GENERAL COMMENTS ON DOCKET NO. 2003N-0539
OTC DESIGNATION FOR VAGINAL LUBRICANTS

Need for improved labeling for use of vaginal lubricants by trying-to-conceive couples

SUMMARY

The authors of these comments do not agree that vaginal lubricants and moisturizers require an OTC drug monograph for safe and appropriate usage. However, existing labels on lubricants and vaginal moisturizers bear claims regarding their effect on sperm which are inadvertently misleading and may be resulting in significant Public Health risks for couples actively trying to conceive children. The following comments address the need for clear testing of sperm function for, and subsequent labeling of, any genitally applied lubricant or moisturizer used by couples during intercourse for procreation.

At least 11 million couples in the United States are actively seeking to conceive a child. Six million of these couples are "infertile", meaning they have been trying to conceive for over one year without a successful pregnancy. Costs of medical intervention for these couples exceed $2 B/ year in the US, with much of this paid directly by couples due to limited health insurance coverage for infertility.

These trying-to-conceive (TTC) couples have a high incidence of vaginal dryness affecting their sexual function. In fact, 75% of TTC couples have reported an increased incidence of personal dryness due to having timed intercourse around ovulation, fertility medications and fear of failure. Unfortunately, three decades of peer reviewed and published research has shown that existing vaginal lubricants harm sperm. Specifically, these studies have shown that sperm motility (swimming) is dramatically reduced after exposure to even small concentrations (<10% v/v) of these products, and that sperm penetration into the cervix can be reduced to almost zero after contact with such compounds.

Several authors in these studies have stated that the use of vaginal lubricants is **contraindicated** in subfertile couples, particularly those 60% of infertile couples with male factor (or sperm) issues. In spite of these reports there remains a great deal of confusion amongst physicians and lay people alike as to the safety of vaginal lubricant use while trying to become pregnant.

⇒ In fact, many doctors are inadvertently recommending lubricants that harm sperm to their infertile patients based on inaccurate information.

This confusion exists in part, because several lubricants claim in their literature that they "will not kill sperm"; that they are sperm "compatible"; and that they have "no effect on sperm motility", in spite of published literature to the contrary. In addition, almost all lubricants that do not contain contraceptive drugs state that they are "not a spermicide". This results in a common misconception, that if a lubricant does not contain a spermicide it will not impair sperm function. Unfortunately, this is contrary to all scientific and medical evidence.

Commercially available vaginal lubricants, many which are FDA approved as Class 1 devices, have pH levels and osmolality levels that are several fold outside of the physiologic range for sperm. This can result in **irreversible damage** to sperm and a loss in motility after exposure

to commercial lubricants ***similar to motility losses after contact with an OTC contraceptive gel*** (nonoxynol-9-containing Gynol II).

⇒ Damage to sperm following contact with lubricants may contribute to conception failure in some couples, resulting in undesired childlessness, or the advancement to invasive advanced reproduction technologies (all of which carry added economic costs and risks).

In order to clarify confusion amongst medical professionals and consumers, vaginal lubricants that deleteriously affect sperm function as determined in repeatable, standard assays should incorporate warning labels stating that they are unsafe for use while trying to conceive. Minimum levels of sperm function following contact with vaginal lubricants should be developed and included in labeling to protect couples that are trying to conceive. This could function similar to the "Special Controls" that are currently in place to ensure safety and efficacy of Reproductive Media that contact gametes in the laboratory during assisted reproduction. Likewise, testing minimums should be developed and implemented to ensure that vaginal lubricants, or other products that could contact sperm during desired procreation, are "acceptable" or "safe" for use by couples actively seeking to have children. Products that fail to meet these standards, resulting in sperm damage that falls below these minimums, should be labeled as "**not intended for use while trying to conceive**". Conversely, products that allow sperm function to continue without harm could be labeled as "safe for use while trying to conceive". Specific indications might also be considered for these products such as: "To alleviate vaginal dryness while trying to conceive", or "To provide added lubrication while trying to conceive", or "Maintains sperm function while treating vaginal dryness when trying to conceive".

BACKGROUND

Incidence of Fertility Disorders in the United States

Infertility affects approximately 15% of all couples in the US. Several studies suggest that rates of infertility may be growing, due to delayed childbearing, prevalence of sexually transmitted diseases and potential declines in male sperm production. At least six million couples in the US have medically defined infertility, meaning they have had unprotected intercourse for over one year without a successful pregnancy. Each year, approximately 750,000 new couples realize that they have infertility issues. Even before the one year date for unsuccessful pregnancy, many couples become concerned if they are failing to conceive. Epidemiology studies suggest that 9 million US women per year approach their physician concerning problems with conceiving.[1-3] In at least half of the cases of infertility, a male factor will be partially or fully causative.

