Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples

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Objective: To evaluate the relationship between duration of sexual abstinence and various characteristics of normal and subnormal semen.

Design: A retrospective study based on computerized data.

Setting: Fertility and IVF unit at a university medical center.

Patient(s): Nine thousand, four hundred eighty-nine semen samples from 6,008 patients were analyzed according to the World Health Organization (WHO) manual and grouped according to sperm concentration (10^6/mL) into severe (0.2–4×10^6), moderate (>4–10×10^6), and mild (>10–19.99×10^6) oligozoospermia, and normozoospermia (≥20–250×10^6) groups.

Main Outcome Measure(s): In each group mean values of semen volume, sperm concentration, percentage of motile sperm and of normal morphology (according to WHO or Kruger criteria), total sperm count, and total motile sperm count per ejaculate were related to duration of abstinence.

Result(s): Among the 3,506 oligozoospermic samples, the peak mean sperm motility of 30.3% was observed after 1 day of abstinence. Similarly, the mean percentage of normal morphology among mild–moderate oligozoospermic samples (n = 2,260) reached peak values of 7.4%–8.6% between 0–2 days of abstinence. The 5,983 normozoospermic samples showed a significant decrease in the percentage of sperm motility and normal morphology to mean values of 33.1% and 7.0%, respectively, on days 11–14 of sexual abstinence.

Conclusion(s): Our data challenge the role of abstinence in male infertility treatments and suggest that to present the best possible semen samples, patients with male factor infertility should collect the semen after just 1 day of sexual abstinence. Patients presenting normal sperm analysis or sperm donors for cryopreservation purposes should be advised not to exceed 10 days of sexual abstinence. (Fertil Steril 2005;83:1680–6. ©2005 by American Society for Reproductive Medicine.)

Key Words: Sexual abstinence, sperm quality, oligozoospermia

The duration of sexual abstinence before fertility treatments that is needed to provide maximum sperm quality is one of the issues commonly discussed between physicians and patients. We assume, regardless of the initial sperm quality, most fertility clinics follow the World Health Organization (WHO) guidelines (1) recommending abstinence for 2–7 days before semen collection for evaluation of infertility.

A wide range of infertility treatments is available, but it is important to obtain the desired pregnancy using a technique that is cost effective and has a minimal complication rate. Very often, and for many different infertility causes, it will be recommended that couples have timed intercourse or intrauterine sperm insemination with or without ovulation induction (2, 3), or IVF with or without micromanipulation (4). Whichever fertility treatment is intended, sperm quality is of paramount importance.

The purpose of this study was to examine, on a large scale, the relationship between duration of abstinence and sperm quality, and to evaluate whether the recommendation for an arbitrary duration of abstinence, regardless of the quality of previous samples, yields the best possible semen.

MATERIALS AND METHODS

This retrospective study was based on 9,489 semen samples obtained from 6,008 patients during the period from January 1995 to September 2003.

All patients were undergoing testing for infertility and the samples were collected for routine examinations or with the intent to perform an IUI. After the approval of the Institutional Review Board at the Faculty of Health Sciences, Ben-Gurion University of the Negev, the results of the semen samples were retrieved from the computerized database of the Male Fertility Laboratory at Soroka University Medical Center.

Seminal samples were analyzed using a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) within 1.5 hours of collection. The analyses were performed by two...
experienced biologists, one performing sperm counts and motility, and the other one examining the sperm morphology. The Male Fertility Laboratory at Soroka University Medical Center is constantly under external quality assessment by UK NEQAS, Sub-Fertility Laboratory, Saint Mary’s Hospital, Manchester, UK, starting July 1999.

