery to Cleveland Clinic’s Andrology Laboratory for cryopreservation and long-term storage. The NextGen® sperm banking kit allows the patient privacy and a comfortable location to collect a sperm sample.

In this study, our objectives were to standardise the contents of the Home Sperm Banking kit called NextGen® and to examine whether the sperm quality, particularly the sperm motility and membrane integrity, was maintained during overnight shipment of this kit. Therefore, in this study, we describe the development process of the sperm banking kit including the initial stages of testing for kit components. A comparison of two transport media and the standardisation of the temperature in the kit are performed to determine the optimal NextGen® kit configuration, its feasibility and the effect of overnight shipping on sperm function.

Materials and methods

This study was approved by the institutional review board of the Cleveland Clinic. Twenty-one semen samples were collected from normospermic men by masturbation into a sterile wide mouth collection cup following a minimum of 48 to 72 h of sexual abstinence. The donors were healthy males, between the ages of 20 and 35 years old and whose semen samples fulfilled the criteria for normal semen parameters established by the World Health Organization, 2010 guidelines for semen analysis. The inclusion criteria for the donors were as follows: (1) normal semen parameters; (2) no sexually transmitted infections; (3) no recreational drug use; and (4) may or may not have initiated a pregnancy in the past. Following liquefaction at 37 °C for 20 min, semen specimens were evaluated for volume, sperm concentration, total cell count, motility and vitality according to the World Health Organization, 2010 guidelines. In this study, each of the 12 men collected a single sample at the Andrology Laboratory at Cleveland Clinic and each of the nine men collected a single sample at home.

Effect of various media on sperm motility, count and vitality

For the initial standardisation, each sample was divided into six equal aliquots. Three aliquots of each sample were supplemented with equal 1 : 1 volumes of refrigeration media (RM; Irvine Scientific, Santa Ana, CA, USA), while the remaining three aliquots were supplemented with equal 1 : 1 volumes of human tubal fluid (HTF) (Sharma et al., 1997). Each aliquot (one from RM-added aliquot and one from HTF-added aliquots) was incubated for 24 h at temperatures of 24 °C, 37 °C and 40 °C respectively. We chose 24 °C and 37 °C due to conflicting study reports on the optimal temperature for the maintenance of sperm motility and fertility (Appell & Evans, 1977; Appell et al., 1977; Esfandiari et al., 2002) and the ability of the spermatozoa to penetrate zona-free hamster eggs (Cohen et al., 1985). We chose 40 °C to determine the effects of high temperatures on seminal parameters that could arise during the actual shipping of samples, especially in extreme climates. Sperm motility, count, vitality by hypoosmotic swelling (HOS) test and percentage recovery of motile spermatozoa were assessed after a 24-h incubation period.

Measurement of sperm vitality

Each semen sample was examined for vitality using the HOS test (Esteves et al., 1996). One hundred microlitres of liquefied semen was mixed with 0.9 ml of HOS media.
solution and incubated at 37 °C for 1 h. After incubation, a 5 μl aliquot of the sample was examined for tail swelling using phase contrast illumination. A total of 200 hundred spermatozoa per sample were scored. Spermatozoa were classified as osmotically competent if tail swelling was observed after exposure to the hypoosmotic solution. Spermatozoa displaying >60% swollen tails were considered as normal with intact membrane.

Optimisation of overnight shipment temperature

The goal was to test the temperature of the ice sleeve surrounding the semen specimen collection container as well as the temperature of the ice packs surrounding the sleeve to determine its ability to maintain the temperature during shipment, melting of the ice bricks and to avoid exposure to extreme temperatures. To examine this, we assessed 5 different ice packs, both gels and ice bricks (Polar Tech Industries Inc., Genoa, IL, USA) in terms of their ability to maintain the desired temperature of the kit and its contents during shipping. Each of the five different kinds of ice packs was placed at the bottom and top of the shipping containers and was tested for their ability to resist temperature elevation when placed frozen inside the kit and incubated at 37 °C for 24 h. Ice packs were frozen for 24 h in a −18 °C freezer prior to usage as per the manufacturer’s instructions. We also assessed the effect of adding a cooling sleeve directly around the semen specimen container and placing ice packs around the cooling sleeve. Temperatures inside the kit were recorded using a digital thermometer – the probe was placed in the centre of the kit, surrounded by the appropriate ice packs. To simulate shipping conditions, the kit was then placed in an incubator to compare the ability of the five ice packs being tested to resist a temperature increase and to maintain the temperature of the kit. Temperature readings were taken by digital output provided by a thermometer unit placed outside the incubator in addition to comparisons between different ice packs.

