Sperm output of older men

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BACKGROUND: Declining fertility of couples from the fourth decade of life is largely attributable to the drop in female fertility. However, increasing numbers of men, whose fertility theoretically lasts until death, are seeking fertility treatment at older ages, yet there is little information on sperm production and function past the age of 50 years. The few studies of such older men have examined men attending fertility clinics, and therefore willing to provide semen samples, but the participation bias of such recruitment hinders extrapolation to the unselected general male population. METHODS: We have taken the opportunity to study a convenience sample of 55 healthy, non-infertile men ranging in age from 52 to 79 years old who provided semen samples as part of a prostate cancer screening project. They were compared with a control group (n = 409) of younger (<52 years) men from among 567 volunteers screened as potential sperm donors for an artificial insemination program. RESULTS: Older men had lower semen volume (mean semen volume 1.8 versus 3.2 ml; P < 0.0001) and total sperm output (median 74 versus 206 million sperm per ejaculate; P < 0.0001), whereas sperm density (median 64 versus 73 million sperm/ml; P = 0.12) was non-significantly decreased. Older men had more abnormal sperm morphology with decreasing numbers of normal forms (mean 14% versus 25%; P < 0.0001) and reduced vitality (mean 51% versus 80%; P < 0.0001), as well as increased numbers of cytoplasmic droplets (median 1 versus 0; P < 0.0001) and sperm tail abnormalities (30% versus 17%; P < 0.0001). Sperm head or neck abnormalities were no different between the groups. CONCLUSIONS: While neither study group may be representative of the general male population, these findings suggest that sperm production, reflected in sperm output but not sperm density, as well as sperm morphology and viability are diminished in this population of healthy, non-infertile older men.

Key words: male ageing/semen analysis/spermatogenesis/sperm morphology/testis

Introduction

It is well known that the fertility of couples declines with age (Leridon, 1977; Schwartz and Mayaux, 1982; Menken et al., 1986). This is mostly attributable to declining female fertility evident from the age of 30 years (Schwartz and Mayaux, 1982) and ceasing altogether by menopause. This precipitate decline in female fertility, together with couples usually being very closely matched in age, overshadows and complicates efforts to determine whether there are also significant declines in male fertility with age (Kidd et al., 2001). Very few studies have addressed actual fertility of older men controlling for declining female fertility (Anderson, 1975), although the time to pregnancy questionnaire (Joffe, 2003) is promising (Hassan and Killick, 2003), but has yet to be applied to older men.

Although testicular function does not exhibit a precipitous age-related decline like the ovary at menopause, there is a gradual and variable decline of modest proportion in testosterone production in older men (Gray et al., 1991b; Harman et al., 2001). Whether there is any real decline in spermatogenesis and/or male fertility in the general male population is much less clear. As direct sampling of the human testis is not feasible for population studies, sperm output is widely used as the surrogate measure of human male fertility. However, men are reluctant to provide semen samples unless actively concerned about their fertility. For example, population-based studies typically recruit <20% of young men willing to provide semen samples (Jensen et al., 2004) constituting an inevitable participation bias in such studies (Handelsman, 1997; Cohn et al., 2002). The limited number of published studies of sperm output in older men are largely restricted to men attending infertility clinics, where few are older than 50 years (Kidd et al., 2001). An uncertain, but probably high, proportion of such men have unrecognized defects in sperm production and/or function. Furthermore, access to such specialized medical services may be strongly influenced by non-biological factors, and findings from infertility clinics may not be reliably extrapolated to the general male
population. Hence, few studies of older men have managed to avoid severe participation and selection biases.

Therefore, in order to provide novel insight into sperm output among healthy, non-infertile older men, we took the opportunity to study a convenience sample of healthy older men without known reproductive disorders or prostate disease who provided semen samples for prostate cytology as part of medical screening for undiagnosed prostate disease (Gardiner et al., 2003).

Materials and methods

Participants

The sample of older men comprised 55 consecutive men who were referred by urologists from private office practices for a prostate cancer detection program based on seminal cytology (Gardiner et al., 1996; Clements et al., 1999). All these men were asymptomatic and had been identified by elevated blood prostate-specific antigen (PSA) concentrations that required prostate biopsy. The men provided a single semen sample to the Clinical Andrology laboratory on the same day immediately prior to their transrectal ultrasound and prostate biopsy for possible in situ prostate cancer. The diagnostic outcomes of the prostate cytology will be reported separately.

