Effect of vaginal lubricants on sperm motility and chromatin integrity: a prospective comparative study

Ashok Agarwal, Ph.D.,[a] *Fnu Deepinder, M.D.,*[a] *Marcello Cocuzza, M.D.,*[a] *Robert A. Short, Ph.D.,*[b] *and Donald P. Evenson, Ph.D.*[c]

[a] Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics-Gynecology, Cleveland Clinic, Cleveland, Ohio; [b] Washington State University, Spokane, Washington; and [c] South Dakota State University, Brookings, South Dakota

Objective: To evaluate the effect of vaginal lubricants Pre∼Seed, FemGlide, Astroglide, and Replens on human sperm motility and chromatin integrity.
Design: Prospective, comparative, in vitro study.
Setting: Andrology laboratory at tertiary care hospital.
Patient(s): Thirteen normozoospermic donors.
Intervention(s): Semen samples from 13 subjects were incubated in human tubal fluid media (HTF) controls and 10% (vol/vol) of Pre∼Seed, FemGlide, Astroglide, and Replens lubricants. After 30 minutes, progressive sperm motility was assessed by light microscopy. Semen samples of 12 patients were placed in positive control (HTF), negative control (10% K-Y Jelly lubricant), and 10% vol/vol Pre∼Seed and FemGlide lubricants. After 4 hours culture, spermatozoa were analyzed for percent DNA fragmentation index with use of the acridine orange–based sperm chromatin structure assay.
Main Outcome Measure(s): Sperm motility and percent DNA fragmentation index.
Results: Percent motility did not differ significantly between HTF controls and Pre∼Seed, whereas FemGlide, Replens, and Astroglide lubricants demonstrated a significant decrease in motility. There was no significant difference in percent DNA fragmentation index between the HTF controls and Pre∼Seed, but a significant decline in sperm chromatin quality occurred with FemGlide and K-Y Jelly.
Conclusion: Pre∼Seed does not cause a significant decrease in progressive sperm motility or chromatin integrity in contrast to other lubricants used by couples. (Fertil Steril® 2008;89:375–9. ©2008 by American Society for Reproductive Medicine.)

Key Words: Vaginal lubricants, sperm motility, chromatin integrity, trying to conceive

Vaginal lubricants are commonly used to self-treat pain or dryness at intercourse in nearly one third to one half of all sexually active couples (1, 2). When mixed with human semen during intercourse, these lubricants may affect sperm integrity and function, thereby decreasing its fertilization potential. It is a common misconception that if a lubricant does not contain spermicide it will not impair sperm function. Several dryness-relief products claim that they do not harm sperm in spite of published data to the contrary. Therefore confusion exists among physicians and lay people regarding the safety and use of vaginal lubricants when couples are trying to conceive. Over the past three decades, a number of studies have reported a deleterious effect of various commercially available lubricants such as K-Y Jelly, Astroglide, and Replens on sperm function and motility (3–9). The proposed sperm damage is profound, and the negative effect has been found with concentrations even as low as 1% of the lubricant. Loss of sperm function has also been reported after exposure to noncommercial products such as saliva (3, 10), glycerin (11), olive oil (3, 6, 7) and vegetable oil (6). What can a woman use to alleviate vaginal dryness while trying to conceive, without harming the sperm?

To answer this question we did a study to evaluate the effects of four commercially available vaginal lubricants, FemGlide, Pre∼Seed, Replens, and Astroglide, on sperm motility and effects of three vaginal lubricants, Pre∼Seed, K-Y Jelly, and FemGlide, on sperm chromatin integrity.

MATERIALS AND METHODS

Semen samples were obtained from 13 healthy donors with proven normal fertility. Samples were produced by

Received December 14, 2006; revised February 21, 2007; accepted February 22, 2007.
Presented at the 61st Annual Meeting of the American Society for Reproductive Medicine, Montreal, Quebec, Canada, October 18, 2005.
Reprint requests: Ashok Agarwal, Ph.D., Professor, Lerner College of Medicine of Case Western Reserve University, Director, Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics-Gynecology, The Cleveland Clinic Foundation, 9500 Euclid Ave., Desk A19.1, Cleveland, OH 44195 (FAX: 216-445-6049; E-mail: agarwaa@ccf.org).

0015-0282/08/$34.00
doi:10.1016/j.fertnstert.2007.02.050

Copyright ©2008 American Society for Reproductive Medicine, Published by Elsevier Inc.

masturbation after 48 to 72 hours of sexual abstinence and collected into a plastic container. All subjects demonstrated a normal semen analysis profile according to the World Health Organization criteria (12). Specifically, two experiments were performed.

