Effect of an isotonic lubricant on sperm collection and sperm quality

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Objective: To assess the influence of an isotonic lubricant used during sperm sample collection on [1] ease of collection and [2] resultant sperm quality.
Design: Paired randomized cross-over design.
Setting: Tertiary hospital.
Donor(s): Healthy men over 18 years old with normal semen analysis as per World Health Organization 2010 guidelines.
Intervention(s): Collection of semen sample from 22 subjects by masturbation with or without the use of Pre-Seed personal lubricant.
Main Outcome Measure(s): Qualitative survey results and quantitative sperm function outcomes were measured to determine resultant sperm quality and collection experience with and without Pre-Seed lubricant.
Result(s): The qualitative questionnaire results showed that 73% of donors prefer the semen collection process with the isotonic lubricant and 55% recommended the use of lubricant in their everyday collection. The motility, viability, membrane integrity, levels of reactive oxygen species, total antioxidant capacity, and percentage of DNA damage in collected semen samples were not affected by the use of the lubricant.
Conclusion(s): More donors prefer, and find it easier, to collect semen samples with the use of the lubricant. The isotonic lubricant Pre-Seed did not compromise sperm quality as evaluated in an array of sperm assays, suggesting its safe use in fertility patients as required during sperm collection. (Fertil Steril® 2013;99:1581–6. ©2013 by American Society for Reproductive Medicine.)
Key Words: Vaginal lubricants, personal lubricant, semen collection, sperm quality, trying to conceive, sperm motility, semen collection
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Vaginal lubricants are used worldwide to decrease vaginal dryness and pain during intercourse. In particular, women trying to conceive (TTC) may have increased intimate dryness from the stress associated with timed intercourse during ovulation (1–3). In fact, 25% of these women report "always" using a lubricant during intimacy (3). Additionally, many TTC couples experience stress around semen/sperm specimen collection during fertility diagnostic and therapeutic interventions. This can result in difficulty or inability to collect a sample by masturbation from the man, especially in hospital or clinic settings (4, 5), where it is not uncommon for men to request a lubricant to facilitate the collection process. However, common lubricants have been reported to be sperm toxic and are contraindicated for semen collection (6–9). The impact of lubricant use on ease of collection and quantitative sperm function outcomes has not been studied.

Even though common lubricants are often labeled as "nonspermicidal," this does not mean they do not impair sperm function and are safe to use by couples who are trying to conceive or during fertility procedures (10). Numerous studies over several decades have consistently shown a sperm toxic effect of common lubricants, even those that are spermicidal agent–free, such as Astroglide (BioFilm Inc.; Replens(Lil'Drug Store Products, Inc.), Surgi-Lube (Savae Laboratories), and K-Y Jelly (Johnson & Johnson Inc.) (2, 6–9). Even human saliva has been reported to harm sperm (8, 11). These data report significant disruptions in sperm motility and viability, which occurs within minutes of direct contact between sperm and the lubricant or saliva. The negative effect of these products is such that sperm function and even fertility could be compromised when these products are used during natural reproduction (1, 3, 7, 8, 12).

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In contrast, recent data show that an isotonic lubricant (Pre-Seed -INGfertility) has minimal impact on sperm motility and chromatin quality (2, 10), suggesting this lubricant's potential value for fertility patients. The goal of this paired cross-over design was to evaluate the effect of using an isotonic lubricant, Pre-Seed, during the sperm collection process on the ease of collection and resulting sperm function.

MATERIALS AND METHODS

After approval from the Institutional Review Board, semen samples were obtained from 22 healthy men of unknown fertility who met the normal semen parameters criteria according to World Health Organization (WHO) 2010 guideline (13). Each donor was randomized for his first collection to either using no lubricant (control) or using Pre-Seed lubricant (INGfertility). The composition of the Pre-Seed lubricant is essentially purified water, sodium chloride, hydroxyethylcellulose, Pluronic 127 (slippery), arabinogalactan (polysaccharide plant; antioxidant properties), sodium phosphate, Carobopol 934, methylparaben, sodium hydroxide, and potassium phosphate.