The control group comprised 409 men under the age of 52 years (lowest age of the older men) from the 567 younger men who volunteered between 1980 and 2000 for screening as potential sperm donors for an donor insemination program, as described previously (Handelsman et al., 1984; Handelsman, 1997). Both groups were studied by the same clinic and laboratory. Owing to changes in sperm morphology recommendations in the WHO Manual, the controls for sperm morphology were the most recent 84 younger men in whom sperm morphology assessment was performed according to the most recent WHO methodology.

Seminal analysis

The standard collection procedures for semen samples as defined by the WHO manual (World Health Organization, 1999) were modified both median semen volume and total sperm output per period of sexual abstinence was specified. As an alternative to collection at the laboratory, men were offered the choice to collect specimens at home. In the latter case, sample handling was unsuitable for valid motility assessment so only sperm concentration and morphology were evaluated, a practical compromise recommended for studies of semen analysis among non-infertile men (Cohn et al., 2002).

Sperm concentration and morphology evaluation were performed according to WHO guidelines (World Health Organization, 1999) using phase-contrast microscopy on unstained specimen, a modified Neubauer-type chamber and a Papanicolaou stain for morphology assessment performed according to ‘strict’ criteria. Defects were divided into head defects (large, small, tapered, pyriform, round and amorphous heads, vacuolated heads, heads with small acrosomal area and double heads), neck and midpiece defects, and tail defects (short, multiple, hairpin, broken tails, bent tails, tails of irregular width, coiled tails). Cytoplasmic droplets greater than one-third of the area of a normal sperm head were also recorded.

Data analysis

Data were analysed by NCSS software and are expressed as mean, SD, quartiles and extremes of the data distribution. Groups were compared by t-test for continuous Gaussian variables and by the non-parametric equivalent for non-Gaussian data. Categorical data were analysed by Fisher’s exact test using StatXact software.

Results

The age and semen analysis findings from 55 older and 567 younger men are summarized in Tables I and II. Among the older men, three were vasectomized, one provided a urine specimen and the volume of one semen sample was too low for adequate sperm counting; therefore, the final sample included results from 54 men for semen volume and from 51 for sperm variables. Two samples were delivered within 3 h and 15 within 5 h of collection.

There was no significant difference in sperm density but both median semen volume and total sperm output per

| Table I. Statistical distribution of semen volume and sperm output in older and younger men |
|---------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|
| Age (years)                               | Minimum         | Q1              | Q2              | Q3              | Maximum         | Mean            | SD             |
| Older men (n = 55)                         | 52              | 57              | 62              | 67              | 79              | 63              | 7              |
| Prostate cancer                           | 52              | 58              | 63              | 69              | 76              | 76              | 4              |
| No prostate cancer                        | 53              | 57              | 62              | 66              | 79              | 62              | 7              |
| Semen volume (ml)                          | 0.05            | 1               | 1.6             | 2.5             | 5               | 1.8             | 1.2            |
| Prostate cancer                           | 0.05            | 1               | 1.2             | 2.1             | 4               | 1.6             | 1.0            |
| No prostate cancer                        | 0.1             | 1               | 2               | 3               | 5               | 2.0             | 1.3            |
| Sperm density (M/ml)                       | 0.21            | 64              | 144             | 585             | 117             | 147             | 54             |
| Prostate cancer                           | 0.41            | 79              | 179             | 585             | 132             | 161             | 54             |
| Total sperm (M/ejaculate)                 | 0.27            | 95*             | 282             | 1395            | 198             | 259             | 107            |
| Prostate cancer                           | 0.43            | 103             | 329             | 585             | 184             | 174             | 107            |
| No prostate cancer                        | 0.18            | 78              | 266             | 1395            | 210             | 322             | 107            |
| Younger men (n = 409)                      |                 |                 |                 |                 |                 |                 |                |
| Age (years)                               | 26              | 31              | 38              | 51              | 32              | 8               |                |
| Semen volume (ml)                          | 0.2             | 3               | 4.2             | 9.9             | 3.2             | 1.7             |                |
| Sperm density (M/ml)                       | 0.44            | 73              | 114             | 400             | 87              | 63              |                |
| Total sperm (M/ejaculate)                 | 0.114           | 203             | 368             | 2560            | 284             | 277             |                |

Tabulated is the range (minimum, maximum) of observed values and quartiles of distribution (Q2 is the median) of semen volume and sperm density and output. For each variable the first line is aggregate data with the data for men with and without histological prostate cancer on subsequent lines. There is no significant difference in sperm density \( (P = 0.12) \), but highly significant differences in semen volume \( (P < 10^{-5}) \) and total sperm output \( (P < 10^{-7}) \). All comparisons between men with and without histological prostate cancer on biopsy were not significant \( (P > 0.25) \) by the non-parametric Mann–Whitney test.
ejaculate, reduced by 47% and 64%, respectively, were lower in older men (P < 10^{-7}).

