

## The effects of coital lubricants on sperm motility *in vitro*

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**Infertility affects approximately 15% of couples, and in about one-third the primary cause is a male factor. Patients undergoing infertility investigations frequently experience sexual dysfunction, which often is due to inadequate vaginal lubrication. This can lead to increased use of coital lubricants. The effects of such lubricants on sperm motility have not been widely studied, although sperm motility is one of the best prognostic indicators of fertilization. Using a prospective longitudinal control-based study, we analysed the effect of adding four lubricants: KY jelly, baby oil, olive oil and saliva on sperm motion in 16 samples from patients undergoing infertility investigations. Sperm samples were prepared by density gradient centrifugation prior to mixing with lubricants. Motility parameters were determined using computer-assisted semen analysis after 5, 15 and 30 min. All lubricants except baby oil significantly decreased percentage progressive motility, progressive velocity, curvilinear velocity and lateral head displacement at 12.5% concentration. At a lower concentration of 6.25%, both olive oil and saliva still significantly reduced progressive motility parameters, while KY jelly diminished head movement parameters. Hence, even at these very low concentrations, coital lubricants impair sperm motility and thus may adversely affect fertility.**

**Key words:** computer-assisted semen analysis/coital lubricants/male infertility/sperm motility

### Introduction

Coital lubricants are generally used for symptomatic relief of dyspareunia. This is one of the most common gynaecological symptoms, with up to 40% of women suffering from it (Semmens and Semmens, 1974). This symptom frequently does not present as a primary complaint to the medical practitioner and, therefore, remains untreated or self-medicated with a lubricant (18% of surveyed group; Semmens and Semmens, 1974). Dyspareunia is not, however, limited to the peri- or postmenopausal groups. Recently it has been shown that dyspareunia can affect up to 46% of 18- to 45-year-olds (Jamieson and Stegge, 1996). This includes the reproductive population, of which one in six couples will seek medical help

because of infertility. It has been reported that these couples frequently experience some sexual dysfunction during the term of their fertility investigations (Mezor, 1978). The emotional impact of involuntary infertility and the necessity of having intercourse 'to order', along with an increasing pressure to conceive, can further increase the incidence of dyspareunia and thus the use of coital lubricants.

To date, there has been little research on the effects of lubricants on sperm motility. Light microscopy studies have indicated that KY jelly (Tagatz *et al.*, 1972) and surgical gels (Schoeman and Tyler, 1983) each have a detrimental effect on sperm motility. Vegetable-based oils and egg whites have been shown to be less toxic to sperm (Tulandi and McInnes, 1984). Goldsberg and Whiter (1975) recommended egg whites as the lubricant of choice for infertile couples, and suggested that light oils had minimal detrimental effect on sperm motility.

Reports on the effects of saliva on sperm motility have been conflicting. Tulandi *et al.* (1982) showed saliva to have a toxic effect, while Amelar *et al.* (1980) recommended that infertile couples use saliva as a coital lubricant. KY jelly remains the most widely used commercial coital lubricant, although it has been shown previously, using conventional light microscopy, to have a toxic effect on sperm motility (Tagatz *et al.*, 1972).

While most studies have been performed with conventional light microscopy, computer-assisted semen analysis (CASA) provides a more reproducible (Barratt *et al.*, 1993; McKinney and Thompson, 1994) and objective measurement of sperm motility (Neuwinger *et al.*, 1990; Liu *et al.*, 1991). The measurement of sperm motility has been shown to be of significant prognostic value for determining fertility (Holt *et al.*, 1989; Barratt *et al.*, 1993).

In this study we have assessed the effects of four commonly used coital lubricants at low concentrations on sperm motility using CASA, over time periods similar to those for which spermatozoa would be in contact with lubricants *in vivo*.