Two weeks after the first collection, each subject performed collection with the reverse treatment (e.g., collection with or without Pre-Seed), and each patient thereby served as his or her own control in the study. For control collections without lubricant, no lubricative product or saliva were used. For sperm collection using Pre-Seed, the donors were instructed to use a 4-mL single-use sachet at room temperature. This sachet was only to be applied to the hands and penis during collection. Subjects presenting normal sperm parameters but with a history of vasectomy, highly viscous samples, or noncompliance with protocol requirements were excluded from the study to prevent bias in the interpretation of the results of advanced semen tests like reactive oxygen species (ROS), total antioxidant capacity, and DNA damage.

All specimens were collected by masturbation at the Andrology Laboratory after a period of sexual abstinence of 48–72 hours. After complete liquefaction for 20 minutes at 37°C, samples were evaluated for volume, semen age, pH, sperm concentration, motility, and viability according to WHO (2010) criteria (13), that is, sperm concentration $>15 \times 10^6$ sperm/mL; motility >40%.

Measurement of Sperm Volume, Semen Age, Concentration, Motility, and Viability

The ejaculate volume was measured, and the age of the sample was calculated by recording the time of collection to the time of analysis. Five microliters of well mixed aliquot of the sample was used for manual evaluation of concentration and motility using MicroCell counting chambers (Vitrolife). Sperm was stained by Eosin Nigrosin staining (13). At least 200 spermatozoa were scored per sample by ×1,000 magnification. The percentage of dead (colored pink) and live (unstained) cells were evaluated.

Measurement of Hypoosmotic Swelling (HOS) Test

Sperm membrane functional integrity was measured by the HOS test (14). One hundred microliters of semen sample was mixed with 0.9 mL of hypoosmotic solution and incubated for 60 minutes at 37°C. After incubation, one drop of semen mixture was evaluated using MicroCell counting chambers (Vitrolife). Using a phase-contrast microscope, a total of 100 spermatozoa were scored in duplicate to calculate the percentage of spermatozoa showing swelling of the tail.

Measurement of ROS

Seminal ejaculates that had not undergone any additional processing (neat samples) were used for ROS measurement by chemiluminescence assay (15). Luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione; Sigma Chemical Co.) was used as a probe. A 100 mmoL/L stock solution of luminol was prepared in dimethyl sulfoxide. For the analysis, 10 μL of the working solution (5 mM) was added to 400 μL of the neat sperm sample. Chemiluminescence was measured for 15 minutes using a luminometer (AutolumatPlus LB 953; Berthold Technologies). Results were expressed as relative light unit (RLU)/second/10^6 sperm.

Measurement of Total Antioxidants

Total antioxidants (16) were measured in the seminal plasma using the antioxidant assay kit Cat no. 709001 (Cayman Chemical), measuring the ability of aqueous and lipid antioxidants in the seminal plasma specimens to inhibit the oxidation of the ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{\bullet+}$. Under the reaction conditions used, the antioxidants in the seminal plasma cause suppression of the absorbance at 750 nm to a degree that is proportional to their concentration. The capacity of the antioxidants in the sample to prevent ABTS oxidation was compared with that of Trolox, a water-soluble tocopherol analog, and is quantified as micromolar Trolox equivalents.

Measurement of DNA Damage

An aliquot of well liquefied seminal ejaculate was used to measure DNA damage using the terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labeling (TUNEL) assay (17). Briefly, 1–2 million spermatozoa were washed in phosphate-buffered saline and resuspended in 3.7% paraformaldehyde; at 4°C. Sperm DNA fragmentation was evaluated using a TUNEL assay with an Apo-Direct kit (Pharmingen) as described earlier (17). Positive and negative kit controls provided by the manufacturer in addition to the lab internal control specimen (specimens from donors and patients with known DNA damage) were included for each run. Spermatozoa were analyzed by flow cytometry using flow cytometer FACScan (Becton Dickinson). A total of 10,000 spermatozoa were examined for each assay at a flow rate of <100 cells/second. The excitation wavelength was 488 nm supplied by an argon laser at 15 mW. Green fluorescence (480–530 nm) was measured in the FL-1 channel, and red fluorescence (580–630 nm) in the FL-2 channel. Gating was done to exclude debris and aggregates using 90° and forward-angle light scatter. The percentage of positive cells (TUNEL-positive) was calculated on a 1,023-channel scale

using the flow cytometer software FlowJo Mac version 8.2.4 (FlowJo, LLC).

