Effects of multiple ejaculations after extended periods of sexual abstinence on total, motile and normal sperm numbers, as well as accessory gland secretions, from healthy normal and oligozoospermic men

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Introduction

Since all pharmacological therapies designed to improve sperm production and fertility fail (Nieschlag et al., 1993), an alternative method of increasing sperm numbers and quality is required. Variations in the times of abstinence and frequency of ejaculations could represent such a method. The advice provided in the literature, however, is contradictory. Because of decreased sperm motility (Lampe and Masters, 1956) or decreased sperm numbers (Levin et al., 1986) following frequent ejaculation, it has been argued that it may be more beneficial for men to have less frequent intercourse in order to father a child, whereas MacLeod and Gold (1952a,b) argued that advising men to decrease their rate of intercourse in order to build up their sperm reserves would have little practical value. Others, impressed by the higher sperm concentrations of a second ejaculation, considered this protocol to be a useful technique (Check and Chase, 1985), but further studies suggested to Zveřina and Pondělíčková (1987) that this method was of little clinical importance. Recently, however, the provision of a second ejaculation has again been recommended for assisted reproduction techniques (Tur-Kaspa et al., 1990), Check et al. (1991) concluded from single ejaculates provided by normal volunteers that abstinence periods longer than 3 days may be preferable and suggested that such studies be repeated with astheno- or oligozoospermic patients. It is expected that longer abstinence times would increase the number of spermatozoa ejaculated, but whether their motility and morphology is preserved during epididymal confinement has not been investigated by emptying of the organ through multiple ejaculate studies of fertile men.

When the effects of abstinence times on spermatozoa have been examined, the unejaculated spermatozoa that remain in the ducts thus providing an older population of spermatozoa in the next ejaculation than anticipated from the abstinence period, have usually been ignored. The effects of 'spill-over' of previous abstinence times was accommodated by Check et al. (1991) by randomization of the order of abstinence times. Furthermore, the relevant control semen samples in these situations are frequently omitted: where these have been included usually only one semen sample is obtained after different abstinence periods (Tyler et al., 1982a; Barratt and Cooke, 1988; Blackwell and Zaneveld, 1992). Tyler et al. (1982a) did conclude, however, that data obtained after depletion of the extra-gonadal reserves and recovery at a set time interval would be useful.

The present study is designed to determine the effect of abstinence time and multiple ejaculation after depletion of sperm reserves on the motility, morphology and intactness of tail membranes of spermatozoa from healthy donors and oligozoospermic men.

Materials and methods

Men and ejaculation protocol

For the initial study, four healthy donors with normal semen analysis were asked firstly to provide on day 0 at the clinic three ejaculates within as short a time as possible (depletion of extra-gonadal reserves) and secondly to abstain from sexual activity for periods of 1, 2, 4, 7, 10 and 14 days, and after each period to provide at the clinic three ejaculates within as short a time as possible. For the feasibility of the study, and in order to maintain the goodwill of the volunteers, the order of providing these ejaculates was left to the discretion of the donors.

For the comparison of patients with healthy donors, two groups of men were recruited. Six patients were recruited from those under consideration for in-vitro fertilization (IVF) and intra-uterine insemination programmes [sperm motility (WHO grade a + b) ≥30% and normal morphology ≥20%] but were rejected as unsuitable because of too low a sperm concentration after swim-up (<10^6/ml). Four donors with normal semen parameters [motility (WHO grades a + b) ≥30% and normal morphology ≥20%, concentration ≥20 × 10^6/ml] were also
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recruited. Data from days 2, 7 and 10 of abstinence from three of the original four donors were also included in this donor group (one volunteer was excluded because his semen analysis did not reach the WHO criteria). For these the sequence of abstinence times was different (see Table I).

These two groups of men were instructed firstly on day 0 to provide at home three ejaculates within as short a period as possible (depletion of reserves), and secondly to abstain from sexual activity for 2, 7, and 10 days and to provide after each period at the clinic three ejaculates within as short a time as possible. In order to fit in with the routine work of the andrology laboratory the order of abstinence was fixed as day 0 (depletion) Sunday; 2 days’ abstinence (the following Tuesday), 7 days’ abstinence (the following Tuesday), 10 days’ abstinence (Friday the following week). The mean, minimum and maximum times to collect all three ejaculates were 2 h, 1 h 5 min and 3 h 35 min for patients and 2 h 6 min, 40 min and 4 h 50 min for donors; other relevant parameters of these two groups of men are listed in Table I.