### Materials and methods

#### Collection and preparation of samples

Semen samples were obtained from 16 patients attending for routine semen analysis as part of fertility investigations at the Regional Fertility Centre, Belfast. Each sample was produced by masturbation into a plastic container after a recommended 48–96 h of sexual abstinence. Samples were allowed to liquefy at room temperature and then analysed by routine light microscopy. All subjects demonstrated a normozoospermic semen analysis profile ( $>20 \times 10^6/\text{ml}$ ,  $>50\%$  forward progression,  $>30\%$  normal forms) according to World Health Organization criteria (WHO, 1992).

### *Sperm preparation*

In order to select the spermatozoa most likely to reach and fertilize the egg, the samples were prepared by two-step discontinuous Percoll gradient centrifugation. Freshly liquefied semen was treated as follows: (i) sperm preparation by two-layer Percoll (95.0–47.5%) centrifugation at 500 *g* for 12 min; (ii) concentration of the 95% layer by centrifugation at 250 *g* for 6 min; and (iii) the resulting sperm pellet was diluted to a concentration of 10<sup>6</sup>/ml with Biggers, Whitten, Whittingham (BWW) medium.

### *Treatment of spermatozoa with lubricant*

Each sperm sample was divided into five aliquots for each concentration. Baby oil (Johnson & Johnson, New Brunswick, NJ, USA), olive oil (Dante, Unilever, Milano-VigN, Italy), KY jelly (Johnson & Johnson) and saliva were adjusted to 12.5% or 6.25% concentration by gently vortexing with BWW culture media on a volume-to-volume basis. The viscosity of each lubricant solution was measured using the Brookfield Digital Cone and Plate Viscometer (model DV-II, Southalls, Harlow, Essex, UK). The osmolality of each lubricant solution was checked before and after addition of sperm samples using the Advanced micro-osmometer (model 3MO, Advanced Instruments Inc., Needham, MA, USA; reference solution 290 mosmol). One 0.04  $\mu$ m aliquot of prepared semen was then added to each 0.04  $\mu$ l aliquot of diluted test substance. The fifth aliquot was added to BWW without any addition of lubricant to act as a control.

Saliva for this study was obtained freshly from a pool of four healthy male volunteers who had abstained from oral intake of food or drink for at least 4 h, were on no medication, and did not smoke. Salivary amylase content was analysed in five randomly selected samples by dry-slide chemistry, using a Johnson & Johnson DitroF analyser (Rochester, N.Y., USA).

From the time of addition of each of the lubricants, the samples were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. All samples were gently shaken by hand throughout the incubation.

### *Determination of sperm motility*

At intervals of 5, 15 and 30 min, 10  $\mu$ l aliquots were withdrawn and placed on a prewarmed 20  $\mu$ m-deep microscope slide (Fertility Technologies Inc., Natick, MA, USA) to prevent restriction of sperm movement and interaction with the walls of the chamber. The microscope slide was then inserted into the Hamilton Thorn Research Motility Analyser (USA HTM model 2030 Version 7.2Y; Beverly, MA, USA). The settings employed for analysis were: frame acquisition rate (Hz), 30; minimum contrast, 7; minimum size, 6; low-size gate, 1.6; low-intensity gate, 0.4; high-intensity gate, 1.6; HTM magnification factor, 2.04. Nine fields, containing at least 100 motile spermatozoa were randomly selected and assessed by the Hamilton Thorn computer for each sample at each time interval.

The order of analysis for each test substance was based on computer-generated random number tables. Values across the nine fields were then totalled. Throughout the analysis an integral heated stage maintained the sperm sample temperature at 37°C. The playback facility was used to ensure that the gates were satisfactory.

The following parameters were recorded for each sample: percentage of progressively motile sperm; progressive velocity (VSL,  $\mu$ m/s, straight-line distance of track from beginning to end, divided by time); curvilinear velocity (VCL,  $\mu$ m/s, total distance travelled by sperm, divided by time elapsed); amplitude of lateral head displacement (ALH,  $\mu$ m, the mean width of sperm head oscillation); beat cross frequency (BCF, Hz, frequency of sperm head crossing the sperm head average path).