Considering the WHO reference ranges, semen volume, sperm density and total sperm output were classified as subnormal in 32/54 (20%), 11/50 (22%) and 11/50 (22%), respectively, of older men. Among the younger control men, the comparable figures were 114/567 (20%), 37/567 (6.5%) and 39/567 (6.9%), respectively. The proportion of men with completely normal semen analysis (all three variables normal: volume, sperm density and total sperm output) was significantly (P < 0.0001) lower among older men (15/50, 30%) than among younger men (409/567, 72%).

The 26 older men with organ-confined, histological diagnosis of cancer present in the prostate biopsy did not differ from the remaining 27 who had no biopsy evidence of prostate cancer in age, semen volume, sperm density or output. None of the men had invasive prostate cancer.

Older men had a higher overall proportion of sperm with abnormal morphology, lower sperm vitality and a higher proportion of sperm tail defects, cytoplasmic droplets and teratozoospermia index. In contrast, defects of sperm head and neck morphology were no different between older and younger men (Table II).

### Discussion

The present opportunistic study of a convenience sample of men over the age of 50 years willing to provide semen samples but not for fertility evaluation shows that sperm concentration was not reduced by age, although semen volume was reduced by nearly 50% and total sperm output by 64%. In addition, sperm morphology and vitality were also more frequently pathological, especially involving sperm tail defects.

The strongest known determinants of semen volume are the positive relationship with time since last ejaculation (Schwartz et al., 1979) and the dependence of prostate and seminal vesicle fluid secretion on androgen exposure (Kitahara et al., 1998; Tash et al., 2000). For practical reasons, the present study was unable to standardize the interval since last ejaculation, so the findings must be interpreted with caution. Anxiety associated with a scheduled prostate biopsy on the same day may have influenced ejaculate volume. Semen samples provided on the day of a vasectomy would be a valuable procedural control in this context, but such data were not available. Given the decreasing intercourse frequency with age (Kinsey et al., 1948; Feldman et al., 2000), the apparently reduced semen volume with increasing age is more likely to reflect impaired androgen action, subclinical accessory gland pathology and/or ejaculatory defects (including treatment for prostate disorders) accumulating with age, rather than shorter abstinence intervals. Indeed, age-related reduction in ejaculation frequency may lead to underestimation of accessory gland hypofunction, whether androgen-dependent or not, in older men.

Sperm output, measured as total sperm per ejaculate, was substantially reduced in the older men, although the concomitant reduction in semen volume led to an apparent preservation of sperm density. This reduced sperm output with ageing is consistent with the impairment of spermatogenesis identified from detailed but small quantitative post mortem studies of sperm production rate (reviewed in Johnson, 1986), as well as a larger study of testis size (Handelsman and Staraj, 1985) of men dying suddenly. These findings highlight the wisdom of considering total sperm output as a variable reflecting sperm production, at least in conjunction with sperm density.

Regarding sperm morphology, our sample of older men showed a decrease of proportions of normal sperm morphology and vitality. The aberrant sperm morphology in older men was most evident in defects of tail morphology, possibly reflecting the complex cellular structural assembly process of

### Table II. Statistical distribution of sperm morphology in older and younger men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Older men (n = 55)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>19</td>
<td>32</td>
<td>38</td>
<td>44</td>
<td>51</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>% normal</td>
<td>0</td>
<td>6</td>
<td>15</td>
<td>19</td>
<td>40</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Head</td>
<td>0</td>
<td>59</td>
<td>63</td>
<td>69</td>
<td>79</td>
<td>61</td>
<td>15</td>
</tr>
<tr>
<td>Neck</td>
<td>0</td>
<td>31</td>
<td>34</td>
<td>38</td>
<td>45</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Tail</td>
<td>0</td>
<td>27</td>
<td>31</td>
<td>35</td>
<td>49</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Cytoplasmic droplets</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>TZI</td>
<td>0</td>
<td>1.47</td>
<td>1.52</td>
<td>1.58</td>
<td>1.76</td>
<td>1.47</td>
<td>0.32</td>
</tr>
<tr>
<td>Vitality</td>
<td>0</td>
<td>37</td>
<td>54</td>
<td>69</td>
<td>85</td>
<td>51</td>
<td>22</td>
</tr>
<tr>
<td><strong>Younger men (n = 84)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>52</td>
<td>74</td>
<td>81</td>
<td>85</td>
<td>96</td>
<td>80</td>
<td>8</td>
</tr>
</tbody>
</table>

Tabulated is the range (minimum, maximum) observed values and quartiles of distribution (Q2 is the median) of sperm morphology and vitality. There is highly significant difference for all variables (P < 0.0001 for all, except for TZI P = 0.008) apart from neck and head pathology, which were not significantly different. TZI = teratozoospermia index.
the axoneme providing more steps susceptible to age-dependent pathology. Such increasing proportion of defects may reflect degenerative changes with ageing in the germinal epithelium and/or in the intrinsicle program directing spermiogenesis.