Assessment of ejaculates

Each ejaculate was assessed by WHO criteria (WHO, 1992) for sperm concentration, percentage motility, motility grades (a, b, c, d), morphology [priority scoring method, see Cooper et al., 1992] in hypotonic media (Jeyendran et al., 1984). Internal quality control was performed as previously described (Cooper et al., 1992). In addition, video recordings of motile spermatozoa were analysed for motility characteristics with a Hamilton—Thorn Analyzer (for settings see Neuwinger et al., 1990). Semen samples were analysed biochemically for markers of the seminal vesicles (fructose), prostate (zinc) and epididymis (neutral α-glucosidase) by methods described previously (Cooper et al., 1991).

Values for average percentage motility, normal forms and swollen cells after each abstinence period were calculated from the total number of such spermatozoa found in the three ejaculates divided by the total sperm count at that time point.

Assessment of daily sperm output

From the total number of spermatozoa recovered in the three ejaculates after each abstinence period it was possible to estimate daily sperm output (DSO) by dividing the total sperm count by the days of abstinence. Providing (i) initial sperm reserves were depleted, (ii) all spermatozoa were recovered by the multiple ejaculation regime and (iii) no spermatozoa were lost in the urine or removed in the epididymis and vas deferens, these values should have approximated daily sperm production (DSP; Amann, 1981). Then by dividing this value by the total weight of the testes, calculated from their volume and specific gravity (Handelsman and Staraj, 1985), the efficiency of spermatogenesis could be estimated. These data are also presented in Table I.

Statistical analysis

Data were analysed by analysis of variance, those with significant F-values were further compared with Tukey’s multiple range tests.

| Table I. Serum hormone concentrations, testicular volumes and estimates of daily sperm output (DSO) and DSO/g from donors and patients providing multiple ejaculates |
|---|---|---|---|---|---|---|
| Code | Duration of abstinence (days, in order) | Age (years) | Testosterone (nmol/l) | FSH (IU/l) | Testis volume (ml)* | Daily sperm output (millions) estimated after abstinence of |
| | | | | | | 2 days | 7 days | 10 days |
| | | | | | | DSO | DSO/g | DSO | DSO/g |
| Donors | | | | | | |
| 1 | 10,7,14,1,2,4 | 25 | 18.8 | 1.6 | 20 | 18 | 113.4 | 2.99 | 75.31 | 1.98 | 98.13 | 2.58 |
| 2 | 4,14,10,2,1,7 | 28 | 33.4 | 1.8 | 28 | 26 | 99.0 | 1.83 | 68.33 | 1.27 | 63.58 | 1.18 |
| 3 | 2,7,10 | 23 | 21.9 | 4.4 | 17 | 20 | 113.68 | 3.07 | 121.87 | 3.29 | 156.63 | 4.23 |
| 4 | 2,7,10 | 34 | 37.3 | 1.2 | 22 | 25 | 277.38 | 5.90 | 193.22 | 4.11 | 201.37 | 4.28 |
| 5 | 2,7,10 | 28 | 22.1 | 1.2 | 30 | 30 | 331.88 | 5.53 | 199.84 | 3.33 | 238.8 | 3.98 |
| 6 | 14,2,7,1,4,10 | 29 | 25.8 | 1.9 | 20 | 20 | 45.40 | 1.13 | 41.45 | 1.04 | 52.54 | 1.31 |
| 7 | 2,7,10 | 33 | 25.8 | 1.9 | 22 | 23 | 58.03 | 1.29 | 33.34 | 0.74 | 33.59 | 0.75 |
| Mean 28.6 | 26.4 | 2.0 | 22.7 | 23.1 | 148.4 | 3.11 | 104.77 | 2.25 | 120.66 | 2.62 |
| SEM 1.5 | 2.8 | 0.4 | 1.6 | 1.8 | 42.0 | 0.73 | 26.03 | 0.50 | 29.98 | 0.59 |
| Patients | | | | | | | | |
| 8 | 2,7,10 | 30 | 16.8 | 3.1 | 25 | 23 | 18.65 | 0.39 | 14.74 | 0.31 | 11.15 | 0.23 |
| 9 | 2,7,10 | 33 | 31.0 | 20.5 | 8 | 24 | 1.71 | 0.05 | 3.12 | 0.10 | 3.40 | 0.11 |
| 10 | 2,7,10 | 31 | 15.6 | 6.0 | 8 | 10 | 8.40 | 0.47 | 8.17 | 0.45 | 6.69 | 0.37 |
| 11 | 2,7,10 | 31 | 31.3 | 6.2 | 12 | 10 | 4.26 | 0.19 | 4.86 | 0.22 | 4.80 | 0.22 |
| 12 | 2,7,10 | 34 | 21.9 | 13.9 | 11 | 9 | 8.42 | 0.42 | 7.81 | 0.39 | 1.58 | 0.08 |
| 13 | 2,7,10 | 28 | 11.1 | 1.7 | 21 | 17 | 27.58 | 0.73 | 18.66 | 0.49 | 22.47 | 0.59 |
| Mean 32 | 21.3 | 8.6 | 14.2 | 15.5 | 11.2 | 0.38 | 9.56 | 0.33 | 8.53 | 0.27 |
| SEM 0.9 | 3.4 | 2.9 | 2.9 | 2.8 | 3.9 | 0.1 | 2.44 | 0.06 | 3.12 | 0.08 |
| Difference between donors and patients (Student’s t-test) | NS | NS | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 |