### *Statistical analysis*

ANOVA was employed to examine differences between control and treated groups. Multiple comparisons with the mean were calculated using the Newman–Keuls test. Analysis was carried out on Arcus Pro-2 (Medical Computing, Aughton, UK) and Statistica (Statsoft Inc., Letchworth, Herts, UK) using means of the raw data from each sample at each of the three time points, and at both concentrations.

## **Results**

### *Effects of coital lubricants at 12.5% on sperm motion parameters*

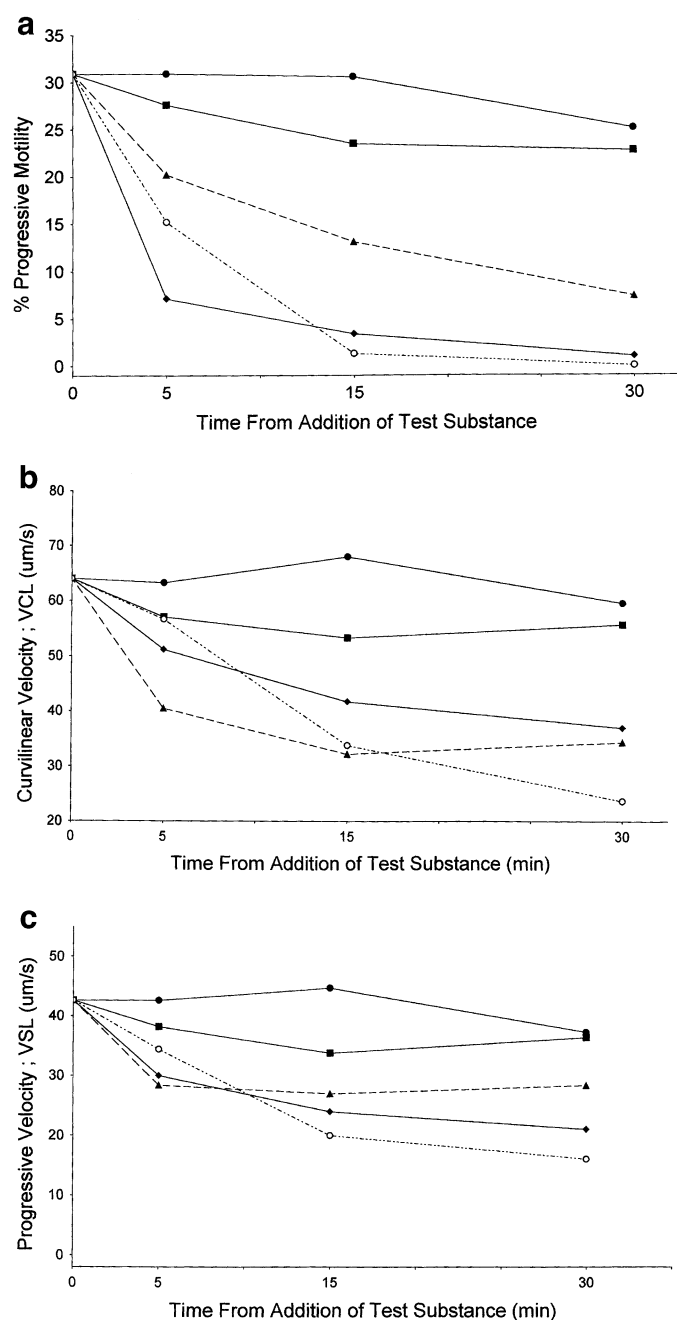
Percentage progressive motility was significantly decreased by 5 min ( $P < 0.05$ ) after the addition of each lubricant except baby oil, the reduction in sperm motility becoming more pronounced with time. However, the decrease seen with baby oil was never significantly different from the control. Saliva caused the greatest reduction in motility, with the percentage of motile sperm reduced by 50% within 5 min, and movement reduced almost to zero (95%) within 15 min. There was no statistically significant difference between the effects of olive oil and KY jelly on percentage progressive motility ( $P > 0.05$ ) until after 5 min. KY jelly had a less deleterious effect on sperm motion, reducing movement by only 74% after 30 min, compared with olive oil, which reduced movement by 42% after 15 min, and by 91% after 30 min (Figure 1a).

