J Assist Reprod Genet (2014) 31:333–339
DOI 10.1007/s10815-013-0168-x

GAMETE BIOLOGY

The effects of vaginal lubricants on sperm function: an in vitro analysis

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Received: 10 October 2013 / Accepted: 26 December 2013 / Published online: 5 January 2014
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Abstract
Background Despite being marketed as "sperm friendly", some vaginal lubricants are known to be detrimental to sperm function and therefore could negatively affect fertility. Many others have not yet been assessed in regards to their effect on sperm function. This issue may concern couples trying to conceive both naturally and via artificial reproductive technologies (ART).
Aim The aim of this research was to analyse the effects that lubricants, commonly used in the setting of natural conception and ART, have on sperm function in an in vitro setting. This was done by assessing sperm motility, vitality and DNA fragmentation following treatment with commercial lubricants or control preparations. We have attempted to mimic the conditions of the vaginal environment in our clinical trial, and so have compiled a list of lubricants that are likely to have minimal negative effect on sperm function in vivo or are "sperm friendly".
Methods Ten samples were obtained for the study from patients attending a fertility clinic. Once collected, the sperm samples were prepared by density gradient centrifugation and incubated with 11 different lubricants including positive and negative controls for 30 min at 37 °C to mimic the temperature inside the female reproductive tract. Sperm motility, vitality and DNA fragmentation were assessed to determine the effects of the lubricants on sperm function and DNA integrity.
Results Nine lubricants were investigated including Sylk™, Conceive Plus®, glycerol, Johnson's® Baby Oil, SAGE® Culture Oil, Yes®, Forelife™, MaybeBaby® and Pre-seed®. The lubricant which had the best results in terms of vitality, at 92 %, was Pre-seed® and the worst was Forelife™ with 28 % vitality. In terms of motility, Pre-seed® resulted in the highest percentage of spermatozoa with progressive motility at 86 % and Sylk™ resulted in the lowest percentage of progressively motile cells in the sample with 31 % of sperm progressively motile. There were no significant effects on DNA integrity.
Conclusions Pre-seed® was the lubricant which had the least negative effect on sperm function, with Conceive Plus® a close second, due to the significantly higher sperm motility and vitality parameters measured following lubricant exposure.

Keywords Vaginal lubricants · Sperm function · Fertility

Capsule The aim of this research was to analyse the effects of common lubricants on sperm function in an in vitro setting. This was done by assessing sperm motility, vitality and DNA fragmentation once sperm was exposed to each of the nine lubricants and the positive and negative controls. The lubricant which had the best results in terms of vitality, at 92 %, was Pre-seed® and the worst was Forelife™ with 28 % vitality. In terms of motility, Pre-seed® resulted in the highest percentage of spermatozoa with progressive motility at 86 % and Sylk™ resulted in the lowest percentage of progressively motile cells in the sample with 31 % of sperm progressively motile. There were no significant effects on DNA integrity.

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Introduction

A lack of vaginal lubrication can be a contributing factor to dyspareunia, which has been shown to decrease chances of successful conception [1]. Young women have high rates of dyspareunia due to vaginal dryness, with up to 25 % of couples using vaginal lubricants [2, 3]. Women trying to conceive are twice as likely to suffer from dyspareunia than couples that are not trying to conceive, possibly in part due to the stress associated with timed intercourse [1].

Vaginal lubricants are used to help overcome this problem and are also used by some clinics for sperm collection for the purposes of ART. There are many lubricants, with varying compositions, marketed around the world. Despite being marketed as "sperm friendly", some have been shown to be detrimental to sperm function [4, 5], which may decrease the chance of successful conception [2]. Ellington et al. [1] found that 40 % of women affected by vaginal dryness choose KY Jelly®, which has been shown to have detrimental effects on sperm function [1, 4–6]. Many of the other available products, including Conceive Plus®, Forelife™, Yes®, Sylk™ and Maybe Baby®, have not been previously studied. Therefore, recommending lubricants to couples trying to conceive is difficult.