According to the WHO criteria, 3,506 samples with sperm concentration below $20 \times 10^6$/mL were defined as oligozoospermic and further subdivided into the following groups: group 1, severe oligozoospermia ($0.2–4 \times 10^6$)—1,246 samples; group 2, moderate oligozoospermia ($>4–10 \times 10^6$)—1,107 samples; and group 3, mild oligozoospermia ($>10–19.99 \times 10^6$)—1,153 samples. The 5,983 semen samples with counts of $\geq20–250 \times 10^6$/mL were categorized as normozoospermic and were included in group 4. Comparison of data with sperm concentrations of $>0–0.2 \times 10^9$/mL and $>250 \times 10^6$/mL was not included into the study because of the low number of samples unable to reach statistical significance. We also have not included information regarding round cells and debris, as it is not part of our database.

Among each group of samples, the mean values of volume of ejaculate, sperm concentration ($10^6$/mL), and percentage of motile sperms were determined according to WHO criteria, as well as the percentage of normal morphology for the group 1 samples. Morphology is investigated among 100 sperms using a magnification of 400 according to WHO criteria and a magnification of 1,000 according to Kruger criteria (5), therefore and to avoid technical difficulties in cases with severe oligozoospermia (slides containing few sperms), our standard of care is to use the WHO criteria. The normal morphology in groups 2, 3, and 4 was determined according to Kruger criteria.

Total sperm count per ejaculate was calculated by multiplying the sperm concentration by the volume of semen in each sample, and the count of total motile sperms (motile density) was obtained by multiplying the total sperm count by the percent motility.

The samples within each group were divided in relation to abstinence duration: 0 (<1 day of abstinence), 1, 2, 3, 4, 5, 6, 7, 8–10, and 11–14 days. Because the number of samples from days 8 through 14 was relatively low, we combined them into two groups: 8–10 and 11–14.

Statistical analyses were performed using Statistical Programs for the Social Sciences (SPSS Inc., version 11.0; Chicago, IL) software programs. Wilcoxon matched-pairs signed-ranks test, $\chi^2$, and one-way ANOVA tests were used when appropriate. The level of statistical significance was assumed as $P<.05$.

RESULTS

The mean age of the 2,495 oligozoospermic and the 3,513 normozoospermic patients included in the study was $31.1 \pm 6.9$ years and $32.2 \pm 7.2$ years, respectively.

As shown in Table 1, the impact of the duration of sexual abstinence on all parameters (except morphology) among the three groups of oligozoospermic semen samples ($n = 3,506$) was found to be similar. Therefore, except for sperm morphology, the three oligozoospermic groups were combined and examined as one with the intention of increasing statistical power.

Semen Volume

The mean semen volume per ejaculate in the oligozoospermic and the normozoospermic samples increased gradually in relation to sexual abstinence (Table 2). In particular, a significant ($P<.001$) increase in the average semen volume was noticed between values of $2.3 \pm 1.4$ mL on days 0–1 and $3.9 \pm 2.0$ mL on days 8–14 of sexual abstinence.

Although the peak mean volume was observed on days 8–10 and 11–14 for oligozoospermic and normozoospermic samples, respectively, the observed additional increase after day 4 of abstinence was statistically insignificant for the oligozoospermic samples, whereas the increase in semen volume between days 5 to 8–14 of abstinence for the normozoospermic samples reached statistical significance ($P<.001$).

Sperm Concentration

The sperm concentration (Table 2) among 3,506 oligozoospermic samples ranged from 0.2 to $19.99 \times 10^6$/mL. Peak mean sperm concentration was observed after 1 day of abstinence among 140 semen samples with an average sperm concentration of $8.4 \pm 5.2 \times 10^6$/mL, a mild and nonsignificant reduction was recorded on succeeding days of abstinence. Despite a significant decrease on day 5 of abstinence, no further decline in the mean sperm concentration during the following days was observed.

Among the 5,983 normozoospermic samples, the sperm concentration ranged from 20 to $250 \times 10^6$/mL. A nonsignificant decrease of the mean sperm concentration was observed from day 0 to 2 days of abstinence, followed by a gradual and statistically significant ($P<.001$) increase starting on day 3 of abstinence with values of $61.1 \pm 37.5 \times 10^6$ continuing up to day 6 with values of $72.3 \pm 46.0 \times 10^6$. Although the peak mean sperm count was observed on abstinence day 7, the increase in values between days 6 and 7 did not reach statistical significance.