Straight-line and curvilinear velocities were not significantly affected by baby oil at any time period, the maximum decrease in velocity being only 10%. There was no significant difference between the effects of the lubricants and control until 15 min, except for KY jelly, which had significantly reduced curvilinear velocity by 5 min. Saliva had a marked effect on both parameters, having decreased progressive motility so much that, at 30 min, accurate velocity readings could not be obtained due to insufficient motile sperm numbers per field. KY jelly and olive oil had less dramatic effects on velocity parameters than saliva, though both caused marked reductions in VSL and VCL, which became statistically significant for both by 15 and 30 min ( $P < 0.005$ ). The decrease was more pronounced for KY jelly, which dampened VCL by 52% compared with 38% deterioration with olive oil. However, there was no statistical significance between their effect on straight-line velocity (Figure 1b and c).

Baby oil had no statistically significant effect on any head movement parameters at the various time intervals. Saliva totally suppressed all sperm head movements after 15 min, while KY jelly significantly reduced lateral head movements by 37% after 5 min. BCF was minimally reduced during this time interval by KY jelly (Figure 2a). Olive oil led to minimal decreases in lateral head movements, but was the only lubricant significantly to decrease BCF after 5 and 15 min (Figure 2b).

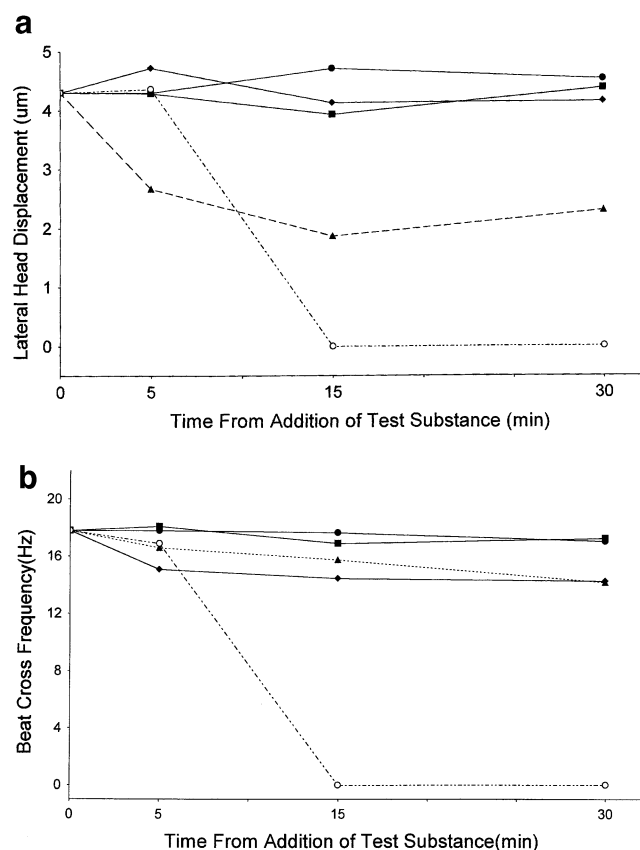
### *Effects of coital lubricants at 6.25% on sperm motility and head movements*

At this lower concentration, only olive oil exerted any statistically significant effect on percentage progressive motility, diminishing movement by 31% after 5 min and by 41% after 30 min ( $P < 0.04$ ). Again, all other lubricants exerted a downward



**Figure 1.** The effects of coital lubricants on (a) % progressive motility, (b) curvilinear velocity (VCL) and (c) progressive velocity (VSL) of sperm samples with time from addition of test substance. Values are means of control spermatozoa (●), and following treatments with KY jelly (▲), baby oil (■), olive oil (◆) and saliva (○), all at 12.5% concentration. For simplicity, SE bars are not included for each data point. The coefficient of variation was <10% for all points, except olive oil at 30 min: progressive motility 13.9% and VCL 14%. A coefficient of variation could not be calculated for saliva after 5 min due to insufficient motile spermatozoa.