Effect of 24-h incubation at 37 °C on sperm motility, count and vitality

Ten semen samples from 10 healthy donors were examined. Each sample was analysed pre-incubation for motility, concentration and vitality. Each sample was then supplemented with 5 ml of RM, placed in the kit with the freezing components and incubated for 24 h at 37 °C. Post-incubation samples were again assessed for motility, count, vitality and percentage recovery for motile spermatozoa.

Effect of overnight shipment in NextGen® kit on sperm motility, count, vitality and percentage recovery for motile spermatozoa

Once the transport media and cooling packs were chosen, we shipped nine kits (Figure 1) to seven different andrology laboratories in the United States. Semen samples were collected from normospermic men (n = 9) by masturbation into a sterile wide mouth collection cup following a minimum of 48–72 h of sexual abstinence from various andrology laboratories. We chose these laboratories located in cities based on its distance from Cleveland as well the variation in temperature/climate in each city (e.g. Washington, DC vs. Orlando, FL). The kits contained the necessary components as well as next-day air return envelope identical to the kind that would be used by a patient. Each sample was analysed pre-shipment for motility, count and vitality according to the WHO, 2010 criteria (World Health Organization, 2010). Each sample was then supplemented with 5 ml of RM, placed in a NextGen® kit and shipped overnight to Cleveland Clinic. Shipments were scheduled overnight such that the sample would be picked up around 5:00 pm at the origin city and would arrive at the Clinic around 10:30 am, marking an average 17.5-h transit time. Post-shipment samples were assessed for motility, count, vitality and percentage recovery for motile spermatozoa.

Statistical analysis

Summaries of quantitative variables are expressed using medians and ranges. Comparisons of RM and HTF were performed using Wilcoxon rank sum tests, in anticipation of non-normal distributions. Comparisons of pre- and post-incubation periods, or pre- and post-shipment, were
performed using the Wilcoxon signed rank test for paired data. Percentage sperm recovery was calculated as:

\[
\text{Post-shipment total motile spermatozoa (TMS)} \times 100 \\
\text{Pre-shipment TMS}
\]

**Results**

Effect of various transportation media on sperm motility, count and vitality

The differences between RM and HTF were assessed on their ability to sustain sperm motility, count and vitality after 24 h of incubation at three varying temperatures: 40 °C, 37 °C and 24 °C.

Samples with RM resulted in excessive clumping at 40 °C and 37 °C post-incubation that made assessment of concentration difficult. The percentage of motile sperm recovery was not different (\(P = 0.31\)) for RM (median 28.6%) compared with HTF (median 54.4%) Post-incubation membrane integrity measured by the HOS test was comparable (Table 1). The post-incubation motility was best preserved at 24 °C with both types of media. Higher temperatures of 37 °C or 40 °C resulted in complete loss of motility function.

Optimisation of overnight shipment temperature and assessment of temperature resistance of various ice packs

The gel-based ice packs were not as successful in maintaining temperature compared to the solid ice bricks (TechPack Bricks, Polar Tech Industries Inc. Genoa, IL, USA). Solid ice bricks were the most efficient at maintaining optimal temperature. The cooling sleeve allowed for a slower melting once the sleeve reached 5 °C. Thus, a cooling sleeve wrapped around the collection container and cushioned with two ice bricks (TechPack) provided the maximum temperature retention.

Effect of 24-h incubation at constant 37 °C on sperm motility, count and vitality

Following the selection of the components for the home shipping NextGen® kit and the appropriate supplement media, we examined the ability of the NextGen® kit to sustain motility and vitality in ten samples incubated at 37 °C for 2 h. After 24 h, there were large decreases in motility, vitality and TMC (Table 2).

Effect of overnight shipment in NextGen kit on sperm motility, count and vitality

Table 3 summarises the differences in sperm motility, concentration and membrane integrity before shipment of the nine kits and after its receipt. The median pre-shipment percentage motility of the samples was 50.0% and post-shipment motility decreased to a median of 31.0%. The percentage recovery in motility was 46.5%.

The average pre-shipment total motile spermatozoa (TMS) were 44.3 \(\times 10^6\) and post-shipment TMS were 24.3 \(\times 10^6\) million as shown in Table 3. Two of the 10 samples had bacterial contamination and very poor motility.

