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Scientific Review

Background:

Infertility affects approximately 1 in 6 couples in New Zealand. Several studies suggest that rates of infertility are growing, due to delayed childbearing, prevalence of sexually transmitted diseases and potential declines in male sperm production.

Even though medical interventions for fertility disorders have developed at a remarkable rate the majority of infertile couples do not access assisted reproduction techniques and instead actively make life style choices and utilise products to support their dreams.

A common complication for trying to conceive couples is vaginal dryness in the female partner, with 75% of these couples reporting increased incidence of vaginal dryness due to: the stress of being infertile; having to have timed intercourse; and/ or fertility medications they are taking¹. Enjoying intercourse can become difficult during daily sexual activity when vaginal dryness and dyspareunia become an issue. In fact, over 25% of trying to conceive couples “always” use lubricants while having intercourse.

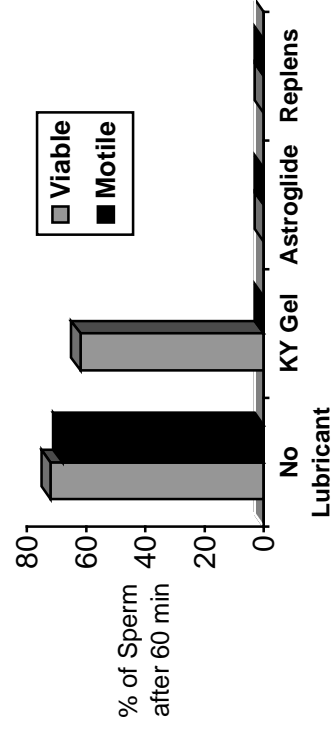
This disruption of the normal reproductive process can lead to decreased ejaculate quality and sperm counts, as well as cause psychological problems for couples. Specifically, poor levels of stimulation and enjoyment by the man during intercourse (as can occur with vaginal dryness) can decrease the number of motile sperm in the ejaculate by as much as 50%.² Because conception requires coitus, even if sexual dysfunction arises, the couple will continue to have intercourse although positive feelings about their sexual relationship diminish. This can result in long-term consequences for the couple with 33% of women and 17% of men having psychological distress from infertility manifesting in sexual maladjustment³. In contrast, couples who are able to manage the stress of infertility, including practicing timed intercourse more reliably and maintaining sexual pleasure even during the ovulatory phase of the cycle, have better outcomes including increased overall sperm counts for the man and better subsequent pregnancy rates.⁴⁻⁸ Using palliative methods to alleviate vaginal dryness and enhance sexual pleasure while trying to conceive would seem ideal; however, existing lubricant products may actually exacerbate a couple's infertility.

Evidence that Existing Vaginal Lubricants Damage Sperm

Vaginal dryness is routinely treated with vaginal lubricant products, of which there is a continually expanding range of products available on the market. However, three decades of peer reviewed, published research has shown that all existing vaginal lubricants studied to date harm sperm, resulting in rapid losses in their viability (% live) and motility (% swimming). Specifically, there have been at least 11 studies on this topic conducted in research settings, reviewing the effect of 16 different lubricants on sperm, including all of the top selling brands.⁹⁻¹⁹ In each study, sperm motility and viability were dramatically reduced after exposure to even small concentrations (<10% v/v) of these products. Notable data from these studies showed:

- A *spermicidal* activity of the leading three brands of lubricants as being *equivalent to contraceptive jellies such as Gyno*.^{12,13} In these studies, sperm had ZERO motility after 30 minutes of contact with the top three selling lubricant products. A similar detrimental effect was observed on viability parameters by 60 min of contact with lubricants¹⁴ as shown in Figure 1.
- These effects are concentration dependent (Figure 2) and were seen even with low concentrations (1–6%) depending on the lubricant product.^{10, 12,14} Even in studies where sperm were washed out of a 1% lubricant exposure after 20 min, sperm motility subsequently fell to zero¹⁰.
- Critically, the deleterious effect of lubricants on sperm includes decreasing sperm penetration into the cervix *in vivo*, as determined by the post-coital test (PCT)¹⁶. For example, numbers of motile sperm per high power field in the cervix dropped from 57 without lubricant to 3 with a common clinical lubricant delivered intra-vaginally prior to coitus.

Figure 1. Effect of 30% Concentrations of Lubricants on Sperm Motility and Viability



decline in sperm chromatin (DNA) quality²⁶, which is significant given the well researched link between sperm chromatin and DNA damage and male infertility.

The following excerpts, from a partial list of lubricant studies done over the past three decades, highlight these investigators' deep concerns with the use of vaginal lubricants by trying to conceive couples. The institutions completing the studies are also cited to emphasize the breadth of this research.

Dept of ObGyn; University of Texas Southwestern Medical Center¹⁴

- "Sperm exposed to (Brand 2) or (Brand 3) were non-motile and nonviable after incubation for 60 minutes, similar to the control nonoxynol-9 containing product Gynol II."
- "For couples with infertility, the use of vaginal lubricants during intercourse is not recommended."
- "Products commercially marketed as vaginal lubricants have marked effects on sperm motility and viability."
- "Our results demonstrate the complete spermicidal action of (Brand 2) and (Brand 3) after a 60 min incubation."
- It is critical to note that (Brand 2 and 3) currently claim on their literature to not effect sperm motility or viability.

Dept of ObGyn; University of Connecticut¹²

- "Both (Brand 1) and (Brand 2) caused an impairment in sperm progressive motility that was immediate and increased with concentration."
- "We conclude that all traditional vaginal lubricants should be avoided in patients desiring conception."