trend on progressive motility. Baby oil had no statistically significant effect on any movement parameters at the various time intervals ( $P > 0.05$ ). Saliva again almost totally suppressed all sperm head movements after 15 min [results not included in Table II as there were insufficient motile spermatozoa (<100) in each field to give accurate values]. Interestingly,



**Figure 2.** The effect of coital lubricants tested on (a) lateral head displacement (ALH) and (b) beat cross frequency (BCF) of sperm samples with time from addition of test substance. Values are means of control spermatozoa (●), and following treatments with KY jelly (▲), baby oil (■), olive oil (◆) and saliva (○), all at 12.5% concentration. For simplicity, SE bars are not included for each data point. The coefficient of variation was <5% for each data point. A coefficient of variation could not be calculated for saliva after 5 min due to insufficient motile spermatozoa.

KY jelly and olive oil decreased both velocity parameters significantly over 30 min, KY jelly having a more detrimental effect than olive oil. KY jelly was the only other lubricant to lead to significantly reduced lateral head movements and BCF at this concentration. Olive oil led to minimal decreases in lateral head movements that were not statistically significant (Table I).

#### Osmolality and viscosity measurements of test solutions

The osmolality of saliva, baby oil and KY jelly changed significantly when spermatozoa were added (Table II). Baby oil and KY jelly were the most viscous solutions when compared with BWB.

#### Discussion

This is the first study to apply CASA to determine the effects of coital lubricants, at low concentrations, on sperm motility parameters. Baby oil had no significant effect on sperm motility, despite having the second highest viscosity and osmolality of the tested lubricants. In contrast, saliva and olive

**Table I.** The effects of coital lubricants at concentrations of 6.25% on sperm motility parameters

Parameters	Time after addition of lubricant (min)	Control	Baby oil	Olive oil	KY jelly
Progressive motility (%)	5	39 ± 5.3	35 ± 6.4	32 ± 4.2 <sup>d</sup>	36 ± 4.2
	15	38 ± 5.5	34 ± 7.1	25 ± 6.25 <sup>b</sup>	34 ± 6.7
	30	28 ± 6.6	32 ± 7.2	28 ± 7.1	27 ± 6.1
VCL (µm/s)	5	62 ± 5.2	60 ± 5.7	50 ± 7.9 <sup>b</sup>	45 ± 5.3 <sup>b</sup>
	15	62 ± 5.4	63 ± 5.6	52 ± 7.8 <sup>b</sup>	48 ± 5.3 <sup>c</sup>
	30	63 ± 4.1	62 ± 7.2	61 ± 5.34 <sup>b</sup>	46 ± 6.4 <sup>d</sup>
VSL (µm/s)	5	40 ± 4.1	41 ± 6.1	38 ± 7.0 <sup>b</sup>	34 ± 5.0 <sup>a</sup>
	15	43 ± 6.1	41 ± 5.2	39 ± 7.1 <sup>a</sup>	37 ± 5.1 <sup>a</sup>
	30	39 ± 5.3	39 ± 6.6	38 ± 7.0	33 ± 4.7
ALH (µm)	5	3.9 ± 0.54	4.0 ± 0.50	3.8 ± 0.53	2.6 ± 0.96 <sup>c</sup>
	15	3.9 ± 0.55	4.3 ± 0.50	3.6 ± 0.86	2.6 ± 1.18 <sup>d</sup>
	30	4.0 ± 0.51	4.0 ± 0.75	3.9 ± 0.48	2.7 ± 1.11 <sup>c</sup>
BCF (Hz)	5	17.3 ± 0.59	17.6 ± 1.0	16.5 ± 1.73	14.9 ± 1.696 <sup>c</sup>
	15	17.4 ± 1.00	17.2 ± 1.32	17.0 ± 1.70	15.6 ± 1.70 <sup>b</sup>
	30	17.2 ± 1.40	16.7 ± 0.62	16.4 ± 2.03	15.7 ± 1.73

Values are means ± SD.