Even though medical interventions for fertility disorders have developed at a remarkable rate, less than 10% of infertile couples undergo in vitro fertilization (IVF) treatment. The limited use of assisted reproduction techniques by infertile couple is due to many factors, including economic constraints, lack of insurance coverage, religious objections, issues of privacy, and concerns about safety and level of intervention. The estimated mean 2002 cost of a single IVF cycle in the United States is $9,500, equivalent to approximately 25% of mean annual household expenditures .[4] With an average success rate of no more than 25% per cycle, advanced reproductive techniques rapidly become unavailable to most American citizens.

Subsequently, over 90% of infertile couples continue to have unprotected intercourse with the hope of becoming pregnant naturally.

In addition to infertile couples, a subset of fertile Americans are also actively seeking to conceive children. Based on annual birth statistics, this number can conservatively be estimated at 5-6 million fertile couples at any given time.

- Therefore, an estimated 11 million US couples are desirous of conceiving a child, and are actively making lifestyle choices and utilizing products to support their dream.

Prevalence of Vaginal Dryness in Infertile Couples

A common complication for these trying-to-conceive couples (often self described as "**TTC**") 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 taking.[5] Enjoying intercourse can become difficult during daily sexual activity when vaginal dryness and dyspareunia become an issue. In fact, over 25% of TTC couples "always" use lubricants while having intercourse (e.g. an estimated 2.25 million Americans).

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%.[6] 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 maladjustment.[7] 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.[8-12] 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 are several hundred products marketed in the US. 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 Medical school settings, reviewing the effect of 16 different lubricants on sperm, including all of the top selling brands (Appendix A).[13-23] In each study, sperm motility and viability were dramatically reduced after exposure to even small concentrations (<10% v/v) of these products.

Specific data from these studies show:

- A *spermicidal* activity of the leading three brands of lubricants as being <u>*equivalent to contraceptive jellies such as Gynol*</u> .[16,17] 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 lubricants[18] as shown in Figure 1.
- These effects are concentration dependent (Figure 2) and were seen even with <u>low concentrations</u> (1–6%) depending on the lubricant product.[14, 16,18] Even in studies where sperm were washed out of a 1% lubricant exposure after 20 min, sperm motility subsequently fell to zero.[14]
- Critically, the deleterious effect of lubricants on sperm includes <u>decreasing sperm penetration into the cervix in vivo</u>, as determined by the post-coital test (PCT)[20]. 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 intravaginally prior to coitus.

Figure 1. Effect of 30% Concentrations of Lubricants on Sperm Motility and Viability (adapted from ref. 18)

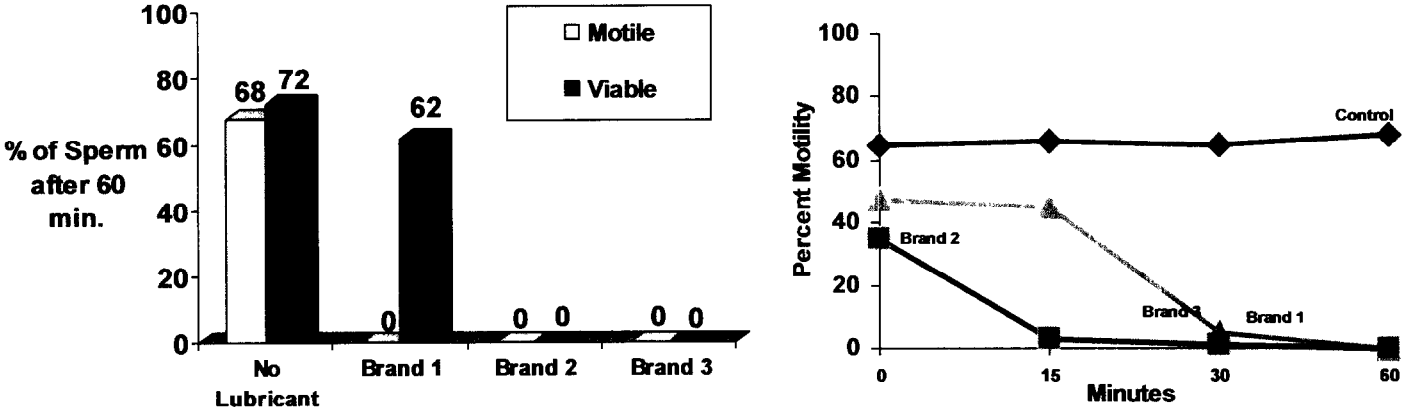
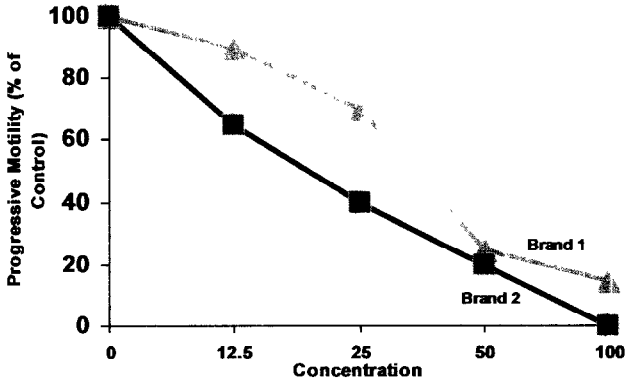