Qualitative Survey

Each donor was asked to complete the same questionnaire twice to obtain qualitative information regarding the use of the lubricant during masturbation. Each questionnaire was completed within 5 minutes of sample production both with and without use of the lubricant (Fig. 1). The qualitative survey was developed by the Andrology Laboratory and approved by the Institutional Review Board before the enrollment of the subjects.

Statistical Analysis

The main outcome measures of this study were sperm function (motility, HOS, sperm viability) and advanced sperm tests (ROS, TAC, and TUNEL). Data are expressed as mean ± SD. Student's *t* test was used to verify the significance. Based on an estimated coefficient of variation for percentage of motile sperm as low as 20% changes from basal values, we estimated that if one setting to another (test groups) provides a 50% difference between test and control samples, then that setting has at least a 90% chance of demonstrating the greater difference in an individual study. Power analysis was done, and 20 subjects per group were considered adequate.

RESULTS

Survey results of the qualitative aspects of the semen collection process showed that 73% (16 of 22) of the donors preferred to use the lubricant during the collection procedure (Fig. 1). In addition, 55% (12 of 22) indicated that they would recommend the use of lubricant in their everyday collections (Fig. 1).

When asked to rate the semen collection with and without the use of lubricant, 68% (13 of 21) of the donors graded the experience without the lubricant as being somewhat or very easy. When using the lubricant, 81% (17 of 21) donors classified the experience as somewhat or very easy (Fig. 1).

In this experiment, all patients used 4 mL of lubricant, regardless of their personal preference or needs. After having

FIGURE 1

A

Circle the lubricant terms that best apply to your experience. The lubricant was		Response to answers (n=34)	Percentage
1	Mostly the right amount	5	15
2	Mostly too little	2	6
3	Mostly too much	1	3
4	Just the right amount of slippery	8	24*
5	Not slippery enough	2	6
6	Too slippery	2	6
7	Helpful to collection	8	24*
8	Hassle for collection	6	18

B

Questionnaire Rate	Yes	%	No	%	total
Preference for use of lubricant	16	72.7	6	27.3	22
Would you recommend use of the lubricant?	12	54.6	10	45.5	22

C

Questionnaire Rate	Very Easy	%	Somewhat easy	%	Neither easy or difficult	%	Somewhat difficult	%	Very difficult	%
Ease of collection without lubricant	9	42.9	4	19	4	19	4	19	0	0
Ease of collection with lubricant	6	28.6	11	52	2	9.5	2	9.5	0	0

Qualitative assessment of user experience during sperm collection using an isotonic lubricant (Pre-Seed): (A) Term that best describes lubricant experience during the collection of the sample. (B) User preference regarding the use of lubricant. (C) Rates of overall collection experience. *Indicates the majority of the answers.

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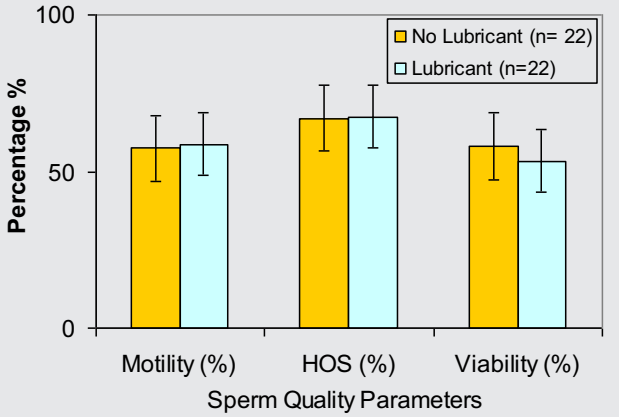
used the lubricant, 39% of the donors categorized this 4 mL dose as "Mostly" or "Just" the right amount of "slippery," and 24% considered the lubricant "Helpful to collection" as shown in Figure 1.

