

Strategies to Avoid Mineral Oil Toxicity during Embryo Culture

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Objectives

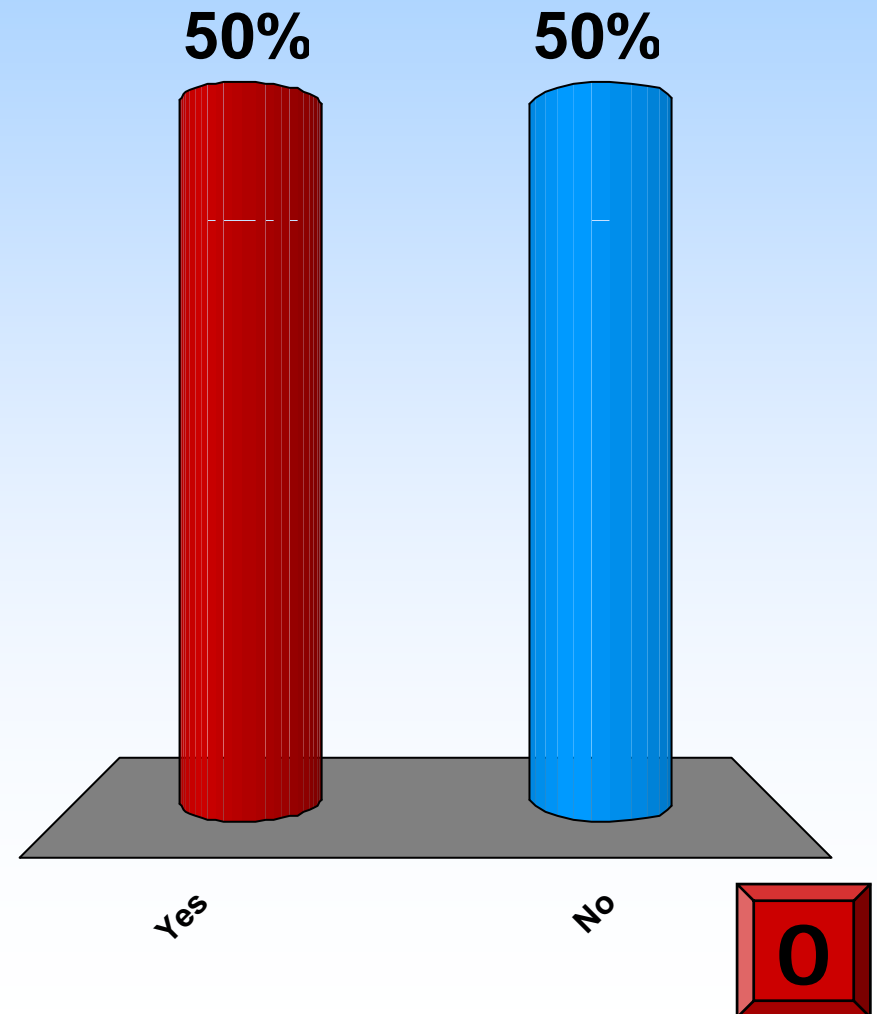
- **Define oil**
- **Evidence for toxicity**
- **Discuss how we use it**
- **Discuss how we can avoid toxicity**

Disclosures

- **None**

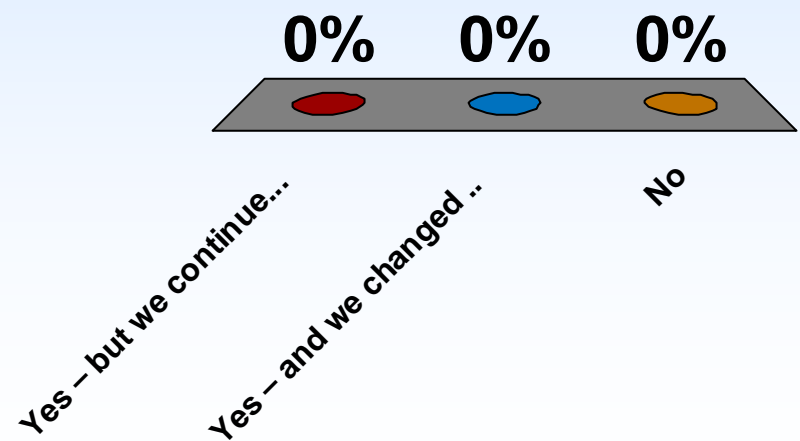
Have you had a shipment of oil that was toxic and returned to the manufacturer?

