

Pre~Seed^{EQ}

“FERTILITY- FRIENDLY” LUBRICATING GEL Science Review

Existing Lubricant Products Used in Animal Breeding Can Harm Sperm

Lubricating gels are part of nearly all animal breeding operations. In particular, concerns over gamete and embryo toxicity can arise with the use of lubricants during semen collection and embryo transfer. Especially during semen collection, gels can mix with the ejaculate and reach significant concentrations.

Most lubricants damage sperm and should be avoided for optimal sperm quality and outcomes. Scientific literature has well-documented the damage to sperm caused by common lubricants (such as Priority Care[®], KY[®] and Surgilube[®]) due to pH or osmolarities that are toxic to mammalian sperm. Surprisingly, many of these damaging lubricants are still widely used in veterinary breeding programs, in spite of numerous publications citing their toxic effects.

Even some so-called “non-spermicidal” lubricants can harm sperm function. Recently, several “non-spermicidal” lubricants have become available for use in animal reproduction, with claims that they do not harm sperm. However, little to no clinical data are available to support these claims. In fact, a recent report suggests that many of these “non-spermicidal” lubricants **DO** diminish stallion sperm motility within minutes and throughout the subsequent 48 hours that sperm may be stored for transport prior to insemination.

⇒ The overall costs of semen collection, shipping, and artificial insemination warrant the use of products that ensure the best sperm function possible during each step of the process.

Evidence that Existing Lubricant Gels Damage Sperm

Numerous studies in both animals and humans have shown that commonly used lubricants harm sperm, resulting in rapid losses in their viability (% live) and motility (% swimming).¹⁻¹⁵

Notable data from these studies show:

- ✚ **Zero** percent fertilization or embryo development after exposure to 10% Priority Care in a bovine in vitro fertilization model (**Figure 1**).¹⁴
- ✚ A motile sperm decline of 50 to 75%, following a 24 hour cold storage exposure of stallion sperm to 5% concentrations of common lubricating gels (**Figure 2**).¹³ This means that ½ to ¾ of the sperm in these ejaculates were no longer able to participate in fertilization after exposure to the lubricants.
- ✚ A motile sperm decline of over 30% within 3 hrs of contact to “non-spermicidal” veterinary lubricants.¹⁵ (**Figure 3**).
- ✚ The detrimental effects of lubricants on sperm function are concentration dependent (**Figure 4**) and have been shown even with low concentrations (1–6%), depending on the lubricant product.^{3,5,7} These changes are also irreversible, such that when sperm are washed out of a 1% lubricant exposure after 20 min, sperm motility subsequently fell to zero.⁵

Figure 1. The percentage of fertilized oocytes (Fig 1a) and normally developed embryos (Fig 1b) after exposure to Pre~Seed^{EQ} (10% & 50%); Aquasonic[®], KY[®], and Priority Care[®] (10%). Asterisk (*) denotes $p < 0.01$ from the control.