Figure 2. Concentration Dependent Effect of Lubricants on Human Sperm Motility Following 1 minute of Exposure (adapted from ref. 16)



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.[24,25] Existing lubricant products rapidly decrease sperm motility, thus possibly decreasing the numbers of sperm penetrating the cervix and being able to participate in fertilization. The following excerpts, from a partial list of lubricant studies done over the past three decades, highlight the investigators' deep concerns with the use of vaginal lubricants by TTC couples. The institutions completing the studies are also cited to emphasize the breadth of this research.

Dept of ObGyn; University of Texas Southwestern Medical Center[18]

- "Sperm exposed to (Brand 2) or (Brand 3) were nonmotile 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 Connecticut[16]

- "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 Belfast[13]

- "...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**."

Dept of ObGyn; University of Minnesota[21]

- "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, MD[20]

- "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 Medicine[14]

- "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 York[19] (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 School[15]

- "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

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.[26] This coincides with the physiologic rise in pH of ovulatory cervical mucus found in women at the time of ovulation.[27] 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.[28,29] Sperm motility decreases linearly with exposure to increasing osmolality, such that sperm motion is basically abolished at 600 mOsm or greater.[29] 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). At a 30% v/v concentrations of these products with raw semen (which could be easily obtained after in vivo exposure to lubricant), semen osmolality is elevated to 820 mOsmo (Brand 1), 1700 mOsmo (Brand 2), and 703 (Brand 3) upon contact[18] (all above the reported threshold for maintenance of sperm motility of 600 mOsmo). 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.[14,15]

Table 1. pH and Osmolalities for Popular Vaginal Lubricants

	pH			Osmolarity (mOsm/kg)		
	Too low	**Physiologic for sperm 7.0 - 8.5**	**Too high**	**Too low**	**Physiologic for sperm 270 - 360**	**Too high**
Brand 3	2.79					>1000
Brand 12	6.44			162		
Brand 13	6.13					>1000
Brand 1	4.52					>1000
Brand 14	4.40					>1000
Brand 1 Liquid	3.82					>1000
Brand 2	3.45					>1000
Brand 15	5.29			187		

Existing vaginal lubricants were designed to provide lubrication to facilitate intromission and alleviate vaginal dryness. Their 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. The ingredients that make up lubricants and that provide these nonphysiologic conditions tend to be drawn from a relatively small group of compounds, 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.

CURRENT PHYSICIAN AND CONSUMER KNOWLEDGE OF LUBRICANT-SPERM INTERACTIONS

In spite of the numerous publications cited above, there remains a great deal of confusion amongst physicians and lay people alike as to the safety of vaginal lubricant use while trying to become pregnant. In fact, many doctors are inadvertently recommending lubricants that harm sperm to their patients based on inaccurate information. While respecting any physician's right to freedom of speech, the following excerpts from medical related internet sites are cited as examples of the confusion amongst the medical community with regards to the safety of lubricant use while TTC (Appendix B). These are placed here purely for example and are in no way inclusive, nor meant to be derogatory to the institutions cited.

Local Channel 12 Health Authority (sponsored by Johnson & Johnson)

"You might have heard the controversy of how lubricants can affect sperm...there's no exact science about which to use. If you're not sure what form of lubrication will help or hinder conception, try alternating methods or talking to your doctor."

Evanston Northwestern Healthcare-

"(Brands 2 and 3) may not be as harmful to sperm."

- shown to be spermicidal in above studies

Gotomydoc.com
"When trying to conceive use water-based lubricants like (Brand 3, 1 or 16)."
- shown to be spermicidal in above studies

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Dr. Spock.com
"While trying to conceive one good lubricant is (Brand 2)...which is not believed to be toxic to sperm."
- shown to be spermicidal in above studies.

UC Davis Medical College
"(Brand 2 and 3).... may not be as harmful to sperm."
- shown to be spermicidal in above studies.

Dept Urology Yale University
"A natural lubricant such as "(Brand 3)" may be substituted and is not sperm toxic."
- shown to be spermicidal in above studies.

Contemporary OB/GYN
"When TTC, Oil-based lubricants may be spermicidal and should be replaced with water-soluble agents (Brands 1 and 2)."
- shown to be spermicidal in above studies.