Statistical comparisons are made between control and treated group at the same time points: <sup>a</sup>*P* < 0.05;

<sup>b</sup>*P* < 0.01; <sup>c</sup>*P* < 0.001; <sup>d</sup>*P* < 0.0001.

ALH = amplitude of lateral head displacement; BCF = beat cross frequency; VCL = curvilinear velocity;

VSL = progressive velocity.

**Table II.** Osmolality and viscosity measurements for each lubricant solution

Lubricant solution	Osmolality (mosmol)		Viscosity
	Before addition of spermatozoa	After addition of spermatozoa	
BWW	295 ± 0.5	296 ± 3.8	1.00
Saliva	259 ± 0.9	274 ± 7.6 <sup>a</sup>	1.39
Olive oil	298 ± 8.8	298 ± 4.9	1.23
Baby oil	422 ± 4.1	306 ± 4.9 <sup>b</sup>	3.09
KY jelly	530 ± 2.5	600 ± 3.7 <sup>b</sup>	5.19

Values are the means ± SD of five samples tested for each solution before and after the addition of spermatozoa (mosmol). Viscosity values are ratios compared with BWW media.

<sup>a</sup>*P* < 0.01; <sup>b</sup>*P* < 0.001.

BWW = Biggers Whitten Whittingham.

oil had highly significant effects, although lower viscosity and osmolality.

Sperm motility alters from day to day and can be affected by many factors, including stress (Takefman *et al.*, 1990). Over the past 20 years, a decline in the number and motility of spermatozoa has been reported by a number of centres (Carlsen *et al.*, 1992; Skakkebaek and Keiding, 1994; Auger *et al.*, 1995). For these men, this may be associated with a possible decline in male fertility and the added insult of a coital lubricant may render their problem pathological. It is also known that stress is associated with infertility; however, it cannot always be distinguished whether the stress results from, or has a causal influence on, the infertility (Christie, 1998). 'Timed intercourse' may lead to further stress and result in coital dysfunction. Both of these can, in turn, lead to increased use of coital lubricants. Hence, in this study, spermatozoa from male partners of couples undergoing fertility investigations was used rather than that from donors of proven fertility, as they are more typical of the couples likely to be using coital lubricants.

Clearly, in a laboratory setting it is difficult to mimic coital

conditions *in vivo*. However, in this study we have attempted to do this by maintaining samples and lubricants at body temperature, diluting semen samples with biological buffer to mimic the dilutions which occur in the vagina, and constant mixing to expose all spermatozoa to lubricants throughout the analysis. Furthermore, aliquots of semen were sampled after preparation in a discontinuous Percoll gradient. This allows analysis of the 'fittest' or 'vanguard' spermatozoa. *In vivo*, the vanguard spermatozoa leave the coagulum of the ejaculate and can reach the oviduct within 5 min (Settlage *et al.*, 1972). Therefore, it is important to determine if even the most motile spermatozoa are affected by coital lubricant use. Once these vanguard spermatozoa have left the coagulum, the remaining spermatozoa make much slower progress. Although it is believed by some that the cervix has the capacity to store spermatozoa for up to 8–10 days post insemination, most of the less motile spermatozoa will be gradually phagocytosed. After 30 min, most of the seminal fluid and approximately 35% of the sperm ejaculate will have been discarded in backflow (Baker and Bellis, 1994).

The concentrations of lubricants used in this study were lower than those used in other trials, with the exception of Frishman *et al.* (1992), who investigated the commercial lubricants Astroglide and KY jelly with concentrations as low as 12.5%, but not at our lower concentration of 6.25%. Such minute concentrations were employed here in an attempt to attain the lowest concentration of lubricant that was sufficient to lubricate, but low enough not to hinder sperm motility. However, since even concentrations as low as 6.25% appear to reduce sperm motility, it is debatable whether a couple could achieve a concentration of lubricant sufficiently low as not to inhibit sperm motility *in vivo*, but which still facilitated lubrication.