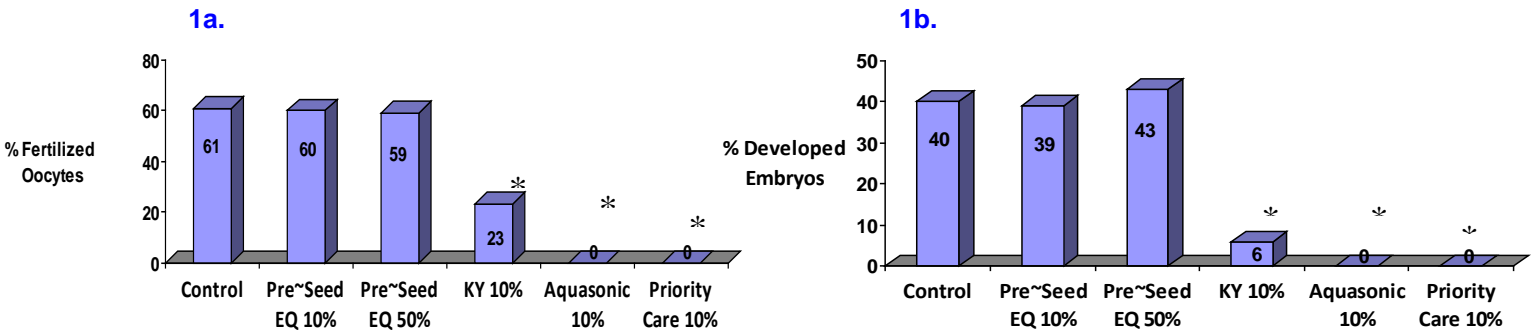


Figure 2. Effects of Artificial Vaginal Lubricants on Stallion Sperm Motion Measures Following 24 Hrs of Cooled Storage (adapted from Ref 13.)

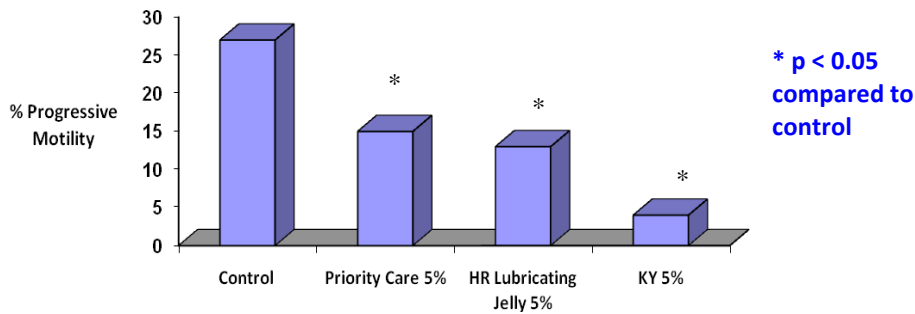


Figure 3. Effect of Different Lubricants (10%) on Motility of Fresh Extended Stallion Spermatozoa (* means differ at $p < 0.05$ compared to control at same time point).

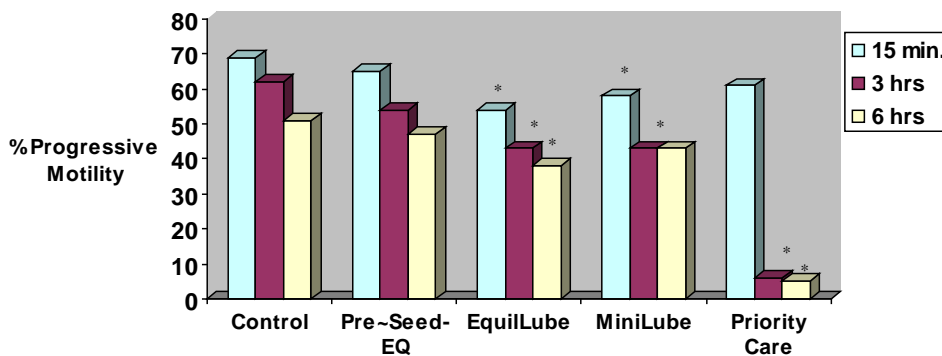
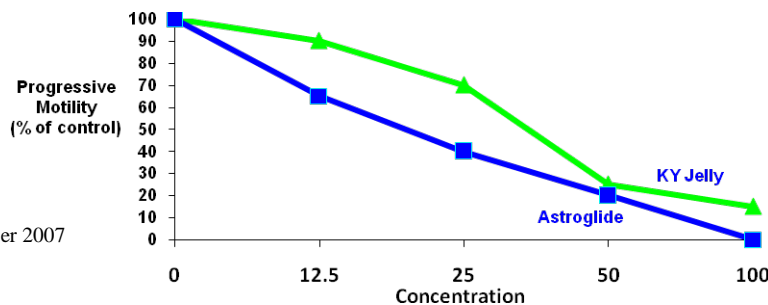


Figure 4. Concentration Dependent Effect of Lubricants on Human Sperm Motility Following 1 minute of Exposure (Adapted from Ref. 5)



