Changes in Semen Analysis over Time: A Temporal Trend Analysis of 20 Years of Subfertile Non-Azoospermic Men

Nahid Punjani1, Omar Al-Hussein Alawamlh1, Soo Jeong Kim1, Carolyn A. Salter1, Gal Wald1, Miriam Feliciano1, Nicholas Williams2, Vanessa Dudley1, Marc Goldstein1

1Center for Reproductive Medicine and Surgery, Institute for Reproductive Medicine and Department of Urology, Weill Cornell Medicine, New York, NY, USA

Purpose: To examine trends of population-level semen quality over a 20-year period.

Materials and Methods: We performed a retrospective review of data from the andrology lab of a high volume tertiary hospital. All men with semen samples between 2000 and 2019 were included and men with azoospermia were excluded. Semen parameters were reported using the World Health Organization (WHO) 4th edition. The primary outcome of interest was changes in semen parameters over time. Generalized least squares (GLS) with restricted cubic splines were used to estimate average-monthly measurements, adjusting for age and abstinence period. Contrasts of the estimated averages based on GLS between the first and last months of collection were calculated.

Results: A total of 8,990 semen samples from subfertile non-azoospermic men were included in our study. Semen volume decreased over time and estimate average at the beginning and end were statistically different (p<0.001). Similarly sperm morphology decreased over time, with a statistically significant difference between estimated averages from start to finish (p<0.001). Semen pH appeared to be increasing over time, but this difference was not significant over time (p=0.060). Sperm concentration and count displayed an increase around 2003 to 2005, but otherwise remained fairly constant over time (p=0.100 and p=0.054, respectively). Sperm motility appeared to decrease over time (p<0.001).

Conclusions: In a large sample of patients presenting to a single institution for fertility assessment, some aspects of semen quality declined across more than two decades. An understanding of the etiologies and driving forces of changing semen parameters over time is warranted.

Keywords: Andrology; Male infertility; Semen analysis; Sperm count

INTRODUCTION

Infertility is a growing problem worldwide, affecting up to 12% of couples, and male factor specifically is present in up to 50% of cases [1]. The most basic test for the assessment of male factor infertility is a standard semen analysis, which reports numerous factors including semen volume, pH, concentration, total count,
morphology, and motility. A confirmatory analysis must be repeated following an abstinence period of 2 to 7 days and at least 4 weeks apart [2]. Male infertility is therefore commonly attributed to abnormalities from these analyses and subsequently classified based on fertile reference ranges published by the World Health Organization (WHO) [3-5].

While some additional adjunctive tests do exist for the assessment of male fertility, the ongoing heavy reliance on semen parameters has prompted investigation as to how semen quality has changed over time. Speculation and controversy exist with respect to these temporal changes. Reports as early as the 1990s have detected a decrease in semen quality, while others during the same period have reported no significant decline [6,7]. Data from more recent and larger study populations from Asia and Europe have similarly suggested worsening semen quality over time with respect to sperm concentration as well as other parameters, while other studies have demonstrated opposite conclusions, including one study suggesting an improvement in sperm count and concentration [8-13]. The largest study assessing a more heterogenous population was a combined analysis of semen analyses from over 100,000 men from the United States and Spain, which showed a decline in semen quality. This study, however, only examined sperm count and concentration [14].

Given the ongoing controversy as to how semen quality is changing over time we elected to review data from our center. More specifically, our objective was to review temporal trends in multiple semen parameters in sub-fertile non-azoospermic men in a large tertiary fertility referral center to further understand semen quality changes over time.

**MATERIALS AND METHODS**

1. **Study population**
   Data were retrospectively reviewed by the study team over a 20-year period from a prospectively collected electronic database between 2000 and 2019. Each consecutively retrieved semen analysis by the laboratory was completed in a written format and then transcribed to an electronic database stored on a secure workstation. All data were entered by a laboratory certified andrologist. Patients providing semen samples were referred from not only within New York City but also nationally and internationally, by male reproduc-
or grade 4 (movement in straight line at high speed). Sperm morphology was recorded as a percentage of sperm classified as morphologically normal following assessment of adequate sperm heads, midpieces and tails. Strict morphology was not presented.