In the first experiment, raw semen samples from 13 donors were diluted to approximately 25 million sperm/mL in human tubal fluid (HTF) medium with 10% human serum albumin (HSA). The samples were then incubated for 30 minutes at 37°C under 5% CO_2 in the medium alone as a control and in 10% solutions of all four lubricants: Pre∼Seed (INGfertility, Valleyford, WA), FemGlide (Wal-Med Inc., Puyallup, WA), Astroglide (Astro-lube, Inc., North Hollywood, CA), and Replens (Parke-Davis, Morris Plains, NJ) plus medium. The 10% lubricant dilution was chosen on the basis of concentrations of lubricant potentially present after intercourse and ejaculation and is reflective of the concentration used in the previously published studies: 5% to 30% (3, 4, 8, 11). These studies assumed a normal ejaculate volume of approximately 3 mL with lubricant volumes estimated by amount of product applied, displacement during intercourse, and subsequent dilution with female secretions. At 30 minutes of incubation, two aliquots from each treatment, in random order, were removed and routinely evaluated for progressive motility by light microscopy. The 30-minute sampling time was chosen on the basis of sperm transport studies suggesting that the majority of fertilizing spermatozoa migrate through the cervix within 15 to 30 minutes after ejaculation (13).

In the second experiment, samples from 12 donors were likewise cultured for 4 hours in the HTF + 10% HSA media at 37°C under 5% CO_2 (as control) and 10% of lubricants Pre∼Seed, FemGlide, and K-Y Jelly in the medium. K-Y Jelly (Johnson & Johnson, New Brunswick, NJ) was used as a negative control on the basis of the numerous published accounts of its damaging effects on sperm function (3, 5, 9). Changes in mammalian sperm can be detected by the sperm chromatin structure assay within hours of culture in vitro. The 4-hour culture time was chosen on the basis of the previous work of one of the authors suggesting that the free radical damage to sperm (as might be seen with sublethally toxic compounds) can manifest in chromatin nicking and damage within 4 hours of exposure (14). Estop et al. demonstrated in mouse sperm that 50% of sperm have increased susceptibility to DNA denaturation within 2 hours, and this level continues to rise over 24 hours (15). Munne and Estop found gross chromosomal abnormality for mouse sperm in culture for 12 hours (16). However, changes in sperm chromatin as detected by the sperm chromatin structure assay can be observed earlier in the culture process than gross chromosomal defects. Similarly, bull sperm has been shown to undergo DNA denaturation within hours in culture, but the timing is dependent on the culture medium used (17). After culture, spermatozoa were flash frozen and analyzed for the percent damaged chromatin (percent DNA fragmentation index) with use of the acridine orange–based sperm chromatin structure assay.

Data are expressed as mean ± SD. Because of expected intersubject variation in baseline sperm motility and sperm chromatin, samples were analyzed by using repeated-measures analysis of variance. Bonferroni's correction was applied when comparing percent change in motility and sperm chromatin damage for each lubricant as compared with the control. Statistical significance was set at $\alpha = 0.05$. The calculations were performed with use of SPSS version 11.0 for Windows statistical software (SPSS Inc., Chicago, IL).

RESULTS

Replens and Astroglide caused dramatic decreases in sperm motility after 30 minutes of contact with semen sample. FemGlide also caused a lesser but still statistically significant decrease in sperm motility. In contrast, sperm in Pre∼Seed had a progressive motility at 30 minutes of culture that did not differ significantly from the sperm in control medium (Table 1 and Fig. 1).

The changes in sperm chromatin quality after treatment were widely variable between individuals as can be seen in the large SDs shown (Table 2). Although population means for the percentage of sperm with DNA fragmentation did not differ between the study and control groups (SD was more than 50% of mean), we found a statistically significant increase in sperm chromatin damage within subject (averaging 10% or more) after culture with FemGlide (P=.02) and K-Y Jelly (P=.04) as compared with sperm in control medium. Although some damage was observed in the sperm chromatin quality (percent DNA fragmentation index) after culture with Pre∼Seed, the increase was not statistically significant (Fig. 2).