Why Lubricating Gels Damage Sperm

The formulation of a lubricant is critical to its impact on sperm function. Other products do not adequately address the following aspects of formulation, with regards to sperm physiology.

pH - The window for optimal **stallion** sperm function is narrower than for other species, being between 7.1 and 7.8, with a rapid decline in motility seen for pH levels above 8.0. Most lubricants have pH values below this optimal level. Sperm motility losses occur with acidic pH exposure. Conversely, pH levels above 8.0 can cause sperm to undergo acrosome loss and death. In order to protect sperm from pH changes, lubricant pH must be stable during contact with air during handling. This requires a buffer, such as the phosphate buffers used in Pre~Seed^{EQ}. Products that utilize sodium bicarbonate as a buffer (e.g. MiniLube) may cause sperm damage through pH increases to alkaline levels (outside of a CO₂ incubator). This may be why sperm motility declines have been reported with 10% concentrations of Minilube[®] (Samper, 2007).

Osmolarity - Sperm are also sensitive to both high and low osmolarity which causes them to either shrink or swell beyond their "critical volume limits". A physiologic osmolarity of around 320 mOsm/kg (that of semen) is best for sperm function. Stallion sperm motility decreases linearly with exposure to increasing osmolarity above 400 mOsm, such that sperm motion disappears at 600 mOsm or greater. The use of Priority Care[®] as a lubricant in an artificial vagina, for example, can cause equine sperm in the ejaculate to be rapidly and suddenly exposed to osmolalities as high as 800 mOsm. Both human and animal model studies suggest that this level of osmotic shock is sufficient to cause irreversible damage to sperm, even if the sperm are moved back into an isotonic situation.

Ionic Concentration - Some veterinary lubricants (EquiLube[®] & Priority Care[®]) are formulated with glycols (such as propylene glycol) which permeate across sperm membranes into the sperm cell, and impact cell function. Although most lubricants with propylene glycol are hyperosmotic, EquiLube's osmolarity is close to physiologic. However, sperm motility still declined during contact with Equilube[®] in both fresh and cooled sperm storage (Samper, 2007). This may have been due to the osmotic pressure coming from propylene glycol molecules, rather than ionically balanced salts, as in Pre~Seed^{EQ}.

Some people have suggested that mineral oil is appropriate for lubricating devices in the breeding shed; however, the pH and osmolality are not physiologic, and studies have shown decreased ability of sperm to penetrate ova in laboratory studies after mineral oil exposure.

pH & Osmolality Levels for Veterinary “Non-Spermicidal” Lubricants

Product	pH			Osmolality (mOsm/kg) # from glycols	
	Too low	Physiologic 7.1 – 7.8	Too high	Physiologic 260 - 365	Too high
Equilube [®]	7.0			368 [#]	
KY [®] Jelly	4.5				2052
Minilube [®]			8.0 ^{**}	312	
Pre~Seed ^{EQ}		7.3		324	
Priority Care [®]	6.0				2199 [#]
Mineral Oil			10.5		1200

[#]Osmotic pressure mostly from Propylene Glycol, not salts

^{**}Buffered by sodium bicarbonate only

What Makes Pre~Seed^{EQ} Unique?

Pre~Seed is the only lubricant formulated with: the right osmolality and ion concentration; a buffer to ensure that pH remains stable; and an antioxidant to protect sperm from free radical damage during handling.

Pre~Seed[®]'s Patented Ingredients Include:

Hydroxyethylcellulose: A non-toxic thickener. Has been shown to be similar to cervical mucus.

Pluronic: A non-toxic slippery agent.

Sodium chloride: Makes the product isotonic to semen.

Arabinogalactan: A unique plant polysaccharide, that **provides antioxidant support** to protect sperm.

Sodium phosphate & Potassium phosphate: Protects, buffers and holds the pH to that of semen for an optimal sperm environment.

Carbomer: A non-toxic thickner.

Methylparaben: One of the most mild and well studied of all preservatives against bacterial growth, parabens in the concentrations we use have no adverse effect on sperm or reproduction.