**Discussion**

Patients with cancer are encouraged to bank their semen specimens prior to the start of their cancer treatment. Due to the fact that the window of opportunity to bank semen samples and the start of the treatment are too narrow, patients are advised to provide multiple ejaculates in the time that is available before the initiation of the treatment. With the availability of assisted reproductive

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**Table 1** Difference in pre- and post-incubation sperm quality after addition of refrigeration medium or human tubal fluid

<table>
<thead>
<tr>
<th>Semen Parameters</th>
<th>Refrigeration medium n = 6</th>
<th>Human Tubal Fluid n = 6</th>
<th>Wilcoxon rank sum P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>50.0 (30.3, 69.8)</td>
<td>50.0 (30.3, 69.8)</td>
<td>0 (0, 57.1)</td>
</tr>
<tr>
<td>Post-incubation</td>
<td>0 (0, 57.1)</td>
<td>0 (0, 62.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Percentage</td>
<td>0 (0, 81.8)</td>
<td>0 (0, 89.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOS (%)</td>
<td>49.1 (36.8, 61.4)</td>
<td>49.1 (36.8, 61.4)</td>
<td>11.3 (6.5, 67.5)</td>
</tr>
<tr>
<td>Post-incubation</td>
<td>27.8 (17.0, 60.0)</td>
<td>27.8 (17.0, 60.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Percentage</td>
<td>28.6 (10.6, 109.9)</td>
<td>54.4 (29.3, 118.4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are median (min, max). \(P < 0.05\) was considered significant.

**Table 2** Pre- and post-incubation differences in sperm quality \((n = 10)\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-incubation</th>
<th>Post-incubation</th>
<th>Wilcoxon rank signed P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>45.9 (40.0, 60.4)</td>
<td>15.8 (3.0, 49.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>HOS (%)</td>
<td>53.6 (37.4, 67.3)</td>
<td>39.0 (14.0, 63.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>TMS (\times 10^6) spermatozoa</td>
<td>19.6 (5.5, 100.3)</td>
<td>5.2 (1.7, 22.1)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are median (range). \(P < 0.05\) was considered significant. HOS, Hypoosmotic swelling test; TMS, Total motile spermatozoa.
Table 3  Sperm quality of specimens shipped via NextGen kit (n = 9)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-shipment</th>
<th>Post-shipment</th>
<th>Wilcoxon signed rank P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>50.0 (27.4, 82.0)</td>
<td>31.0 (7.0, 47.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Count (x 10^6/ml)</td>
<td>90.1 (15.0, 210)</td>
<td>64.0 (14.1, 182)</td>
<td>0.004</td>
</tr>
<tr>
<td>TMS (x 10^6 spermatozoa)</td>
<td>44.3 (9.0, 138)</td>
<td>24.3 (1.7, 57.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>TMS Recovery (%)</td>
<td>44.0 (3.8, 66.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility</td>
<td>46.5 (8.5, 94.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are median (range). P < 0.05 was considered significant.

TMS, Total motile spermatozoa.

Shipping semen samples by Home Sperm Banking kit

Motility
TMS Recovery
9
TMS (9 Motility (%) 50.0 (27.4, 82.0) 31.0 (7.0, 47.0) 0.004

In the earlier studies examining the effect of temperature on sperm motility (Appell & Evans, 1977), the researchers examined semen specimen from pre-vasectomy patients that were maintained at 4 °C, 20 °C and 37 °C and were evaluated at 3 h, 6 h, 12 h and 18 h after collection. They concluded that samples should be kept at room temperature (20 °C) and not at 37 °C as any increase in temperature will lower motility. In another study by Cohen et al. (1985), spermatozoa stored in refrigerator had significantly higher fertilisation rates compared to samples stored at room temperature. In a study by Esfandiar et al. (2002), samples stored at 37 °C maintained the highest motility at 37 °C. Therefore, in the standardisation of the NextGen kit, we chose to use 24 °C as an optimal ambient room temperature, and 37 °C and 40 °C were selected as temperatures that the kit may be exposed to during the actual shipping of samples, especially in extreme climates or weather conditions.

Refrigeration medium TYB from Irvine Scientific is designed for short-term storage of human spermatozoa at 2° to 5 °C, and it contains TYB and gentamicin. It has been used for overnight storage and shipment of samples. It contains TES and Tris and heat-inactivated egg yolk. It is intended for refrigeration of semen for use in assisted reproduction. Human tubal fluid from Irvine Scientific is a synthetic solution for use as a culture media for processing of gametes. It also contains gentamicin as an antibiotic. In an earlier study (Sharma et al., 1997), we compared the quality of spermatozoa prepared in RM and HTF medium by examining the motility after thawing. Results from the use of both mediums were comparable. Similarly, in this study, we found comparable results in motility, percentage recovery and HOS testing of both pre- and post-incubation in samples transported in HTF and RM. Refrigeration medium has been suggested as a preferred medium for overnight shipment and storage of semen samples. Therefore, although both media appear to be equally good in preserving motility, we preferred to use RM for transportation of spermatozoa in the NextGen® home banking kit.