1. Yes
2. No



Have you had concerns about a batch of oil but were unable to confirm the problem?

- A. Yes – but we continued to use it
- B. Yes – and we changed to a different lot
- C. No

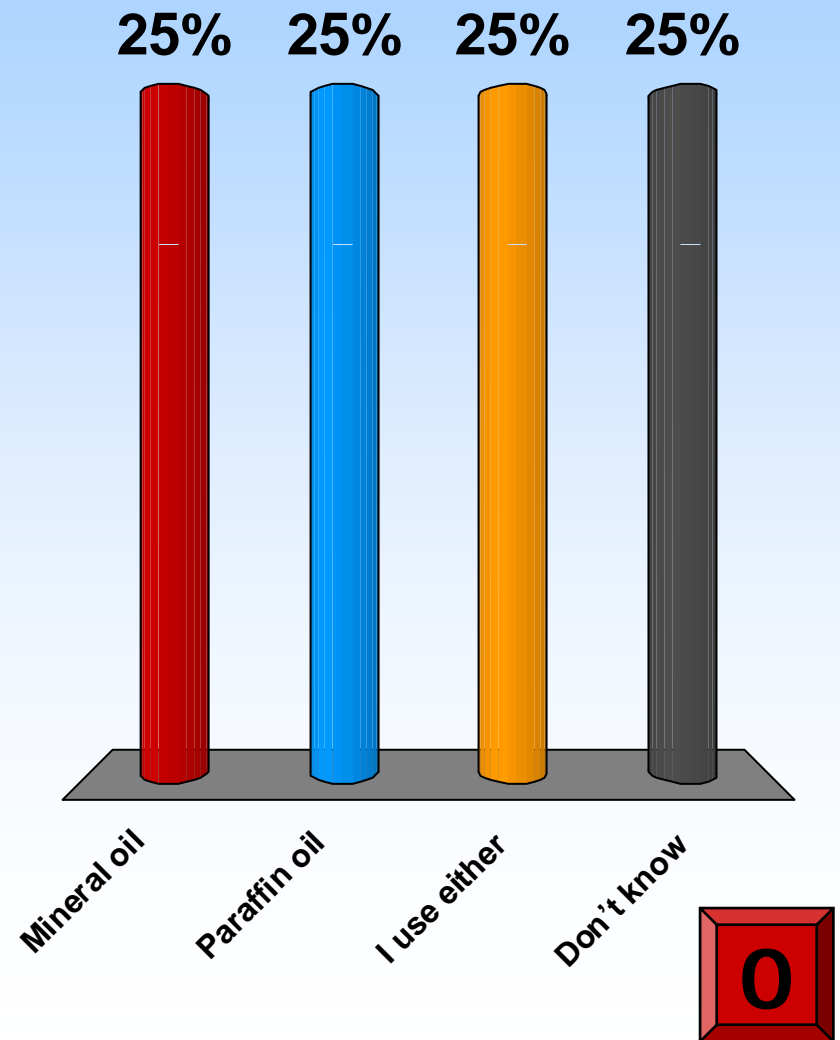


Why is mineral oil such a problem?



When purchasing oil, which type do you prefer?