Cornell University Medical College
"If lubrication is necessary we recommend (Brands 2 and 3)."
- shown to be spermicidal in above studies.

These quotes represent only a fraction of the professional medical sites addressing lubricant use while trying to conceive. However, they show a fundamental disconnect between the published data of the effects of lubricants on sperm function and the physician's knowledge.

This lack of clarity on an issue of critical public health importance, causes tremendous confusion within the consuming public. A search for the terms lubrication and conception turns up over 6,500 internet matches. Virtually every one of the hundreds of preconception websites has discussions and recommendations on the safety of lubricant use while TTC. A few of these discussions are cited here for example (Appendix C). It is CRITICAL to note that each of these preconception sites is **recommending and/or selling lubricant brands for use while TTC that have been shown to be spermicidal in peer reviewed studies.**

Babycenter.com (This site is sponsored by Brand 1 maker)
Question: "What if the lubricant says "this product does not contain a spermicide? Is it still toxic to sperm?"
Answer 1: "My physician says as long as it is water based and says does not contain a spermicide you are fine
Answer 2: "I called the (Brand 1) 1-800 number on the back of the bottle and they confirmed that it does not kill sperm."
Answer 3: "I went to Gotomydoc.com. They say if it does not have a spermicide then it is OK, they specifically **say water liquid lubricants are fine** and they list three including (Brand 1)."

Parents Nest.com
"(Brand 2) really helps during intercourse. It is known to be safe for those trying to conceive. Find (Brand 2) on ParentsNest.com. You can buy (Brand 2) here along with many preconception products."

Mom'sview.com
Question: "Does anyone know if (Brand 2) hurts sperm"
Answer: "(Brand 2) is fine to use. My doc even recommended it"
Site links to sale of Brand 2.

Resolve (the national infertility support group!)
"Avoidance of spermatotoxic lubricants such as (Brand 1)...is important. A natural lubricant such as (Brand 3) may be substituted and is not toxic".

The Fertility Shop
"(Brand 3) is perfect for conception. (Brand 3) does not alter sperm motility or ovum penetration unlike some lubricants, because it is not petroleum based."
Brand 3 is for sale at this site.

TTC Dreams
"(Brand 2) is the only lubricant that does not interfere with conception".
This site offers a click through to (Brand 2) for a free sample.

WHY THERE IS CONFUSION REGARDING LUBRICANT USAGE WHILE TTC?

This confusion exists in part,

⇒ ***because the leading Brands state in their marketing material that they "will not kill sperm", or that they have "no effect on sperm motility" in spite of published literature to the contrary***.

In spite of published data to the contrary the following claims are made by lubricant manufacturers without any available supporting scientific evidence that the academically trained authors could locate (Appendix D).

Example 1) Brand 2 states in its website Question and Answers section:
Q. "Can I use (Brand 2) while trying to conceive?"
A. "(Brand 2) is not a spermicide and **will not kill sperm**."
They then do go on to say "Studies have shown, however, that (Brand 2) as well as other topical lubricants do inhibit the motility of sperm." "Discontinue if conception is not achieved in a reasonable amount of time."

⇒ Published studies by others show no live sperm with Brand 2 after 30 minutes of contact with a 30% solution of this product.

Example 2) Brand 3, in their Product Information section of their website under a heading of "Clinically Proven and Effective", states:

"Does not alter sperm motility or ovum penetration[2] unlike some lubricants."

However, the reference they site as reference #2 in the above sentence[16] is for a published study on Brand 2's damage to sperm on contact. This sentence is misleading in that it appears

to cite a paper supporting Brand 3's benign effects following sperm contact. The paper, however, describes sperm damage by a competitive lubricant and mentions nothing about Brand 3.

Again at this website, a "scientific" abstract in its Commonly asked Questions section, Titled "Effects of (Brand 3) on Sperm Motility and Ova Penetration" cites: "Results from preliminary and expanded studies show (Brand 3) had no effect on sperm motility and does not affect sperm ability to penetrate hamster ova. "

⇒ Published studies by others show no live sperm after 30 minutes of contact with a 30% solution of this product.

Numerous attempts to locate a copy of the actual abstract cited for Brand 3 have been unsuccessful. This has included contacting authors, journal editors and extensive library searches. No copy of the abstract has been found, so data can not actually be reviewed. The citation at Brand 3's website does not disclose any study design or numbers.

Example 3) Brand 12 states that it is "sperm compatible". However, repeated written and phone requests to the manufacturer and distributor for data to confirm this have resulted in only the statement that Brand 12 is "reportedly sperm compatible". Again no data from the Company or independent investigators can be provided.

Example 4) Nurses staffing consumer calls for Brand 1, state that Brand 1 "does not harm sperm". Similar statements are present on a preconception web site sponsored by Brand 1.