The aim of this research was to analyse the effects that common lubricants have on sperm function in an in vitro setting. This was done by assessing sperm motility, vitality and DNA fragmentation following treatment with commercial lubricants or control preparations. We have attempted to mimic the conditions of the vaginal environment in our clinical trial, and so have compiled a list of lubricants that are likely to have minimal negative effect on sperm function in vivo or are "sperm friendly". The majority of the lubricants investigated in this study have not been previously examined and include alternative preparations not acknowledged as commercial lubricants such as glycerol, Johnson's® Baby Oil and SAGE® Culture Oil.

Motility and vitality are the major semen parameters characterised when assessing male fertility. In this experiment, motility was analysed as it is the most important predictor of sperm transport and subsequent fertilisation [7, 8]. Vitality was also measured to determine whether lubricant exposure is toxic to sperm cells. Sperm DNA damage does not appear to affect fertilization though it has been associated with abnormal embryo development, failed implantation and miscarriage [9, 10]. Exposure to various environmental toxins, and illicit and pharmaceutical drugs can induce the formation of reactive oxygen species, which can in turn cause oxidative stress, which is known to cause DNA damage [10, 11]. Therefore, this study also investigated whether the selected lubricants have the potential to act as toxins capable of inducing DNA fragmentation.

Methods

The present study was based on the work previously reported by Agarwal et al. [4], with several modifications. Prospective participants were recruited through Assisted Conception Australia (ACA) situated at Greenslopes Private Hospital, Brisbane, Australia. New patients who attended ACA between July 2012 and September 2012 were invited to participate in the study. All male patients were undergoing seminal fluid analysis as part of the investigation of infertility. Semen samples were collected after an average of 3 days of abstinence by masturbation into a sterile container. Routine semen analysis was performed on all samples within 0.5 to 2 h of production to determine baseline parameters.

Ten samples were obtained for the study that included a combination of normal and below normal vitality (average of 89.90 %±14.93 SD live cells) and motility (average of 57.20 % ±13.82 SD progressive motility) with the majority of samples exhibiting teratozoospermia (average of 3.65 %±4.06 SD normal forms) [12]. The only samples that were excluded from the study were those with insufficient concentrations to complete all experiments. All experiments were completed in triplicate. The study was approved by the Human Research and Ethics Committee of Greenslopes Private Hospital and all patients gave informed written consent.

Sample and lubricant preparation

All samples were prepared using a PureCeption™ 40 %/80 % discontinuous density gradient (Gytech, Melbourne, Australia) within 2 h of ejaculation to select the most viable sperm fraction, which represents the fraction of spermatozoa that would reach the oocyte in vivo through the natural selection processes that occur [12]. The resulting motile sperm fractions were diluted to a working concentration of 25×10^6 ml with Quinn's Advantage Hepes Media® (Gytech) supplemented with 5 % Human Serum Albumin (HSA; Gytech). This concentration was chosen as an effective working concentration; a higher or lower working concentration would reduce the accuracy due to the difficultly in counting the number of spermatozoa in the field of view if too low or high. Each lubricant was diluted and mixed thoroughly with Quinn's Advantage Hepes Media® supplemented with 5 % HSA to a 10 % (v/v) concentration, which is consistent with the concentration used in previous studies and is thought to approximate the concentration of lubricant within the ejaculated semen following intercourse [13]. The diluted motile sperm fractions were mixed in a 1:1 ratio with equivalent concentrations of each diluted lubricant and incubated for 30 min at 37 °C to mimic the temperature of the female reproductive tract.

Motility analysis

Motility was analysed following lubricant exposure to determine the effect of lubricants on sperm motility. Motility was graded in four categories: fast progressive motility (A), slow progressive motility (B), non-progressive motility (C) and immotile spermatozoa (D) [14]. For ease of interpretation, the results were analysed in terms of total progressive motility (A + B).

Vitality analysis

Sperm vitality was measured in terms of the percentage of live cells after exposure to each lubricant. Eosin stain (5 g/l of eosin Y and 9 g/L of NaCl; Sigma-Aldrich, Sydney, Australia) was utilised to differentiate between live and dead cells [13], with live cells excluding the stain and remaining white while dead cells were stained red.