Total Sperm Count

Among oligozoospermic samples (Table 2), the mean total sperm count per ejaculate was found to increase significantly ($P<.001$) from day 2 to day 3 and from day 3 to day 4 of abstinence due to an increase in semen volume. A decline observed between the peak mean value of $30.1 \pm 28.3 \times 10^6$ on day 4 and $26.5 \pm 27.6 \times 10^6$ on day 5 of sexual abstinence was related to a reduction of sperm concentration. Further variations were not found to be statistically relevant.

Normozoospermic samples showed a relatively low and very similar mean total sperm count for the first days of abstinence.
<table>
<thead>
<tr>
<th>Abstinence days</th>
<th>Severe (group 1) n = 1,246</th>
<th>Moderate (group 2) n = 1,107</th>
<th>Mild (group 3) n = 1,153</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume $^{a1}$ (mL)</td>
<td>Concentration $^{b1}$ (10^6/mL)</td>
<td>Motility $^{c1}$ (%)</td>
</tr>
<tr>
<td>0</td>
<td>57</td>
<td>2.4 ± 1.7</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>2.4 ± 1.2</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>126</td>
<td>2.7 ± 1.5</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>386</td>
<td>3.2 ± 1.6</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td>292</td>
<td>3.5 ± 1.8</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>5</td>
<td>159</td>
<td>3.6 ± 1.8</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>3.8 ± 1.6</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td>7</td>
<td>83</td>
<td>3.5 ± 2.1</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td>8–10</td>
<td>30</td>
<td>4.0 ± 2.3</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>11–14</td>
<td>38</td>
<td>3.5 ± 2.4</td>
<td>2.2 ± 1.2</td>
</tr>
</tbody>
</table>

Note: The data are expressed as mean ± standard deviation.
Severe (group 1) = 0.2–4 × 10^6/mL; moderate (group 2) = >4–10 × 10^6/mL; mild (group 3) = >10–19.99 × 10^6/mL.
a1, $P < .001$, from days 0–3 to days 4–10; a2, $P < .001$, from days 2–6 to days 11–14; a3, $P < .001$, from days 0–2 to days 3–14.
b1, $P < .001$, from days 2 and 6 to days 11–14; b2, $P < .001$, from day 0 to day 7; b3, $P < .001$, from day 1 to day 3.
c1, $P < .001$, from days 0–4 to days 7, 11–14; and from days 1–3 to day 5; c2, $P < .001$, from days 0–4 to days 7, 11–14; and from day 3 to day 4; c3, $P < .001$, from days 1–4 to days 7, 11–14; and from days 1–3 to day 5.

Semen volume, sperm concentration, and total sperm count per ejaculate in relation to sexual abstinence duration in oligozoospermic and normozoospermic semen samples.

<table>
<thead>
<tr>
<th>Abstinence days</th>
<th>Oligozoospermic samples (n = 3,506)</th>
<th>Normozoospermic samples (n = 5,983)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Volumea1 (mL)</td>
</tr>
<tr>
<td>0</td>
<td>162</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>1</td>
<td>140</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>382</td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>1,081</td>
<td>3.3 ± 1.7</td>
</tr>
<tr>
<td>4</td>
<td>843</td>
<td>3.7 ± 1.8</td>
</tr>
<tr>
<td>5</td>
<td>364</td>
<td>3.7 ± 1.8</td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>3.7 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>218</td>
<td>3.6 ± 1.9</td>
</tr>
<tr>
<td>8–10</td>
<td>90</td>
<td>3.9 ± 2.0</td>
</tr>
<tr>
<td>11–14</td>
<td>133</td>
<td>3.7 ± 2.2</td>
</tr>
</tbody>
</table>