Sodium hydroxide: Raises the pH to that of semen and fertile cervical mucus.

Purified water: Very pure for low bio burden.

Pre~Seed’s Lot Release Testing. Each Lot is tested before release to ensure the following:

pH	Between 7.1-7.6
Osmolality	260- 365 mOsmo/kg
Viscosity	Between 11,000 – 15,000 cps
Sperm motility	Sperm retain 80% or more of motility in control medium (w/o Pre~Seed)
Microbial count	0 cfu/ml pathogenic bacteria, others < 100 cfu/ml

COMPARE PRE~SEED TO OTHER “NON-SPERMICIDAL” LUBRICANTS

	Sperm Motility Unaffected	Fertilization Normal	Contains Antioxidant	Buffered pH + isotonic	Clinical Data on Irritation	Lot Release Report
EquiLube	no*	n/a	no	no	n/a	no
MiniLube	no**	n/a	no	no	n/a	no
Pre~Seed ^{EQ}	yes	yes	yes	yes	yes	yes
Priority Care	no	no	no	no	n/a	no

Independent study (Samper, 2007) used 10% concentrations of each lubricant over 6 hrs for fresh storage and over 48 hours for cold stored, using 22 ejaculates. Unpublished technical data from individual companies reported no effect of these products on sperm at *10% over 10 minutes with 3 ejaculates; and **5% with no time, or sample numbers provided.

Routine Biocompatibility Studies Performed with Pre~Seed^{EQ}

Animal Biocompatibility Testing

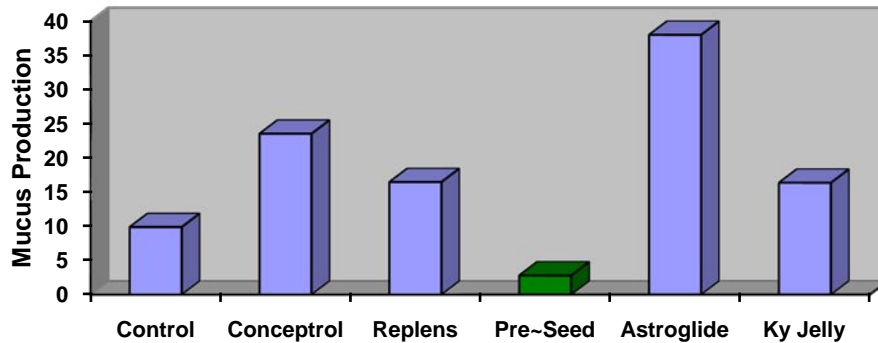
Routine skin and mucous membrane biocompatibility studies of Pre~Seed^{EQ} have been performed in humans and animals. These studies have demonstrated that Pre~Seed^{EQ} has a much lower potential for mucosal irritation than leading lubricants like KY®.

Slug Mucosal Irritation Test (MIT)

The Mucosal Irritation Test (Pharmaceutical Technology Laboratory, Ghent University, Belgium) uses slugs to screen gel formulations for local tolerance. This method appears to be more sensitive than the rabbit vaginal irritation model in predicting subclinical vaginal mucosal damage. Slugs (*Arion lusitanicus*) were evaluated daily for 5 days, following 30 min exposures to Pre~Seed^{EQ} and other lubricants (including a known negative control, hydroxyethylcellulose; and a positive irritating control, Conceptrol). After each exposure mucus production was assessed to determine irritation potential.

A summary of the findings is shown in **Figure 5** below. Pre~Seed^{EQ} caused less mucus production than even the negative control, suggesting limited potential for mucosal irritation. In contrast, other products such as Replens®, Astroglide®, and KY® Jelly were associated with an increased degree of mucosal irritation and even tissue damage.

Figure 5. Mucosal Irritation Potential of Lubricating Gels



Special Fertility Biocompatibility Studies Performed with Pre~Seed^{EQ}

Effects of Pre~Seed^{EQ} on Sperm Function & Embryo Development.

