Distribution of Semen Parameters Among Adolescent Males Undergoing Fertility Preservation in a Multicenter International Cohort


OBJECTIVE
To determine the distribution of semen parameters among adolescent and adult males presenting for fertility preservation.

METHODS
A retrospective, cross-sectional cohort study of adolescent males age 11-19 who underwent semen analysis for fertility preservation at 3 centers in 2 countries with a comparison cohort of adult men presenting for fertility preservation. Prevalence of azoospermia and distribution of semen parameters was compared across groups.

RESULTS
A total of 197 adolescents and 95 adults underwent semen analysis for fertility preservation. Azoospermia was present in 17 (8.6%) adolescents and 3 (3.2%) adults. There was a decline in the prevalence of azoospermia with increasing age. After exclusion of patients with azoospermia, the adolescent and adult cohorts were comprised of 180 and 92 patients, respectively. Median age at presentation among adolescents vs adults was 16.5 years (interquartile range [IQR] 15.2-17.6) and 30.8 years (IQR 22.7-43.8), respectively. Median semen volume was 1.0mL (IQR 0.5-2.0) vs 2.5mL (IQR 1.5-3.5), P < .001. Median sperm concentration was 30 million/mL (IQR 10-57) vs 39 million/mL (IQR 14-57), P = .2. Median sperm motility was 39% (IQR 20-55) vs 45% (IQR 35-55), P = .01. Median total motile sperm count was 11 million (IQR 1.4-33) for adolescents vs 29 million (IQR 13-69) for adults, P < .001.

CONCLUSION
Young adolescent males had a higher prevalence of azoospermia and lower semen parameters compared to adults. In conjunction with physical examination, Tanner stage, and specific clinical context, these data can help to inform patients and their families about potential for fertility preservation, even in very young adolescent patients.

While semen parameters are not diagnostic of male infertility, they are crucial drivers of treatment decisions in the management of adolescent male patients with fertility concerns. Varicocele is associated with impaired semen parameters, and varicocele repair is often pursued with the goal of improved semen parameters and fertility. Likewise, semen parameters may impact fertility preservation among adolescents with malignancy, such as the decision to pursue testis biopsy and the extent of sperm cryopreservation. However, the lack of established reference values for adolescent semen parameters substantially impairs their utility in these clinical settings.

The commonly referenced World Health Organization (WHO) criteria are based on fertile adult men only, and semen parameters consistent with subfertility in adults may not harbor the same prognostic significance in adolescents on the spectrum of puberty and Tanner stage development. Attempts to collect semen analyses from healthy adolescents to establish reference ranges will be hampered by barriers pertaining to practical and ethical concerns of internal review boards, patient, and parental consent.

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While prior studies have characterized adolescent semen parameters in the setting of fertility preservation, the lack of a comparison cohort renders it difficult to discern the extent to which impaired adolescent semen parameters are due to puberty or the presence of malignancy.

Therefore, we sought to compare the distribution of semen parameters in a multicenter, international cohort of adolescent and adult males presenting for fertility preservation in order to inform patients and providers confronted with fertility preservation prior to cancer therapy, as well as to potentially offer insight into the management of other conditions, such as varicocele, pertaining to fertility in the adolescent male population.

**MATERIALS AND METHODS**

We performed a retrospective review of all adolescent males age 11 through 19 years who had semen analysis (SA) at 1 of 3 tertiary care centers in 2 countries (United States and United Kingdom). Patients presented for sperm banking prior to treatment of malignancy from 2010 to 2017. Those with a history of testis cancer or systemic chemotherapy prior to SA were excluded as a result of power concerns secondary to the low number of young adolescents, though no statistical testing was performed as an inherent variation in timing and progression of puberty or genetic abnormalities. Cohort demographic and clinical characteristics including age and duration of abstinence prior to SA were described for all nonazoospermic adolescent patients.

Semen parameters for the overall cohort were described according to quartile with additional reporting of the fifth and 95th percentile as a reference given the methodology of the aforementioned WHO guidelines, which utilize the fifth percentile as the lower bound of “normal.” Distribution of semen parameters between adolescents and adults was compared using the Wilcoxon rank-sum test.

Statistical significance for all testing was determined at a $P$ value of $0.05$. All statistical analysis was performed using Stata version 13.0 (StataCorp, College Station, TX). Institutional review board approval was obtained for each participating institution.

**RESULTS**

A total of 197 adolescent males underwent SA for fertility preservation during the study period of whom 17 (8.6%) had azoospermia. As expected, there was a decline in the prevalence of azoospermia with increasing age (Fig. 1). In comparison, a total of 95 adult men with cancer had SA in whom 3 (3.2%) had azoospermia ($P = .08$).