Dept of ObGyn; Queen's University of Belfast⁹

- "...since even concentrations as low as 6.25% (of a lubricant) 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."
- "We recommend that couples – especially those having difficulty conceiving- should be aware of the detrimental effects of such lubricants and avoid their use."

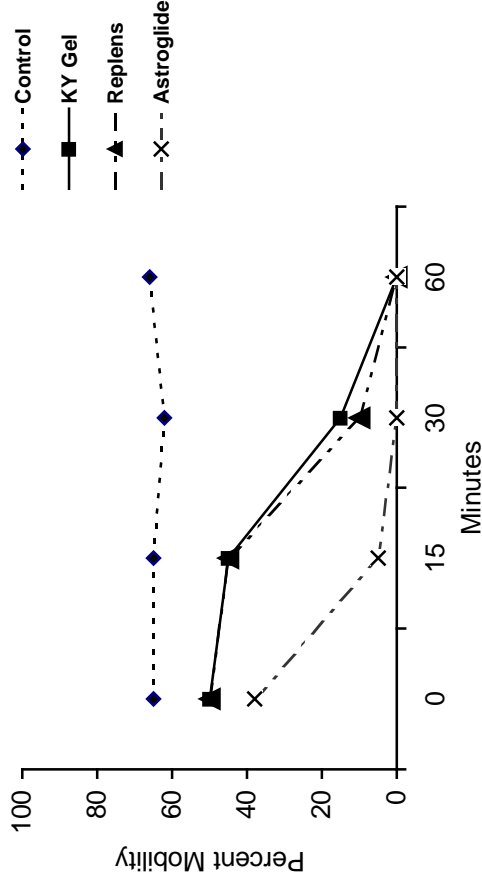
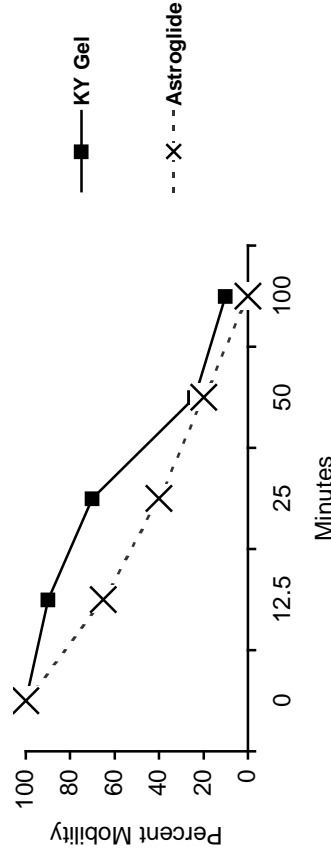


Figure 2. Concentration Dependent Effect of Lubricants on Human Sperm Motility Following 1 Minute of Exposure



In order for conception to occur, motile, functionally competent sperm must be able to penetrate into the cervical mucus and proceed to the Fallopian tubes in adequate numbers.^{20, 21} Existing lubricant products rapidly decrease sperm motility, thus possibly decreasing the numbers of sperm penetrating the cervix and being able to participate in fertilization.

More recently research has also revealed an actual and severe decline in the ability of embryos to form in vitro (using a bovine model) when the sperm are exposed to the leading personal lubricants.²⁷ Research from the Cleveland Clinic has clearly demonstrated that many commonly used lubricants cause a significant

Dept of ObGyn; University of Minnesota¹⁷

- “The spermicidal effect of the lubricants was statistically significant ($p < 0.001$). The lubricants had a similar effect on both normal and abnormal semen specimens.”

Dept of ObGyn; Uniformed Service University, Bethesda, MD¹⁶

- “In vivo data demonstrated severe impairment to sperm penetration of mid-cycle human cervical mucus by the lubricant.”
- “*Coital lubricants may impair the fertility of some couples.*”

Dept of ObGyn; Yale School of Medicine¹⁰

- “Even after a brief (Brand 5) exposure, motile migrated sperm deteriorated rapidly making it unlikely that sperm migrating to the upper reproductive tract might escape (Brand 5’s) action.”
- (Brand 5) has significant *spermicidal activity, which contraindicates its use in couples who are trying to conceive*”.

In addition to the above studies with fresh human ejaculates, quotes from two recent animal model studies corroborate that the affects of common vaginal lubricants on sperm may be irreversible and contraceptive.

Population Council, New York¹⁵ **(studying mouse sperm in contact with a vaginal lubricant)** “

- (Brand 3) hindered forward (sperm) progression and may suggest some *contraceptive activity*”.

Dept of Urologic Surgery, University of Minnesota Medical School¹¹

- “The loss of motility of stallion sperm (after contact with a commercial water based lubricant) *caused by hyperosmotic stress is permanent with no recovery of motility upon return to iso-osmolality.*”

Why Vaginal Lubricants Damage Sperm

Existing vaginal lubricants were designed to provide lubrication to facilitate intromission and alleviate vaginal dryness. With the exception of Pre~Seed™, lubricant formulations all have pH levels and osmolality levels that are outside of the physiologic range for sperm thus exerting pH and osmotic shock to sperm cells that can cause contraceptive level losses in sperm viability and motility (Table 1). The ingredients that make up lubricants and that provide these nonphysiologic conditions tend to be drawn from a relatively small group of compounds (i.e. glycerin & propylene glycol), and have many themselves, been found to harm sperm function. Therefore, the adverse effect on sperm of most vaginal lubricants can be extrapolated from these studies.

TABLE 1. pH and Osmolalities for Popular Vaginal Dryness Remedies.

