Quantitative sperm mucus penetration: modified formulae for calculating penetration efficiency

Gary N. Clarke¹, Claire Garrett² and H.W. Gordon Baker²

¹Andrology Unit, The Royal Women’s Hospital, Carlton 3053 and
²Department of Obstetrics and Gynaecology, Melbourne University, Parkville 3052, Australia

In 1980 Katz et al. derived a formula for the percentage of successful collisions (PSC) as a quantitative measure of sperm–cervical mucus penetration efficiency. The use of PSC waned after its validity was questioned by reports of values >100% and the observation that PSC varied with the cross-sectional area of the mucus column. The aim of the present study was to develop a more accurate measure of mucus penetration efficiency by correcting the original formula for the effects of sperm depletion in the semen reservoir. Two formulae were derived using different models for the sperm–mucus interaction: (i) each motile spermatozoon was assumed to have an equal chance of mucus penetration on collision; (ii) a select subpopulation of spermatozoa was assumed to penetrate with 100% efficiency on collision. Both modified formulae gave PSC values higher than the original estimates. Under the experimental conditions employed in this work, where large capillaries were used, the depletion corrections ranged from 4 to 46% \( (n = 8, \text{mean } 20\%) \) for model (i) and from 190 to 320% \( (n = 8, \text{mean } 250\%) \) for model (ii). The invariance of PSC(ii) results with respect to capillary cross-sectional area \( (1.52 \text{ mm}^2, \text{31.1\%; } 5.4 \text{ mm}^2, \text{28.2\%}) \) suggests that the assumptions of model (ii) provide the more accurate description of the sperm–mucus interaction.

Key words: cervical mucus penetration/human spermatozoa/percentage successful collisions

Introduction

The first detailed investigations of sperm–cervical mucus interactions in vitro were performed by Kurzrok and Miller (1932). Subsequently, several groups studied mucus penetration using quantitative procedures. For example, Botella-Llusia and Ruiz-Velasco (1960) studied the migration of human spermatozoa through normal human cervical mucus loaded into a calibrated glass syringe of 20 mm length and 2 mm diameter. Their scattergram showed a strong correlation between the number of spermatozoa which had penetrated through the 20 mm mucus column into a 0.2 ml chamber, and a derived index of semen quality incorporating motility, sperm concentration, velocity and sperm morphology. Bergman and Ferbas (1961) used a similar quantitative system to study sperm migration through an artificial medium. Their results showed that the number of spermatozoa penetrating an isotonic, weakly alkaline fructose solution was dependent on the sperm motility, concentration and morphology. Although highly promising, these procedures were not widely accepted and were soon overshadowed by the simple quantitative capillary test introduced by Kremer (1965). Virtually all subsequent research and clinical testing proceeded with variations of the Kremer test. An important modification to this system came with the use of flat capillaries, which allowed easier, more accurate counting of spermatozoa in the mucus (Mills and Katz, 1978). The subsequent re-introduction of a quantitative procedure for measuring sperm penetration (Katz et al., 1980), coupled with the percentage successful collisions (PSC) formula for calculating sperm penetration efficiency, opened the way for several studies which attempted to clarify the relationship between semen quality and mucus penetration.

The most significant semen variables related to mucus penetration included concentration of progressively motile spermatozoa and variables describing aspects of the lateral head displacement of the spermatozoa (Aitken et al., 1985; Mortimer et al., 1986; Clarke, 1997). However, the PSC gave much weaker correlations with sperm movement variables than did the sperm concentration in mucus. Aitken et al. (1985) suggested that the relatively weak relationship between PSC and sperm movement variables may have resulted from the fact that the behaviour of spermatozoa was not accurately described by the kinetic theory of gases, which was the main assumption used in the derivation of the PSC formula. The validity of the assumptions inherent in the PSC calculation was further questioned by researchers who found PSC results to be dependent on the choice of cross-sectional area of the capillary used in the test (Reid and Aitken, unpublished observations, cited in Schats et al., 1984) and, in some instances, PSC values exceeded the meaningful physical maximum of 100% (Katz et al., 1984; Aitken et al., 1985). However, the concept behind the definition of PSC is to isolate the intrinsic mucus penetration ability of a spermatozoon from its ability to arrive at the semen–mucus interface. Thus it is encouraging, rather than surprising, that PSC results are not well correlated with motility parameters, for this suggests that the PSC provides a quantitative, independent measure of the functional competence of spermatozoa. Katz et al. (1980) make this point clear by reporting a semen–mucus cross penetration test in which semen samples from a patient with poor sperm motility and concentration exhibited low absolute penetration numbers, yet the calculated PSC values were equivalent to those for donor semen incubated with the same 