After exclusion of adolescents with azoospermia, the adolescent cohort was comprised of 180 patients from the United States ($N = 84$, 46.7%) and the United Kingdom ($N = 96$, 53.3%). Median age at presentation was 16.5 years (interquartile range [IQR] 15.2-17.6).

After exclusion of adults with azoospermia, the adult cohort was comprised of 92 patients. Median age at presentation was 30.8 years (IQR 22.7-43.8).

Semen parameters for the adolescent and adult cohorts are presented in Table 1. Median abstinence prior to SA was 4 days (IQR 3-5) for adolescents and 5 days (IQR 3-7) for adults. Median semen volume was 1.0mL (IQR 0.5-2.0) for adolescents vs volume 2.5mL (IQR 1.5-3.5) for adults, $P < .001$. Median sperm concentration was 30 million/mL (IQR 10-57) for adolescents vs 39 million/mL (IQR 14-57) for adults, $P = .2$. Median sperm motility was 39% (IQR 20-55) for adolescents vs 45% (IQR 35-55) for adults, $P = .01$. Median total motile sperm count was 11 million (IQR 1.4-33) for adolescents vs 29 million (IQR 13-69) for adults, $P < .001$. A boxplot presenting the distributions of total motile sperm count is presented in Figure 2.

The fifth percentile for all semen parameters among adolescents and adults is presented in Table 2. Across all semen parameters, both adolescent and adult parameters were lower than the WHO reference ranges.

**DISCUSSION**

There is a paucity of data examining semen parameters in adolescent males, which is further complicated by the inherent variation in timing and progression of puberty among adolescents. The complexity of this issue and absence of adolescent-specific parameters is particularly challenging for management of adolescent conditions such as varicocele and malignancy, wherein future fertility
potential is a crucial component of clinical decision-making. Studies of adolescent semen parameters have been hindered by practical and ethical concerns from internal review boards, providers, and parents. The ideal study would evaluate middle- and high-school volunteers with history, physical exam, and 2 semen samples after a predefined period of abstinence. This would also enable the exclusion of patients with cancer, varicocele, or other potential confounding medical history. As such a study is not technically feasible due to the aforementioned barriers, we sought to retrospectively compare semen parameters in adolescents and adults presenting for fertility preservation in the context of malignancy.

Two recent retrospective studies have examined semen parameters in an adolescent fertility preservation cohort. Dinoia et al identified adolescents who were at least Tanner stage III and newly diagnosed with malignancy, some of whom had already received chemotherapy. The study was limited by exclusion of adolescents with early Tanner stage who may have had spermatogenesis, as spermarche is an early pubertal event. Daudin et al reported data from a large, multicenter, national cohort over 3 decades. While the largest series in the literature, the study did not report the prevalence of azoospermia in the adolescent fertility preservation cohort. Furthermore, the data were accrued across many different laboratories and over the course of a long time period, which may render the results subject to substantial variation in laboratory techniques and data collection. Nonetheless, both studies described baseline semen parameters in adolescents with malignancy, thereby helping to establish a reference range for these patients. The current study supports and expands upon the findings of these prior studies.

We found that the prevalence of azoospermia in adolescents decreased substantially with age and ultimately approached those of adults. We hypothesized that the prevalence of azoospermia in the adolescent population was likely driven by puberty. However, to exclude the possibility that azoospermia in the adolescent cohort was driven by a diagnosis of malignancy in a substantial portion of patients, we assembled a comparison cohort of adult men with malignancy. Using age as a surrogate for pubertal state, the higher prevalence of azoospermia in the adolescent cohort suggested that this was likely due to variations in onset of puberty. If azoospermia in adolescents was driven by malignancy, we would have expected similar rates of azoospermia across all adolescent and adult age groups, though this assumption is confounded by the inherent differences in the effects of adolescent and adult malignancies on spermatogenesis and the lack of physical

<table>
<thead>
<tr>
<th>% azoospermia</th>
<th>0.0%</th>
<th>66.7%</th>
<th>31.3%</th>
<th>10.7%</th>
<th>9.7%</th>
<th>2.3%</th>
<th>6.7%</th>
<th>0.0%</th>
<th>0.0%</th>
<th>3.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No azoospermia</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>28</td>
<td>42</td>
<td>42</td>
<td>13</td>
<td>17</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Azoospermia</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Adolescent and adult semen parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>25th Percentile</th>
<th>50th Percentile</th>
<th>75th Percentile</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adolescent</td>
<td>Adult</td>
<td>Adolescent</td>
<td>Adult</td>
</tr>
<tr>
<td>Concentration (mil/mL)</td>
<td>10</td>
<td>14</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>0.5</td>
<td>1.5</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>20</td>
<td>35</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Total motile count (mil)</td>
<td>1.5</td>
<td>13</td>
<td>11</td>
<td>29</td>
</tr>
</tbody>
</table>

* Wilcoxon-Mann-Whitney test.
examination (Tanner stage and varicocele). These findings are consistent with DiNoia et al showing decreasing prevalence of azoospermia across increasing age groups, though the absolute rates of azoospermia are lower in the current study.