DISCUSSION

Most couples who try to conceive experience vaginal dryness at some time during ovulatory intercourse. A self-reporting Internet study of 1,500 "trying-to-conceive couples" found that 75% of these couples had increased incidences of vaginal dryness because of having timed intercourse on repeated days around ovulation, the fear of failing at conception, and fertility medications. Twenty-five percent of these trying-to-conceive couples reported always using a vaginal lubricant during intercourse (18). However, use of various commercial products available has been strongly discouraged by the previous investigators, who found significant reduction in motility and viability of sperm with the use of these products in a number of in vitro and in vivo studies (3–11). In contrast to the previous studies, we studied the effects of vaginal dryness products on sperm chromatin quality, in addition to the sperm motility.

Our study suggests that even the water-soluble lubricants containing no spermicide, such as FemGlide, Astroglide, and Replens, have negative effects on sperm motility and chromatin integrity. Kutteh et al. achieved results similar to

TABLE 1

Sperm motility 30 minutes after incubation with vaginal lubricants.

Treatment intervention	Sperm motility (%)	Percent decrease in motility from controls
Control (HTF + 10% HSA)	66 ± 12	NA
10% FemGlide	51 ± 16[a]	23 ± 15[a]
10% Pre∼Seed	64 ± 14	3 ± 10
10% Replens	25 ± 12[a]	60 ± 19[a]
10% Astroglide	2 ± 1[a]	99 ± 1[a]

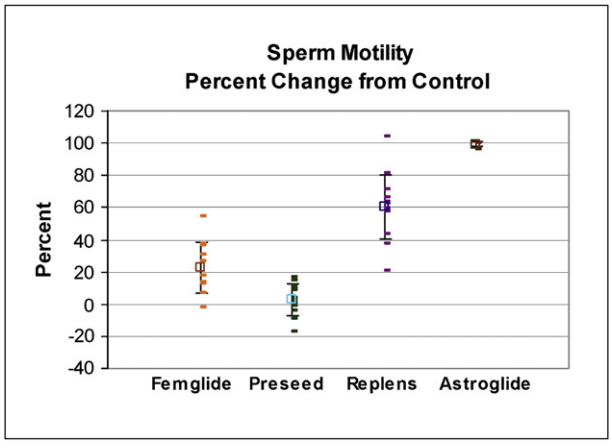
Note: Values are expressed as mean ± SD. NA = not applicable.
[a] $P<.01$ compared with controls, by repeated-measures ANOVA.

Agarwal. Vaginal lubricants and semen quality. Fertil Steril 2008.

ours showing complete spermicidal action of Astroglide and Replens after 60-minute incubation with the semen samples (7). Glycerin, the main ingredient of these lubricants, has been found to cause profound inhibition in motility and forward progression by a number of investigators (4, 7, 11); however, Goldenberg and White achieved contradictory results and suggested glycerin to be a lubricant of choice for infertile couples (6). A possible explanation for their finding may be that glycerin and semen were not adequately mixed because of glycerin being coated only on the bottom of the test tubes (11). Tagatz et al. found no motile or viable spermatozoa after incubating the semen sample with K-Y Jelly for 30 minutes (9). Anderson et al., in a prospective study, also found decreased percentage of progressive motility, progressive velocity, curvilinear velocity, and lateral head displacement with K-Y Jelly, olive oil, and saliva (3). Frischman et al. demonstrated that the impairment of sperm's progressive motility with Astroglide and K-Y Jelly were dose dependent but time independent (5). The physiologic relevance of such lubricant damage on sperm was shown by Miller et al., demonstrating that cervical mucus penetration of sperm after intercourse was profoundly disrupted after lubricant contact during coitus (8).

FIGURE 1

Scatter plot of the difference in sperm motility for each subject in each treatment as compared with the control. The mean difference for each treatment is the *square box*, and the SD is the *line* in each column of data.



Agarwal. Vaginal lubricants and semen quality. Fertil Steril 2008.

The sperm damage seen with these vaginal dryness treatments is most likely due to the nonphysiologic osmolality and pH of these products (7). The optimum pH value for sperm migration and survival in the cervical mucus is between 7.2 and 8.5 (12). At the time of ovulation, the pH of cervical mucus secretions in the woman elevates to a similar range to promote sperm transport through the cervix (19). Outside this range, pH levels are damaging to sperm. Ion concentration or osmolality is also strictly regulated in body fluids, and a physiologic osmolality between 270 and 360 mOsm/kg is best for sperm function. As osmolality of semen approaches 600 mOsm, all sperm motility is lost (20, 21). Osmolality levels of three products evaluated here are high (>1,000 mOsm/kg), specifically K-Y Jelly, Astroglide, and Replens. Interestingly, hypoosmotic concentrations are even more damaging to sperm than hypertonicity, when coupled with an acidic environment (22). The osmolality of FemGlide is lower than the physiologic levels. Tulandi et al. postulated that the spermicidal activity of saliva is due to the presence of thiocyanate ions and amylase enzyme (10).