Product	pH		Osmolality (mOsm/kg)	
	Too Low	Physiologic for Sperm 7.2 – 8.5	Too High	Too High
KY Jelly	4.52			2052
Sylk	4.50			1092
Replens	2.79			2610
FemGlide		7.22	162	
Astroglide	3.45			5848
Pre~Seed		7.30		324
Egg White			8.95	238
Saliva	7.08		151	
Tap Water		8.01	98	
Mineral Oil			10.50	1735
Canola Oil	683			2250

The optimum pH value for sperm migration and survival in cervical mucus has been well established as reported by the World Health Organization guidelines at between 7.0 and 8.5.²² This coincides with the physiologic rise in pH of ovulatory cervical mucus found in women at the time of ovulation.²³ In contrast, vaginal lubricants tend to have pH values below 7, and often as much as half of this value. Sperm are very sensitive to low (acidic) pH. Below pH levels of 6.9, sperm die at a rate that increases with lowering pH. Table 1 below summarizes the low pH values of commercial lubricants. These pH

values are not consistent with optimal sperm function, and can cause sperm death.

Sperm are also sensitive to both high and low osmolality (concentration of osmotically active particles in solution) because these can cause the cells to either shrink or swell beyond their "critical volume limits". A physiologic osmolality around 320 mOsm/kg (that of semen) is best for sperm function.^{24, 25} Sperm motility decreases linearly with exposure to increasing osmolality, such that sperm motion is basically abolished at 600 mOsm or greater.²⁵ Table 1 shows the very nonphysiologic osmolality of a list of common (leading) over-the-counter vaginal lubricant products. Most of these products have osmolality 3-4 times that of semen. Some have osmolality exceeding 3,000 mOsmo/ml (or almost 10 fold that of semen). Both human and animal model studies suggest that these levels of osmotic shock are sufficient to cause irreversible damage to sperm, even after the sperm are moved into an isotonic situation, such as swimming out of the vagina.^{10, 11}

Pre~Seed™ – The Right Solution for Vaginal Dryness When Trying to Conceive

The Inventor:

Dr. Joanna Ellington, an internationally recognized scientist with over 75 publications and co-founder of INGfertility developed the formulation for Pre~Seed™ after seeing a need for a vaginal dryness relief product that would not harm sperm during her 20 years of federally funded research into male infertility and sperm physiology.

Dr. Ellington has received the prestigious National Institutes of Health's Physician Scientist Award and First Independent Research in Science Training Award. The scientific community honored her research into male reproductive physiology when she received the internationally recognized Young Andrologist Award in 2003. In 2004, She has also held leadership positions in several medical societies, including an Executive Council position in the American Society of Andrology (sperm physiologists).

The Product:

Pre~Seed™ is the first "fertility-friendly" vaginal lubricant that mimics natural body secretions to relieve vaginal dryness while providing an optimal sperm environment.

Pre~Seed's moisture is delivered in a fluid with the same pH and osmolality as semen and **fertile cervical mucus**, so as not to harm sperm, and to be mild to a woman's body.

Pre~Seed™ contains a naturally occurring bioactive plant sugar (arabinogalactan) which provides antioxidant support to sperm cells.

Pre~Seed comes in pre filled applicators designed to coat the external cervix and vagina with moisture where it is most needed to provide the optimal environment for sperm.

Pre~Seed™, unlike other lubricants does not create a barrier that interferes with sperm swimming out of the semen. Sperm are able to move freely from the semen into Pre~Seed.

Pictures taken in laboratory at 200X magnification after 10 minutes of contact between semen and products. Dead sperm remain behind in semen.

Pre~Seed™ is the first and only lubricant approved by the FDA for use during fertility interventions and to have FDA approval to claim it is “safe for use while trying to conceive”.

Pre~Seed™ has been extensively studied and clinically tested by the inventors and third party independent laboratories. The results of which have been published in many medical journals and presented at major medical meetings worldwide (See Section B: Scientific Reports)

Pre~Seed™ has been clinically tested for irritation in people and rabbits. These tests show no evidence of reactivity or irritation. In fact, an independent study by Ghent University, found Pre~Seed™ formulation to cause NO membrane irritation, whereas KY, Replens, and Astroglide all caused some irritation. Interestingly, in this study one well-known brand caused a LOSS of mucus from the membranes.

Pre~Seed™ is carefully screened to assure a fertility-friendly environment before it is released for sale. INGfertility is the only vaginal lubricant manufacturer that performs “Lot Release” testing on each batch to ensure the product provides an optimal environment for sperm.

Each lot is tested to ensure:

1. pH between 7.1 - 7.4
2. Osmolarity between 260 and 365 mOsmo/kg
3. No harm to Mouse Embryos: assures that greater than 85% of one cell mouse embryos develop to the blastocyst stage when cultured with **Pre~Seed™**.
4. Sperm-friendliness: freshly ejaculated human sperm are cultured with **Pre~Seed™** in media to ensure that the percentage of swimming sperm, and their swimming speed is not negatively affected by the **Pre~Seed™**.
5. Cultured to ensure there are no pathogen bacteria.

Pre~Seed™ has been classified as a Class II a medical device, in New Zealand by Medsafe, Ministry of Health on account of it's therapeutic purpose, which is clinically supported.