Figure 1 is very useful for counseling younger patients age 11-13 who present with malignancy or other fertility-related concerns. In the former circumstance, these data demonstrate that even very young adolescent boys may have spermatogenesis. Whereas some providers or family members might overlook the potential for fertility preservation in these young adolescent boys, the current data reinforce the importance of discussing and offering fertility preservation in even the youngest patients. In the latter circumstance, most commonly a clinical varicocele, these data can provide some reassurance that azoospermia is likely related to puberty rather than varicocele pathology, particularly when this is corroborated by Tanner stage. Though lack of Tanner stage and varicocele data in the current study unfortunately precludes the specific association of azoospermia with puberty, the high prevalence of azoospermia in younger patients relative to older patients suggests that azoospermia in this age range is likely to be puberty related.

We found that the distribution of semen volume, sperm motility, and total motile sperm count in adolescents was significantly different from those of adults. These data are consistent with those of DiNoia et al and Daudin et al, who previously demonstrated a correlation between these semen parameters and age, which likely reflects progression of puberty. In contrast, whereas both Dinoia et al and Daudin et al did observe a slight association between age and sperm concentration, we did not observe a difference in sperm concentration between the 2 groups. In the current study, there does appear to be a much lower sperm concentration in both adult and adolescent fertility preservation groups compared to the WHO reference ranges, which may be due to the inherent effects of malignancy on testicular function. Similar to these findings in the context of malignancy, sperm concentration is lower but not correlated with age amongst adolescent varicocele patients, whereas semen volume and motility are positively correlated with age. In aggregate, these findings suggest that sperm concentration may be most dependent upon nonage-related gonadotoxic risk factors such as malignancy and varicocele, whereas semen volume and motility are age-dependent phenomenon, increasing with age and progression of puberty irrespective of potential gonadotoxic insult.

Our results must be evaluated in the context of the strengths and limitations of our study design. The multi-institutional and international nature of our cohort mitigates confounders and renders the results broadly applicable and generalizable. We attempted to minimize heterogeneity by excluding adolescent and adult men with known

Table 2. Fifth percentile lower bound of “normal” SA for adolescent, adult, and World Health Organization (WHO) cohorts

<table>
<thead>
<tr>
<th></th>
<th>Adolescent</th>
<th>Adult</th>
<th>WHO*</th>
<th>WHO†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mil/mL)</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>0.2</td>
<td>0.6</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>0</td>
<td>1.3</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>Total motile count (mil)</td>
<td>0</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* General population of unscreened men.
† Fertile men whose partners had time-to-pregnancy of less than 12 months.
varicoceles or pertinent history such as testis cancer, cryptorchidism, or orchiectomy that may affect spermatogenesis. Additionally, the inclusion of a comparison adult cohort sheds light upon the etiology of azoospermia in the adolescent population. In this regard, the findings further support and expand upon those of prior studies.

The results are limited by the lack of specific clinical data such as Tanner stage, type of malignancy, and physical examination for the presence of varicocele. Ultimately, the interpretation of these results is contingent upon use of age as a surrogate for pubertal status in the absence of these data and without controlling for these potential confounders. Variability in laboratory sample processing and reporting (eg, counting chambers) across sites introduced data and without controlling for these potential confounders. We evaluated semen parameters from a single SA in the setting of fertility preservation. Young adolescent males had a higher prevalence of azoospermia compared to adults, and the distribution of semen parameters was significantly lower in adolescents compared to adults. The current data build upon prior studies to establish a frame of reference for providers, patients, and families who are confronted with fertility concerns in the context of adolescent malignancy and may further provide insight for patients presenting with azoospermia in the context of adolescent varicocele. In conjunction with physical examination, Tanner stage, and specific clinical context, these data can help to inform patients and their families about potential for fertility preservation, even in very young adolescent patients.

**CONCLUSION**

We present the first international, multi-institutional population of patients, which likely decreased the precision of semen parameter measurement given the substantial variation in semen parameters between consecutive samples from the same patient. Additionally, the distinct malignancy profiles of adolescents vs adults likely contributed to variation between the 2 groups.

**References**