Only two previous studies have examined semen findings in substantial numbers of non-infertile men over 50 years of age. One study of 23 grandfathers over 60 years of age compared with 20 young fathers reported a non-significant 20% decrease in semen volume, a significant increase in sperm density, and no significant change in total sperm output or sperm morphology (Nieschlag et al., 1982). The small sample size of that study may have limited its power to detect changes in semen volume and sperm output. The lack of detailed morphological analysis according to ‘strict’ criteria as used in this study may also have led to an inability to detect minor defects in sperm morphology. Another study examined a healthy occupational cohort of 97 present or former non-smoking and generally healthy laboratory employees, including 42 men over the 50 years of age (Eskenazi et al., 2003). That study found a progressive decline in semen volume and total sperm output, while the sperm density demonstrated a non-significant downward trend, especially in men over 70 years of age. When compared with the WHO reference norms, the proportion with abnormal semen volume, sperm density and total sperm output all significantly increased progressively with age, with most striking effects over the age of 70 years. These findings are consistent with the present study, as well as with post mortem findings that demonstrated ageing effects on testis size were only significant in the eighth decade of life (Handelsman and Staraj, 1985). The present study’s findings are also consistent with the conclusions of a systematic literature review of ageing effects on semen analysis (Kidd et al., 2001). Following evaluation of 28 studies of semen analysis, it was concluded that ageing is associated with a decline in semen volume and sperm morphology, but not sperm density. However, that review did not evaluate total sperm output, a parameter less affected by abstinence interval, a variable in turn not well controlled in most studies. Furthermore, most studies reviewed comprised men attending fertility clinics, with very few over 50 years old. In that context the present study extends these findings to a larger group of older, non-infertile men.

The present opportunistic study suffers from the significant limitation of non-representative sampling that is virtually unavoidable in studies requiring semen analysis (Cohn et al., 2002). Similar issues essentially invalidate claims regarding historical changes in sperm output where non-representative samples were extrapolated to their epoch or geographical location (Handelsman, 2001). It would be equally mistaken to extrapolate the present data to older men without strong caveats and independent replication. Nevertheless, by sacrificing sperm motility assessment, using this convenience sample we were still able to analyse ejaculate volume, sperm density, output, vitality and morphology in older men and compare with younger men recruited and examined in the same clinic and laboratory. However, the requirement for a semen sample will have limited the participation in the present study to not only potent men, but also to those with different attitudes, the impact or correlates of which on preservation of testicular function with ageing remains unknown.

An additional caveat on this sample is that the older men were biased by selection for requiring a prostate biopsy to evaluate possible prostate cancer. Prostate disease can theoretically obstruct the excurrent ductular system or have more general systemic effects on spermatogenesis. However, men with and without biopsy-proven prostate cancer did not differ with regard to semen variables. The general good health of the participants in this study together with the minimal extent and low grade of the biopsy-proven cancers make it unlikely that systemic effects of the cancer would be sufficient to influence sperm production. Although it is well established that chronic, even asymptomatic, disease may accelerate the age-dependent decline of blood testosterone (Gray et al., 1997; Lemcke et al., 1997; Chia et al., 1998; Andersen et al., 2000) or too low (Bonde et al., 1998; Zinaman et al., 2000), the WHO criteria nevertheless represent a widely understood set of independent and conventional criteria for putatively normal semen findings for fertile men. By these criteria, the overall reduction in semen quality among the older men was modest and still largely conducive to fertility. Nevertheless, on average, men of this age may take longer to produce a pregnancy, and a higher proportion may warrant treatment for male infertility by conventional standards.

We conclude that in this convenience sample of older non-infertile men, sperm density is not reduced compared with younger men, but that reduced semen volume masks a decline in total sperm output. In addition, sperm morphology and vitality decline with age. Nevertheless these age-related declines are only modest in degree, and the extent to which they would increase delayed conception and treatment for male infertility remains uncertain. Considering the increasingly later age of marriage, the increasing frequency of remarriage and longer life expectancy of men who are theoretically capable of achieving paternity lifelong, the paucity of studies in this area is striking, and more detailed studies of non-infertile older men are desirable.
References


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