

# Sperm toxicity of “nonspermicidal” lubricant and ultrasound gels used in reproductive medicine

Three commercial, nonspermicidal gels used in fertility practice were found to be toxic to sperm in a 24-hr sperm survival assay; these included Felis, Replens, and Aquasonic Gel, which is used for transvaginal ultrasound during ovulation monitoring. In contrast, Pre-Seed did not cause any sperm toxicity, suggesting its appropriate use by patients who are trying to conceive, as well as clinicians during fertility procedures. (Fertil Steril® 2011;95: 835–6. ©2011 by American Society for Reproductive Medicine.)

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During human reproduction, spermatozoa (sperm) deposited in the vagina migrate through the cervix and uterus to reach the oocyte in the fallopian tube. Human sperm are capable of surviving exposure to unfavorable environmental conditions, such as those in the vagina, provided their motility remains unaffected. Sperm transport through the female genital tract is optimal at a pH of 7.0–8.4 (1), whereas pH levels <7.0 result in sperm immobilization. Sperm function is also best maintained at the physiologic osmolality of semen (approximately 320 mOsm/kg), whereas osmolalities above this level decrease sperm motility, with a 50% reduction seen in solutions >400 mOsm/kg (2).

Lubricant gels are used to relieve vaginal dryness for comfort during intimacy and in the clinic to facilitate insertion of medical devices. Ultrasound gel is used during transvaginal ovarian echography, to act as a lubricant for the introduction of the transducer probe and as a transmitter of ultrasound waves. Many commercial lubricant and ultrasound gels contain traditionally harmless ingredients such as glycerin (glycerol) or propylene glycol. Because they do not contain chemical spermicides, they are often labeled as *nonspermicidal*. Most of these gels have pH levels <6.5 and osmolalities >1,000 mOsm/kg, making them suboptimal for sperm function (3). In fact, studies have shown that lubricants and ultrasound gels can have a toxic effect on sperm and are contraindicated for couples who are trying to conceive (3–6). The reported sperm-toxic effects of gels are related to dose and duration of exposure, but previous studies have primarily used concentrations of gels (>10%), which may not reflect the levels sperm would encounter in the vagina after ejaculation.

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The toxic properties of substances coming into contact with human sperm can be screened using a 24-hour sperm survival assay (7). To optimize the sensitivity of this test, albumin products should be removed from the culture medium, so as not to mask deleterious aspects of test solutions. This bioassay provides a sperm motility index (SMI) calculated by dividing the percent of progressively motile sperm in the test solution by that in control solution at specific times. SMI values <0.75 are predictive of poor assisted reproduction outcomes and indicate sperm toxicity (7).

The isotonic, pH-neutral lubricant (Pre-Seed Lubricant; INGfertility, Valleyford, WA) has indications of use as being safe for couples who are trying to conceive. An initial study showed no effect of Pre-Seed on sperm motility in 30-min cultures (3). However, albumin was included in this study, thereby decreasing the sensitivity of the assay.

The purpose of this study was to compare sperm toxicity of four commercial nonspermicidal gels, using an albumin-free, 24-hour human sperm survival assay and low concentrations of the gels (<10%) to better reflect proposed in vivo exposure.

Fresh, normospermic semen samples were obtained from donors and analyzed according to the World Health Organization guidelines (1). Institutional review board approval was obtained from the FABER Foundation Scientific Board. A computer-assisted semen analyzer (CASA; Microptic, Barcelona, Spain) assessed sperm concentrations and motility using 20- $\mu$ m-deep counting chambers (Leja Products, Nieuw-Vennep, The Netherlands). Semen samples were washed through density gradients (Spermfilter; NMS Biomedical, Praroman, Switzerland) and motile sperm pellets washed twice with human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) plus 10% Synthetic Serum Supplement (Irvine Scientific). Final pellets of sperm were pooled from three to five donors, as previously validated (7), and resuspended in HTF to achieve sperm concentrations of 10–20 million/ml.

Test solutions included HTF with four different concentrations (0.10%, 1.0%, 5.0%, and 10%) of each of the following: Pre-Seed Lubricant, Felis Lubricant (Lagap SA, CH-6943; Vezia, Switzerland), Replens Vaginal Moisturizer (Vifor SA; Villars-sur-Glâne, Switzerland), and Aquasonic Ultrasound Gel (Parker Labs, Fairfield, NJ), in 0.5-mL volumes equilibrated for 1.5 hours in

5% CO<sub>2</sub> and 95% humidity in a 37°C incubator. The osmolality of each test solution was determined by the freezing-point method (Micro Osmometer Model 3300; Advanced Instruments, Norwood, MA).