Pre~Seed™'s Patented Ingredients Include:

- Purified water: Very pure with low endotoxin from bacterial cell walls, and low metal or salt content
- Hydroxyethylcellulose: A non-toxic thickener. Has been shown to be similar to cervical mucus in laboratory studies.
- Pluronic: A non-toxic slippery agent.
- Sodium chloride: Makes the product isotonic to semen.
- Arabinogalactan: A unique plant polysaccharide from larch trees, that provides antioxidant support for cells. Arabinogalactan is a very large sugar and cannot be digested by bacteria or yeast to cause an infection, as can small molecules like glycerin.
- Sodium phosphate & Potassium phosphate: Protects and holds the pH to that of semen and fertile cervical mucus for an optimal sperm environment.
- Carbomer: A non-toxic thickener.
- Methylparaben: One of the mildest and well studied of all preservatives against bacterial growth. There is no evidence that this paraben at the levels we use has any adverse effect on sperm or reproduction.
- Sodium hydroxide: Raises the pH to that of semen and fertile cervical mucus

Beware of Pre~Seed™ Imitations

Recently a number of lubricants have made their way into the Australian market with claims of creating “an optimal environment for sperm”, for trying to conceive couples. Independent studies carried out on these products, by the Northwest Andrology Clinic and Faculty of Washington State University have revealed that these products caused significant declines in in-vitro fertilisation and embryo development using an animal model which has been reviewed and accepted by the FDA as an appropriate model for human gametes. (See “Effect of Lubricant Development” in Scientific Reports Section)²⁷. In addition to this study additional research has revealed that Forelife FertilityCare™ lubricant also causes a statistically significant reduction in the percentage of motile sperm with only 54% being motile after exposure to this lubricant.²⁸

The results of these studies are currently before the Medsafe, NZ Ministry of Health and at this time neither of these products have been released for sale into the New Zealand market.

A. Independent Third Party Published Reports on Pre~Seed

Fertility & Sterility, 2008 Feb;89(2):375-379 Agarwal A, Deepinder F, Cocuzza M, Short RA, Evenson DP. Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics-Gynecology, Cleveland Clinic, Cleveland, Ohio.

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.

Mucosal Irritation Potential of Personal Lubricants Relates to Product Osmolality as Detected by the SMI (Slug Mucosal Irritation Assay).

Sexually Transmitted Diseases, 2008 May;35(5):512-516 Els ADRIAENS, PhD; Jean Paul REMON, PharmD. Laboratory of Pharmaceutical Technology, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium

Background: The slug mucosal irritation assay has recently been used as a sensitive measure of mucus membrane tolerance for vaginal microbicide products and carriers. In the current study, it was determined whether mucosal irritation potency of personal lubricants is related to varying product osmolalities.

Methods: Five commercial lubricants with an osmolality range were evaluated using the previously validated slug mucosal irritation assay. Specifically, Arion lusitanicus were treated with lubricants over 5 days to quantify mucus production and tissue damage, allowing assignment of each product into an irritation potency category (none, mild, moderate, or severe).

Result: The irritation potency (assessed by the mucus production) of the lubricants showed a significant, quadratic relationship with the product osmolality ($P = 0.001$; $R(2) = 0.99$). Femglide, a hypo-osmotic lubricant (32 mOsm/kg), caused a negative mucus production. Pré, an iso-osmotic lubricant (316 mOsm/kg), caused no changes. Two moderately hyperosmotic lubricants, Replens and K-Y jelly (2143 and 2463 mOsm/kg), induced mild and moderate irritation, respectively. The highly hyperosmotic lubricant Astroglide (5848 mOsm/kg) resulted in severe irritation and tissue damage.

Conclusion: Commonly used personal lubricants show a full range of mucosal irritation potential, which is related to product osmolality.

Toxicity Tests Carried Out On the PRE-SEED Lubricant Compared With Other Commercially Available Brands (FELIS, REPLENS, AQUASONIC)

In Preparation for Submission to *Human Reproduction*, 2007

Josefina Vargas, Dr Alfred Senn , Prof. Marc Germond. Fondation Faber, Lausanne Switzerland.

Several studies have shown that gels used to treat vaginal dryness are liable to affect the motility, chromatin integrity and kinetic parameters of human spermatozooids to varying degrees. The chemical-physical elements that play a role in the mechanism for reducing motility are principally a drop in pH and a change in ionic strength. In our study, we compared different commercially available lubricants (Felis, Replens and Pre-Seed) with a gel routinely used by gynaecologists during vaginal US (Aquasonic). Several concentrations of these products were tested in an HTF medium to which albumin was added (10% SSS), and were also subject to two periods of exposure (1 hr and 24 hrs). In order that all of the solutions tested should be subject to identical handling, we selected the weakest C1 concentration (0.083%) as the control. Taken together, these conditions allow the motility of spermatozooids to be maintained in the controls for 24 hours at values close to those at time 0. In our study, Replens lubricant and the Aquasonic US gel have a negative effect on the motility of spermatozooids even at the weakest concentrations (C2, C3 and C4). The Felis lubricant, in contrast, exercises a negative action at higher concentrations, i.e. C3 and C4. Pre-Seed, on the other hand, has no negative effect on the survival of spermatozooids at any of the concentrations tested. In conclusion, of all of the gels and lubricants tested, Pre-Seed appears to guarantee optimal conditions for the survival of spermatozooids in vitro and can be recommended by gynaecologists to infertile patients suffering from vaginal dryness. The negative role of the gels used for vaginal US poses a significant problem, because these are regularly used for echographs to monitor ovarian stimulation in infertile patients.