SCSA

A sperm chromatin structure assay (SCSA) was used to investigate DNA fragmentation to determine whether lubricant exposure increases DNA damage. Treated motile sperm fraction aliquots were batch frozen in accordance with the protocol used by Evenson and Jost [15] prior to completing SCSA. Cells were treated with an acid-detergent solution, which induces further DNA denaturation at already damaged DNA sites stained by fluorescent dye acridine orange (BioScientific, Sydney, Australia). Samples were prepared following the protocol detailed by Evenson and Jost [15] with the inclusion of the modifications detailed by Boe-Hansen et al. [16]. Boe-Hansen et al. modified the method initially described by Evenson and Jost [15] by incubating the samples on ice for 5 min post-thaw based on their previous observations that variation in the SCSA measures within samples was significantly larger when they were analysed immediately after thawing. It is thought that the post-thaw incubation time allows for the spermatozoa to adapt to the rapid temperature changes allowing for more accurate SCSA measurements to be taken.

Samples were analysed using a FACSCanto II flow cytometer (Becton Dickinson, Sydney, Australia) with the blue laser operating at 488 nm at 20 mW of power. Acridine orange intercalates double stranded DNA and emits green fluorescence at 530±30 nm. Single stranded DNA that is intercalated with acridine orange emits red fluorescence at >630 nm. DNA damage was quantified by measurements taken from the metachromatic shift from green fluorescence conveying native DNA to red fluorescence conveying damaged DNA. These measurements were displayed as red vs. green fluorescence intensity cytograms, which were gated to differentiate between cell populations to identify the percentage of DNA damaged cells [15].

Statistical analysis

All statistics were performed using IBM SPSS version 20 software package. The variance of lubricant effects on motility, vitality and DNA fragmentation were analysed by a one-way ANOVA with any outliers excluded from statistical analysis. ANOVA was followed by post-hoc Tukey analysis to determine variance between groups. In the case of unequal variances, determined by the test of homogeneity of variances, Tunnett T3 post-hoc analysis was used. If both ANOVA assumptions were violated, Kruskal-Wallis was performed.

Results

The nine lubricants investigated were Sylk™, Conceive Plus®, glycerol, Johnson's® Baby Oil, SAGE® Culture Oil, Yes®, Forelife™, MaybeBaby® and Pre-seed®. K-Y Jelly® has been reported to have detrimental effects on sperm function and so was used as the positive control [4–6]. Sperm wash media (Quinn's Advantage Hepes Media® supplemented with 5 % Human Serum Albumin), which is used routinely in the laboratory for semen preparation, was used as the negative control. KY Jelly® and Glycerol were excluded from vitality analysis due to an interaction between the lubricants and eosin stain causing the cells to fluoresce, which has not been previously reported. Due to this phenomenon, the red (dead) and white (alive) cells were unable to be differentiated.

The study revealed significant differences in vitality following exposure to different preparations, ranging from 27 to 91 % live spermatozoa, as illustrated in Fig. 1. Sperm vitality following exposure to Pre-seed® was significantly higher than all other lubricants at over 90 %. Vitality of sperm treated with Conceive Plus® was over 70 % which was significantly higher than the vitality of sperm treated with Yes®, Sylk™, SAGE® Culture Oil, Johnson's Baby Oil®, Media and Maybe Baby® which were all significantly similar at between 50 and 65 %. Sperm treated with Forelife™ had the lowest measured vitality assessments at 27 %.. The positive control was also significantly lower (53 %) than both Pre-seed® and Conceive Plus®, suggesting that it decreases sperm vitality. It unknown how the negative control, KY Jelly® compares since it was excluded from this analysis.