Note: The data are expressed as mean ± standard deviation. Concentr. = concentration. a1, P<.001, from days 0 and 1 to any of the following days, until days 8–10; a2, P<.001, from days 0, 1, 2 to any one of the following days, until days 8–10 and 11–14; b1, P<.001, from day 4 to day 5; b2, P<.001, starting day 3, between each one and the following day, until day 6; c1, P<.001, from days 0 and 1 to any of the following days, until days 8–10; c2, P<.001, from days 0, 1 and 2 to 3, 4, 5, 6 and 7; and starting day 3, between each one and the following day, until day 6.


abstinence, whereas a significant (P<.001) increase was observed between 130.6 ± 113.0 × 10⁶ on day 1 and 267.3 ± 187.6 × 10⁶ on day 7 of sexual abstinence. Starting on day 3 of abstinence and continuing through day 6, the mean total sperm count on every additional day of abstinence was significantly higher (P<.001) when compared with any one of the preceding days. The further increase observed on days 7 through 14 did not reach statistical significance.

Sperm Motility

The oligozoospermic sperm samples showed a peak mean percentage of sperm motility of 30.3% ± 21.0% after 1 day of sexual abstinence (Table 3). A gradual and statistically significant (P<.0001) reduction in the percentage of mean sperm motility was noticed when comparing day 1 values with 26.1% ± 19.4% on days 2 and through 14. After 5 days of abstinence, sperm motility seemed to reach a plateau and then dropped sharply to values of 17.8% ± 14.6% on days 11–14.

Likewise, the normozoospermic samples showed a significantly improved mean percentage of sperm motility after 1 day of abstinence with values of 42.1% ± 22.9%, but differently than the oligozoospermic samples, the high level of motility was preserved until day 7. A decrease in percentage of sperm motility started on days 8–10 of abstinence with a further and a significant (P<.001) decrease after 11–14 days reaching values of 33.1% ± 20.2%. Therefore, the mean motility of normozoospermic samples on any abstinence day was significantly higher when compared with the values at 11–14 days of abstinence.

Total Motile Sperm Count

The mean total motile sperm count per ejaculate (Table 3) among oligozoospermic samples started to increase at 1 day of abstinence but statistically significant (P<.01) and peak mean values of 8.9 ± 13.3 × 10⁶ were observed after 4 days of abstinence, followed by a significant (P<.01) decline beginning on abstinence day 5 with values of 7.1 ± 11.6 × 10⁶.

The normozoospermic samples showed a gradual and statistically relevant (P<.001) increase in mean total motile sperm count between days 0, 1, and 2 and days 3–7 of sexual abstinence. Lowest values of 49.3 ± 48.8 × 10⁶ were observed on day 0, whereas peak values of 122.7 ± 118.5 × 10⁶ were recorded after 7 days of abstinence.

Sperm Morphology

Sperm morphology among the 1,246 severe oligozoospermia samples (group 1) was examined according to WHO criteria (Table 4). The mean values of normal morphology ranged from 9.4% ± 8.0% after 1 abstinence day to 6.7% ± 5.5% after 8–10 days of abstinence. However, a statistically significant (P<.05) decline was observed between values of
Kruger criteria for sperm morphology (Table 4) were applied to 2,260 mild–moderate oligozoospermic and 5,983 normozoospermic samples. The mild and moderate oligozoospermic samples showed a mean peak percentage of normal sperm morphology after 1 and 0–2 days of abstinence, respectively. A statistically significant ($P<.001$) decline was observed between the values of 7.4% ± 5.6% on day 0 to values of 5.1% ± 5.0% on day 4 in the moderate (group 2) oligozoospermic samples, and from values of 8.6% ± 7.2% on day 1 to any of the following abstinence days ($P<.05$), except days 8–10, for the mild (group 3) oligozoospermic samples.

Among normozoospermic samples, the peak level of 9.3% ± 6.7% for the mean percentage of normal sperm morphology was observed in semen samples collected without any abstinence. A lower but a steady level of normal sperm morphology was observed on the succeeding abstinence days until day 10 of sexual abstinence. On days 11–14 the mean percentage of normal sperm morphology dropped to 7.0% ± 5.5%, a level that was significantly lower ($P<.0001$) if compared to values from any of the preceding days of abstinence.