1. Mineral oil
2. Paraffin oil
3. I use either
4. Don't know

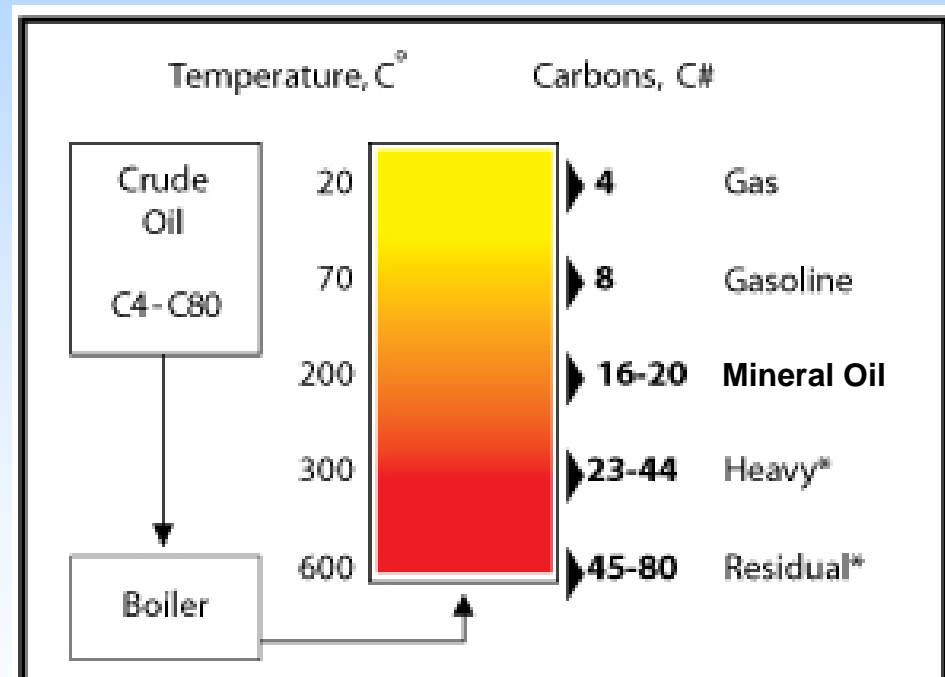


What's in a name?

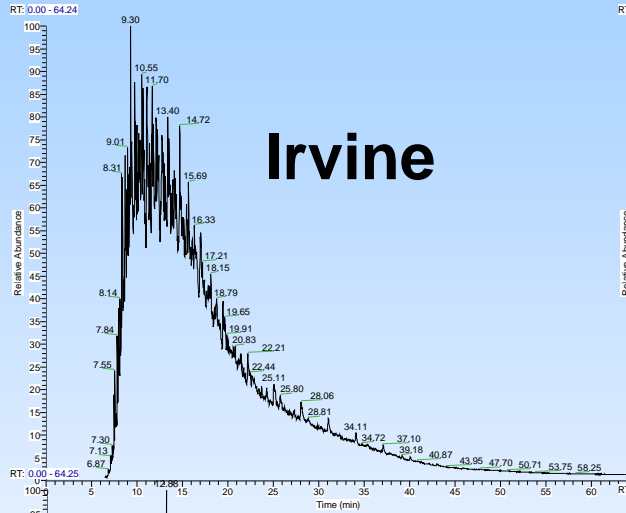
- CAS # 8042-47-5
 - Mineral oil
 - Light mineral oil
 - Paraffin oil
 - Liquid Paraffin
 - White oil
 - White mineral oil



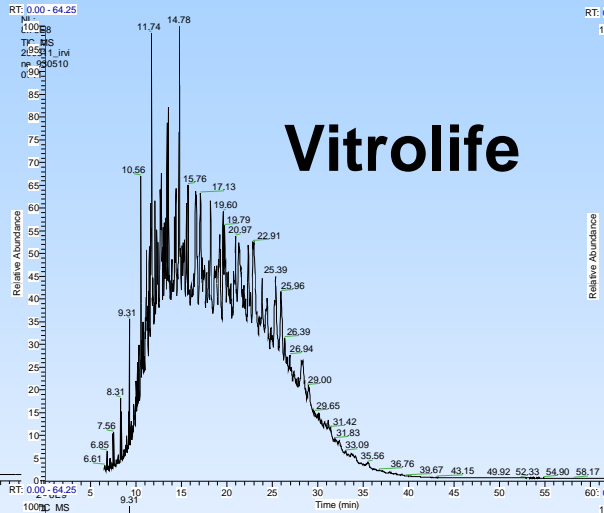
Crude oil distillation



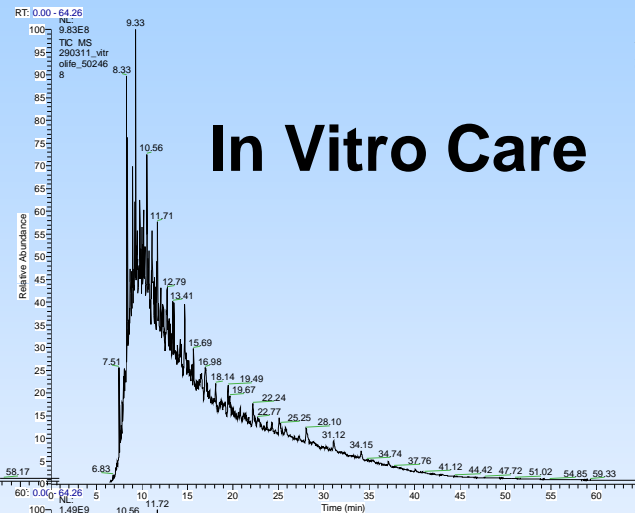
What do the oils look like with GC-MS?