2008 Practice Committee Report on Optimizing Natural Fertility

The November 2008 Fertility and Sterility Journal Supplement of the 2008 Compendium of Practice Committee Reports, included a report on "Optimizing Natural Fertility". In this report, Pre-Seed was the only commercial lubricant specifically mentioned for use while trying to conceive and as not harming sperm. These reports represent best practice guidelines from the American Society of Reproductive Medicine. Specifically, this article abstract states, "This Committee Opinion provides practitioners with suggestions for optimizing the likelihood of achieving pregnancy in couples attempting conception who have no evidence of infertility." Fertil Steril 2008;90:S1-6.

B. Studies Presented at Major Medical Meetings:

Effect of Lubricants Developed for Fertility Markets on In Vitro Fertilization and Embryo Development

Accepted for presentation at the 34th Annual Meeting of the American Society of Andrology Philadelphia, PA April 4-7, 2009
Raymond W. Wright Jr, PhD, Washington State University, Center for Reproductive Biology, WSU Pullman, WA

Introduction: Traditional lubricants damage sperm and should not be used while pregnancy is desired. Newer products have been developed for this consumer/patient group. Bovine and human embryos share similar paternal sperm regulatory pathways, making this species a model for detection of sublethal sperm damage (RepBioMedOn 2002;4:170)

Objective: Experiments were done to evaluate in vitro fertilization and embryo development following bull sperm exposure to lubricants developed for the fertility market.

Method: Cryopreserved bull sperm was washed, resuspended in medium and placed into one of 5 treatments. These were: 1) Control medium; 2) Pré[®] Lubricant (Pré); 3) FertilityCare[™] (FC); 4) ConceiveEase[™] (CE) or 5) PREConceive plus[™] (PC). Lubricants were mixed with sperm at 10% v/v & incubated for 30 min at body temperature. Then sperm from each treatment was placed into fertilization wells with mature bovine oocytes. At 8 hr, putative zygotes were transferred into development medium and further incubated. At 32 hr of culture, dividing embryos were counted (% fertilized oocytes)

Results: Embryo development (%) was determined by the number of morulae and blastocysts on Day 7. Friedman's test compared percent fertilization and embryo development in each treatment versus control.

In Vitro Fertilization & Embryo Development

Treatment	Total Oocytes	% Fertilized Oocytes (+sd)	% Embryos Developing (±sd)	p value fertilization / developed
Control	160	63(8)	45(12)	/
Pré	160	62(11)	42(5)	0.900 0.470
FC	160	53(12)*	28(20)*	0.003 <0.0001
CE	160	56(9)*	27(16)*	0.033 <0.0001
PC	160	56(10)*	26(16)*	0.025 <0.0001

* denotes treatment means differ from control

Conclusion: Sperm contact with Pré did not interfere with fertilization or embryo development, whereas other lubricants caused significant declines in these end points. The reasons for these differences require additional study.

Pré® Lubricant - INGfertility, Valleyford, WA
 FertilityCare™ - Marco D'Polo, Ingleburn, NSW, AU
 ConceiveEase™ - Sepal, Boston, MAPREConceive plus™ - Lake Consumer Products, Inc., Jackson, WI

Animal Model Study of a New Patient Lubricant's Affect on In Vitro Fertilization & Embryo Development American Society of Andrology Annual Meeting, Orlando FL, April 2007 RW Wright Jr, Center for Reproductive Biology, WSU Pullman, WA

Experiments were done to evaluate in vitro fertilization and embryo development following sperm exposure to products used to lubricate devices in fertility medicine including: KY Gel, Aquasonic Ultrasound Gel and Pré' (a new Patient Lubricant recently cleared for use during fertility interventions). Bovine in vitro fertilization and embryo culture methods are standard and have been proposed as an excellent model for gamete toxicity studies (ReprodBioMed Online 2002;4:170-5). In this study, cryopreserved bull sperm (from a single bull) were routinely washed, resuspended in a TALP medium and placed into one of 5 treatments. These included: 1) Control sperm in medium alone; or sperm medium suspensions with the following added (v/v) 2) 10% Pré' lubricant; 3) 50% Pré' lubricant; 4) 10% KY; or 5) 10% Aquasonic Gel. Sperm were incubated in treatments for 30 min at body temperature, and placed into fertilization wells with mature oocytes (1 x 10⁶ sperm cells per well). At 8 hrs, putative zygotes were transferred into embryo culture medium and further incubated. At 32 hr of culture, dividing embryos were counted (% fertilization in each treatment). Final development rates were evaluated on Day 7 (post IVF) to determine the % of total oocytes that had developed to the morula or blastocyst stage. ANOVA was used to compare the % fertilization of oocytes & the % of normal embryo development resulting from sperm in each treatment (as seen in Table below, data are mean +/- sd). In Vitro Fertilization & Embryo Development After Sperm Exposure

Treatment	Total Oocyte Number	% Fertilized Oocytes (± sd)	% Embryos Developing (± sd)
Control Medium	80	61(5) ^a	40(9) ^a
Pre' 10%	80	60(8) ^a	39(8) ^a
Pre' 50%	80	59(6) ^a	43(10) ^a
KY 10%	80	23(6) ^b	6(5) ^b
Aquasonic10%	80	0 ^c	0 ^c

a,b,c denote means that differ within column by p<0.0001(ANOVA).

Pre' Patient Lubricant did not interfere with the ability of sperm to fertilize oocytes or support embryo development in vitro (using a bovine model) even at high concentrations. Conversely, KY and Aquasonic significantly impacted the ability of sperm to fertilize oocytes, and allow normal embryo development.

Changes in Sperm Motility and Chromatin Integrity Following Contact with Vaginal Lubricants American Society of Reproductive Medicine Annual Meeting, Montreal Quebec, October 2005 (now a full publication in Fertility & Sterility, 2007: Effect of vaginal lubricants on sperm motility and chromatin integrity: a prospective comparative study).