© European Society for Human Reproduction and Embryology
samples of donor and patient mucus. The PSC ideally provides standardization of concentration, percentage motility and sperm velocity in a quantitative sperm–mucus penetration test. These considerations, together with some initial studies in our laboratory, prompted us to investigate other sources of error in PSC performance and calculations, rather than abandon the concept of PSC as a meaningful measure of sperm–mucus penetration efficiency. Re-examination of the derivation of PSC has indicated the need for consideration of sperm depletion effects in the semen reservoir. Two approaches to this problem have produced modified formulae for the calculation of PSC. One model specifically allows for reduced penetration due to depletion of the general motile sperm population, and the other focuses on depletion of an assumed subset of the sperm population which is identified as being capable of mucus penetration. Effectively, the difference between these approaches can be interpreted as: (i) each motile spermatozoon has an equal chance of penetration on collision and must make, on average, 100/PSC collisions before penetration occurs; (ii) the motile sperm population comprises a select proportion (PSC%) of spermatozoa which penetrate on collision with 100% efficiency, whilst the remaining (100 – PSC)% of the population fail to penetrate, irrespective of the number of collisions made at the semen–mucus interface.

During this study, formulae were derived for each of the depletion models and then evaluated experimentally.

### Materials and methods

#### Cervical mucus

Human peri-ovulatory cervical mucus was collected using collection catheters (Rocket Ltd, London, UK) or tuberculin syringes. Mucus samples were assessed and stored as described previously (Clarke, 1997).

#### Kremer test

The qualitative penetration test was performed and scored essentially as described by Kroeks and Kremer (1980). Mucus samples which scored ‘good’ penetration with husband or donor spermatozoa were used in this study.

#### Semen

The semen samples used in this investigation were from prospective donors to our donor insemination programme or from infertility patients. Routine semen analyses (count, motility, morphology) and computer-assisted semen analyses (CASA; sperm motion characteristics) were performed using a Hamilton-Thorne motility analyser (Hamilton-Thorne Research, Beverley, MA, USA) as described previously (Johnston et al., 1995).

The semen samples used in this study were selected on the basis of normal sperm concentration (median = 80×10^6 sperm/ml, range 40–232), progressive motility (median = 54%, range 38–75) and straight line velocity (VSL; median = 40 μm/s, range 30–62).

#### Sperm antibody testing

Each semen sample used was negative for sperm-bound antibodies using the direct immunobead test (Clarke et al., 1985).

#### Quantitative mucus penetration test

The quantitative mucus penetration test (QMPT) was performed as described previously (Clarke, 1997), except for the use of larger capillaries (100–200 μl, catalogue no. 341; Socorex, Renens, Switzerland). After loading the capillary with mucus, care was taken to remove any excess mucus, and the mucus meniscus was checked and adjusted so that it was level with the capillary end. This procedure ensured that the surface area of interaction was, as far as possible, equal to the cross-sectional area of the capillary.

### Semen reservoir depletion correction to PSC

PSC is defined simply as the measured number of spermatozoa in the mucus expressed as a percentage of the number of calculated collisions at the semen–mucus interface. The original formula for PSC was derived by Katz et al. (1980) based on the collision frequency calculations of Ojakian and Katz (1973) and is given by:

$$PSC = 400(C_M/C_S) [L/v_S t]$$

where $C_S$ (spermatozoa/ml) is the concentration of motile spermatozoa in the semen reservoir, $C_M$ is the sperm concentration in a column of cervical mucus of length $L$, μm after $t$ seconds incubation and $v_S$ (μm/s) is the mean swimming speed of spermatozoa in the semen. Since this derivation is based on the kinetic theory of gases, spermatozoa are assumed to swim in straight lines between collisions and thus $v_S$ is taken as the straight line velocity measure VSL of the Hamilton-Thorne motility analyser.