⇒ Published studies by others show Brand 1 resulted in no motile sperm after contact in the above studies.

The information available from lubricant manufacturers themselves suggests, at best, confusion and, at worst, a lack of full disclosure in advertising for these products. This disconnect between published effects of lubricants on sperm and the lubricant industries statements make it difficult for physicians and consumers to make well informed choices regarding the use of lubricants for those trying to conceive.

Additionally, a common misconception is that if a lubricant does not contain a spermicide or if it is water soluble, it will not impair sperm function. Unfortunately, this is completely inaccurate. As discussed above, water based lubricants are lubricious due to formulations based on glycerin (which has been shown to be toxic to sperm)[17,22] and propylene glycol both of which are highly hyperosmotic. This results in irreversible damage to sperm and a loss in motility after exposure to commercial lubricants *equivalent to motility losses after contact with a contraceptive gel*. Damage to sperm and subsequent inability of the sperm to penetrate into the cervix following contact with lubricants may prevent conception in some couples, resulting in consequences such as undesired childlessness, or the advancement to more invasive advanced reproduction technologies (all of which carry added economic costs and medical risks).

THE NEED FOR VAGINAL LUBRICANT LABELING

It is striking that lubricant products which significantly impact sperm function and viability in every published study to date do not carry a warning label for couples that are trying to conceive. Many consumers are shocked when they discover that products they use every time

they have intercourse could actually be impairing sperm penetration into the cervix and their subsequent fertility.

The following excerpts from preconception chat rooms throughout the internet highlight the thoughts of many American couples (Appendix E).

- " I am shocked to hear (that lubricants damage sperm). I use lube all of the time. I wonder if there is some kind of warning on the bottle. If not there definitely should be."
- "It should be labeled more clearly if you are trying to conceive."
- "I can't believe this is the first time I have heard that using a lubricant will affect conception!!!!! Why isn't this more widely publicized or have I been just living under a rock?"
- "I am really upset that now I learned that apparently it (Brand 1) can kill sperm, but on the bottle it says does not contain a spermicide. So I thought it was safe. It should be labeled more clearly if you are trying to conceive."

PROPOSED LABELING CHANGES FOR VAGINAL LUBRICANTS

In order to clarify confusion amongst medical professionals and consumers, vaginal lubricants that deleteriously effect sperm function **should be labeled as such**. Minimum levels of sperm function following contact with vaginal lubricants should be developed and identified in labeling to protect couples that are trying to conceive. Standard, routine assays can be implemented to determine if vaginal lubricants are "acceptable" or "safe" for use by couples seeking to become pregnant. Products that fail to meet these standards, due to sperm damage following contact, should likewise be labeled as "not intended for use while trying to conceive".

The proposed requirements are similar to the existent Special Controls currently in place for Part 884 Obstetrical and Gynecological Devices, Subpart G Assisted Reproduction Devices (Sec 884.6180 Reproductive media and supplements). These are products that "come in direct physical contact with human gametes...including (their) maintenance" while being handled in vitro. These Special Controls "have been identified in order to provide reasonable assurance of the safety and effectiveness of devices used in assisted reproduction procedures". FDA has placed controls in effect to modify safety and efficacy for Reproductive Media that only about 20% of all infertile couples utilize to conceive. It would seem warranted to develop controls for products such as lubricants that could come in contact with gametes (i.e. sperm) during natural intercourse by the remaining 80% of American TTC couples.

Based on published data and clinical observations, it is possible that the use of vaginal lubricants could be limiting normal sperm transport in women following coitus and thus causing or exacerbating fertility disorders. The consequences of such disturbances in fertility can be profound for couples, with regards to: economic costs in diagnosis and treatment of fertility disorders; maternal and perinatal medical risks resulting from assisted reproduction techniques to overcome fertility problems; mental health issues associated with depression and sexual maladjustment from fertility disorders; and relationship costs for couples coping with infertility. If there is <u>any evidence that vaginal lubricants could interfere or limit a couple's fertility, they should carry a warning label to avoid their use while trying to conceive</u>. The authors believe this evidence does exist and that it is clear and compelling.

In contrast, vaginal lubricants that show no interference in post-ejaculatory sperm function could be labeled as "safe for use while trying to conceive"; or with specific indications of "To alleviate vaginal dryness while trying to conceive", "To provide added lubrication while trying to conceive", or "Maintains sperm function while treating vaginal dryness when trying to conceive".

PROPOSED SPECIAL TESTING FOR LUBRICANTS TO BE USED WHILE TRYING TO CONCEIVE

Specifically we propose the following minimum standards for vaginal lubricant formulation, and subsequent sperm function outcomes following sperm contact with any vaginal lubricant claiming to be "safe for use while trying to conceive".