Sperm motility was also significantly affected by exposure to different lubricants, as illustrated in Fig. 2. Exposure to Pre-seed® resulted in a significantly higher percentage of spermatozoa with progressive motility (over 85 %) compared with all other lubricants, whereas Sylk™ and Forelife™ resulted in the lowest percentage of progressively motile cells in the sample at 31 % and 47 % respectively. The positive control had significantly higher progressive motility than Yes®, Forelife™ and

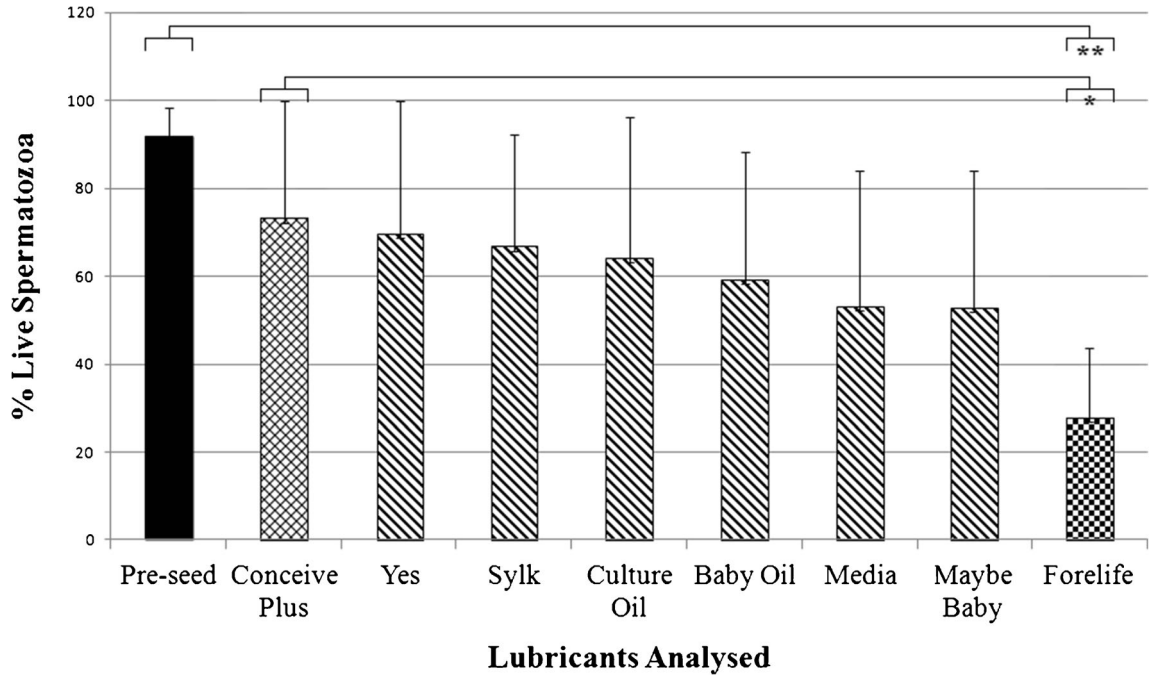

Fig. 1 Exposure to different lubricants significantly affects sperm vitality. The lubricants have been ranked in order of means (+/− SD) and colour coded in groups to visualise significant differences (* $p<0.05$; ** $p<0.001$). KY Jelly and Glycerol were excluded from the vitality analysis, see text)

Sylk™, while the negative control, KY Jelly®, had significantly lower motility than Pre-seed®.

The proportion of DNA fragmentation of sperm following exposure to different lubricants, as analysed by SCSA, did not differ significantly (Fig. 3). The highest percentage of DNA damaged cells was measured following exposure to Pre-seed® and the lowest following exposure to media (control), however these results were not significant ($p = 0.729$, ANOVA).