**DISCUSSION**

To the best of our knowledge, this is the largest report ever published comparing oligozoospermic and normozoospermic sperm populations in relation to duration of sexual abstinence. It was aimed at evaluating the relationship between duration of sexual abstinence and various semen parameters of normal and subnormal semen. The samples, therefore, were divided within the groups into 10 groups according to abstinence duration and, in spite of that, the statistical power within the groups remained adequate due to the relatively large number of samples.

We have observed among oligozoospermic semen a gradual but consistent reduction in the mean percentage of motile sperms and normal sperm morphology inversely related to abstinence duration. Avoiding sexual abstinence or abstinence lasting just 1 day was related to peak sperm quality. Analysis of these same parameters in the normozoospermic cases showed an increase from day 0 to any of the following days until day 7 for the percent motility, whereas the percent of normal morphology was found to be steady in relation to abstinence duration until day 10. It should be emphasized that the percentage of normal sperm morphology is positively related to the oocyte fertilization rates in an IVF setting (5).

Similarly, oligozoospermic samples showed an increasing mean total sperm count and total motile sperm count until day 4 with a reduction observed from days 4–5 of abstinence. The same parameters among normozoospermic samples steadily and significantly increased up to day 7 with an observed deterioration on days 11–14 of abstinence for the mean total motile sperm count only. Among oligozoospermic semen samples, because the initial increase in these parameters should be assigned exclusively to the increase in

**TABLE 3**

Percentage of motile sperms and total motile sperm count per ejaculate in relation to sexual abstinence duration in oligozoospermic and normozoospermic semen samples.

<table>
<thead>
<tr>
<th>Abstinence days</th>
<th>Oligozoospermic samples (n = 3,506)</th>
<th>Normozoospermic samples (n = 5,983)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Motile sperms$^a$</td>
<td>Total motile sperms$^b$ (10$^6$)</td>
</tr>
<tr>
<td>0</td>
<td>162 24.5 ± 18.9</td>
<td>5.1 ± 7.6</td>
</tr>
<tr>
<td>1</td>
<td>140 30.3 ± 21.0</td>
<td>7.5 ± 12.6</td>
</tr>
<tr>
<td>2</td>
<td>382 26.1 ± 19.4</td>
<td>7.0 ± 11.4</td>
</tr>
<tr>
<td>3</td>
<td>1,081 26.4 ± 19.0</td>
<td>8.6 ± 12.9</td>
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<td>364 21.5 ± 17.6</td>
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<tr>
<td>6</td>
<td>93 22.6 ± 15.5</td>
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<td>7</td>
<td>218 20.5 ± 17.5</td>
<td>7.5 ± 10.4</td>
</tr>
<tr>
<td>8–10</td>
<td>90 23.6 ± 17.0</td>
<td>7.9 ± 10.0</td>
</tr>
<tr>
<td>11–14</td>
<td>133 17.8 ± 14.6</td>
<td>7.1 ± 11.7</td>
</tr>
</tbody>
</table>

Note: The data are expressed as mean ± standard deviation.

$^aP<.0001$, from day 0 to 1; from day 1 to any one of the following days; from day 3 to 4, 5 and 11–14; from day 4 to 5, 7 and 11–14; $^bP<.01$, from day 0 to 3 and 4; from day 4 to 5; $^cP<.001$, from day 0 to any of the following days, until day 7; from days 11–14 to any of the preceding days; $^dP<.001$, from days 0, 1 and 2 to any of the following days, until day 7; from day 4 to 5 and from day 5 to 7.

the mean volume of semen in relation to abstinence duration, for the normozoospermic samples both the sperm concentration and the semen volume continued to increase.

Several pathophysiological explanations have been offered to clarify the difference in the response of these two sperm populations to the abstinence period. Johnson and Varner (6) reported that decreased rates of sperm production did not reduce the number of sperm in the human epididymis. However, the sperm transport time through the epididymis was three times longer in oligozoospermic than in normozoospermic men. In addition, it was suggested that some cases with supposed idiopathic testicular failure might, in fact, have partial obstruction (7, 8). These pathologies may explain the prolonged sojourn of sperm within the genital tract of patients with male factor infertility.