1. **Physical Properties of TTC Safe Lubricants.**
   pH: The lubricant shall conform to the guidelines for optimal sperm penetration through the cervix as stated in the World Health Guidelines specifically, having a pH value between 7 and 8.
   Osmolality: The lubricant shall conform to the published guidelines of normal human fluids of about 320 mOsmo with a +/- 25% range of deviation from this. Specifically, a range of 240 mOsmo to 400 mOsmo would be required. This is no evidence that sperm contact with a product in this range would result in significant detriment.

2. **Bioactive Effects of TTC Safe Lubricants on Sperm Function.**
The following assays will be completed to identify lubricants conforming to labeling as "safe for use while trying to conceive" or "Maintains sperm function while treating vaginal dryness when trying to conceive".

A) Mouse Embryo Assay (MEA)
Rationale: The mouse embryo assay (MEA) is used currently for toxicity and functionality testing of reproductive media, **or any device coming into contact with gametes**.[30] The rationale for requiring information on this test as a special control for class II assisted reproduction devices is that it is a good surrogate indicator of potential toxicity of materials used in assisted reproduction devices to gametes and/or embryos.

Assay: Specifically, as proposed here, 1-cell mouse oocytes are exposed to a 5% solution of the lubricant in question and cultured for 30 minutes. Thereafter, the oocytes are washed out of the lubricant mixture and routinely cultured for 72 hrs. As with other compounds tested, a minimum percentage of oocytes >/= 86% need to develop normally for a lubricant to be viewed as non-toxic in this assay.

Example: Preliminary studies on several leading lubricants suggest that normal oocyte development occurs at levels below the 86%. These compounds would not be approved for in vitro gamete contact, and likely therefore should not be used while trying to conceive.

B) Bovine In Vitro Fertilization Assay (bIVF)
Rationale: The bovine in vitro fertilization and embryo culture model has been used extensively to evaluate the effects of various parameters and conditions on embryo fertilization and development in vitro.[44] This model has been demonstrated to be useful

for screening conditions for human IVF. This assay differs from the mouse embryo assay in that it evaluates the effect of lubricants on sperm prior to and during the fertilization process. For example, the bovine IVF model has shown inferior embryo development resulting from fertilization by sperm that had damaged DNA, even though fertilization itself was normal.

Assay: Bovine oocytes are routinely matured in vitro. Frozen-thawed semen from bulls is washed as is standard and diluted in a fertilization medium. Oocytes are then moved from the maturation medium to the fertilization medium, and incubated at 39° C for 18 hours either with no lubricant present or with a 10% v/v of the test lubricant. After this fertilization step, putative zygotes are washed into culture media and allowed to develop for seven days as is routine. At the end of culture, developmental stage of each embryo is recorded with a dissecting microscope at 70x. Lubricants should cause no more than a 15% drop in percentage of oocytes fertilized and subsequent development to blastocysts as compared to the control medium.

Example: Embryos were exposed to several products during fertilization and then cultured for 7 days prior to scoring for normal development to the blastocyst (multi-cell) stage.[43] Data are expressed as Mean (SEM). More information is shown in Appendix F.

Treatment	# Oocytes	% Fertilized	% Blasts
Brand 1	100	12 (2.0)[a]	2 (1.2)[a]
Brand 12	200	72 (3.4)[b]	42 (0.7)[b]
Brand 16	200	73 (4.6)[b]	47 (0.9)[c]
Control (no lube)	200	77 (3.4)[b]	44 (0.8)[c]

Brand 1 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 after contact for 18 hours with a 10% solution of the product.

C) Sperm Chromatin Structure Assay (SCSA)

Rationale: Sperm cells are very sensitive to oxidative stress resulting in sperm chromatin (DNA) damage.[31-33] This damage can be profound in sperm cells because they contain little to no mechanisms to repair DNA damage when it occurs. Substantial scientific data over the last decade has shown that sperm chromatin damage can result in severe disruptions in fertility and adverse consequences for offspring. For example, at least 15% of childhood cancers are thought to be due sperm DNA damage resulting from paternal smoking. No modern assessment of sperm function would be complete without testing for sperm chromatin damage as upheld by the World Health organization guidelines on semen analysis.

Assay: The SCSA is based on the metachromatic staining of single- and double-stranded nucleic acids by acridine orange.[34,35] Excitation with an argon laser causes acridine orange intercalated into double-stranded DNA to emit a green fluorescence, whereas red fluorescence occurs with single-stranded DNA. To assess sperm chromatin damage

following contact with lubricants, freshly ejaculated human sperm should be added to culture wells with a 10% solution of lubricant in a balanced salt solution for 4 hours at body temperature. After culture, sperm are flash frozen and assayed for DNA breakage. Levels of DNA damage in sperm exposed to lubricant cultures should maintain 85% or more of the level of sperm DNA damage seen in control media without lubricant product.