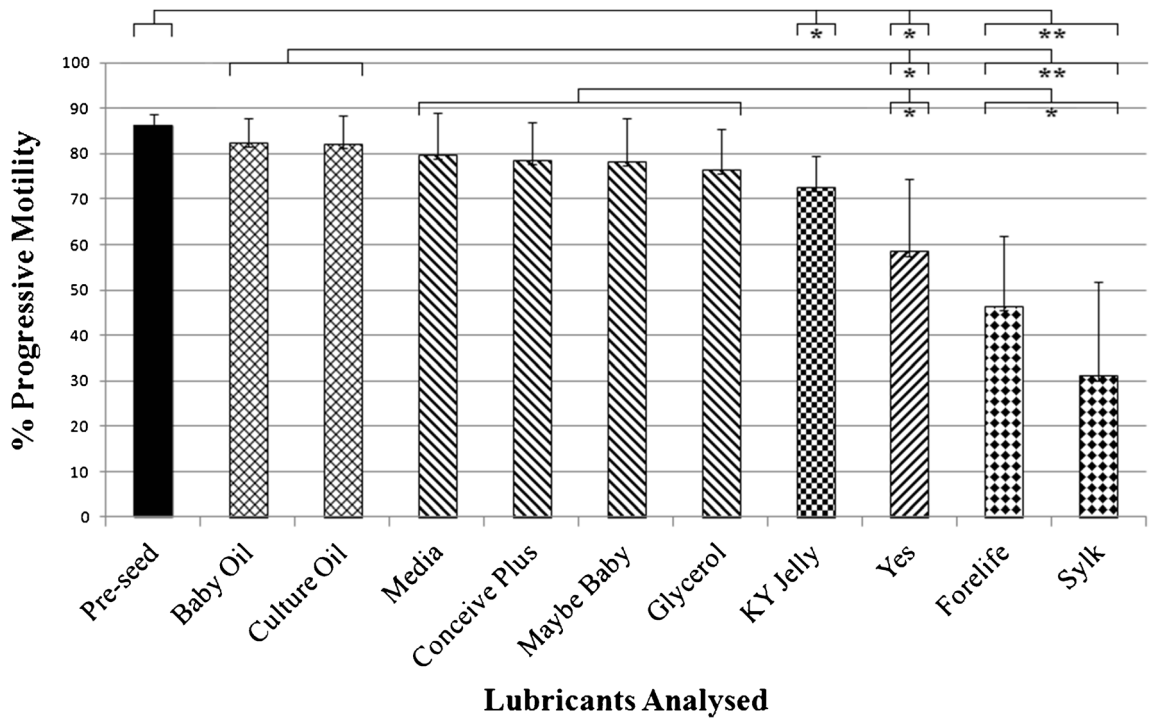

Fig. 2 Exposure to different lubricants significantly affects sperm motility. The lubricants have been ranked in order of means (+/− SD) to visualise significant differences (* $p<0.05$; ** $p<0.001$)