The derivation of Eq. 1 also assumes that $C_S$ is constant, ignoring the continual loss of spermatozoa from the semen reservoir during the incubation period. This depletion effect can be incorporated in a revised calculation of PSC by equating the reservoir depletion rate with the rate of penetration of spermatozoa into the mucus column and solving the resultant first-order differential equation. However, the explicit application of this correction depends on whether $p$ = PSC/100 is interpreted as (i) a probability of penetration common to each motile spermatozoan in the sample, or (ii) that it represents an average probability for a sperm population comprised of two subpopulations, which are classified as penetrators and non-penetrators. In the latter approach, individual sperm probabilities of penetration on collision with the mucus interface are assumed to be either zero or one, and thus the effective PSC for the sample can be equated with the initial percentage of motile spermatozoa capable of penetration. That is

$$p = C_P(t = 0)/C_S(t = 0)$$

where $C_P$ is the concentration of the subpopulation of spermatozoa with 100% chance of penetration once in contact with the mucus interface.

During a time interval $dt$, the number of collisions at a semen–mucus interface of area $A$ and sperm concentration $C$ is given by the formula derived by Ojakian and Katz (1973) as

$$dN_{\text{collisions}}(t) = \frac{1}{4}C(t)v_S A dt$$

If in the same time interval the number of spermatozoa which penetrate the mucus is $dN_{\text{P}}$, then substituting for the appropriate collision rate from the general Eq. 3 the penetration rate for approaches (i) and (ii) is given by

$$dN_M(t)/dt = \frac{1}{4} pC_S(t)v_S A$$

and

$$dN_M(t)/dt = \frac{1}{4} C_P(t)v_S A$$

respectively. But the semen reservoir is depleted by the number of spermatozoa penetrating the mucus, and thus at any time $t$

$$C_S(t) = C_0 - N_M(t)/V_R$$
The number of sperm ($N_M$) that penetrate the mucus column as a function of incubation time, calculated for each of the three models of sperm–mucus interaction. Values of $N_M$ are calculated for the experimental conditions and mean semen characteristics of the data reported in Table I. Horizontal lines at $6.0$ and $2.3$ M/ml indicate saturation values for $N_M$ in the model (i) and (ii) calculations respectively. PSC = percentage of successful collisions; $C_0$ = initial sperm concentration; $L$ = capillary length; $A$ = capillary area; $v_s$ = mean swimming speed of spermatozoa in semen; $V_R$ = volume of semen in reservoir.

$$C_P(t) = pC_0 - N_M(t)/V_R \tag{ii}$$

where $V_R$ is the volume of semen in the reservoir and $C_0 = C_S(t = 0)$. Substituting for $C_S$ or $C_P$ in Eq. 4, the mucus penetration rates become:

$$dN_M(t)/dt = \left(\frac{1}{2}\right)(v_S A_V R) [C_0 V_R - N_M(t)] \tag{i}$$

and

$$dN_M(t)/dt = \left(\frac{1}{2}\right)(v_S A_V R) [pC_0 V_R - N_M(t)] \tag{ii}$$

These first order differential equations are of the form where the rate of change of a quantity is linearly related to the quantity itself and hence the solutions incorporate an exponential growth or decay. In this instance, the solutions are represented by an exponential growth of the number of spermatozoa in the mucus, asymptoting with incubation time to a maximum of (i) all motile spermatozoa or (ii) all possible penetrators from the original semen reservoir. Mathematically, the solutions are expressed by the equations:

$$N_M(t) = C_S V_R [1 - \exp(-at_1)] \tag{5(i)}$$

and

$$N_M(t) = pC_0 V_R [1 - \exp(-at_2)] \tag{5(ii)}$$

where $a = \frac{1}{2}v_S A_V$ and their form is illustrated graphically in Figure 1 for typical semen parameters and a QMPT set-up using a large cross-sectional area capillary.