Over a number of years various authors have suggested different products to be used by infertile couples as vaginal lubricants. Miller et al. demonstrated that in vitro sperm motility inhibition by vaginal lubricants is time and concentration dependent, thereby suggesting use of smaller intravaginal aliquots of lubricant, which might provide adequate coital lubrication without significantly impairing sperm penetration of cervical mucus (8). Kutteh et al. observed that sperm motility is maintained with canola oil and Ham's F-10. This could be due to an observable bilayer of canola oil and Ham's F-10 providing

TABLE 2

Sperm DNA fragmentation results after culture with vaginal lubricants.

Treatment intervention	Percent DNA fragmentation index	Percent increase in damage over controls
Control (HTF + 10% HSA)	14.8 ± 8	NA
10% Pre∼Seed	15.5 ± 8	7 ± 13
10% K-Y Jelly	16.0 ± 9	10 ± 15[a]
10% FemGlide	16.4 ± 8	14 ± 16[a]

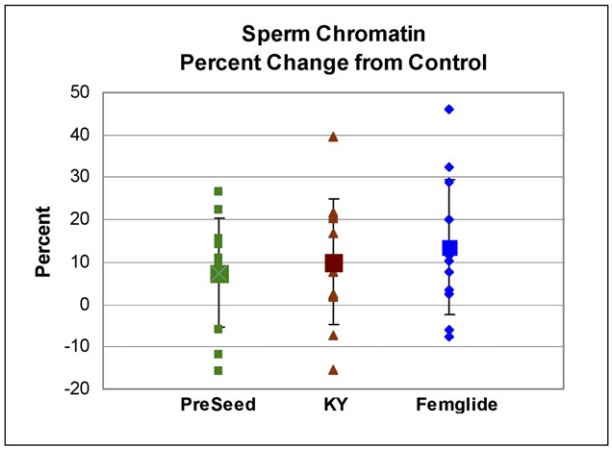
Note: Values are expressed as mean ± SD. NA = not applicable.
[a] $P<.05$ compared with controls, by repeated-measures ANOVA.

Agarwal. Vaginal lubricants and semen quality. Fertil Steril 2008.

a barrier to oxidation, pH fluctuation, and other microenvironmental changes (7). Edvinsson et al. suggested using raw egg white as vaginal lubricant for infertile couples who need a lubricant (23). However, the possibility of development of allergic reaction to the egg protein and the fear that it might act as a culture medium for vaginal flora has impeded its use as a vaginal lubricant. Anderson et al. found baby oil to cause only small reductions in sperm motility, despite having a high viscosity and osmolality (3). Despite all these findings, none of the above-mentioned products has received universal acceptance and commercial marketing. However, on the basis of inaccurate information, many physicians are still recommending lubricants or saliva to the infertile couples who are dealing with vaginal dryness.

FIGURE 2

Scatter plot of the difference in sperm chromatin quality for each subject in each treatment as compared with the control. The mean difference for each treatment is the *square box*, and the SD is the *line* in each column of data.



Agarwal. Vaginal lubricants and semen quality. Fertil Steril 2008.

In this study, we found that Pre∼Seed intimate moisturizer had minimal negative effect on both sperm motility and chromatin quality, which may be due to its more physiologic pH and isotonic formulation as compared with other products. It provides moisture in a pH and osmolality that are matched to those of semen and fertile mucus, so that sperm can transport through the reproductive tract without damage. In addition, it contains a plant antioxidant, arabinogalactan. Ellington et al. in an in vitro study in 25 normozoospermic donor samples using computer-assisted semen analysis achieved results similar to ours (24). They found that sperm in 10% vol/vol solution of Pre∼Seed had a higher likelihood of penetrating cervical mucus based on their higher mean average path velocity, straightness, and amplitude of lateral head displacement as compared with FemGlide, Astroglide, K-Y Jelly, and Replens (24). Our results suggest that Pre∼Seed may be a promising treatment for vaginal dryness in infertile couples who are trying to conceive; however, large-scale in vivo trials are needed to support our findings.

Acknowledgment: The authors gratefully acknowledge the technical assistance of the Andrology Laboratory technologists. Research support was provided by the Glickman Urological Institute, Cleveland Clinic.