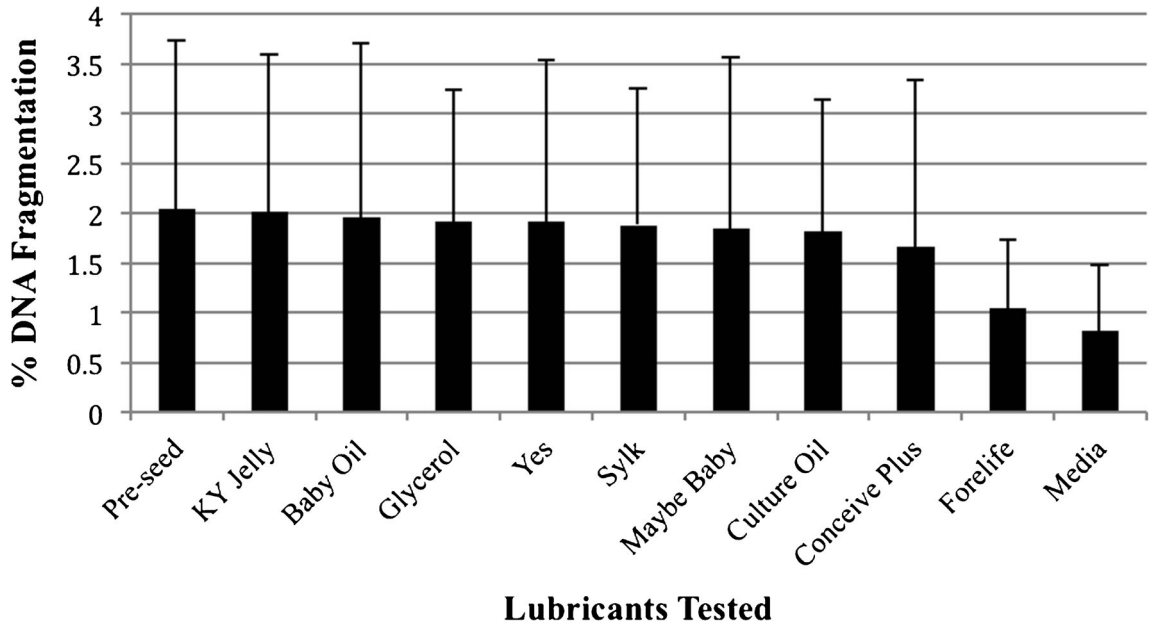

Fig. 3 Sperm DNA fragmentation is not significantly affected by exposure to different lubricants. The lubricants have been ranked in order of means (+/− SD), however no significant differences were obtained (p=0.729)

Discussion

The most clinically relevant results obtained from the study were those regarding motility as motile spermatozoa are able to fertilise oocytes whereas immotile but viable spermatozoa are not. Spermatozoa treated with Pre-seed® showed significantly higher progressive motility than all other lubricants at over 85 %. Spermatozoa treated with Johnson's® Baby Oil and SAGE® Culture Oil showed the second highest progressive motility at just over 80 % and this was significantly better than the other lubricants (apart from Pre-seed®). These results are consistent with Agarwal et al. [4, 13] who reported that Pre-seed® and Johnson's® Baby Oil did not significantly reduce progressive motility when compared to controls. In contrast, spermatozoa treated with Yes®, Sylk™ and Forelife™ had significantly lower progressive motility than the other lubricants, including KY Jelly®.

A clear difference in vitality of sperm exposed to different lubricants was also evident, with spermatozoa treated with Pre-seed® and having a significantly higher percentage of live cells compared with other lubricants, closely followed by sperm treated with Conceive Plus®. In contrast, spermatozoa treated with Forelife™ showed the lowest proportion of live cells. Due to technical difficulties with the vitality stain (reading positive results was particularly subjective), these results were less accurate than the motility results. There are no previous studies specifically looking at the effects of lubricants on sperm vitality. Pre-seed® has the least effect on sperm motility and vitality and also has a simple composition, consisting mostly of purified water and hydroxyethylcellulose (Table 1).

The lubricants that negatively affect sperm function have an extensive list of constituents and consist of mostly vegetable gums and fruit extracts. As there is controversy regarding the inclusion of many components of modern lubricants including surfactants such as pluronic, antioxidants such as arabinogalactan, acid polymers such as carbomer, ionic supplements as well as natural and organic ingredients, there is a need for further studies of individual constituents and their effect on sperm function. It would therefore be beneficial to isolate these factors and repeat the study to determine which specific components have a deleterious effect on sperm function. This study also suggests that further studies are needed to investigate the inclusion of glycerol in lubricants as Pre-seed®, which does not contain glycerol, appears to be the lubricant which has the least negative effects on sperm function while Conceive Plus®, which includes glycerol, appears to be a close second. There are conflicting reports regarding the effect of both glycerol and the pH of lubricants on sperm function in the literature [17–19]. Unfortunately in our study we did not control for varying lubricant compositions when analyzing the data for pH and so the results were not accurate. It would be beneficial to conduct further studies into the pH of commercial lubricants as there is much debate regarding the correct pH range to use.

Agarwal et al. [4, 13] reported that Pre-seed® had minimal negative effects on DNA integrity but did not significantly differ from the control, while KY Jelly® significantly decreased DNA integrity. This was not found to be the case in our study where exposure to KY Jelly® did not increase DNA fragmentation. This may be due to the acquisition of such small changes in the percentages of DNA fragmentation following exposure to the different preparations, making a