The number of spermatozoa in the mucus at the end of the incubation period is simply the product of the measured concentration $C_M$ and the volume of mucus in the capillary. Thus, assuming the sperm–mucus interface corresponds to the cross-sectional area of the capillary,

$$N_M(t) = C_M A_L$$

and can be equated with Eq. 5 and rearranged to give the revised formulae for PSC as

$$PSC(i) = 100[p(400V_M/(v_S A_V R))]\ln[1 - C_M A_L/(C_S V_R)] \tag{6(i)}$$

and

$$PSC(ii) = 100[p(100C_M A_L/C_S V_R)]/[1 - \exp(-aL)] \tag{6(ii)}$$

Using the mathematical approximations that $\ln(1 + x) = x$ and $1 - \exp(x) = -x$ when $x << 1$, each of the revised PSC formulae can be shown to reduce to the original formula of Katz et al. (1980). That is, when $C_M A_L/(C_S V_R) << 1$, Eq. 6(i) approaches to

$$PSC(i) = -[400V_M/(v_S A_V R)]/[C_M A_L(C_S V_R)]$$

$$= 400[C_M A_L/(C_S V_R)] [V_M A_V R = 100] \tag{Eq. 1}$$

Similarly, when $aL = \frac{1}{2}V_S A_V V_R << 1$, Eq. 6(ii) approaches to

$$PSC(ii) = [100C_M A_L/(C_S V_R)]/[V_M A_V V_R]$$

$$= 400[C_M A_L/(C_S V_R)] [V_M A_V R = 100] \tag{Eq. 1}$$

These limiting approximations indicate that, for mucus columns with small cross-sectional areas coupled with large reservoir volumes, the depletion corrections are minimized and the original formula of Katz et al. (1980) provides accurate PSC values. In practice, however, the use of very small capillaries enhances other sources of error, particularly those associated with the implicit assumption that the area of the semen–mucus interface is equivalent to the cross-sectional area of the capillary.

A comparison of PSC results using the formula of Katz (Eq. 1) and both reservoir depletion corrected formulae of Eq. 6 was made for the QMPT performed on eight different semen and mucus samples using round capillaries (cross-sectional area = 5.4 mm$^2$) containing 100 µl of cervical mucus ($L = 18$ mm), with 100 µl semen reservoir volumes and incubation times of 60 min at 37°C. Similarly, comparisons were made for our previously published QMPT data obtained for 21 semen samples using the same QMPT set-up except for a substantially smaller capillary ($A = 0.82$ mm$^2$, $L = 30$ mm) and twice the reservoir volume. The magnitude of the depletion correction for the PSC data reported by Katz et al. (1980) was also calculated.

In addition, the $A$ dependence of PSC for the three different calculations was explicitly examined by performing paired QMPT for five different semen and mucus samples using capillaries with cross-sectional areas of 1.52 and 5.4 mm$^2$ and column lengths of 33 and 18 mm respectively. A semen reservoir volume of 200 µl and incubation time of 60 min at 37°C were used for both capillaries.

Statistical procedures

The data were analysed using the Wilcoxon signed-ranks test (Statgraphics 6.0; Statistical Graphics Corporation, Rockville, MD, USA).

Results

Table I presents the results of the comparison between the three different PSC calculations for eight QMPT experiments using different semen and cervical mucus samples. By definition, both depletion corrected formulae give higher PSC values than the Katz formula, with correction magnitudes ranging from 4 to 46% (mean = 20%) for PSC(i) and from 190 to 320% (mean = 250%) for PSC(ii). For the substantially smaller capillary dimensions employed in the QMPT results reported in our previous work (Clarke, 1997), the modified PSC results correspond to corrections of 1–9% (mean = 4%)
5.4 mm². By comparison, the PSC(ii) formula gives the same results for both sizes of capillary. Since a meaningful PSC dependence can be resolved if the original PSC formula incorporates two estimates of sperm count plus a reservoir volume. The magnitude of this correction depends strongly on the geometry of the QMPT set-up employed and is greatest for large cross-sectional area capillaries and small reservoir volumes. The magnitude of the correction also increases with incubation time and motile sperm velocity. Because the reservoir is depleted only of those spermatozoa capable of penetration, large percentage corrections to PSC are possible, even when the penetration efficiency is low (experiment no. 3, Table I). Dependence of the magnitude of the PSC correction on QMPT set-up explains some of the discrepancies that have been reported in the literature. Schats et al. (1984) noted that their PSC values (mean = 43.6%) were considerably higher than those obtained by Katz et al. (1980) with a mean of 24%, which is consistent with smaller depletion corrections corresponding to their smaller capillary cross-section and larger reservoir volume.