*By palpation.

FSH = follicle stimulating hormone.
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(after transformation of percentages to arcsin of square root values) and Student's $t$-test, with the level of statistical significance set at 5%. Linear regression analysis of marker concentrations was assessed to be significant at the 5% level.

Results

Preliminary study of four healthy volunteers

Characteristics and numbers of spermatozoa

The percentages of spermatozoa that were capable of swelling in hypotonic medium remained high (70–80%) over the 14 day abstinence period. Motile spermatozoa (WHO grades a + b) remained in the range 45–55% and normal forms were between 20 and 35%. Overall no statistically significant changes were found over 14 days for any of the parameters (Figure 1a) although values increased with time up to 10 days with maxima as follows: total number of spermatozoa ($600 \times 10^6$), total cells with intact membranes ($400 \times 10^6$), total motile cells ($300 \times 10^6$) and total normal cells ($200 \times 10^6$). No further increase was noted by 14 days of abstinence (Figure 1b).

Disposition of spermatozoa within the three ejaculates

By 4 and 7 days of abstinence significantly more spermatozoa (>60% of the total) were voided in the first of the three ejaculates obtained; no such significant differences were observed after 1, 2, 10 and 14 days of abstinence (data not shown).

Production of seminal markers

Calculation of the total ejaculated seminal markers per day (sum of the three ejaculates) after each abstention period revealed a great variability between volunteers in the amount of products accumulated with time (Figure 2). Total semen volume increased with time but in a variable fashion; all volunteers produced less semen volume after day 14 than on day 10. Fructose secretion increased in all patients, plateauing between days 4 and 7 for different volunteers. Total zinc followed a similar pattern to semen volume, but in only three volunteers was there a definite increase in zinc secretion with abstinence. The secretion of glucosidase only definitely increased with time in one volunteer; in the others the secretion was constant after day 2.

There were marked differences between the donors in the relative amounts of accessory gland secretions. For example, the donor producing the highest total fructose ($300 \mu$mol) output over the 14 day study period had the lowest zinc output (5 $\mu$mol) and glucosidase (150 mU). The donor with the highest zinc output ($30 \mu$mol) and glucosidase (350 mU) had a low value for fructose (150 $\mu$mol). The donor with the lowest fructose (100 $\mu$mol) had intermediate values for zinc (18 $\mu$mol) and glucosidase (200 mU).

Irrespective of the days of abstinence or ejaculate number, only the positive relationship between glucosidase and zinc concentrations was significant although the correlation was weak ($r = 0.230$).

Comparison of seven healthy donors and six oligozoospermic patients

Characteristics of spermatozoa

Because of the acceptability and practicability of this regime in the donors, an abstinence schedule of 2, 7 and 10 days and the provision of three ejaculates on each occasion (and day 0) were chosen for a comparison of donors and patients. An initial difference in motility and normal forms between patients and donors was anticipated from the selection criteria. Figure 3 shows that the percentage of spermatozoa that swelled in hypotonic media was identical in both groups and that there were no significant trends towards reduced motility or percentage of normal forms either with increased abstention time or with subsequent ejaculates on the same day after any period of abstinence. Motility parameters estimated objectively by the computer aided sperm analysis (CASA) system remained unaltered (Table II).

Daily sperm production

For the donors, estimates of DSO were constant over the 10 day period (Table I) and fell within a range of $100–150 \times 10^6$ per day. Providing this reflected daily sperm production by the testicles, spermatogenic activity ranged from $2–3 \times 10^6$ sperm.
cells per gram testis tissue. DSO values for the patients were significantly lower (11–14 times smaller at \(8-11 \times 10^6\) per day) and remained 7–10 times smaller when testicular size was taken into account (~300 000 cells per gram testis).

**Multiple ejaculation and total sperm numbers**

Figure 4 shows the increases in total sperm numbers provided in two and three ejaculates compared to the first after each period of abstinence. After provision of two ejaculates, values were 45, 71 and 73% greater at 2, 7 and 10 days of abstinence, respectively, and for three ejaculates the increases were 79, 96 and 99%.

For total normal forms and total motile spermatozoa, the mean increases in numbers after 2, 7 and 10 days’ abstinence were respectively, 46, 81, 67% for normal forms and 44, 85, 69% for motile spermatozoa with the provision of two ejaculates compared to a single ejaculate after the same period. With provision of three ejaculates, values were increased by 79, 129 and 88% for normal forms and 86, 114 and 82% for motile cells (Figure 4).

**Disposition of spermatozoa within the three ejaculates**

A difference in the delivery of spermatozoa in the successive ejaculates between patients and donors was apparent. For donors after 7 and 10 days’ abstinence, statistically more spermatozoa (>60%) were delivered in the first than in the subsequent ejaculates. In contrast, the patients delivered similar proportions of spermatozoa in the three ejaculates after each abstinence period. After 2 days of abstinence, the distribution of spermatozoa in the ejaculates of the donors was more like that of the patients.

**Production of seminal markers**

Regarding the total semen volumes and total ejaculated amounts of fructose, zinc and glucosidase, there was little difference between the patients and donors (Figure 5). Comparing all the samples, only weak correlations between concentrations of markers were found. Statistical significance was achieved for the positive relationship between glucosidase and zinc (\(r = 0.479\) for donors) and the inverse relationship between fructose and zinc (\(r = 0.389\) for patients).

**Discussion**

The present study examines the number and quality of spermatozoa produced in multiple ejaculates after different times of abstinence. The results indicate that spermatozoa residing in the human extra-gonadal reserve, initially emptied of most of its ageing spermatozoa, are maintained in a viable state for up to 14 days. This appears to be the case in the men investigated.
Fig. 3. The percentage of spermatozoa (mean + SEM; ordinate) in each ejaculate obtained after different times of abstinence (abscissa) from seven healthy donors (filled circles) and six oligozoospermic patients (open triangles). **Upper panel:** spermatozoa that swelled in hypotonic medium; **middle panel:** spermatozoa that were progressively motile (WHO grades a + b); **lower panel:** spermatozoa that were morphologically normal.

Here who had normal circulating testosterone. Patients in whom some spermatozoa do not survive epididymal confinement (Wilton et al., 1988) are clearly different since, although circulating testosterone was normal, its action on the epididymis seems to have been ineffective.

If the number of normally formed motile spermatozoa is related to fertility, the simple expedient of providing more than one ejaculate after initial depletion of reserves and abstinence for 10 days prior to sequential collection of ejaculates may well be beneficial for infertility treatment. Considering the low efficiency of recovery of motile spermatozoa in the swim-up procedure (~ 12% of total sperm cells in our laboratory) at least 10 × 10⁶ motile spermatozoa are required to provide sufficient motile spermatozoa for an insemination programme (our programme defines 1 × 10⁹/ml after swim-up as the critical concentration). Indeed, this number of motile spermatozoa was provided by five of the six patients after two ejaculates and by all six patients after three ejaculates after 7 days of abstinence.

The concept of stabilizing the reserves of spermatozoa prior to altering abstinence times in this study was contemplated to reduce variability in results, by attempting to synchronize the ageing process of stored spermatozoa. Considerable evidence that the storage capacity of the human epididymis is limited is based on data (i) showing the low sperm content of epididymal tissue (Amann and Howards, 1980; Amann, 1981; Johnson and Varner, 1988), (ii) on the rapid stabilization (2 or 4 days) of the sperm reserve after daily ejaculation (Freund, 1963; Johnson, 1982; Tyler et al., 1982a,b; Zimmerman et al., 1965; Levin et al., 1986) and (iii) on the rapidity of reserve stabilization after prior depletion (2 days: Johnson, 1982). Nevertheless, as a small, rapidly emptied sperm store may also be filled quickly between ejaculations (for which there is evidence: Levin et al., 1986), collecting daily ejaculates may be too infrequent to prevent significant ingress and ageing of spermatozoa during the stabilization period itself.

How the frequency of ejaculation could be increased to deplete the sperm reserve has been suggested by studies where daily sperm production was to be estimated from daily sperm output. Freund (1963) suggested either three ejaculates on 2 sequential days or as many as possible on 2 successive days, and Johnson (1982) suggested two per day on successive days or one per day for 2 days and three per day on the third day. We chose three ejaculates per day in as short a time as possible (1–4 h). The few reports in which multiple ejaculations are obtained attest to the difficulty in recruiting donors willing to perform these procedures. Nevertheless, that volunteers could provide these samples in our pilot study encouraged us to examine infertile patients who have a greater incentive to try new techniques.

As anticipated, the greatest difference between the patients and donors was their daily sperm output. The use of consecutive ejaculates after depletion of initial sperm reserves to provide estimates of total sperm output is novel. As with other methods it may approximate daily sperm production providing that all spermatozoa are recovered, none are absorbed by the male tract during storage or lost in the urine. Given these provisos, the estimates of sperm output by the donors are in close agreement with estimates made by other methods [Amann and Howards (1981) 45–207 × 10⁶; Amann (1981) 120 × 10⁶; Johnson (1982) 166 × 10⁶]. In the only other study where three ejaculates were obtained on 1 day (after 13 days' abstinence), DSO calculated in this way was similar: 45, 106 and 116 × 10⁶ for three donors (Barratt and Cooke, 1988). DSO values for patients were 7–14 times lower than for the donors even when testicular size was taken into account, indicating the less efficient spermatogenesis in these men.

Another difference between donors and patients was the proportion of the total spermatozoa ejaculated that appeared in the first of the three ejaculates. These observations confirm studies with donor semen which also indicated that the majority of spermatozoa are voided in the first ejaculate: 76–78% in the first of four or five ejaculates (Murphy, 1962) and 63–68% from three ejaculates (Barratt and Cooke, 1988) after abstinence periods of 5 and 13 days, respectively. Where a smaller percentage of spermatozoa were collected in the first ejaculate, abstinence times were only 3 days (50% of three ejaculates: Oldereid et al., 1984) or not given (54% of six ejaculates: Bedford, 1990). Lower percentages of the total ejaculated spermatozoa were also found...
in the first of two ejaculates obtained from patients (46%) compared to donors (75%) by Tur-Kaspa et al. (1990). This phenomenon is more likely to represent the normal emptying properties of a partially filled epididymis, rather than a difference in the neuromuscular activity of patients' ducts, since the patients' sperm reserves were much smaller than those of the donors, and the donors had a sperm distribution similar within consecutive ejaculates to that of the patients when their epididymides were less full after 2 days of abstention following depletion.

Concerning the changes in sperm quality between successive ejaculates following any one abstience period, no consistent trends were found. Others have found no changes in sperm morphology in two or three ejaculates after 3–6 day abstention periods (Lampe and Masters, 1956; Oldereid et al., 1984; Zvěřina and Pondělčková, 1987), either a decrease in velocity (30% between two ejaculates per day; Zvěřina and Pondělčková, 1987) or no decrease in velocity and reduced percentage motility (four ejaculates per day from one fertile donor: Murphy, 1962).

With regard to changes in spermatozoa occurring with time of abstinence, none were found in our study. Again, the literature holds conflicting reports. Most demonstrate no changes in motility with abstention times of 4–5 days (Heuchtel et al., 1981; Jouannet et al., 1981; Tyler et al., 1982a) but there are suggestions (from subjective evaluation) of both increased motility (weak correlation: Poland et al., 1985) and decreased motility (MacLeod and Gold, 1952a,b; Le Lannou et al., 1986). Many of these discrepancies may relate to the provision of only one ejaculate and chance fluctuations in measurements of subjective parameters.

Our data on objective measurement of sperm motility confirm other reports with CASA that there are no changes in any motility parameters of spermatozoa stored for 5–10 days (Sauer et al., 1988; Check et al., 1991). Insofar as these techniques provide objective information on subtle differences in sperm motility kinematics, sperm motion parameters can be said to be normal after abstinence of this duration.

When considering the subjective nature of morphological assessments, it is perhaps not surprising that most reports find no changes with abstention time up to 21 days (MacLeod and Gold, 1952b; Jouannet et al., 1981; Tyler et al., 1982a; Schwarz et al., 1979; Poland et al., 1985; Le Lannou et al., 1986). Increases from initial values followed by decreases were found by Blackwell and Zaneveld (1992), but no changes in the percentage of dead spermatozoa were found by Tyler et al. (1982b). The percentage of cells swelling in hypotonic media

### Table II. Summary of motility parameters of spermatozoa, measured objectively by the computer aided sperm analysis (CASA) system in sequential ejaculates from patients and donors after different abstention times

<table>
<thead>
<tr>
<th>Donors (n = 7)</th>
<th>Patients (n = 6)</th>
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<tr>
<td>Day 2</td>
<td>Day 7</td>
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<td></td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Curvilinear velocity (μm/s)</td>
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<tr>
<td>Mean</td>
<td>62.2</td>
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<tr>
<td>SD</td>
<td>18.3</td>
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<tr>
<td>Straight line velocity (μm/s)</td>
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<tr>
<td>Mean</td>
<td>37.8</td>
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<tr>
<td>SD</td>
<td>14.2</td>
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<tr>
<td>Average path velocity (μm/s)</td>
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</tr>
<tr>
<td>Mean</td>
<td>46.2</td>
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<tr>
<td>SD</td>
<td>13.9</td>
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<tr>
<td>Linearity</td>
<td>Mean</td>
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<tr>
<td>SD</td>
<td>8.9</td>
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<tr>
<td>Straightness</td>
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<td>SD</td>
<td>7.6</td>
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<td>Amplitude of lateral head displacement (μm)</td>
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<td>Mean</td>
<td>5.1</td>
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<td>SD</td>
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<sup>a</sup>Sequential ejaculate number.
remained the same over 10 days (Check et al., 1991; Blackwell and Zaneveld, 1992), as in the current study.

Although the mean serum testosterone concentration was slightly lower in the patients than in the donors (not significant) it was sufficient to maintain normal accessory gland function. The secretion of glucosidase, fructose and zinc appeared to be regulated in both donors and patients by the concentration within the respective accessory organs, since their daily production decreased with time of abstinence by day 4 for glucosidase, day 7 for fructose and day 10 for zinc. This probably demonstrates feedback inhibition at the site of synthesis or transport as soon as a certain intraluminal concentration is reached. This differing rate of accumulation of accessory gland secretions contrasts with their relatively constant ratio after depletion of accessory organ secretions through frequent ejaculation (Eliasson, 1965; Rui et al., 1984).

The findings here suggest that single ejaculates obtained after abstinence times of 48 h to 7 days recommended by WHO (1992) may provide misleading information about the ability of patients to produce acceptable numbers of normally formed progressively motile spermatozoa. Others have also suggested that longer periods of abstinence may be preferable (Check et al., 1991; Blackwell and Zaneveld, 1992). Whether the protocol will be useful as therapy for oligozoospermic men may depend on the maintenance of other parameters of sperm function. Studies of spermatooza aged by long abstinence periods have indicated that resistance to dissolution in the detergent SDS increases slightly (Le Lannou et al., 1986) and that the acrosin content of

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**Fig. 4.** Data from six oligozoospermic patients. The total number (mean + SEM; ordinate) in ejaculate 1 (filled columns), the sum of ejaculates 1 + 2 (open columns) and sum of ejaculates 1 + 2 + 3 (hatched columns) of all spermatozoa (upper left panel), of all spermatozoa that swell in hypotonic medium (upper right panel), of all progressively motile spermatozoa (WHO grades a + b; lower left panel) and of all normally formed spermatozoa (lower right panel) obtained at different times of abstinence (abscissa).

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**Fig. 5.** The total semen volume and accessory gland output of markers in three ejaculates per day (mean + SEM; ordinate) after various times of abstinence (abscissa). Data from seven healthy donors (filled columns) and six oligozoospermic patients (open columns) are shown.
spermatozoa decreases slightly (Blackwell and Zaneveld, 1992) but in neither study was prior depletion performed. Such disturbances may reveal alterations in sperm physiology, unidentified in this and most studies, which are incompatible with fertility.

To establish this ejaculation protocol as beneficial, a clinical study is required in which pregnancy rates are determined after natural intercourse, artificial insemination or IVF programmes.

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References

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