The following third party studies have been done to evaluate Pre~Seed^{EQ} effects on sperm function and embryo development.

- A. Effects on fertilization rate and embryo development
- B. Sperm Motility
- C. Sperm Chromatin Structure Assay (SCSA)

A. Effects on fertilization and embryo development

1. Bovine In Vitro Fertilization Assay (bIVF)

Rationale: The bovine in vitro fertilization and embryo culture model has been used extensively to screen for sublethal toxicity of assisted reproduction media or supplies on fertilization and embryo development in vitro. This assay evaluates the effect of lubricating gels on sperm prior to and during the fertilization process.

Methods: This study was performed by Dr. RW Wright, Department of Animal Sciences, Washington State University. In this study, cryopreserved bull sperm were routinely washed, resuspended in medium and placed into one of the following 6 treatments:

- 1) Control: sperm in medium alone
- 2) sperm medium suspension with 10% (v/v) Pre~Seed^{EQ} lubricating gel;
- 3) sperm medium suspension with 50% (v/v) Pre~Seed^{EQ} lubricating gel;
- 4) sperm medium suspension with 10% (v/v) KY[®] gel
- 5) sperm medium suspension with 10% (v/v) Aquasonic[®] gel
- 6) sperm medium suspension with 10% (v/v) Priority Care[®] lubricating gel

Sperm were incubated in treatments for 30 min at body temperature, and placed into fertilization wells with mature oocytes (1×10^6 sperm cells per well). Eighty oocytes per treatment were used over 4 replicates. At 8 hrs of culture, 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 appropriately 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.

Results: The effects of Pre~Seed^{EQ} and other commonly used lubricating gels on oocyte fertilization and blastocyst formation are shown in **Figures 6 & 7**.

Figure 6. The percentage of fertilized oocytes after exposure to Pre~Seed^{EQ} (10% & 50%); Aquasonic[®], KY[®], and Priority Care[®] (10%). Asterisk (*) denotes statistically significant difference ($P < 0.01$) from the control treatment.

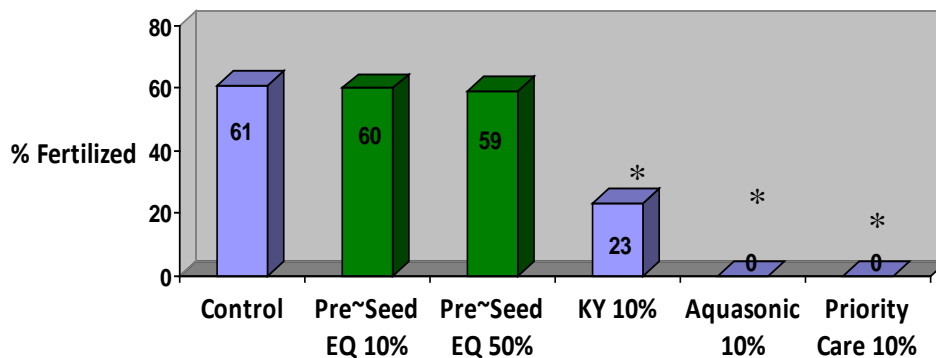
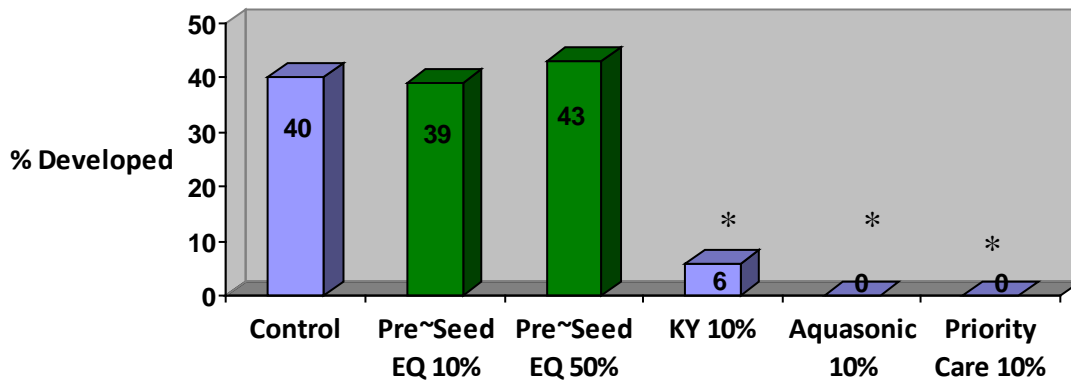