Further understanding of previous results may be obtained by analysing the various sources of experimental error inherent in the QMPT and associated PSC calculations. The coefficient of variation (CV) for sperm counts in semen is ~10% (Katz et al., 1984; Johnston et al., 1995) and the CV for sperm velocity is ~5% (Johnston et al., 1995). Because the PSC formula incorporates two estimates of sperm count plus a velocity estimate, these errors are additive. Such errors may have partly contributed to the PSC results of ~140 and 175% reported by Katz et al. (1984) in samples diluted to 5–10×10⁶/ml. However, the serial dilutions performed in their experiments may have also produced cumulative errors in the PSC results. Counting errors can be reduced by counting more spermatozoa or by taking the mean of several replicate sperm counts. For example, the approximate CV for a sperm count could be reduced to 5% by counting a minimum of 200 spermatozoa per chamber and using the mean of two such counts. Therefore, a more accurate estimation of PSC could readily be obtained by improving the precision of sperm counts, in addition to application of the modified PSC formula given by Eq. 6(ii). The potential use of CASA systems to improve counting precision further is now being evaluated in our laboratory.

Discussion

One of the main anomalies arising from the application of the original PSC formula of Katz et al. (1980) was the unpublished observation cited by Schats et al. (1984) that PSC values increased with decrease in cross-sectional area of the capillary used in the QMPT. This unpredicted A dependence in PSC (Katz) was confirmed by our experiments in which direct comparison was made between the results for the same semen and mucus samples measured using capillaries with different dimensions (Table II). We also established that this anomaly of A dependence can be resolved if the original PSC formula is modified to include semen reservoir depletion effects, provided the depletion calculations are based on the assumption that the motile sperm population of a semen sample can be divided into two subpopulations, defined as penetrators and non-penetrators [PSC(ii)].

Inclusion of a depletion correction necessarily provides a more accurate estimate of PSC and, by definition, gives higher PSC values than those obtained using the formula of Katz et al. (1980). The magnitude of this correction depends strongly on the geometry of the QMPT set-up employed and is greatest for large cross-sectional area capillaries and small reservoir volumes. The magnitude of the correction also increases with incubation time and motile sperm velocity. Because the reservoir is depleted only of those spermatozoa capable of penetration, large percentage corrections to PSC are possible, even when the penetration efficiency is low (experiment no. 3, Table I). Dependence of the magnitude of the PSC correction on QMPT set-up explains some of the discrepancies that have been reported in the literature. Schats et al. (1984) noted that their PSC values (mean = 43.6%) were considerably higher than those obtained by Katz et al. (1980) with a mean of 24%, which is consistent with smaller depletion corrections corresponding to their smaller capillary cross-section and larger reservoir volume.

Further understanding of previous results may be obtained by analysing the various sources of experimental error inherent in the QMPT and associated PSC calculations. The coefficient of variation (CV) for sperm counts in semen is ~10% (Katz et al., 1984; Johnston et al., 1995) and the CV for sperm velocity is ~5% (Johnston et al., 1995). Because the PSC formula incorporates two estimates of sperm count plus a velocity estimate, these errors are additive. Such errors may have partly contributed to the PSC results of ~140 and 175% reported by Katz et al. (1984) in samples diluted to 5–10×10⁶/ml. However, the serial dilutions performed in their experiments may have also produced cumulative errors in the PSC results. Counting errors can be reduced by counting more spermatozoa or by taking the mean of several replicate sperm counts. For example, the approximate CV for a sperm count could be reduced to 5% by counting a minimum of 200 spermatozoa per chamber and using the mean of two such counts. Therefore, a more accurate estimation of PSC could readily be obtained by improving the precision of sperm counts, in addition to application of the modified PSC formula given by Eq. 6(ii). The potential use of CASA systems to improve counting precision further is now being evaluated in our laboratory.

Table I. A comparison of percentage of successful collisions (PSC) results using the formula of Katz et al. (1980) versus the newly derived reservoir depletion corrected formulae