The results of the quantitative measures of the sperm function (motility, HOS, sperm viability) and advanced tests (ROS, TAC, and TUNEL) of the samples collected with and without the use of the Pre-Seed lubricant are shown in Table 1. The average ejaculate volume was comparable for collection without and with use of lubricant, respectively (3.25 ± 1.4 mL vs. 3.46 ± 1.42 mL). The average semen age was 40 ± 10 minutes for both groups. No significant differences were observed between sperm samples collected with and without lubricant with respect to motility, viability, and membrane integrity (Fig. 2). Furthermore, ROS, TAC, and percentage of DNA damage for sperm samples collected without lubricant versus those collected with lubricant did not differ (Fig. 3).

FIGURE 2



Effect of an isotonic lubricant (Pre-Seed) on sperm quality outcomes versus sperm in controls (no lubricant) as a percentage of motility, HOS, and vitality of samples collected with and without lubricant.

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DISCUSSION

This study is the first to our knowledge to evaluate the impact of lubricant use on qualitative and quantitative outcomes regarding sperm sample production. Sperm sample collection through masturbation for fertility diagnostic or therapeutic interventions is a commonplace procedure for medical practitioners, yet it can be a stressful and daunting occurrence for men (4, 5). The stress and tension surrounding sperm sample collection, especially in a hospital or clinical setting, may impact the quality and quantity of the sperm sample and therefore the accuracy of testing or the numbers of sperm available for therapeutic interventions (18, 19). In fact, some patients may not be able to produce a sample at all in this setting. The use of lubricant is often requested by patients before masturbation; however, previous to this study physicians had no evidence-based outcomes to draw on for recommendations for lubricant use by collecting patients. The current data suggest that the isotonic lubricant used in this study does not interfere with sperm function or quality and that it may facilitate the collection process for some men. However, in this study we did not enroll donors who indicated prior difficulty during sperm collection.

To date, previous studies evaluating sperm function after lubricant contact have used an in vitro incubation model (2, 7–10, 20). These studies found that common lubricants, even those labeled as nonspermicidal, decrease sperm motility in concentrations ±10% vol/vol In fact, several common lubricants have been found to be as toxic to sperm in vitro as contraceptive gels (9). Such decreases in sperm function after lubricant contact may be a consequence of specific ingredients in the lubricants or due to chemical properties of these products, such as extremely elevated osmolarity or low pH (2, 10). This sperm toxic aspect of many lubricants has led to their being classified as contraindicated for use in fertility patients (6–9). In contrast, Pre-Seed is an isotonic lubricant that has received Food and Drug Administration clearance for labeling as "safe to use when trying to conceive" (21).

The in vivo impact of common lubricant use in TTC couples is not known. A recent survey study found no impact of lubricant use on fecundity; however, this study did not ensure lubricant use occurred during ovulatory coitus, nor did it evaluate lubricant impact on subfertile couples, making the conclusions of the study limited (22).

The current study did not quantify the amount of lubricant present in the semen sample beyond a consistent volume of lubricant (i.e., 4 mL) being provided to each donor. Therefore, the study provided a limited understanding of a possible direct effect on sperm from the Pre-Seed at higher concentrations, as could occur in vivo. At the concentrations studied here, it appears donors can use this lubricant without altering the resultant sperm sample, further suggesting the safety of this lubricant in a fertility clinic setting (1, 2, 10). Negative controls (lubricants known to be toxic to sperm) were not used in this study as it is difficult to justify using any product in fertility patients that has been consistently shown

TABLE 1

Effect of lubricant Pre-Seed on semen parameters.

Samples	Motility (%)	HOS (%)	Viability (%)	ROS (RLU/second/10^6 sperm)	TAC (μM of trolox)	DNA damage (%)
No lubricant (n = 22)	57.3 ± 10.6	67.1 ± 14.8	58.1 ± 11.7	157.5 ± 223.6	1,936.8 ± 366.2	16.9 ± 7.3
Lubricant (n = 22)	58.6 ± 8.3	67.4 ± 10.7	53.3 ± 9.9	115.6 ± 140.9	2,112.7 ± 424	17.7 ± 8.8
P value	.8	.9	.2	.5	.2	.8