REFERENCES

1. Jamieson DJ, Steege JF. The prevalence of dysmenorrhea, dyspareunia, pelvic pain, and irritable bowel syndrome in primary care practices. Obstet Gynecol 1996;87:55–8.
2. Oberg K, Fugl-Meyer AR, Fugl-Meyer KS. On categorization and quantification of women's sexual dysfunctions: an epidemiological approach. Int J Impot Res 2004;16:261–9.
3. Anderson L, Lewis SE, McClure N. The effects of coital lubricants on sperm motility in vitro. Hum Reprod 1998;13:3351–6.
4. Boyers SP, Corrales MD, Huszar G, DeCherney AH. The effects of Lubrin on sperm motility in vitro. Fertil Steril 1987;47:882–4.
5. Frishman GN, Luciano AA, Maier DB. Evaluation of Astroglide, a new vaginal lubricant: effects of length of exposure and concentration on sperm motility. Fertil Steril 1992;58:630–2.
6. Goldenberg RL, White R. The effect of vaginal lubricants on sperm motility in vitro. Fertil Steril 1975;26:872–3.
7. Kutteh WH, Chao CH, Ritter JO, Byrd W. Vaginal lubricants for the infertile couple: effect on sperm activity. Int J Fertil Menopausal Stud 1996;41:400–4.
8. Miller B, Klein TA, Opsahl MS. The effect of a surgical lubricant on in vivo sperm penetration of cervical mucus. Fertil Steril 1994;61:1171–3.

9. Tagatz GE, Okagaki T, Sciarra JJ. The effect of vaginal lubricants on sperm motility and viability in vitro. Am J Obstet Gynecol 1972;113: 88–90.
10. Tulandi T, Plouffe L Jr, McInnes RA. Effect of saliva on sperm motility and activity. Fertil Steril 1982;38:721–3.
11. Tulandi T, McInnes RA. Vaginal lubricants: effect of glycerin and egg white on sperm motility and progression in vitro. Fertil Steril 1984;41: 151–3.
12. World Health Organization. Laboratory manual for the examination of human semen and semen–cervical mucus interaction. 4th ed. New York: Cambridge University Press, 1999;56–61.
13. Settlage DS, Motoshima M, Tredway DR. Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitation study. Fertil Steril 1973;24:655–61.
14. Ellington JE, Evenson DP, Fleming JE, Brisbois RS, Hiss GA, Broder SJ, et al. Coculture of human sperm with bovine oviduct epithelial cells decreases sperm chromatin structural changes seen during culture in media alone. Fertil Steril 1998;69:643–9.
15. Estop AM, Munne S, Jost LK, Evenson DP. Studies on sperm chromatin structure alterations and cytogenetic damage of mouse sperm following in vitro incubation. Studies on in vitro-incubated mouse sperm. J Androl 1993;14:282–8.
16. Munne S, Estop A. The effect of in-vitro ageing on mouse sperm chromosomes. Hum Reprod 1991;6:703–8.
17. Karabinus DS, Evenson DP, Kaproth MT. Effects of egg yolk–citrate and milk extenders on chromatin structure and viability of cryopreserved bull sperm. J Dairy Sci 1991;74:3836–48.
18. Ellington JE, Short RA. Prevalence of vaginal dryness in trying to conceive couples. Pacific Coast Reproductive Society Meeting, April 23–27, 2003; Rancho Mirage, California.
19. Guttmacher AF, Shettles LB. Cyclic changes in cervical mucus, and its practical importance. Hum Fertil 1940;5:4–9.
20. Rossato M, Balercia G, Lucarelli G, Foresta C, Mantero F. Role of seminal osmolarity in the reduction of human sperm motility. Int J Androl 2002;25:230–5.
21. Makler A, David R, Blumenfeld Z, Better OS. Factors affecting sperm motility. VII. Sperm viability as affected by change of pH and osmolarity of semen and urine specimens. Fertil Steril 1981;36:507–11.
22. Blackshaw AW, Emmens CW. The interaction of pH, osmotic pressure and electrolyte concentration on the motility of ram, bull and human spermatozoa. J Physiol 1951;114:16–26.
23. Edvinsson A, Bergman P, Steen Y, Nilsson S. Characteristics of donor semen and cervical mucus at the time of conception. Fertil Steril 1983;39: 327–32.
24. Ellington JE, Schimmels J. The effects of vaginal lubricants and moisturizers on computer assisted sperm analysis (CASA) parameters associated with cervical mucus penetration. Fertil Steril 2004;82:S145–6.