Table 1 List of ingredients and pHs

Lubricant	pH	Composition
Pre-seed®	7.29	Water, Hydroxyethylcellulose, Pluronic, Sodium Chloride, Arabinogalactan, Sodium Phosphate, Potassium Phosphate, Carbomer, Methylparaben, Sodium Hydroxide
Johnson's Baby Oil®	7.26	Mineral Oil, Fragrance
SAGE® Culture Oil	7.40	Parafin Oil, Mineral Oil
Media	7.40	Sodium Chloride, Potassium Chloride, Magnesium Sulfate, Potassium Phosphate, Calcium Lactate, Sodium bicarbonate, HEPES, Glucose, Sodium Pyruvate, Alanyl-glutamine, Taurine, L-Asparagine, L-Aspartic Acid, Glycine, L-Proline, L-Serine, Sodium Citrate, EDTA, Gentamicin, Phenol Red
Conceive Plus®	7.26	Water, Hydroxypropylmethylcellulose, Sodium Chloride, Glycerol, Sodium Phosphate, Disodium Phosphate, Methylparaben, Potassium Chloride, Magnesium Chloride, Calcium Chloride
Maybe Baby®	7.34	Water, Hydroxyethylcellulose, Arabinogalactan, Disodium Hydrogen Phosphate, Sodium Chloride, Potassium Phosphate, Methylparaben, Propylparaben
KY Jelly®	7.34	Water, Glycerin, Hydroxyethylcellulose, Chlorhexidine, Gluconate, Gluconolactone, Methylparaben, Sodium Hydroxide
Yes®	7.16	Water, Aloe Vera, Guargum, Locust Bean Gum, Flax Extract, Phenoxyethanol, Potassium Sorbate, Xanthan Gum, Citric Acid
Forelife	7.35	Water, Carmellose Sodium, Glycerol, Propylene glycol, Sclerotium Gum, Sodium Methylhydroxybenzoate, Sodium Phosphate, Sodium Propylhydroxybenzoate
Sylk™	6.9	Water, Kiwifruit Vine Extract, Vegetable Glycerine, Xanthan Plant Extract, Potassium Sorbate, Sodium Citrate, Citric Acid, Grapefruit Seed Extract
Glycerol	7.26	Glycerol

significant difference between lubricants difficult to ascertain. However Agarwal et al. compared DNA fragmentation in whole ejaculate samples using SCSA, which might not accurately reflect the DNA status of the motile sperm fraction that would ultimately go on to fertilise the oocyte [20]. This suggests that the result obtained in our study, that the lubricants investigated do *not* increase DNA fragmentation, are more clinically relevant. However the number of samples included in the current study was relatively small and it is possible that a larger sample size might reveal small increases in DNA fragmentation following exposure to different lubricants as well as giving further information on the effect of lubricants on semen with normal and abnormal parameters. Despite the small sample size statistically significant results have been obtained regarding sperm motility and vitality enabling some clinical recommendations to be made.

In conclusion, this study identifies lubricants that have minimal negative effects on sperm function in vitro. Further research is needed to confirm that these results can be applied to the in vivo scenarios of couples trying to conceive. Table 2 summarises which lubricants, based on the results of this in vitro clinical trial, are best avoided when attempting to conceive.

Table 2 "Sperm friendly" lubricants

Lubricant	Vitality	Motility	Sperm friendly
Baby oil	(Medium 40–60 %)	(Good >70 %)	
Conceive plus	(Good >60 %)	(Good >70 %)	Sperm friendly
Culture oil	(Good >60 %)	(Good >70 %)	Sperm friendly
Forelife	(Poor <40 %)	(Poor <50 %)	Avoid
Glycerol	Excluded	(Good >70 %)	
KY jelly	Excluded	(Good >70 %)	
Maybe baby	(Medium 40–60 %)	(Good >70 %)	
Media	(Medium 40–60 %)	(Good >70 %)	
Pre-seed	(Good >60 %)	(Good >70 %)	Sperm friendly
Sylk	(Good >60 %)	(Poor <50 %)	Avoid
Yes	(Good >60 %)	(Medium 50–70 %)	

Each lubricant has been rated as Good, Medium or Poor following vitality and motility analysis to determine which lubricants are "Sperm Friendly"

Conflict of interest The authors declare that they have no conflict of interest.

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Contributions

Alex Mowat: wrote the article

Cora Newton: performed literature review, carried out laboratory trial and contributed to writing of article

Dr Clare Boothroyd: designed and supervised the laboratory trial, recruited patients, reviewed the written article

Dr Kristy Demmers: Co-supervised the laboratory trial, reviewed the written article

Dr Steven Fleming: Co-supervised the laboratory trial, reviewed the written article