D) Post Coital Test

Rationale: The post coital test most accurately assesses sperm functioning in the vaginal vault after intercourse. By collecting cervical mucus from inside the cervix and counting the number of motile sperm, fundamental aspects of sperm survival and transport can be determined. Numerous studies have shown correlations between extremely poor post coital studies with few motile sperm in the cervix and pregnancy outcomes. This test is routinely used to evaluate vaginally delivered contraceptive medication efficacy. It also has been previously utilized in published studies to evaluate sperm motility after exposure to lubricants, where profound decreases in cervical mucus penetration have been reported.[20]

Assay: The post coital studies to test lubricants should be done in a randomized cross over design with at least 200 couples, similar to already developed protocols for contraceptive efficacy. Randomly, couples first or second cycles in this study are assigned to no lubricant or the test lubricant. Volumes of at least 2 mls of lubricant should be applied as indicated by the manufacturer or as is most often done by the public with product applied to the penis and the vulvar lips. Sampling of cervical mucus after intercourse is done during the periovulatory phase of the cycle. The mucus is collected within 12 hours after intercourse. Mucus is expelled onto slides for observation at 100 um deep preparations using a 40X objective to view 10 random fields. The number of progressively motile sperm in each field and their motility status is counted. Numbers of sperm and their motility is used to assign a grade of poor, average, good and excellent as per WHO guidelines. A "TTC safe" lubricant should cause no more than one level downgrade as compared to post coital tests done with no lubricant present.

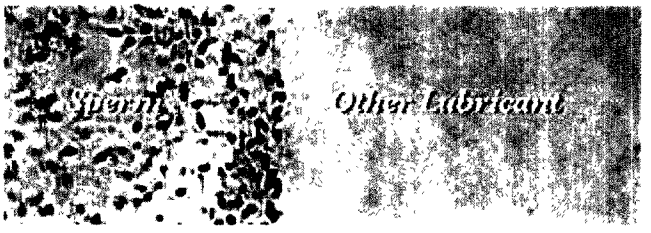
E) Lubricant Barrier Assay

Rationale: Lubricants (especially those provided by vaginal applicator in a bolus) could encounter raw semen post-ejaculation in an undiluted form. In order to evaluate the direct effects of pure lubricant on sperm in raw semen this assay places these two components side by side. Sperm penetration into and survival alongside undiluted lubricant is quantified and assessed.

Assay: Approximately 250 ul of undiluted test lubricant product is placed on a glass slide warmed to human body temperature. An equal size drop of liquefied freshly ejaculated human semen is placed next to the product on the same slide and observed for sperm migration into each lubricant over 20 minutes. Lubricants that are not toxic to sperm will show migration of sperm into the lubricant droplet within 10 minutes of contact. Further, sperm that are in contact with undiluted lubricant will maintain greater that 50% of the original percent motile sperm present prior to lubricant addition.

Example: These photographs from the laboratory show Brand 5 lubricant precluding sperm penetration into the undiluted product (top photo). Whereas Brand 11 provides

no barrier to sperm penetration (bottom picture).[36] Photos taken in the laboratory at 200X after 10 minutes of contact.



Sperm are able to move freely through both semen and Brand 16

F) Computer-Aided Sperm Analysis (CASA)

Rationale: Sperm motility characteristics are the most important parameter related to in vivo cervical mucus penetration, and CASA-based multi-parametric sperm classification provides an objective assessment of functionally relevant categories of sperm motility.[37-39]

Assay. In order to use computer assisted sperm analysis to record effects on sperm motility and velocity parameters, freshly ejaculated semen is diluted to a standard concentration in 1 ml of balanced salt solution (HTF) and incubated at body temperature for 30 min in either the control balanced salt solution or in a 10% lubricant and basal media mixture. Between 15 and 30 minutes of culture, motility and velocity parameters are then analyzed for sperm in each treatment. Duplicate assessments are made on each sample in 20 μm chambers, analyzing at least 200 motile sperm per determination, using computer assisted analysis such as by the Hamilton Thorn IVOS system. In addition to the commonly-used population-averaged kinematic measures, the proportion of sperm showing "good mucus penetrating" kinematic characteristics will be evaluated using gating functions. These parameters identify the percentage of sperm with progressive velocity (VAP) $\geq$25 μm/s + straightness (STR) >80% + amplitude of lateral head displacement (ALH) $\geq$2.5 μm. In addition, the concentration of progressively motile sperm given by the CASA will be recorded, along with a concurrent visual assessment of the percent motile sperm. Sperm in culture with lubricants maintaining at least 75% of the percent motile sperm, mean velocity parameters and good penetrating score as compared to sperm in a balanced salt solution control shall suggest a lack of lubricant toxicity on sperm, and should be acceptable. This level of change in velocity should not measure false positives due purely to viscosity differences between the basal media and the media with lubricant present.