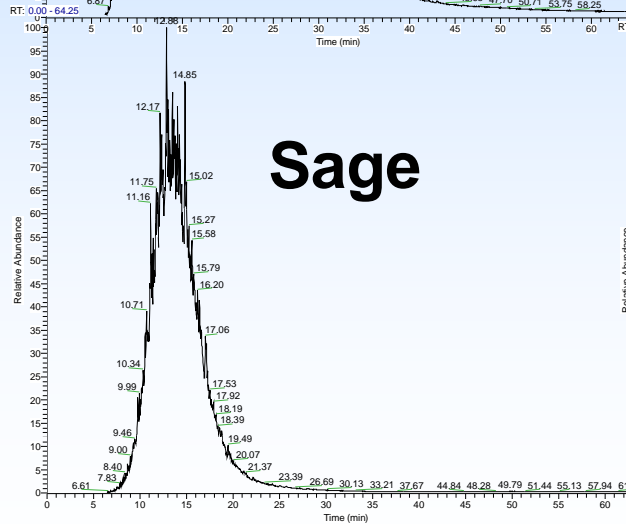
Irvine



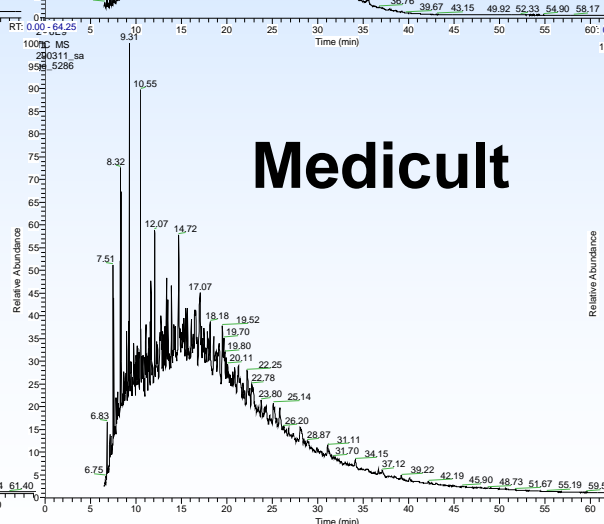
Vitrolife



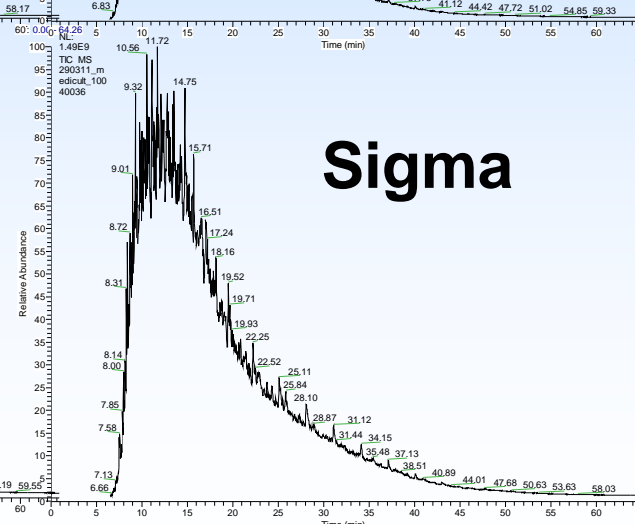
In Vitro Care



Sage



Medicult



Sigma

NL: 1.33E9
TIC MS
290311_01
C_10M11H
10

NL: 7.91E8
TIC MS
290311_sig
ms_047K0
069

Mineral Oil Toxicity

- Zinc (Erbach et al., 1995)
- Peroxide (Otsuki et al., 2007)
- Peroxides and Triton X-100 (Morbeck et al., 2010)



Classes of Toxins

- **Reactive by-products**
 - **Peroxides**
 - **Aldehydes, alkenals**
 - **Volatile organic compounds?**
- **Contaminants**
 - **Detergents**
 - **TX-100**
 - **Metals**
 - **Zinc**

Clinical Significance

- **2010 Two manufacturers recalled lots with reported toxicity**
 - Turner, Fertility Magazine 2010, Vol 12
- **2012 One manufacturer recalled a lot with demonstrated toxicity**
- **Cause of toxicity never reported**

How Did This Happen?