Agarwal A, Deepinder F, Cocuzza M, Short RA, Evenson DP. Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics-Gynecology, Cleveland Clinic, Cleveland, Ohio.

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.

The Effects of Vaginal Lubricants and Moisturizers on Computer Assisted Sperm Analysis (CASA) Parameters Associated with Cervical Mucus Penetration
American Society of Reproductive Medicine Annual Meeting, Philadelphia PA, October 2004

JE Ellington, and J. Schimmels, INGfertility, Spokane, WA and Washington State University, Spokane, WA

Objective: The incidence of vaginal dryness is increased in trying-to-conceive (TTC) couples; however, numerous papers have cited the detrimental effect of common vaginal lubricants and moisturizers on sperm motility. To date, studies have not been done using CASA to evaluate the effects of lubricant products on the motion characteristics of sperm thought to be associated with cervical mucus transport. Specifically, samples of sperm with mean average path velocity (VAP), % straightness (STR) and Amplitude of Lateral Head Displacement (ALH) exceeding a predetermined level have recently been proposed to have a superior likelihood of good cervical mucus penetration in vivo. Numerous studies have found a correlation between such ability of sperm to penetrate cervical mucus and pregnancy outcomes. The current study evaluated sperm motion parameters following contact with several vaginal lubricants/moisturizers, to determine their impact on CASA outcomes associated with good cervical mucus penetration, as well as overall motility.

Design: Prospective, comparative, in-vitro study.

Materials and Methods: Raw semen from 25 normospermic donors was diluted 1:1 with Human Tubal Fluid. Each sample was then divided into one ml aliquots and placed into multiple culture wells. Vaginal lubricants/moisturizers as shown in the Table below were applied across these wells to achieve a final 10% v/v concentration, and incubated at 37°C for 30 min. CASA of sperm from these treatments and those in a control well (with no lubricant) was then performed. Samples in each treatment fulfilling all of the following parameters were given a positive penetration score (e.g. likely to penetrate cervical mucus well): VAP > 25 micron/sec; STR >80%; and ALH >2.5 microns. Positive penetration scores were reported as a

percent of all samples tested and compared between the treatments. Additionally, mean outcomes in each treatment were determined and compared for the individual CASA parameters, as well as for the overall percentage of motile sperm.

Results: The mean motion characteristics for these specific CASA criteria (+/- SEM) and percent samples with a positive penetration score are shown in the Table below. Means with differing superscripts differ from the control at p<0.05.

Sperm Motility Following Contact with Vaginal Lubricants					
Treatment	VAP	STR %	ALH	Overall % Motile	% Samples w/ + Score
Control media	56 +/-3	81 +/-2	3.9 +/-0.3	70 +/-4	61b
FemGlide	50 +/-2	81 +/-4	3.7 +/-0.4	61 +/-3	68b
Pre ~Seed	53 +/-3	85 +/-3	3.6 +/-0.2	76 +/-3	90a
KY Jelly	49 +/-4	80 +/-4	2.4 +/-0.6a	29a +/-2	20c
Astroglide	39a +/-2	74 +/-3	2.6 +/-0.5a	5a +/-0.4	10c
Replens	*	*	*	15a +/-2	*

Replens caused the media to abruptly turn very acidic and opaque. Further, sperm clumping occurred to the point that meaningful CASA data could not be generated.

Conclusion: Within 30 minutes of exposure, a 10% v/v concentration of the most commonly used lubricant products significantly decreased sperm motility and penetration scores. The percentage of samples with a positive penetration score was significantly improved with Pre-Seed as compared to the other treatments. Products used to alleviate vaginal dryness that negatively effect sperm motility and transport should be avoided by trying to conceive couples. Studies to determine the in vivo impact of vaginal lubricants/moisturizers on cervical mucus penetration are ongoing.

Prevalence of vaginal dryness in trying to conceive couples
Pacific Coast Reproductive Society Annual Meeting, Rancho Mirage CA, April 2003

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Dyspareunia, primarily due to vaginal dryness, has been reported to occur “sometimes” or “more often”, in at least 46% of all reproductive age women. However, it is currently not known if vaginal dryness is increased in trying-to-conceive (TTC) couples. Additionally, it is not known how trying to conceive

couples are managing symptoms of vaginal dryness, given numerous reports on the sperm-toxic nature of most personal lubricants and even saliva. This study was done to determine the prevalence of vaginal dryness among trying to conceive couples, and their level of understanding of appropriate interventions for such dryness. An opt-in internet survey of 900 TTC couples was conducted over 5 months. Thirty questions regarding fertility and vaginal dryness were asked of each participant. Summary statistics for the group were compiled and analyzed.

Average TTC time for the group was 7 months, with 33% TTC 1 year or more. Medical care for their fertility issues included: 23% no doctor, 13% PCP, 43% ObGyn, 16% Fertility Specialist, 4% Urologist. Most couples (78%) had no definitive diagnosis for cause of fertility problems. Most (69%) routinely used some ovulation prediction method. Only 16% were currently taking “fertility medications”.

While TTC, vaginal dryness negatively affected sexual intimacy for most couples: 11% always, 35% often, 42% sometimes, 9% rarely, 3% never. Vaginal dryness episodes also increased while TTC: 19% a lot, 57% some, 23% not at all. Although 30% knew not to use a lubricant while TTC, another 26% often or always used such products. Use by this later group included mostly that of KY (40%) and Astroglide (19%). **Only 20% of couples had ever discussed their dryness problem with a doctor.** Of those that had, 75% of the doctors reiterated the sperm-toxic effects of lubricants.