5. Statistical analysis
Statistical analyses were performed to examine temporal trends in all semen parameters both descriptively and quantitatively. Mean values and associated standard deviations were calculated for pH, progressive motility and motility as they appeared to have a normal distribution. Medians and interquartile ranges (IQRs) were reported for all other parameters as they did not appear to have normal distributions. Parameters with missing data were excluded and therefore complete-case analysis of each individual variable was performed. Daily data was aggregated at the month using the mean. Generalized least squares (GLS) was then used to estimate trends in average-monthly sperm quality measurements (volume, pH, concentration, total sperm count, motility, morphology, and progressive motility) from 2000 to 2019 adjusting for average-monthly age and abstinence days. Autocorrelation of residuals was accounted for. Non-linearities were modeled using restricted cubic splines with six knots. Wald tests of non-linear terms were performed to assess if the use of restricted cubic splines were appropriate. Contrasts of the estimated average sperm quality measurements based on GLS models were performed between the first month and last months of data collection. Sensitivity analysis were completed repeating analysis using only first time semen analysis data. Statistical significance was evaluated at the 0.05 alpha level and model estimates are presented with 95% confidence intervals. Analyses were performed using Stata v14 (Stata Corp, College Station, TX, USA) and R version 3.6.3.

RESULTS
A total of 10,865 semen analyses were reviewed by a single laboratory over the study period. After excluding those with a sperm concentration of zero, a total of 8,990 samples remained over a total of 20 years (Fig. 1). Data were missing for <0.01% of patients for volume, concentration, and total sperm count, motility, and morphology, but 7.0% (n=664) for pH.

1. Demographics
Demographic data for the entire cohort (n=8,990), including those with some missing semen parameter data, are summarized in Table 1. The median age for the complete cohort was 37.0 years (IQR, 33.0–43.0 y) and was similar amongst yearly groups between 36 and 39 years of age. Overall, median abstinence period was 3.0 days (IQR, 2.5–4.0 days) for the entire cohort, and was between 3 and 4 days for all time periods.

2. Semen parameter trends
Semen parameters based on years are shown in Table 1. Given the non-linearity of the data, restricted cubic spline modeling was used to model the data graphically based on monthly averages (Fig. 2). A significant downward trend is demonstrated for volume, with the most pronounced changes between 2005 and 2010. Sperm pH displayed a variable upward trend towards a more basic pH. Sperm morphology appeared to display a significant decline over time. For both sperm count and concentration, a peak is visualized between 2002 and 2006, otherwise the quality appears relatively unchanged over time. Both progressive motility and motility appeared relatively stable over the study period. Table 2 contrasts the average estimated outcomes at
the beginning and end of the study period. Only semen volume, motility, and morphology were significantly different over time ($p<0.001$).

**DISCUSSION**

We demonstrated that some semen parameters including volume, motility, and morphology, are worsening over time, while sperm count and concentration appear stable in a large heterogeneous population of subfertile non-azoospermic men. These findings support previous studies which concluded that semen quality may be declining or remains unchanged, but refutes those which suggest quality is improving [8-14].

Sperm count and concentration appear unchanged over the study period. However, there was a distinct peak in sperm concentration and total sperm count between the periods of 2002 and 2006. No laboratory specific factors or technical elements including sperm processing and assessment were identified that could account for our findings. However, an amendment to New York state law regarding improved insurance coverage for fertility treatment in women occurred in 2002, which may explain the influx of more fertile male partners seeking care while their partners were being evaluated [15].

As seen in previous studies, semen volume significantly decreased over time, while semen pH increased over time, but not this was not statistically significant [16]. Technical factors such as inadequate collection would not explain this change over such a significant time period. Abstinence period is known to influence semen volume, but this was adjusted for in our study [17]. A hypothesis to explain this observation may include increased rates of partial obstruction in some portion of the male reproductive tract from iatrogenic procedures, infections, or idiopathic sources [18]. Additional studies have also explored the impact of seminal vesicle characteristics and ejaculate volume. Reduced seminal vesicle size or abnormal seminal vesicle ejection fraction may provide an alternative explanation for our findings [19].

Sperm morphology significantly decreased over time, which is consistent with previously reported studies [20,21]. During the study period there were two changes to the WHO criteria for sperm morphology. However,
these changes were only modifications to the normal cut-off values and not changes to the actual criteria for which morphology assessment was completed [22]. Given the subjectivity in sperm morphology assessment, amendments to technical assessment over time could explain the discrepancies, but in our study this change was consistent despite having four different andrologists over the study period. However, it has been suggested that the introduction of new criteria over time may have caused a classification drift as technicians became more critical of their morphological assessments [21].

Sperm motility did decrease significantly over time. Previous studies have suggested that sperm motility
is decreasing for men with normal sperm counts, but increasing for those with low counts [14]. Progressive 
motility in our study was generally reported as a range 
(i.e. 2 to 3) and therefore it was captured as an average 
of these values. Given the predominant reporting as 
a range as opposed to discrete values, this parameter 
should be interpreted with caution.