Figure 7. Percentage of normally developed embryos after seven days of culture following exposures in Figure 6. Asterisk (*) denotes $p < 0.01$ from the control.



Conclusion: Pre~Seed^{EQ} at concentrations of 10 and 50% did not significantly affect fertilization or embryo development over that observed in controls without lubricant present ($p = 0.80$). Conversely, sperm exposure to KY[®], Aquasonic[®], and Priority Care[®] at concentrations of only 10% significantly and profoundly ($p < 0.01$) reduced fertilization and/or embryo development compared to the control treated sperm.

B. Motility and Velocity Analysis of Stallion Spermatozoa

Rationale: Collection of spermatozoa for artificial insemination requires that the stallion be stimulated with an artificial vagina (AV) using proper pressure, temperature and lubrication. However, a detrimental effect of lubricants on sperm function has been widely reported; largely due to the hyperosmotic formulation of most commercial lubricants and the resultant cellular damage occurring after sperm contact with even small concentrations (i.e. <10%). Recently, several “non-spermicidal” lubricants have become available for equine reproductive work. This study was done to compare the affect of these “non-spermicidal” lubricants on stallion sperm, by Dr. JC Samper of JCS Veterinary Reproductive Services, Langley BC .

Methods: Extragonadal reserves were stabilized and Expt 1) 7 ejaculates from 4 stallions; or Expt 2) 3 ejaculates from 5 stallions of proven fertility were collected with a Missouri AV lubricated with 5 mls of petroleum Vaseline®. Immediately after collection, the number of sperm per ml was determined using a Spermacue and 1 part v/v of NFG semen extender was added to the ejaculate for every 50 million sperm present. Each ejaculate was then split 5 ways and 10% v/v of each of 4 different lubricants was added. A sample with no lubricant added was kept as a control. The lubricants evaluated were Priority Care® (First Priority Elgin, IL); Pre~Seed^{EQ} (INGfertility Valleyford, WA), MiniLubeTM (Minitube Verona, WI) and EquiLubeTM (Boehringer Ingelheim St. Joseph, MO). In Expt 1, samples were held at room temperature and evaluated at 15 min, 3 hr and 6 hrs. In Expt 2, samples were evaluated immediately, and placed into an Equitainer for cold storage and sample evaluation at 24 and 48 hrs. At each test time, aliquots of sperm were reheated to body temperature for motility analysis.

Results: Analysis by two-way ANOVA showed that overall there was a significant treatment effect over time. In Expt 1, sperm progressive motility (PM) differed from that seen with the control sperm at two or more time points for all lubricants except Pre~Seed^{EQ} (Figure 9). In Expt 2, PM did not differ between the treatments after initial contact; however, sperm motility did differ between controls and lubricant treatments at 24 and 48 hrs, with Pre~Seed^{EQ} being the only lubricant to show no difference from control sperm (Figure 10).

Figure 8. Effect of Different Lubricants (10%) on Motility of Fresh Extended Stallion Spermatozoa (* means that differ at $p < 0.05$ compared to control at same time point).