Example: The following table compares CASA parameters and overall motility for sperm in culture with a variety of lubricant products versus media alone. The percentage of sperm with the good mucus penetrating kinematics after contact with each product is also shown.

Sperm Motility Following Contact with Vaginal Lubricants[36]

Treatment	VAP	STR %	ALH	Overall % Motile	% Samples w/ + Score
Control media	56 +/-3	81 +/- 2	3.9 +/- 0.3	70 +/-4	61[b]
Brand 12	50 +/-2	81 +/- 4	3.7 +/- 0.4	61 +/-3	68[b]
Brand 16	53 +/-3	85 +/- 3	3.6 +/- 0.2	76 +/-3	90[a]
Brand 1	49 +/-4	80 +/- 4	2.4 +/- 0.6[a]	29[a] +/-2	20[c]
Brand 2	39[a] +/- 2	74 +/- 3	2.6 +/- 0.5[a]	5[a] +/-0.4	10[c]
Brand 3	*	*	*	15[a] +/-2	*

** optically not possible to complete studies

G) Bovine Cervical Mucus Penetration (BCM)

Rationale: Numerous studies have demonstrated that BCM is a useful surrogate for studying human sperm penetration into cervical mucus.[40-42] It is produced by cattle at estrus in far larger volumes than can be obtained from women and therefore allows better control of penetration tests across entire experiments. A significant relationship has been found between BCM and pregnancy failures not evident by routine semen analysis alone.[41]

Assay: Estrus mucus from nulliparous cattle is used in this study. This can be either commercially purchased or prepared in house. Each batch of frozen BCM must be quality-controlled using control donor semen and compared with control, anti-sperm antibody negative, Human Cervical Mucus obtained from women undergoing stimulation for ART. Only BCM batches showing vanguard migration distances of $\geq$65% of the HCM control will be retained for use. After thawing, BCM-filled capillaries are left at ambient temperature for at least 3 h to permit rehydration of the gel structure and then warmed to 37°C for use. After measuring the length of each mucus column, capillaries will be placed vertically in Eppendorf tubes containing 50 µl of control or treated semen. Freshly ejaculated human sperm will be tested, either in raw semen alone or after mixing 1:3 parts lubricant:semen. The Eppendorf tubes will be sealed with parafilm to limit dehydration and the tests incubated at 37°C under 5% CO_2 for 60 min[40-42]; all tests will be run in duplicate. After incubation, the distance of penetration by the vanguard sperm (in mm) will be assessed microscopically before expelling the contents of the capillary tube for determination of the total number of penetrating sperm using a Makler chamber. Mean values from the pairs of tubes will be used as the result for each test. Lubricants that decrease sperm penetration into bovine cervical mucus by no more 20% will be acceptable for use while trying to conceive.

Summary of Proposed Assays

Taken together, the above tests should validate safety of vaginal lubricants for use while trying to conceive. Specifically, safety shall refer to findings that a "TTC safe" lubricant will not: 1) significantly impede sperm motility or penetration into the lubricant or cervical mucus both in laboratory and in vivo settings; 2) increase DNA damage to sperm after exposure; nor 3) present a risk to fertilization and embryo development (as determined in animal studies) if low levels are present on the gametes at fertilization.

CONCLUSION

The current FDA review of vaginal lubricants offers an opportunity for the FDA to improve safety and efficacy of these types of products. At least 46% of American women have episodes of vaginal dryness that require the use of products to facilitate intercourse. In truth, almost every home in the United States has these products available for occasional use as needed. It is difficult to imagine that such widely used products, with such a long history of human application require drug designation. However, a vast majority of the time couples using vaginal lubricants are not trying to become pregnant.

There is a unique and life changing time of reproductive years when couples desire above all to conceive children. The authors believe that although vaginal lubricants can alleviate dryness without drug indications or over site, the inadvertent effect of these products on sperm function warrants labeling against their use while trying to conceive, unless they can be tested and shown to be non-harmful to sperm. This way, no couple need have exacerbation of fertility issues due to a lack of knowledge amongst consumers or medical care providers regarding the safe use of lubricants while trying to conceive.

The Authors:

Dr. JE Ellington has a PhD in reproductive Physiology from Cornell University. She was awarded the 2003 Young Andrologist Award from the American society of Andrology that advances the clinical and basic study of male reproduction. This award recognizes her federally funded research in gamete biology and infertility, and her over 75 publications that have resulted from this work. She has received the Physician Scientist and the FIRST awards from the National Institutes of Health, and has also had funding from the Untied States Department of Agriculture. Currently, Dr. Ellington is founder and CEO of INGfertility a Company dedicated to development and commercialization of products for trying to conceive couples.

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