Furthermore, oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants, known as reactive oxygen species (ROS). Human spermatozoa are rich in polyunsaturated fatty acids, and therefore are susceptible to damage induced by reactive oxygen species (ROS). It was reported that excessive production of ROS in spermatozoa is associated with abnormal physiological functions leading to an infertile status. Infertile men have decreased sperm variables induced by higher ROS levels in semen (10–12). Damaged spermatozoa (13) or infiltrating leukocytes (14) are likely to be the source of ROS, which is correlated with decreased sperm motility. In fact, higher levels of ROS have been found in semen of infertile men if compared with semen from fertile and azoospermic patients (13, 15). Therefore, this may be one of the reasons for the relatively fast reduction in sperm motility and morphology related to prolonged abstinence observed among oligozoospermic samples in our study.

Likewise, an increase in seminal oxidative stress related to sperm DNA fragmentation index was observed in patients with male factor infertility compared with cases of fertile donors (16). Apoptosis, or programmed cell death due to DNA fragmentation, in gonadal tissue during spermatogenesis has attracted much research interest (17, 18). Recently, a positive relationship was found between increased sperm damage by ROS and higher levels of cytochrome c and caspase 9 and 3, which indicates positive apoptosis in sperm donors (16). Apoptosis, or programmed cell death due to DNA fragmentation, in gonadal tissue during spermatogenesis has attracted much research interest (17, 18). Recently, a positive relationship was found between increased sperm damage by ROS and higher levels of cytochrome c and caspase 9 and 3, which indicates positive apoptosis in sperm donors (16).

In support of these data, our study has uncovered a significant deterioration in sperm parameters inversely related to a prolonged sexual abstinence period, expressed by a reduction in the percentage of motility and normal morphol-
ology. Although it was observed in the whole study group, the oligozoospermic samples showed decreased parameters after only 2 days of abstinence, whereas the parameters of the normozoospermic samples decreased later on.

In addition, our findings are in concert with previously published studies (22–24) that noted that avoiding abstinence improves the percentage of sperm motility, normal morphology, and particularly the total motile sperm counts among patients with male factor infertility. Therefore, pooling sequential ejaculates was recommended to increase the success rate (24).

Makkar et al. (22) have observed a reduction in semen volume when samples collected after various abstinence periods (from 2–7 days) were compared to those obtained without any abstinence. In agreement with those findings, our data suggest that a pattern of mean semen volume positively related to abstinence duration with a significant increase observed from day 0 (no abstinence) up to day 4 and remaining high, at approximately the same level, despite increasing the abstinence duration. No benefit, therefore, in terms of semen volume, could be obtained from recommending abstinence beyond 4 days in patients with oligozoospermia.

In conclusion, based on our findings, we assume that the various semen parameters are strongly related to abstinence duration, particularly regarding subnormal semen. After only 2 days of abstinence, sperm from patients with male factor infertility initiate a process of quality degradation. Therefore, our data challenge the empirical approach to the issue of sexual abstinence duration for infertility treatment purposes and suggest an approach, which will differentiate the recommendations for abstinence duration according to the initial sperm quality based on previous sperm analysis.

Patients with male factor infertility, defined as oligozoospermia, intending to undergo fertility treatments, should collect semen samples after only 1 day of sexual abstinence to present the highest percentage of sperm motility and best morphology. The total sperm counts and total motile sperm counts may increase with prolonged sexual self-restraint, but abstinence beyond 4 days should not be recommended so as to contain the deterioration in sperm morphology related to a prolonged abstinence period.

Patients with normal semen analysis, or sperm donors for cryopreservation purposes, could further improve their semen through 7 days of abstinence. However, more than 10 days of sexual abstinence should not be recommended, beyond this time frame the fertility potential may be undermined.

**REFERENCES**