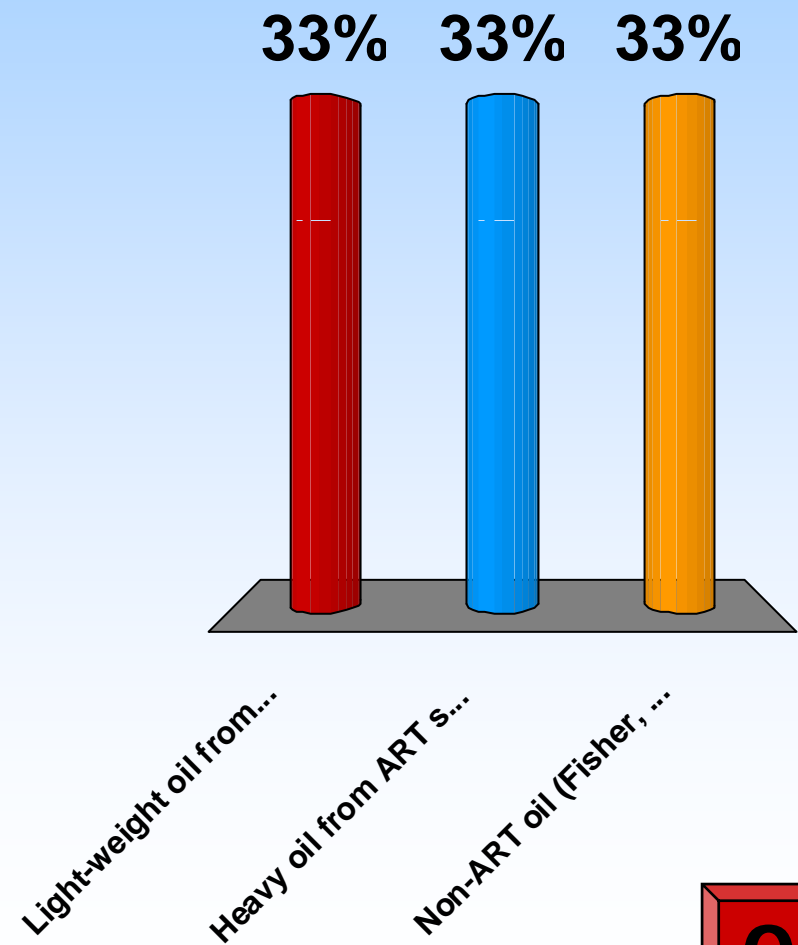
- **Manufacturers lack analytical tools**
- **Within lot variation difficult to test**
- **Oil can degrade in heat and light**
- **Toxicity is condition dependent**

Factors that may affect oil toxicity.

- **Type of oil**
- **Storage conditions**
- **Culture system – media:oil**
- **Oxygen tension**
- **Protein**
- **Air quality**

What type of oil do you use for embryo culture?