Rates of vaginal dryness in TTC couples appears to be as much as twice that seen in the general population. Patients are not discussing this problem with their care providers adequately. **Fully one-quarter of TTC couples are utilizing personal lubricant products which reportedly are as toxic to sperm as are contraceptive jellies.** Products designed specifically to relieve vaginal dryness without harming sperm, such as Pre~Seed, are needed for use by TTC couples.

Effects of Personal Lubricants on In Vitro Fertilization and Embryo Development

American Society of Andrology Annual Meeting, Phoenix AZ, March 2003 RW Wright¹, PhD; RA Short², PhD; & JE Ellington³ DVM, PhD
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Use of personal lubricants is not recommended for couples that are trying to conceive based on several studies reporting their deleterious effect on sperm motility. In spite of this, 43% of all trying-to-conceive couples use personal lubricant products due to a high frequency of vaginal dryness. The current

study was designed to compare in vitro fertilization and embryo development of bovine oocytes in the presence of moderate doses (10%) of several different products. In vitro matured cow oocytes were fertilized by bull sperm with: 10% KY Jelly; 10% FemGlide (labeled as “sperm compatible”); 10% Pre~Seed (a new moisturizer developed to provide an optimal sperm environment); and control TALP IVF media. Lubricants were only present during the fertilization incubation of sperm and oocytes. The bovine IVF model allows for detection of sperm DNA damage which can inhibit embryo development. Embryos were cultured for 7 days and then scored for normal development for blastocyst (multi-cell) stage. Data are expressed as Mean (SEM). Treatment

	# Oocytes	% Fertilized	% Blasts
KY Jelly	100	12 (2.0)a	2 (1.2)a
FemGlide	200	72 (3.4)b	42 (0.7)b
PreSeed	200	73 (4.6)b	47 (0.9)c
Control	200	77 (3.4)b	44 (0.8)b,c

KY Jelly in the fertilization medium had a very negative effect on fertilization and development (a,c differ by p<0.001), with only 2% of all eggs developing to the blastocyst stage. FemGlide decreased embryo development as compared to the Pre~Seed treated sperm (b,c differ by p=0.05). Pre~Seed did not effect embryo development as compared to the control media in this model, in fact a trend for improved development was seen. Mouse embryo development studies with 10% volume of test product are routinely done as a toxicology screen for assisted reproduction media. A similar design, using cow embryos detected a harmful effect of KY Jelly and FemGlide on embryo development after sperm exposure to these products.

Effect of New Intimate Moisturizer on Sperm Motility American Society of Andrology Annual Meeting, Phoenix AZ, March 2003

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 Numerous publications cite the deleterious effect of existing commercial lubricants on sperm motility. Additionally, 75% of trying-to-conceive couples have an increased incidence of vaginal dryness. This study compared motility parameters for human sperm (n=25 ejaculates) cultured for 30 min in HTF media with HSA (control), to which either 10% KY Jelly; 10% Astroglide; 10% FemGlide (marketed as “sperm compatible”); or 10% Pre~Seed (specifically developed to not harm sperm) were added.

culture at 37° C. At 4h, sperm motility was determined and aliquots were removed to determine membrane lipid peroxidation.

Results: More sperm were motile ($p=0.04$) and had lower oxidative damage ($p=0.01$) in TALP with added PCAG, than in TALP alone. Follow-up studies identified an active fraction of the PCAG between 20K and 100K which promoted sperm motility and membrane stability. Preliminary studies have shown decreased oxidative stress and chromatin damage for human sperm in culture with HTF including the PCAG.

Conclusion: PCAG stabilize sperm during assisted reproduction techniques. Specifically, they appear to decrease oxidative stress and chromatin damage.

a,b,c Superscripts show means (SEM) within a column that differ at $p<0.05$.

Treatment	% Motility	Progressive VSL (µm/s)	VCL (µm/s)	VAP (µm/s)
Control	100a	53 (2)a	89 (3)a	59 (2)a
KY	62 (6)b	37 (2)b	67 (2)b	40 (2)b
FemGlide	92 (4)c	44 (4)c	79 (3)c	50 (4)c
PreSeed	100 (5)a	51 (2)a	79 (2)c	56 (3)a,c
Astroglide	<5	NA	NA	NA

Due in part to viscosity change, all lubricants slowed sperm velocity as compared to control medium. However, sperm in Pre~Seed retained motility equivalent to the control over the 30 min of culture, whereas sperm in the other lubricants had decreased motility ($p<0.05$). This effect was profound with Astroglide. Placed side-by-side, FemGlide and KY created a distinct barrier whereby sperm in raw semen had difficulty penetrating into the products (photos available). In contrast, sperm moved freely between raw semen and the Pre~Seed.

Polysaccharides Containing Arabinose and Galactose Decrease Oxidative Damage to Sperm In Vitro

American Society of Andrology Annual Meeting, Montreal Quebec, June 2002

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Polysaccharides containing arabinose & galactose (PCAG), such as arabinogalactan, are abundant in plant gums. These PCAG have a membrane stabilizing effect in a variety of cell types. Studies were done to determine the effects of PCAG on bull sperm during freezing and culture.

In Experiment 1, ejaculates from 4 bulls at a commercial AI stud were frozen in standard egg yolk buffer (EYB) or in egg yolk buffer with PCAG (PEYB). Six straws of sperm from each bull and treatment were thawed and: 1) held at 37° C for 10 min, then evaluated for membrane lipid peroxidation (TBARS assay) and sperm chromatin damage (Sperm Chromatin Structure Assay); or 2) cultured in routine TALP medium for 24h to determine sperm survival rates.