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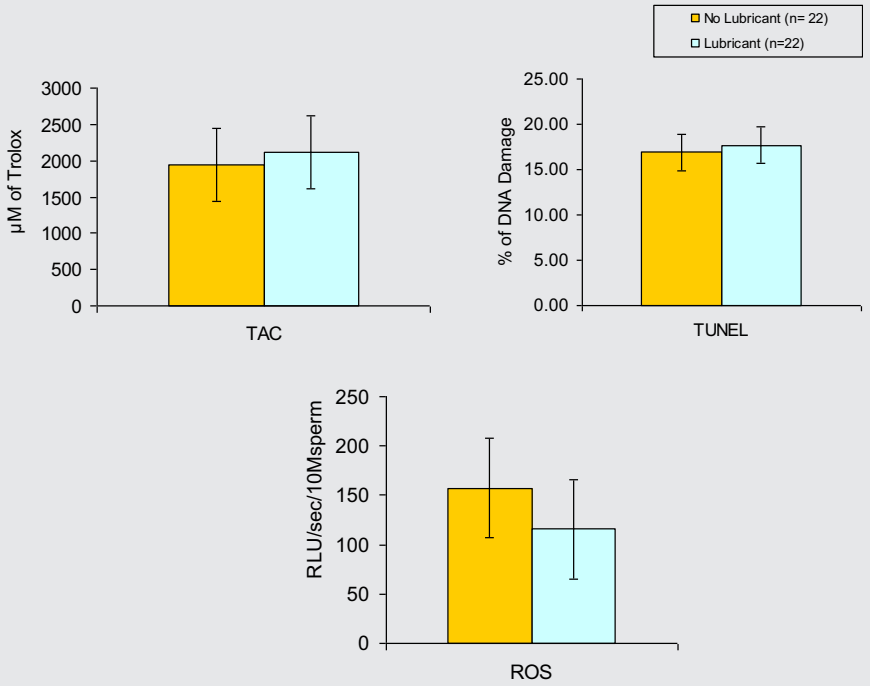
FIGURE 3



Effect of an isotonic lubricant (Pre-Seed) on sperm quality versus sperm in controls (no lubricant) as discrete outcomes for sperm ROS, TAC, and DNA damage in samples collected with and without lubricant.

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in medical studies to harm sperm or interfere with their function.

Most past publications evaluating the lubricant effect on sperm have only studied limited aspects of sperm function (e.g., vitality or motility). The current study used a robust battery of assays including sperm membrane integrity, antioxidant capacity, and DNA damage. Studies are still controversial regarding a relationship between the level of sperm DNA damage and fertility potential in a sample (21); however, suggesting that fertility patients avoid products known to damage DNA would seem prudent. Higher levels of sperm chromatin damage have been reported after common lubricant exposure (2). While we acknowledge that there is a variability in semen parameters especially in sperm count and motility, we would like to point out that the time interval was short (<2 weeks) and the abstinence period was 2–3 days as defined by the American Society for Reproductive Medicine guidelines (1). This was consistently observed among all the donors. Furthermore, in the current study, no difference in sperm membrane integrity, DNA damage, or oxidative stress was observed for samples collected with or without the isotonic lubricant, suggesting that Pre-Seed can be used as requested by donors and patients during sperm collection without negatively affecting the sample for analysis or functionality. In addition, the qualitative experience of donors using the lubricant suggested that the majority of the donors preferred lubricant use during masturbation and found it somewhat or very easy to use. Not all subjects in the study responded to all the questions in the questionnaire. Information on whether the donors had prior experience with any other lubricant during masturbation or intercourse was not recorded. Pre-Seed is reasonably priced and comparable with similar "nonspermicidal" lubricants available in the market. It does not really add to the overall cost of testing.

In conclusion, the results of this study suggest that more donors preferred collecting sperm samples by masturbation with the use of the isotonic lubricant. Furthermore, Pre-Seed lubricant did not compromise sperm quality or functional outcomes as studied, including a battery of assays (e.g., motility, viability, membrane integrity, ROS, total antioxidant capacity, and DNA damage). The use of the isotonic Pre-Seed lubricant caused no detrimental impact on sperm quality and appeared safe for use as needed during sperm sample collection in fertility patients.

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