Various potential etiologies have been proposed to 
explain worsening semen quality over time, of which 
only age was included in our study. Aside from tech-
cnical errors, increasing age has been shown to have an 
inverse association with total sperm motility, progres-
sive motility, normal sperm morphology, and sperm 
concentration [23]. Other factors have been hypoth-
esized, which were not included in our study, but may 
explain some of our associations. Obesity for example, 
is associated with reduced sperm morphology and sperm 
concentration [23]. Environmental factors such as 
 exposure to pollutants including methyl mercury, 
pesticides, organic solvents, radiation, endocrine dis-
rupting compounds, and even mobile phones have also 
been hypothesized to compromise male reproductive 
function [24-26]. Other factors include smoking and al-
cohol, and even cell phone usage [27].

The findings of our study do have important clinical 
implications. There is increasing evidence of an asso-
ciation between male infertility and systemic disease, 
including malignancies, autoimmune conditions, and 
high risk behavior [28,29]. There is also evidence of 
worsened overall health with worse semen parameters 
[30]. If these trends are accurate, this overall suggests 
the need for an increased focus on a thorough history 
and physical evaluation in infertile men, and that if 
certain parameters are worsening perhaps this is re-
flective of additional previously undiagnosed medical 
disease, especially in young men who may otherwise 
not seek medical care.

There are several limitations to our study. Our ret-
rospective study design limits obtaining any additional 
demographic or environmental data for analysis, in-
troducing potential misclassification bias from errone-
ous data entry, and inability to obtain missing data. 
However, our study had a large sample size over a 
significant time period, so although missing data was 
prevalent for almost every parameter, the missing data 
was <1% for all but one parameter (pH), suggesting 
minimal impact from the missing data. Our study also 
includes all men obtaining semen samples for infertil-
ity workup, which may lend itself to selection bias, and 
did not discriminate referrals from primary care physi-
cians, reproductive endocrinologists, general urologists, 
or reproductive urologists. An increased number of 
referrals from male reproductive urologists may poten-
tially explain the decrease in semen parameters as they 
may represent more severe cases. Our study did not use 
the same andrologist over the entire study period but 
instead included four separate andrologists for vari-
ous periods of time. While this suggests a potential role 
for observer bias, the timing of change in andrologists 
did not impact or correlate with any trends presented 
in our study. Finally, duplicate samples from the same 
patient were not removed from the study. However, 
sensitivity analysis (Supplement Table 1, Supplement 
Fig. 1) completed including only men with first time se-
men analysis provided similar results and also suggests 
additional statistically significant differences in total 
sperm count and pH.

CONCLUSIONS

In a large subset of non-azoospermic sub-fertile men 
presenting to a single institution for fertility assess-

Table 2. Difference in estimated average outcomes at end of data collection vs. beginning of data collection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beginning</th>
<th>End</th>
<th>Contrast</th>
<th>95% CI</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>3.04</td>
<td>2.54</td>
<td>0.50</td>
<td>0.23 to 0.77</td>
<td>3.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>8.21</td>
<td>8.29</td>
<td>-0.08</td>
<td>-0.16 to 0.00</td>
<td>-1.88</td>
<td>0.060</td>
</tr>
<tr>
<td>Concentration (millions/mL)</td>
<td>64.28</td>
<td>46.19</td>
<td>18.09</td>
<td>-3.48 to 39.66</td>
<td>1.64</td>
<td>0.100</td>
</tr>
<tr>
<td>Total sperm count (millions)</td>
<td>179.86</td>
<td>116.06</td>
<td>63.8</td>
<td>-1.17 to 128.77</td>
<td>1.92</td>
<td>0.054</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>25.75</td>
<td>5.68</td>
<td>20.07</td>
<td>15.79 to 24.34</td>
<td>9.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>0.64</td>
<td>0.52</td>
<td>0.12</td>
<td>0.07 to 0.17</td>
<td>4.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>2.92</td>
<td>2.66</td>
<td>0.26</td>
<td>-0.02 to 0.53</td>
<td>1.80</td>
<td>0.072</td>
</tr>
</tbody>
</table>

CI: confidence interval.
ment at a single high-volume laboratory, some aspects semen quality has significantly declined over the past twenty years but sperm concentrations appear unchanged. Further research exploring the etiologies and driving forces impacting altered semen quality over time are warranted.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: NP, SJK, MG. Data curation: NP, OAHA, SJK, CAS. Formal analysis: NP, NW. Investigation: NP, SJK, MG. Methodology: NP, MG. Supervision: MG. Visualization: NW. Writing – original draft: NP, MG. Writing – review & editing: All authors.

Supplementary Materials

Supplementary materials can be found via https://doi.org/10.5534/wjmh.210201.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES