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Quantitative post-coital test: sperm counts in cervical mucus after enzymatic liquefaction

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The post-coital test involves direct microscopic examination of sperm number and motility in cervical mucus. The results depend on the quality of the mucus and the distribution of spermatozoa within the sample. To progress from such qualitative data to quantitative measurements of the spermatozoa present in post-coital mucus, we have developed methods to measure sperm concentrations in enzymatically liquefied post-coital cervical mucus. The mucus score and sperm motility were measured prior to mucus liquefaction, and, together with sperm concentration, they allowed the calculation of the total number of motile spermatozoa present. A combination of bromelin and glycosidases proved to be more efficient in achieving reliable mucus liquefaction than treatment with bromelin alone, and was used to liquefy a series of 36 post-coital test samples. Total sperm numbers ranged between 19×10^3 and 16.8×10^6. Of the samples, 75% contained $<3\times10^6$ spermatozoa, and 39% contained $<1\times10^6$ spermatozoa. Sperm motility was very high in these samples, except for a distinct subset of samples (19%) in which the total sperm motility was markedly decreased (<20%). The measurement of sperm concentration in liquefied cervical mucus will help to determine normal values for the post-coital test, and to estimate the number of motile spermatozoa reaching the upper female genital tract.

Key words: cervical mucus/enzymatic liquefaction/infertility/post-coital test/spermatozoa

Introduction

The post-coital test (PCT) allows assessment of the physiological interactions occurring between cervical mucus and sperm cells. The quality of the mucus and the number and motility of spermatozoa in the cervical mucus environment are scored to evaluate the efficiency of sperm penetration into the uterine cavity. Despite the fact that the PCT has been part of the classical infertility workup for many years, it is still the subject of conflicting reports, due to its subjective nature (Griffith and Grimes, 1990; Markham, 1991).

The accuracy of sperm quantification in cervical mucus is poor because spermatozoa are not evenly distributed throughout the hydrogel, but rather form migration columns. Moreover, because of the high viscosity of cervical mucus, the thickness of the sample examined under the microscope is both variable and unknown, and it is therefore impossible to convert counts to total sperm content or to sperm concentrations. This inaccuracy prevents the establishment of reliable normal values, and yields highly variable test outcomes. As a result, the relationship between PCT results and fertility (i.e. pregnancy) is reportedly poor and the prognostic value of the PCT for fertility remains the subject of ongoing controversy (Stone, 1983; Barnea and McInnes, 1986; Matson *et al.*, 1986).

In an effort to improve the quantification of sperm cells in PCT, we have developed methods to liquefy the mucus hydrogel enzymatically, thereby allowing accurate sperm quantification in a Makler chamber (Makler, 1978). The measurement of sperm motility in the sample prior to liquefaction allows estimation of the total number of motile spermatozoa present in cervical mucus at the time of PCT.

Cervical mucus liquefaction using the cystein protease, bromelin, was used originally to detect antisperm antibodies and leukocytes in the mucus (Ingerslev and Poulsen, 1980; Thompson *et al.*, 1991; Shulman and Hu, 1992). Recently, bromelin liquefaction was proposed as a means of quantifying spermatozoa in PCT (Campana *et al.*, 1991).

The objective of the present study was to optimize conditions in which sperm concentration can be measured accurately in PCT samples. We have observed that mucus liquefaction with bromelin alone yielded poor sensitivity in sperm concentration measurements, and have examined the effects of a combined treatment with bromelin and glycosidases to liquefy cervical mucus. The results presented indicate that this improved method allows the total number of spermatozoa as well as the number of motile spermatozoa present in cervical mucus at the time of PCT to be quantified.

Materials and methods

Cervical mucus was collected in an Aspiglaire device (Biotechnologie International, l'Aigle, France). The Aspiglaire consists of a thin transparent polyvinyl chloride capillary tube 2.8 mm inner diameter and 25 cm in length. The capillary has an inner piston tightly fitted to it allowing smooth aspiration of cervical mucus. The thinness of the tube and its transparency allow sperm motility to be observed directly in the sample without further manipulation. Bromelin (EC 3.4.22.4) and hyaluronidase (EC 3.2.1.36) were purchased from Sigma (St Louis, MO, USA). α-Amylase was obtained from Merck (Darmstadt, Germany). All enzymatic digestions were performed in modified Biggers, Whitten and Whittingham's (BWW) buffer without albumin (Biggers *et al.*, 1971). For determination of sperm concentrations spermatozoa were counted in Makler chambers (Sefi Medical

Instruments, Haifa, Israel) according to the manufacturer's instructions. Sperm observation within the Aspiglaire was performed on a Nikon TMS phase-contrast inverted microscope at a total magnification of ×400 using a long distance ×40 objective (Nikon, Tokyo, Japan).

Patients

A total of 77 infertile couples who were attending the Infertility Clinic were subjected to PCT as an integral part of the infertility workout. All the women included in this study had one or both Fallopian tubes patent as shown by hysterosalpingography. The male partners had neither suggestive history nor clinical evidence of incapability to deliver semen intravaginally. PCT for each couple was performed 1 day before the expected time of ovulation, on the basis of follicular measurement (≥18 mm diameter) by vaginal ultrasonography.

The couples were asked to observe sexual abstinence for at least 3 days before performing the test. The women were asked to avoid the use of any vaginal douches or lubricants until after the test. Samples were collected in the outpatient clinic. All the patients reported a time-lapse of 6–10 h between intercourse and specimen collection.

Method of sample collection

With the patient in the lithotomy position, a dry sterile Cusco's speculum was used to expose the cervix whose external os was then gently wiped with a dry sterile cotton swab. While the piston of the Aspiglaire was completely pushed forward, the tip of the Aspiglaire was then introduced through the external os and advanced through the cervical canal very slowly and gently until the tip reached the level of the internal cervical os. At this point slow aspiration of the cervical mucus started while slowly and carefully retracting the Aspiglaire. Air aspiration and bubble formation was avoided as far as possible, while at the same time collecting as much as possible of the whole mucus. When the procedure was completed, the Aspiglaire was immediately handed to the andrology laboratory. This method was similar to that described in the World Health Organization (WHO) guidelines manual using a tuberculin syringe without needle (WHO, 1992).

Post-coital test

Shortly after collection the cervical mucus was analysed in the andrology laboratory in the following manner:

1. Method proposed by the WHO Special Programme in Human Reproduction (WHO method)

The classical WHO method involved scoring of cervical mucus, and microscopic examination of the sample mounted between slide and coverslip at high magnification (×400). The number of spermatozoa per high power field was counted (sperm/hpf) and the percentage sperm motility estimated (WHO, 1987, 1992).

2. Quantitative method with mucus liquefaction

To allow the measurement of sperm concentration in liquefied samples the WHO method was modified as follows:

Observation of the mucus in Aspiglaire. Microscopic inspection of the cervical mucus was performed directly within the Aspiglaire using an inverted microscope. The Aspiglaire was immobilized on the microscope stage using rubber bands or tape and the sample examined across its depth and length at a ×400 magnification using an ultra-long focus objective (Nikon).

This setting was used to assess sperm motility (%) and count (sperm/hpf) as well as total number of cells (cells/hpf). Motility could be accurately measured in the Aspiglaire, but sperm counts were not very accurate, due to the thickness of the sample and the superimposition of successive focal planes. In contrast to the direct observation of spermatozoa between slide and coverslip, where the distance varies according to the mucus characteristics, in the case of measurements made in the Aspiglaire the thickness was constant, but too large to be scanned within the same focal plane.

We have verified that sperm counts obtained by the WHO method are proportional to those obtained directly within the Aspiglaire. Sperm counts in cervical mucus were first obtained directly within the Aspiglaire, using an inverted microscope (magnification ×400). The sample was then extruded from the Aspiglaire, mounted between slide and coverslip and sperm cells were counted (magnification ×400). Linear regression of the counts obtained using both methods revealed that sperm counts within the Aspiglaire and on the slide were correlated ($y = 0.595x + 0.679$, $r = 0.871$, $n = 49$). The differences in absolute counts obtained using the two methods were due to the different depths of the microscopic fields observed. Thus we showed that sperm counts (spz/hpf) from the Aspiglaire and from the slide were interchangeable for comparisons with sperm concentrations. To avoid unnecessary handling of the samples, we have therefore compared sperm counts obtained in the Aspiglaire (sperm/hpf) with sperm concentrations measured after liquefaction. Similar results were obtained for motility measurements in the Aspiglaire and on slide (data not shown).

The volume of the sample was determined by measuring the length of the Aspiglaire filled with mucus (0.06 ml/cm). Samples with volumes <0.1 ml, samples with no spermatozoa observed in the Aspiglaire and/or samples insufficiently transparent to allow cell counting within the Aspiglaire were discarded from the study and were analyzed using the WHO method for PCT as described above. Mucus samples of adequate quality were then processed for enzymatic liquefaction and sperm quantification.

Cervical mucus score

Macroscopic parameters of cervical mucus score (pH, consistency, spinnbarkeit and ferning) were measured on the endocervical part of the mucus, i.e. the part close to the piston of the Aspiglaire, at the time of extrusion of the sample into 3 ml polycarbonate tubes. The criteria or scoring were as described in the WHO guidelines (1992).

Enzymatic liquefactions

Cervical mucus from PCT was liquefied by incubation (i) with bromelin alone (2 mg/ml) or (ii) with an enzyme cocktail containing bromelin (2 mg/ml), α-amylase (1.5 mg/ml) and hyaluronidase (3 IU/ml). The enzymes were prepared as 2-fold concentrated stock solutions in modified BWW buffer and equal amounts of cervical mucus and enzymes were admixed on a vortex mixer and incubated at 37°C. Liquefaction was assessed every 10 min by gently tilting the tube, and liquefaction was considered complete when the entire sample behaved as one non-viscous liquid phase. Sperm cells were counted in duplicates immediately after complete liquefaction in a Makler chamber, and counts converted into concentration (sperm/ml cervical mucus). PCT samples were randomly distributed in two groups, one treated with bromelin, the other with the enzyme cocktail. The samples were assigned alternately to one of the two groups according to their arrival sequence, before the initial examination of cervical mucus, and the two groups of PCT samples had comparable mucus score values (unpaired *t*-test, not significant).

Statistical methods

The Spearman rank test was used to estimate the correlation between sperm counts in Aspiglaire and sperm concentration measured after cervical mucus liquefaction. Two-way variance analysis was per-

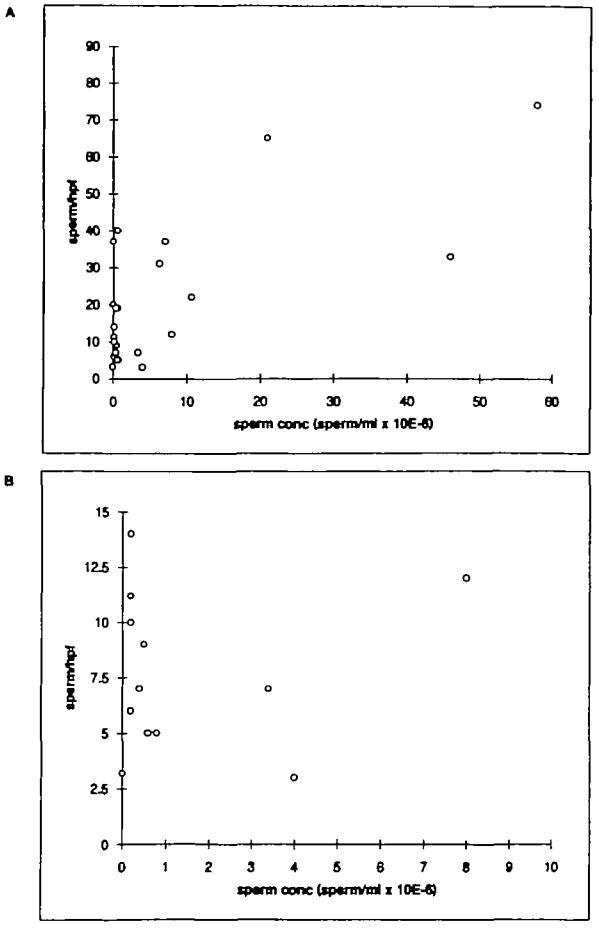

Figure 1. Post-coital test (PCT) liquefaction with bromelin. Sperm concentrations measured in Makler cells after PCT liquefaction with bromelin (sperm/ml$\times 10^{-6}$) is compared to sperm counts in Aspiglaire before liquefaction (sperm/hpf). **A**: series of 23 samples, liquefied with bromelin. Spearman rank correlation: rho = 0. 38, $P < 0.1$. **B**: selection of data from **A**, with counts in mucus prior to liquefaction between 0 and 15 sperm/hpf, plotted at higher resolution. hpf = high power field.

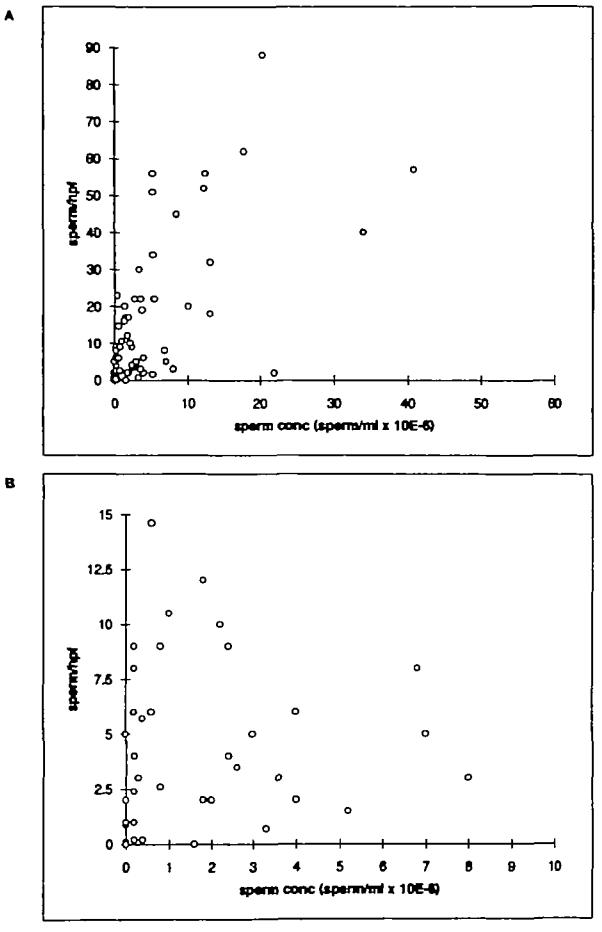

Figure 2. Post-coital test (PCT) liquefaction with enzyme cocktail. Sperm concentrations measured in Makler cells after PCT liquefaction with enzyme cocktail (sperm/ml$\times 10^{-6}$) is compared to sperm counts in Aspiglaire before liquefaction (sperm/hpf). **A**: series of 70 samples, liquefied with enzyme cocktail. Spearman rank correlation: rho = 0. 66, $P < 0.001$. **B**: selection of data from **A**, with counts in mucus prior to liquefaction between 0 and 15 sperm/hpf, plotted at higher resolution. hpf = high power field.

formed to compare cervical mucus liquefaction time according to enzyme treatment and mucus score. When interaction between these two factors was present, the enzyme treatment was compared in each category of mucus score (<10, 10–12, >12), using non-parametric Wilcoxon test or *t*-test, depending on normality of the data. Variability of the different groups was compared by Levene's test. $P<0.05$ was considered significant.

Results

PCT liquefaction with bromelin

Comparison between sperm counts obtained from the Aspiglaire before liquefaction and the counts in Makler cells of the same samples after bromelin liquefaction is shown in Figure 1. The data show that while some samples gave elevated counts in Aspiglaire as well as elevated sperm concentrations in liquefied mucus, in a large number of cases low sperm concentrations were obtained in liquefied samples ($<1\times10^6$ sperm/ml) (Figure 1A), whereas these samples had significant numbers of spermatozoa observed in the Aspiglaire (>10 sperm/hpf) (Figure 1B).

PCT liquefaction by combined enzymatic treatment with bromelin and glycosidases

Cervical mucus from PCT was liquefied using a combination of bromelin, α-amylase and hyaluronidase (hereafter referred to as 'enzyme cocktail') to allow the measurement of sperm concentrations. Figure 2 shows a comparison of sperm counts in the Aspiglaire (sperm/hpf) with the sperm concentrations measured after liquefaction. As seen in Figure 1, some samples had elevated sperm counts in Aspiglaire which corresponded to elevated sperm concentrations in liquefied mucus (Figure 2A). In contrast, samples with low sperm counts in Aspiglaire behaved very differently when liquefied with the enzyme cocktail or with bromelin alone. Figure 2B shows that PCT samples with <10 sperm/hpf detected in the Aspiglaire displayed variable sperm concentrations ($0–22\times10^6$ sperm/ml) after liquefaction with enzyme cocktail. In addition, some samples had consistently low counts in Aspiglaire and low concentrations in liquefied mucus. The variability observed between the sperm counts obtained before and after liquefaction reflects the heterogeneous distribution of sperm cells in the mucus hydrogel.

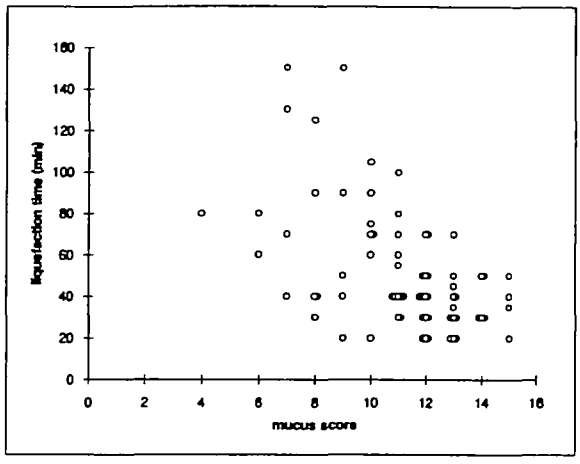

Figure 3. Cervical mucus score plotted against post-coital test (PCT) liquefaction time. PCT samples (n = 77) were liquefied with enzyme cocktail, and the incubation time required for complete liquefaction was compared to the cervical mucus score. Linear regression analysis gave the following equations: 6. 50x + 123. 84, r = –0.52 (P = 0.01).

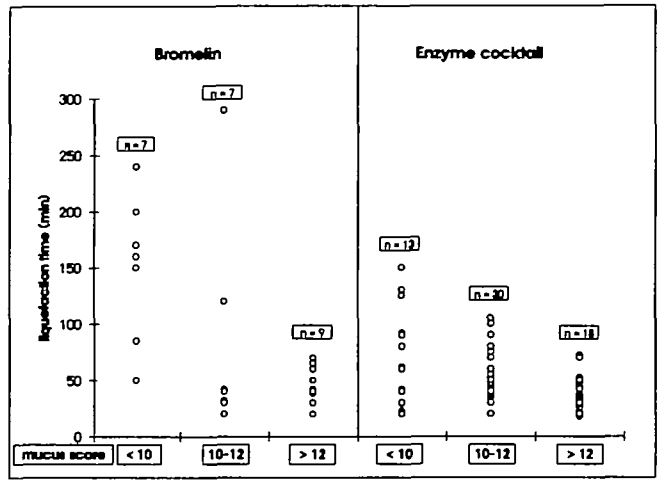

Figure 4. Liquefaction times of post-coital test (PCT) with bromelin or enzyme cocktail. PCT samples were classified into three groups according to their mucus score (group 1: score <10, group 2: score 10–12, group 3: score >12) and this was plotted against liquefaction times for bromelin and enzyme cocktail treatment.

Relationship between cervical mucus score and liquefaction time

The times required for mucus liquefaction by the enzyme cocktail were plotted against mucus score for 77 post-coital mucus samples. Most of the prolonged liquefaction times were associated with low mucus scores (Figure 3). All post-coital mucus scoring ⩾12 liquefied within 70 min, and out of 20 samples scoring between 10 and 11, only two required liquefaction times >90 min (100 and 105 min), while four of the 17 samples scoring <10 had markedly prolonged liquefaction times (125–150 min). Overall, 92% of the post-coital mucus samples liquefied within 90 min when incubated with the enzyme cocktail.

To compare liquefaction times obtained after treatment with bromelin alone or the enzyme cocktail, we have classified PCT samples according to the cervical mucus score. We have examined the distribution of liquefaction times for each group and the results are presented in Figure 4. Liquefaction times observed for high score mucus (>12) were comparable for bromelin and enzyme cocktail treatments but in the groups of samples with lower mucus scores (10–12 and <10), liquefaction times were considerably more variable, particularly after bromelin treatment. Two-way variance analysis showed significant differences in variability according to the enzyme treatment and to the mucus score (Levene's test). The variance analysis also showed an interaction between mucus score and enzyme treatment for the liquefaction times. Therefore we performed Wilcoxon tests between the two treatments in each category of mucus score. In the low mucus score category (<10) liquefaction times proved to be significantly (P = 0.0126) shorter when the mucus samples were treated with the enzyme cocktail than with bromelin. In the other mucus score categories, these differences did not reach statistical significance. Thus, the enzyme cocktail was found to yield less variation in mucus liquefaction times than bromelin alone, and also achieved faster mucus liquefaction in the samples with a score <10.

Estimation of the total number of spermatozoa present in cervical mucus

The measurement of sperm concentration in liquefied mucus allows an estimation of the total number of spermatozoa present in the mucus at the time of PCT. Since the amount of mucus in the cervix shows considerable individual variability, the evaluation of the total number of spermatozoa present in the cervix may be useful to estimate the number of spermatozoa reaching the female upper genital tract and therefore potentially able to fertilize. Such data are readily available from the measurement of sperm concentration after enzymatic liquefaction of the cervical mucus. We have calculated the total sperm number in a series of 36 PCT treated with the enzyme cocktail and found values ranging from 19×10^3 to 16.8×10^6 sperm cells. The percentage of motile spermatozoa was evaluated in the Aspiglaire before liquefaction of the mucus, making it possible to calculate the total number of motile spermatozoa (categories 1 + 2 + 3, cf. WHO, 1992) as well as the number of progressive motile spermatozoa (categories 2 + 3, cf. WHO) present in cervical mucus at the time of PCT. These values ranged from 0 to 14.45×10^6 and from 0 to 14.20×10^6 for total motile spermatozoa and progressive motile spermatozoa respectively. It should be noted that these values may have been somewhat underestimated, due to incomplete collection of the mucus, where a loss of ~20% was estimated.

To analyze the distribution in sperm content of these 36 samples, they were categorized according to their sperm content (i.e. 0 spermatozoa = 0; 0.001–10^6 spermatozoa = 1, 1.001–2×10^6 spermatozoa = 2, etc.) and the case numbers were plotted as a function of the count categories (Figure 5). Figure 5A shows the distribution of 36 PCT samples in terms of total sperm cells, total motile spermatozoa and progressive motile spermatozoa. The histogram shows that these distributions were closely related, reflecting the high percentage of motile spermatozoa characteristically found in PCT. The lowest sperm counts recorded were 19 000, 18 000 and 9000 sperm

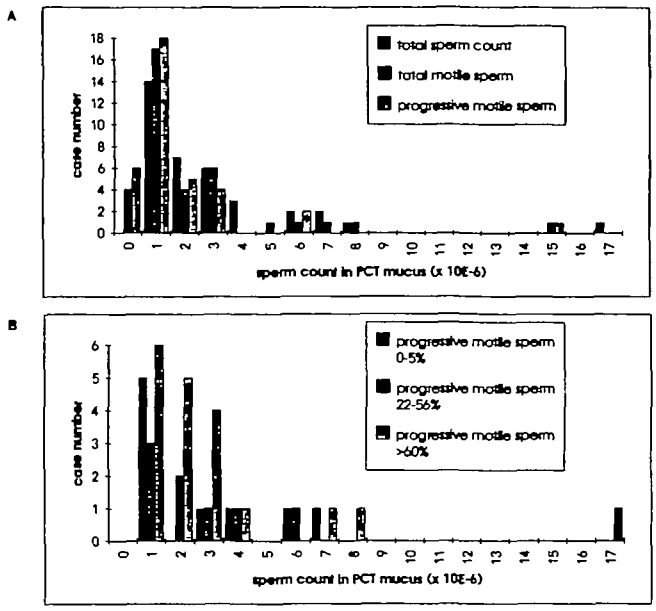

Figure 5. Distribution of total sperm numbers and motile sperm numbers in post-coital test (PCT) mucus samples (n = 36). Total sperm numbers were derived from sperm concentrations measured in PCT liquefied with enzyme cocktail. Total motile and progressive motile sperm numbers were calculated from total motility (categories 1 + 2 + 3, cf. WHO, 1992) and progressive motility (categories 2 + 3, cf. WHO, 1992) measured in the Aspiglaire respectively. Samples were classified according to their content in total spermatozoa, total motile spermatozoa and progressive motile spermatozoa as follows: 0 spermatozoa = 0; $0.001–1\times10^6$ spermatozoa = 1; $1.01–2\times10^6$ spermatozoa = 2; $2.001–3\times10^6$ spermatozoa = 3, etc. **A**: Histogram showing distribution of 36 samples with respect to total sperm count, total motile spermatozoa and progressive motile spermatozoa. **B**: Histogram showing 36 samples with regard to distribution of the total sperm counts in samples of low (0–5%, n = 9), intermediate (22–56%, n = 8) and high (>60%, n = 19) progressive motility.

cells for total spermatozoa, total motile spermatozoa and progressive motile spermatozoa respectively (data not shown). In 27 cases (75%) sperm counts were $<3\times10^6$ spermatozoa for all three parameters. Moreover, 14 samples (39%) contained $<1\times10^6$ spermatozoa, while 17 and 18 samples (47 and 50%) contained $<1\times10^6$ total motile spermatozoa and progressive motile spermatozoa respectively. In six cases (17%) no forward motility was observed, among which four (11%) contained only immotile spermatozoa. To examine whether the distribution of total sperm count was different in samples with high versus low sperm motility, samples with very low (0–5%), intermediate (22–56%) and high (>60%) progressive motility (Figure 5B) were plotted separately. The data show that the distribution in sperm content was similar for all three groups, indicating no apparent correlation between sperm progressive motility and number. It should be noted, however, that the two cases containing $>8\times10^6$ spermatozoa had 65 and 84% progressive motile spermatozoa respectively.

To examine further the variations in motility observed in the different samples, the total motile sperm counts were plotted as a function of total sperm count (Figure 6). Most of the samples showed an excellent correlation between these two parameters, underlining their high percentages of motile

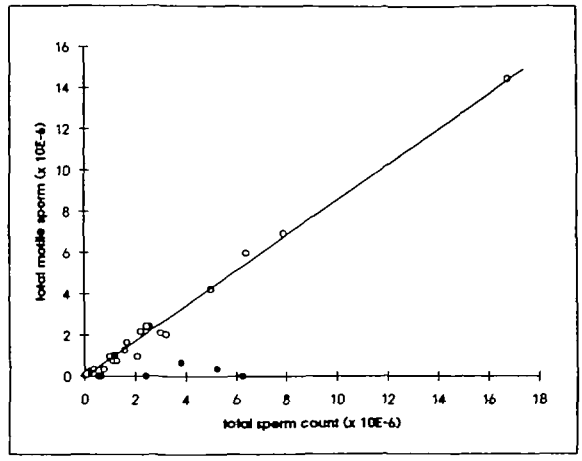

Figure 6. Total sperm counts and total motile spermatozoa were compared using total sperm content derived from concentrations measured after post-coital test (PCT) mucus liquefaction with the enzyme cocktail. Values were obtained for a series of 36 samples; seven samples with a ratio of motile spermatozoa/total spermatozoa <0. 2 appear as closed circles. Linear regression curve of values with total motility >20% (open circles): y = 0. 868 – 0. 073, r = 0. 99, (n = 29) (P = 0.01).

spermatozoa (open circles). In contrast, a distinct group of samples gave drastically different results with total motilities inferior to 20% of the total sperm counts (closed circles). The latter group included seven of the 36 samples analysed (19%). In six of these cases data from semen analyses were available, and showed normal motility and the absence of antisperm antibodies.

Discussion

The PCT was first reported in 1866 by Sims (1866). Since then the PCT has been widely used but there is still no consensus as to its validity and reliability as it has never been adequately standardized with regard to methodology and interpretation (Hartman, 1957; Griffith and Grimes, 1990; Markham, 1991). These considerations prompted us to develop a quantitative method to measure sperm numbers in cervical mucus. We therefore set out to provide a means to allow a more accurate definition of the range of normal PCT results in groups of fertile couples.

Previous reports have described the use of bromelin for cervical mucus liquefaction (Ingerslev and Poulsen, 1980; Shulman and Hu, 1992). We have successfully dissolved cervical mucus with bromelin and have compared the counts obtained before and after liquefaction. We have noticed that we obtained biased results for a fraction of the samples, which contained significant numbers of spermatozoa, as observed in the Aspiglaire, but underestimated sperm concentrations. Whereas direct conversion of counts per high-power field to concentration is not possible because of undefined width of the sample examined in the Aspiglaire, we still expect to find a positive correlation between the absolute values for the two sets of measurements. Therefore our data indicate that cervical mucus liquefaction with bromelin yielded sperm concentration measurements of poor sensitivity.

Since the detection of low numbers of sperm cells in PCT is of particular clinical relevance, we have explored alternative ways to liquefy cervical mucus in order to improve the reliability of our measurements. Cervical mucus is composed of proteins and glycosidic macromolecules such as mucins and has rheological properties similar to hyaluronic acid (McCoshen, 1987; Aitken *et al.*, 1992). Glycosidases such as α-amylase and hyaluronidase have been reported to be present in the environment of the spermatozoa, and α-amylase is present in cervical mucus (Schumacher, 1975). α-Amylase can be used to accelerate spontaneous semen liquefaction, and hyaluronidase has been used to dissociate corona radiata follicular cells from the oocyte in in-vitro fertilization (IVF) procedures (Trounson, 1989; Vermeiden *et al.*, 1989). We used an enzyme cocktail containing bromelin, α-amylase and hyaluronidase to liquefy cervical mucus in PCT and compared the sperm concentrations obtained to the sperm counts obtained in the Aspiglaire before liquefaction. Our data demonstrate that the use of combined enzymes allows sperm concentrations ranging between 0.2 and 22.0×10^6 spermatozoa/ml to be measured in samples with low sperm counts (<10 sperm/hpf) before liquefaction. Therefore it is possible to measure sperm concentrations in cervical mucus both above and below the cut-off point for normality referred to by the WHO (1992) guidelines. Such concentrations are in agreement with those recently reported by Doody and Good (1993), who have used calibrated beads to even the thickness of cervical mucus preparations. The scattered correlations observed between sperm concentrations and counts reflect the heterogeneous distribution of spermatozoa in native cervical mucus rather than poor reproducibility of the concentration measurements. This issue has been further addressed by de Agostini and Campana (1996) using donor cervical mucus to which known amounts of spermatozoa have been added before liquefaction.

The time required for complete liquefaction of PCT by the enzyme cocktail was ≤90 min in 92% of cases. Our results suggest that the time required for enzymatic liquefaction of post-coital mucus might be inversely proportional to the mucus score. This trend was present when plotting liquefaction times with individual parameters of the mucus scoring system, such as spinnbarkeit, consistency, or ferning, but not volume, leukocyte colonization or pH (data not shown). In addition, PCT samples with low score mucus had a much greater variability in liquefaction time than mucus scoring >10 and the enzyme cocktail proved to be significantly more efficient in achieving liquefaction of low score mucus than bromelin. Overall, our data demonstrate that incubation with the enzyme cocktail induced cervical mucus liquefaction in the vast majority of the samples in a time frame suitable for the measurement of sperm concentration.

The total number of spermatozoa, the total number of motile spermatozoa and the number of progressive motile spermatozoa present in the cervical mucus at the time of PCT were found to be widely variable. Altogether, 75% of the samples analysed contained $<3\times10^6$ spermatozoa and 39% of these samples contained $<1\times10^6$ spermatozoa. The numbers of spermatozoa measured in PCT were not correlated to the mucus score or to the mucus volume and were not correlate with sperm concentration or motility in semen (data not shown). This underlines the complexity of sperm–cervical mucus interactions which include the physico-chemical characteristics of the mucus as well as the functional fitness of the sperm cells. The percentage of spermatozoa with normal morphology has been reported to be significantly higher in endocervical mucus than in ejaculates, suggesting that spermatozoa with normal morphology penetrate the cervical mucus more efficiently (Insler, 1977; Hanson and Overstreet, 1981; Katz *et al.*, 1990). McCoshen (1987) has also suggested that spermatozoa secrete enzymes which digest mucin fibres, thereby facilitating their progression in the hydrogel. Abnormalities of the flagellar wave form and lateral head displacement have been associated with infertility and poor PCT results (Feneux *et al.*, 1985), particularly in cases of immunological infertility (Wang *et al.*, 1985). Abnormal sperm motility associated with abnormal head morphology suggests flagellar dyskinesis as the probable explanation for mucus penetration failure (Overstreet *et al.*, 1981; Overstreet, 1986).

The net result of the sperm selection achieved by cervical mucus is a dramatic decrease in the numbers of spermatozoa entering the female upper genital tract and thereby potentially able to fertilize, as compared to the numbers of spermatozoa deposited in the vagina at ejaculation. It therefore seems likely that the number of spermatozoa present in cervical mucus could constitute a useful parameter to judge the normality of sperm–cervical mucus interactions.

The total number of spermatozoa which traverse the mucus barrier after an intercourse is difficult to estimate, as the number of spermatozoa crossing the cervical mucus at any time post-coitally is known to be variable (Kremer and Jager, 1988). In addition, sampling of the mucus might be unavoidably incomplete, due to the convoluted anatomy of the cervical crypts. Nevertheless, evaluation of PCT cervical mucus in terms of motile and immotile sperm content and of sperm concentration can serve as a standard comparative test. Given the fact that few if any spermatozoa with abnormal head morphology can pass through the cervical mucus (Insler, 1977; Hanson and Overstreet, 1981; Katz *et al.*, 1990), we assume that most of the motile spermatozoa within cervical mucus have a normal morphology. Therefore the quantification of spermatozoa in liquefied cervical mucus should allow precise determination of the total number of morphologically normal and motile spermatozoa present in cervical mucus at the time of PCT. Standardization of the test could be achieved by performing the test in a group of couples with proven fertility from which normal values for sperm numbers in cervical mucus could be derived. These values would account for variability in cervical mucus quality and in sperm count and motility in the ejaculate.

In the present study we have examined the possibility of improving the accuracy of sperm quantification in cervical mucus. We have found that liquefaction can be achieved by using a combination of protease and glycosidases, yielding a non-viscous homogeneous solution in which sperm cells can be quantified. This allows for the first time an estimation of the total number of spermatozoa present in post-coital cervical mucus conditions. The liquefaction procedure described here

is simple and can be easily conducted in any standard andrology laboratory where semen analysis and PCT are routinely performed. The liquefaction times observed are in most cases <90 min, making it easy to handle in the laboratory.

The data presented in this study show that the quantification of sperm numbers in liquefied PCT mucus allows the identification of a subpopulation of samples where sperm motility in PCT mucus is largely reduced. Interpretation of the biological significance and possible prognostic value of such an observation must await further studies underway in our laboratory.

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References

Aitken, R.J., Bowie,H., Buckingham, D., Harkiss, D., Richardson, D.W. and West, K. (1992) Sperm penetration into a hyaluronic acid polymer as a means of monitoring functional competence. *J. Androl.*, **13**, 44–54.

Barnea, E. R. and McInnes, R. (1986) Reappraisal of the post-coital test: a controlled study. *Int. J. Fertil.*, **31**, 46–49.

Biggers, J.D, Whitten, W.K. and Whittingham, D.G. (1971) The culture of mouse embryos *in vitro*. In: Daniel, J.C. (ed.), *Methods in Mammalian Embryology*, W.H. Freeman and Co., San Francisco, pp. 86–116.

Campana, A., Ferla, E., Marossi, L., Radici, E., Stalberg, A. and Balerna, M. (1991) Eine neue Methode zur Beurteilung des Postkoitaltests. *Fertilität*, **7**, 181–184.

de Agostini, A. and Campana, A. (1996) Sperm counts in enzymatically liquefied cervical mucus: quantitative validation using donor cervical mucus. *Hum. Reprod.*, **11**, 318–324.

Doody, M.C. and Good, M.C. (1993) The post-coital test: a quantitative method. *J. Androl.*, **14**, 149–154.

Feneux, D., Serres, C. and Jouannet, P. (1985) Sliding spermatozoa: a dyskinesia responsible for human infertility? *Fertil. Steril.*, **44**, 508–511.

Griffith, C.S. and Grimes, D. (1990) The validity of the post-coital test. *Am. J. Obstet. Gynecol.*, **162**, 615–620.

Hanson, F.W. and Overstreet, J.W. (1981) The interaction of human spermatozoa with cervical mucus *in vivo*. *Am. J. Obstet. Gynecol.*, **140**, 173–178.

Hartman, C.G. (1957) How do sperm get in the uterus? *Fertil. Steril.*, **8**, 403–427.

Ingerslev, H.J. and Poulsen, F. (1980) Bromelin for liquefaction of cervical mucus in sperm antibody testing: its effect on sperm agglutinating immunoglobulin G. *Fertil. Steril.*, **33**, 61–63.

Insler, V. (1977) The evaluation and treatment of cervical mucus diseases leading to infertility. *Adv. Exp. Med. Biol.*, **89**, 477–488.

Katz, D., Morales, P., Samuels, S.J. and Overstreet, J.W. (1990) Mechanisms of filtration of morphologically abnormal human sperm by cervical mucus. *Fertil. Steril.*, **54**, 513–516.

Kremer, J. and Jager, S. (1988) Sperm-cervical mucus interaction, in particular in the presence of antispermatozoal antibodies. *Hum. Reprod.*, **3**, 69–73.

Makler, A. (1978) A new chamber for rapid sperm count and motility estimation. *Fertil. Steril.*, **30**, 313–318.

Markham, S. (1991) Cervico-utero-tubal factors in infertility. *Curr. Opin. Obstet. Gynecol.*, **3**, 191–196.

Matson, P.L., Tuvik, A.I., O'Halloran, F. and Yovich, J. (1986) The value of the post-coital test in predicting the fertilization of human oocytes. *J. In Vitro Fert. Embryo. Transf.*, **3**, 110–113.

McCoshen, J.A. (1987) The role of cervical mucus in reproduction. *Contemp. Ob/Gyn.*, **26**, 94–116.

Overstreet, J.W. (1986) Evaluation of sperm–cervical mucus interaction. *Fertil. Steril.*, **45**, 324–326.

Overstreet, J.W., Price, M.J., Blazak, W.F., Lewis, E.L. and Katz, D.F. (1981) Simultaneous assessment of human sperm motility and morphology by videomicrography. *J. Urol.*, **126**, 357–360.

Schumacher, G.F.B. (1975) Soluble proteins in cervical mucus. In: Blandau, R.J. and Moghissi, K. (eds), *The Biology of the Cervix*. University of Chicago Press, Chicago, pp. 201–233.

Shulman, S. and Hu, C. (1992) A study of the detection of sperm antibody in cervical mucus with a modified immunobead method. *Fertil. Steril.*, **58**, 387–391.

Sims, J.M. (1866) *Uterine Surgery*. Wm Woods Co., New York.

Stone, S.C. (1983) Peritoneal recovery of sperm in patients with infertility associated with inadequate cervical mucus. *Fertil. Steril.*, **40**, 802–804.

Thompson, L.A., Tomlinson, M.J., Barratt, C.L., Bolton, A.E. and Cooke, I.D. (1991) Positive immunoselection – a method of isolating leukocytes from leukocytic reacted human cervical mucus samples. *Am. J. Reprod. Immunol.*, **26**, 58–61.

Trounson, A. (1989) Fertilization and embryo culture. In: Wood, C. and Trounson, A. (eds), *Clinical In Vitro Fertilization*. Springer-Verlag, London, pp. 34–50.

Vermeiden, J.P.W., Bernardus, R.E., ten Brug, C.S., Statema-Lohmeijer, C.H., Willemsen-Brugma, A.M. and Schoemaker, J. (1989) Pregnancy rate is significantly higher in *in vitro* fertilization procedure with spermatozoa isolated from nonliquefying semen in which liquefaction is induced by alpha-amylase. *Fertil. Steril.*, **51**, 149–152.

Wang, C., Baker, H.W.G., Jennings, M.G., Burger, H.G. and Lutjen, P. (1985) Interaction between human cervical mucus and sperm surface antibodies. *Fertil. Steril.*, **44**, 484–488.

WHO (1987) *WHO Laboratory Manual For The Examination Of Human Semen And Semen-Cervical Mucus Interaction*, 2nd edn. Cambridge University Press, Cambridge, pp. 1–67.

WHO (1992) *WHO Laboratory Manual For The Examination Of Human Semen And Sperm-Cervical Mucus Interaction*, 3rd edn. Cambridge University Press, Cambridge, pp. 1–107.

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