Furthermore, based on the tests on different kinds of ice packs, the cooling sleeve allows for a slower melting at 5 °C. Therefore, this was wrapped around the specimen collection cup. The cooling sleeve was cushioned between two solid ice bricks (TechPack). This combination was most effective in retaining the optimal temperature required for overnight shipment of the NextGen® kit.

To evaluate the effects of a potential shipping delay or accidental moderate warming of the samples, we investigated the effects of 37 °C and room temperature (24 °C) on spermatozoa attributes pre- and post-incubation. There was a significant decrease in post-incubation motility (P = 0.004) and post-incubation vitality (P = 0.004) at samples exposed to room temperature (24 °C). This decrease could be attributed to bacterial contamination inside two of the ten samples during incubation. A decrease in motility and increase in bacterial counts was reported in samples maintained at 37 °C (Appell &
Results indicated that samples shipped from various andrology laboratories in the United States to determine the effects of actual shipping on the sperm parameters. This is an important finding, indicating that if the patient were to have an infection during the time of collection, the quality of the semen sample could be affected (Rybar et al., 2012).

We also shipped nine NextGen® kits to seven different andrology laboratories in the United States to determine the effects of actual shipping on the sperm parameters. Results indicated that samples shipped from various distances and temperatures using the NextGen® kit were successful in sustaining sperm quality. Our results show that if the home shipping kit is assembled correctly and shipment is optimised for overnight delivery in a time-appropriate manner, then the sperm banking kit can provide a safe, reliable, efficient and most of all convenient manner for men to transport their samples to the Cleveland Clinic from all parts of the United States.

The home sperm banking kit (NextGen®) presents a potential strategy to decrease barriers to sperm banking such as privacy, discomfort and access to sperm banking facilities (Young et al., 2003). By standardising the kit components, we can provide a standard of care for fertility preservation to men with cancer and infertility issues. Producing a semen sample on site is the best option. However, the kit offers patients who cannot come to a sperm bank facility with an alternative to collect a semen sample in the privacy of their own home and ship the samples overnight to the andrology laboratory for further processing and storage. Collecting semen at home and transporting the sample overnight reduces emotional anxiety, the need to travel from geographically distant places (different cities/states), and is cost-effective in terms of travel, hotel, taking time off from work, etc.

To our knowledge, this is the first study that has determined the effects of remote collection and shipping on semen parameters. Studies on other similar remote collection kits have only simulated the overnight shipping conditions or have evaluated only post-shipping semen parameters and thus are lacking comparative results (Royster et al., 2000).

Shipping delays resulting in accidental moderate warming of the samples might induce chromosome/DNA damage after remote collection and overnight shipping of semen samples (Young et al., 2003). Poor quality samples generally do not freeze as well, and this method will decrease the initial quality. So while this method allows men to bank who would otherwise not bank, if possible, it should not be used as a substitute to on-site production of samples.

There were some limitations to our study. One of our study limitations was the small sample size. We will be conducting another follow-up study with a larger number of patients in the future. Furthermore, we did not examine the samples for oxidative stress, apoptosis or DNA fragmentation that can be detrimental to the fertilising potential of the spermatozoa. Future plans include offering the NextGen® kit to our patients requesting collection of spermatozoa at home and shipping the kit overnight to determine rates of sperm banking among patients at our centre and from other centres that are lacking in sperm banking services.

Conclusion

In conclusion, we have standardised and designed a specialised sperm collection and transport kit, NextGen® at the Andrology Center at the Cleveland Clinic. This is an innovative, first-of-its kind product that is evaluated in a clinical setting and is specially designed for men with cancer and other clinical diagnosis of infertility that requires sperm banking. Semen parameters were maintained by remote collection and shipping utilising the NextGen® kit. Patients can collect semen sample in the privacy of their own home and ship the samples overnight to the andrology laboratory for further processing and storage. Although sperm quality is reduced, an adequate number of good quality spermatozoa are available that can be used by ART, such as ICSI for procreation. Collecting semen at home and transporting the same overnight reduces barriers such as emotional anxiety and the need to travel from remote locations.

Acknowledgements

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References