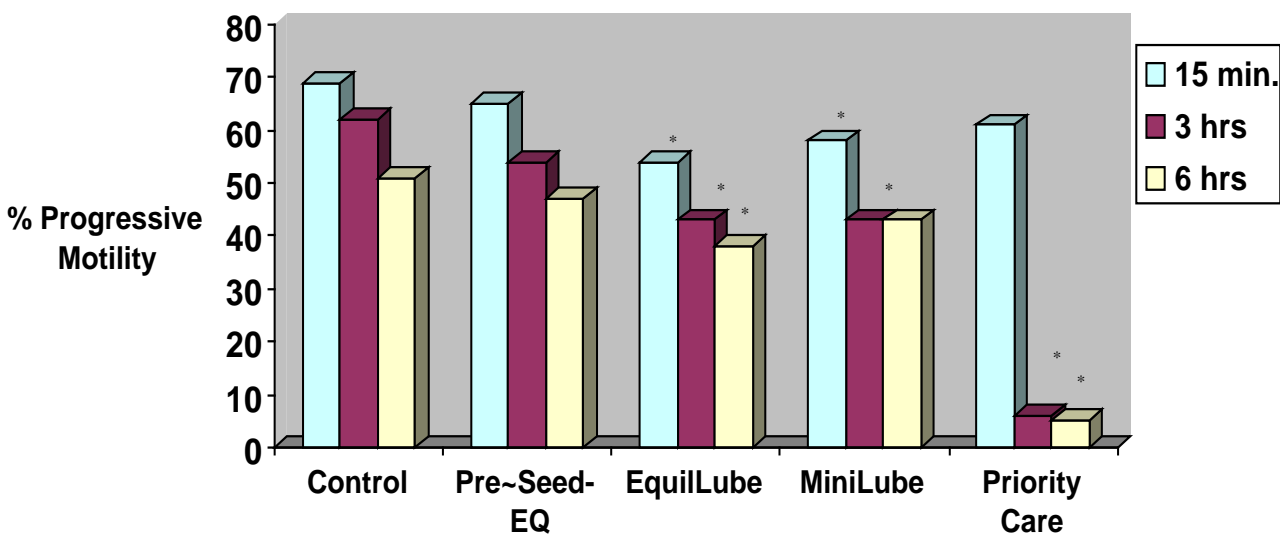
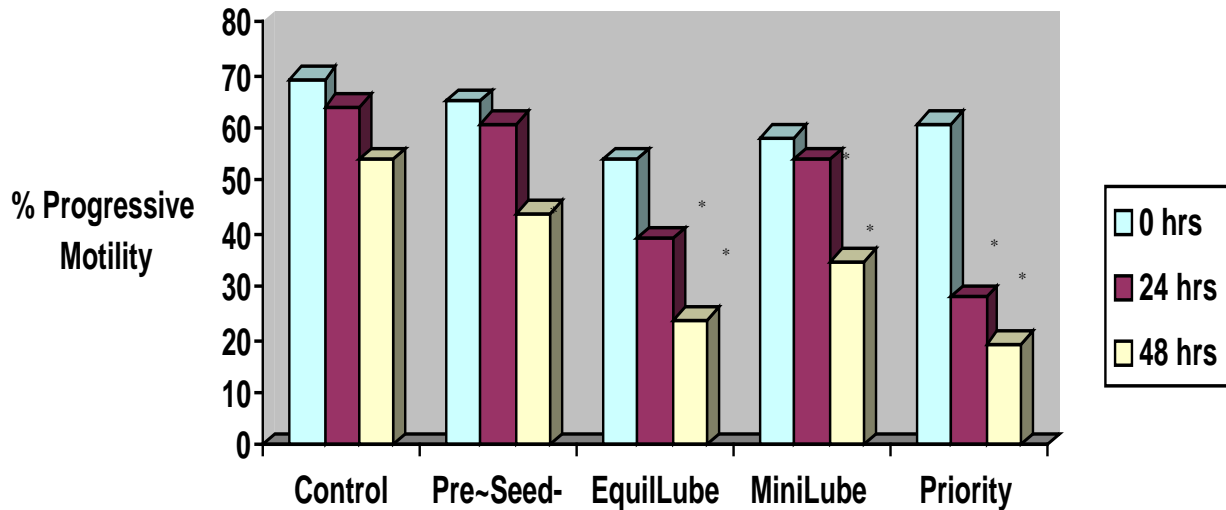


Figure 9. Effect of Different Lubricants (10%) on Motility of Cold-Stored Stallion Spermatozoa (* means that differ at $p < 0.05$ compared to control at same time point).