It is probable that the mechanisms by which lubricants affect sperm motility vary between the different lubricants. Saliva, which has the most damaging effects, is probably the

most commonly used of all lubricants and often recommended by clinicians as a 'safe choice'. However, we have shown that saliva was the most toxic lubricant tested, the lag period of 15 min before its full effect suggesting perhaps that the toxic activity might be enzymatic, rather than mechanical in nature. Interestingly, in the past amylase was used by some andrology laboratories to liquefy viscous semen samples, but with no knowledge that it might impair sperm motility. Dougherty *et al.* (1978) showed previously that salivary amylase was deleterious only after 30 min and at higher concentrations (5 mg/0.5 ml), but noted specifically that it was not toxic at low concentrations. In contrast, in the present study we observed damaging effects with saliva within a much shorter time span (5 min) and at lower concentrations (0.32 mg/0.5 ml) of amylase. Antimicrobial activity or disruption of the disulphide bonds which are essential for sperm tail architecture (Bedford and Calvin, 1974) may be alternative mechanisms for the inhibitory effects of saliva on sperm motility.

KY jelly is known to be detrimental to sperm motility (Tagatz *et al.*, 1972) and appears specifically to dampen the lateral head movements and curvilinear velocity. It is made up of water and glycerine, which may contribute to its damaging properties as glycerine has been shown to dissolve the flagellar membrane on sperm tails (Amelar *et al.*, 1980). Thus, although glycerine leaves the 9+2 axoneme intact, sperm movement becomes highly inefficient because of a loss of optimal concentrations of ATP and other essential ions around the axoneme of the sperm tail which are crucial for sperm motility. Since the osmolality of KY jelly is higher than that of the other lubricants, it may be the changes in osmolality which have led to damage of tail membranes. In addition, the highly viscous nature of KY jelly may also contribute particularly to the damping effect on lateral head movement, as this will require a much greater effort by the spermatozoa to move through a medium of higher viscosity.

Baby oil and olive oil were incorporated into this study as they are widely available, and light oils have been recommended as less toxic to spermatozoa. Our study confirms the findings of Goldsberg and Whiter (1975), who suggested that light oils had minimal detrimental effect on sperm motility. Baby oil caused only small reductions in sperm motility parameters. In contrast, we have shown that while less toxic than KY jelly or saliva, olive oil still had significant damaging effects on spermatozoa. Kutleh *et al.* (1996) found that the light vegetable oil, canola oil, did not inhibit sperm motility, whereas the more dense olive oil had detrimental effects.

The mechanism by which olive oil exerts its toxicity is unknown, but considering that olive oil is closer in osmolality to control than baby oil, and exhibits almost no change in osmolality after the addition of spermatozoa, olive oil should be less toxic than baby oil. However, baby oil—which has the least detrimental effect on sperm motility—shows a significant change in osmolality on addition of spermatozoa and also is three times more viscous than both olive oil and the control. Baby oil also appears to contain paraffin as its major constituent. Although, to our knowledge, no studies have been made on the effects of paraffin on sperm motility parameters, one might have presupposed a toxic nature based on the known

toxicity of Vaseline (Amelar *et al.*, 1980), another petroleum product. The reasons for this and the differences between the effects of baby oil and olive oil require further research. Such studies would need to focus on the constituent ingredients of both oils and their respective properties in an attempt to establish spermicidal ingredients and, potentially, to create a non-spermicidal coital lubricant.

This study was initially driven by the observation that practising clinicians are recommending coital lubricants for some couples undergoing fertility investigations. In fact, saliva or olive oil were both considered 'safer' lubricants than commercial lubricants which are known to be toxic to spermatozoa. From the results of this study, this is not the case. Since damaging effects to sperm motility are induced by these lubricants, even at minute concentrations, we would recommend that couples—especially those having difficulty in conceiving—should be aware of the detrimental effects of such lubricants and avoid their use.

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