A 100-μL aliquot of pooled sperm sample was added to each of the 0.5-mL test solutions, resulting in final gel concentrations (v/v) of 0.083%, 0.830%, 4.150%, and 8.300%. The 0.083% solution was assigned as the control for each gel, based on low concentrations of gel, and the consistent handling across all treatments. At 0, 1, and 24 hours of incubation, 5-μL samples from each solution were evaluated in triplicate for percent progressively motile sperm and solution pH. The pH was measured using indicator paper (Merck 1.09557.0003; Merck, Whitehouse Station, NJ).

Sperm toxicity of test solutions was determined using the SMI to compare percent motility in each test solution to that in the control solution at 1 and 24 hours (Table 1). SMI values <0.75 indicated sperm toxicity, as previously reported (7). The experiment was replicated three times, and means ± SD were used in a chi-square analysis to compare the SMI observed in each gel.

Only Replens at 4.15% and 8.3% caused a decrease in mean solution pH (pH = 6.60 and 5.53, respectively). Mean osmolality levels of solutions rose above 400 mOsm/kg only in 4.15% and 8.3% Felis and 8.3% Aquasonic (mean of 511, 734, and 408 mOsm/kg, respectively).

The initial percent motile sperm did not differ for controls across gels at time 0 (mean percent motility ± SD: Aquasonic, 52 ± 9%; Felis, 54 ± 9%; Pre-Seed, 59 ± 14%; Replens, 49 ± 18%). The percent motile sperm in control incubations for each gel did not decrease over the 24 hours ( $P>0.05$ ), supporting the use of the 0.083% solution as the control. During the 24-hr assay, Aquasonic, Felis, and Replens caused declines ( $P<0.05$ ) in mean percent motile sperm, resulting in SMI values <0.75 (Table 1) and a designation of *sperm toxic*. Loss of sperm motility was seen with Replens, even at the 0.83% concentration. In contrast, sperm motility did not decrease with Pre-Seed, indicating a lack of sperm toxicity for this gel.

These data confirm that even gels labeled *nonspermicidal* can significantly impair sperm motility, and at lower concentrations than previously reported (<10%). It is unlikely that safe concentrations of Aquasonic, Felis, and Replens can be determined reliably. Of the products evaluated, only Pre-Seed was not toxic to

**TABLE 1**

**SMI values following 1 or 24 hours of incubation as a ratio of percent sperm motility in culture with 8.33% gel over percent sperm motility in controls (0.083% gel).**

Lubricant gels	1 h	24 h
Aquasonic	1.13	<b>0.54</b>
Felis	1.11	<b>0.52</b>
Pre-Seed	1.05	0.93
Replens	<b>0.03</b>	<b>0.12</b>

Note: Boldface text denotes SMI <0.75, indicating sperm-toxicity.

Vargas. Sperm toxicity of reproductive gels. *Fertil Steril* 2011.

sperm. The American Society for Reproductive Medicine Practice Committee consensus guideline *Optimizing Natural Fertility* urges physicians to discuss the importance of lubricant choice for couples who are trying to conceive (8).

Reduced sperm motility following gel contact has been thought to be due to shifts in pH, osmolality changes, or both (3–5). This study found that even in treatments without large changes in pH or osmolality, sperm toxicity was observed. A direct toxic effect of ingredients cannot be ruled out. Both Replens and Felis contain glycerin (Aquasonic does not list ingredients). Pre-Seed alone in this study is known to not contain glycerin. Glycerin penetrates across sperm membranes and even at low concentrations (e.g., 2%) can disrupt cell function and motility (9, 10).

Aquasonic Gel is commonly used for vaginal ultrasound examination to monitor follicle development before intercourse or insemination. Based on sperm toxicity found in this study and others (6), this gel should not be used for transvaginal procedures in fertility patients.

Labeling of gels as *nonspermicidal* does not ensure that a product will not impair sperm function. Even low concentrations (<10%) of gels in this study were toxic to sperm and interfered with their motility. Among the gels evaluated, only Pre-Seed did not show any sperm toxicity, making it the most appropriate lubricant choice for women who are trying to conceive or for use during clinical procedures, including vaginal insertion of diagnostic and therapeutic probes.

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