Results: sperm frozen in EYB had more oxidative ($p=0.03$) and chromatin ($p=0.01$) damage after thawing than sperm in PEYB. Only 1 of 4 bulls had > 10% motile sperm at 24h of culture for sperm frozen in EYB; whereas 3 of 4 bulls had >10% motile sperm for sperm frozen in PEYB.

In Experiment 2, sperm from 5 bulls (4 straws each) frozen in standard EYB were thawed, washed and placed in TALP either w/or w/o PCAG for

Use of a Plant Polysaccharide Gradient to Wash Bull Sperm Improves Fertilization & Embryonic Development

International Embryo Transfer Society Annual Meeting, Salt Lake City UT, Jan 1996

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Experiment 1. A continuous gradient of 22% plant polysaccharides (arabinogalactan) in a buffered salt solution (Sperm Concept-INGfertility, Spokane, WA) was compared to a standard Percoll density gradient of 45 % and 90% using frozen-thawed bull sperm (Select Sires). Studies were designed to evaluate sperm recovery and performance in a routine IVF system as determined by fertilization rates and subsequent embryonic development. Frozen sperm from 4 bulls (2 replicates each) were washed through 4 ml of Sperm Concept (SC) or gradient Percoll (P) for 30 min at 300 x g. The pellet of sperm from P was washed again in a TALP medium prior to use. No extra wash step was used in the hemocytometer. Sperm motility was also evaluated both subjectively and objectively with an HTM Analyzer. All data are expressed as the mean SEM for SC or P treated sperm, respectively. Statistical analyses were conducted using ANOVA.

Conclusions: Recovery of sperm was significantly higher ($p=0.02$) after SC washing than with P (72 6% vs 53 5%). SC also tended ($p= 0.08$) to recover more of the motile sperm than did P (88 9% vs 73 6%). Overall percent motilities were high for both groups and did not differ ($p=0.18$; 95 0.3 % vs 90 3%).

Experiment 2. Pooled frozen bull sperm was also washed through either SC or P as above, treated routinely with 10 IU heparin/ml and placed with total of 1100 IVM oocytes over three replicates. Oocytes were obtained from slaughtered beef heifers. Cleavage rates were determined visually on Day 3 of culture in CZB medium. Embryo quality was recorded on Day 9 of coculture on BRL cells, followed by embryo staining (Hoechst 33258) and cell counts. Embryo evaluators were blind to the sperm washing treatment utilized prior to IVF.

Conclusion: Fertilization rates for the oocytes were improved after washing sperm through SC versus P (p=0.001; 73 2% versus 53 3%). The percentage of fertile oocytes able to develop to blastocysts by Day 9 of culture was higher after washing sperm through SC versus P (p=0.009; 59 4% vs 26 5%). Overall production of blastocysts as a percentage of total oocytes introduced into the IVF system was greater after washing sperm through SC versus P (p=0.004; 43 4% vs 18 3%). Total cell counts for blastocysts formed after fertilization with either SC or P treated sperm did not differ (p=0.25; 85 2 vs 88 2).

Overall Conclusion: Washing frozen thawed bull sperm through SC appears to offer several advantages over P gradients. A pellet of highly motile sperm cells can be obtained in one centrifugation step. Sperm exposed to the arabinogalactan-containing SC wash show improved ability to fertilize oocytes and contributed to better embryonic development to the blastocyst stage.

A Novel One Step Sperm Wash Product

Pacific Coast Fertility Annual Meeting, Palm Springs CA, March 1995

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A continuous gradient sperm wash product containing arabinogalactan, Sperm Concept (sold as IsoCare One Step) was compared against a Percoll wash in 3 experiments.

Experiment 1: In Expt. 1, fresh semen from 4 men was divided and washed either in SC or 80% Percoll (P) for 30" at 300 x g. Pellets were then washed again in media alone and sperm evaluated. All data are expressed below as mean SEM for SC versus P. There was no difference in the % motility for recovered sperm (83 4% vs 83 3%), the motility of sperm cultured for 24 h (64 6% vs 75 6%) or in the numbers of sperm recovered (p=0.6). Based on animal model data showing a lack of toxicity of SC to sperm throughout the IVF process, the next 2 experiments were done on sperm removed directly from the SC pellet, without a second media wash step.

Experiment 2: In Expt. 2, SC was compared to bilayer P (90%:45%), to wash fresh semen from 8 men. The % normal morphology (83% 3% vs 81 1%) and motile sperm (74 6% vs 76 3%) did not differ between treatments. Both treatments improved (p<0.09) these parameters over that found for prewashed sperm morphology (72 2%) and motility (62 5%). The % of motile sperm recovered tended to be higher (p=0.1) after SC wash (93 5% vs 80 5%).

Experiment 3: In Expt. 3, semen from 12 men with fertility disorders was evaluated after washing in SC or P. There was no difference in motility of recovered sperm (59 12% vs 60 16%); however, normal membrane function (hypo-osmotic swell test) was better (p=0.045) in SC (70 13% vs 46 10%).

Hamster egg SPA was also done on sperm washed from 4 donors in both SC and P. There was no difference in % eggs penetrated (41 2% vs 40 2%) or in the penetration factor (0.48 0.02 vs 0.41 0.03). One man did show a 30% improvement using SC versus P. The SC product is made from a polysaccharide approved for human consumption. It offers a non-toxic one step wash alternative to Percoll.

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