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Cₛ (10⁶/ml)</th>
<th>Cₘ (10⁶/ml)</th>
<th>vₛ (μm/s)</th>
<th>PSC (Katz)</th>
<th>PSC (i)</th>
<th>PSC (ii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.6</td>
<td>22.3</td>
<td>30</td>
<td>15.9</td>
<td>18.1</td>
<td>30.5</td>
</tr>
<tr>
<td>2</td>
<td>110.2</td>
<td>61.5</td>
<td>51</td>
<td>21.9</td>
<td>31.5</td>
<td>59.9</td>
</tr>
<tr>
<td>3</td>
<td>37.0</td>
<td>2.7</td>
<td>38</td>
<td>3.8</td>
<td>4.0</td>
<td>8.5</td>
</tr>
<tr>
<td>4</td>
<td>27.8</td>
<td>6.5</td>
<td>41</td>
<td>11.4</td>
<td>12.9</td>
<td>26.4</td>
</tr>
<tr>
<td>5</td>
<td>116.0</td>
<td>20.9</td>
<td>31</td>
<td>11.6</td>
<td>12.8</td>
<td>22.7</td>
</tr>
<tr>
<td>6</td>
<td>31.2</td>
<td>17.9</td>
<td>62</td>
<td>18.5</td>
<td>27.1</td>
<td>59.6</td>
</tr>
<tr>
<td>7</td>
<td>15.2</td>
<td>2.4</td>
<td>35</td>
<td>7.2</td>
<td>7.8</td>
<td>19.2</td>
</tr>
<tr>
<td>8</td>
<td>51.0</td>
<td>15.6</td>
<td>50</td>
<td>12.2</td>
<td>14.5</td>
<td>32.9</td>
</tr>
</tbody>
</table>

Cₛ = concentration of motile spermatozoa in the semen reservoir; Cₘ = sperm concentration in a column of cervical mucus of length L (μm) after t seconds; vₛ = mean swimming speed of spermatozoa in the semen.

Table II. Effect of capillary size on percentage of successful collisions (PSC) results

<table>
<thead>
<tr>
<th>Capillary size</th>
<th>PSC (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Katz et al. (1980)</td>
</tr>
<tr>
<td>(A mm²) (L mm)</td>
<td>26.8²</td>
</tr>
<tr>
<td>1.52 33</td>
<td>17.2²</td>
</tr>
</tbody>
</table>

²Wilcoxon signed-ranks test, P < 0.05.
²Wilcoxon signed-ranks test, NS.
It is important to note that, although the surface area of sperm–mucus interaction (A) is explicitly required only in the revised PSC formulae, the derivation of both the old and modified formulae assumes equivalence between A and the cross-sectional area of the capillary. Thus, if care is not taken when loading the capillary to ensure this assumption is valid, PSC values calculated using any of the formulae will be overestimated because the effective surface area of the sperm–mucus interface will exceed its assumed value. It is for this reason that the use of very small capillaries for the QMPT is not recommended, as this practice simply reduces the magnitude of a calculable correction at the expense of introducing an indeterminate random error. In fact, the apparent lack of sensitivity to A in the original PSC formula, created by mathematical cancellation of the two areas, may well have encouraged experimental indifference to this consideration, possibly accounting for some of the previous PSC estimates that exceeded 100%.

Although the corrected PSC formula provides a more accurate estimation of the efficiency of cervical mucus penetration, several other possible sources of error should be investigated. In particular, the assumption that the motion of spermatozoa in the semen reservoir can be represented by the kinetic theory of gases is presumably compromised in that only a percentage of the motile sperm population in a given semen sample exhibits the linear motion assumed in the model. In fact, the positive geotaxis demonstrated by sperm motion (Roberts, 1972) will result in a vertical gravitational sperm density gradient in the semen reservoir, giving rise to an effective C · at the interaction surface which is lower than the mean reservoir value which is applied in the PSC formulae. These factors are expected to provide relatively small additional corrections to the PSC estimation, but are the subject of continuing investigation. Technical improvements are also being investigated, including the use of enzyme cocktails for cervical mucus liquefaction prior to sperm counting (de Agostini et al., 1996; de Agostini and Campana, 1996).

We have now begun to use the modified QMPT/PSC system to study the sperm–cervical mucus interaction. It may help us to understand the mechanism of the sperm antibody blockade of mucus penetration, the apparent selectivity of cervical mucus for spermatozoa of normal morphology, the relationship between mucus penetration and fertilizing ability, and the specific dynamics of sperm penetration of cervical mucus.

Acknowledgements
The authors thank Peter Elliot, B.Sc., Henry Oh, Dip. App. Sc. and Jing An, B.Sc. of the Andrology Laboratory for performing semen analyses, and Kay Kerrison for typing the manuscript.

References

Received on September 15, 1997; accepted on January 13, 1998