Conclusion: These results demonstrate that stallion sperm motility is affected by different “non-spermicidal” lubricants. This effect could not be attributable solely to osmolarity of the lubricant, as osmolarity (in mOsm/kg) of the lubricants in this study were: EquiLube® 368; Priority Care® 2199; Pre~Seed^{EQ} 328 and MiniLube® 336. A low concentration (10%) of EquiLube®, Priority Care® and MiniLube®, caused declines in sperm motility at various timeframes in both experiments, even though only the Priority Care® was substantially hyperosmotic.

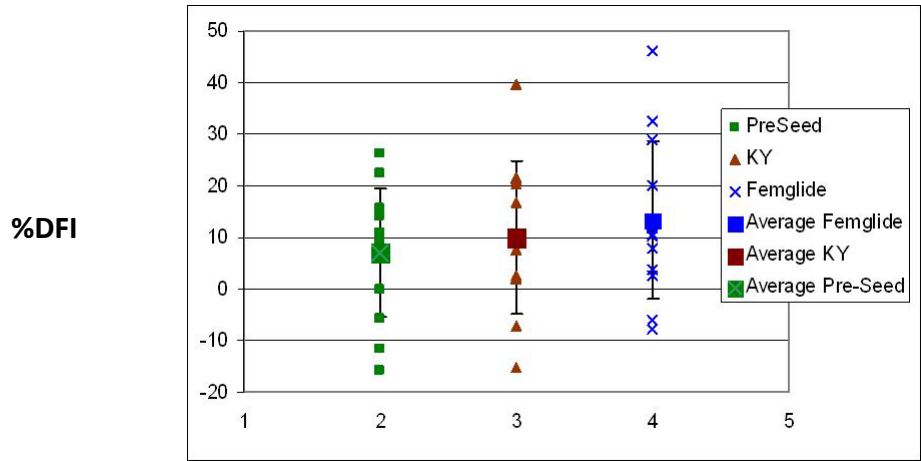
C. Sperm Chromatin Structure Assay (SCSA)

Rationale: Sperm cells are very sensitive to oxidative stress resulting in sperm chromatin (DNA) damage.²⁵⁻³⁰ 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.

Assay: The Sperm Chromatin Structure Assay (SCSA) was performed by SCSA Diagnostics, Brookings, ND. In order to assess sperm chromatin damage following contact with either control medium, or 10% (v/v) solutions of lubricants (Pre~Seed^{EQ}, FemGlide® KY®), freshly ejaculated human sperm from 13 donors were incubated for 4 hours at body temperature in the specified treatments. After culture, sperm were flash frozen and subsequently assayed for DNA breakage.

Results: As shown in Figure 11, there was no difference in the percentage of sperm with damaged chromatin (DNA fragmentation index or ‘DFI’) between the HTF control & Pre~Seed^{EQ} ($p=0.23$), whereas a decline for sperm chromatin quality occurred in FemGlide® ($p=0.02$) & KY® ($p=0.04$).

Figure 11. Effects of Vaginal Lubricants (10%) on the Percent of Human Sperm with DNA Damage (% DFI).



Conclusion: Pre~Seed^{EQ} did not induce sperm chromatin changes after contact, unlike the other lubricant products tested.

Product Quality Assurance

Manufacturing Facility

INGfertilityTM operates according to Good Manufacturing Practices (GMP). Pre~Seed^{EQ} is made under contract at neighboring UnicepTM Packaging in Idaho. UnicepTM has the highest international quality certification of ISO 9001 2000.

Lot Release Testing

Each and every lot of Pre~Seed^{EQ} is tested to ensure no deleterious effect on mammalian sperm.

Stability Testing

Stability testing of Pre~Seed^{EQ} was performed in accordance with federal guidelines. Based on data collected, the projected shelf-life from the date of manufacture of the products is 2 years.

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