1. Light-weight oil from ART supplier
2. Heavy oil from ART supplier
3. Non-ART oil (Fisher, Sigma, Squibb, etc.)

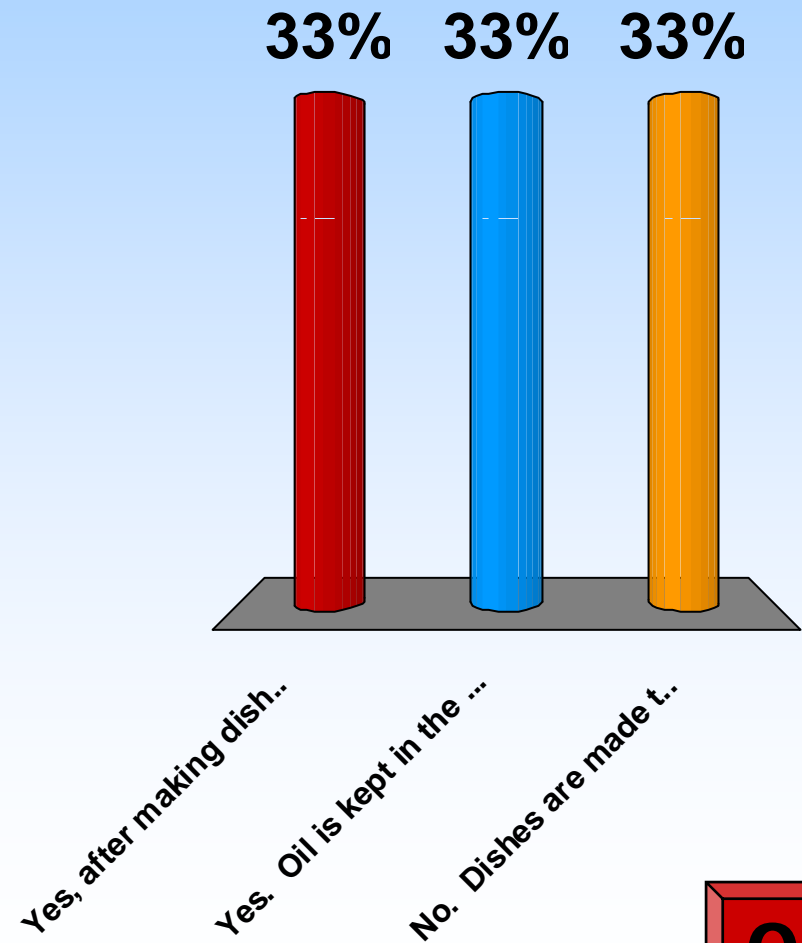


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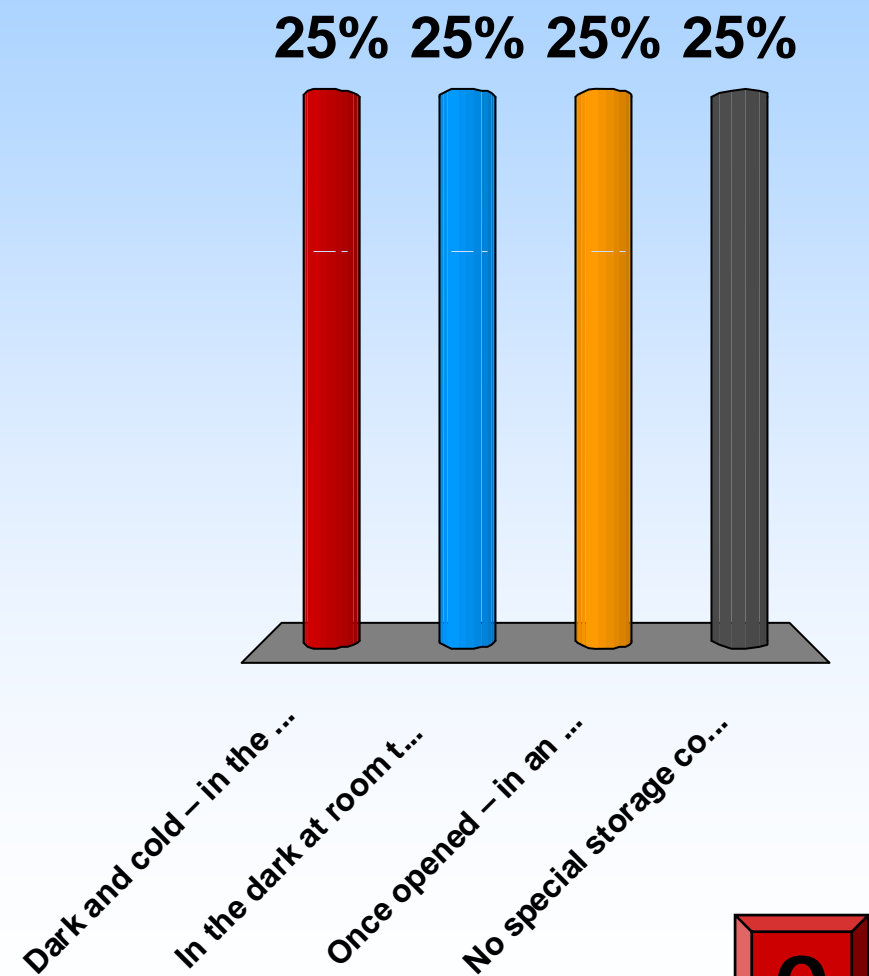
Do you pre-equilibrate oil before use?

1. Yes. Oil is kept in the incubator until needed for dish prep.
2. Yes, when making dishes for the next day.
3. No. Dishes are made the same day they are needed.



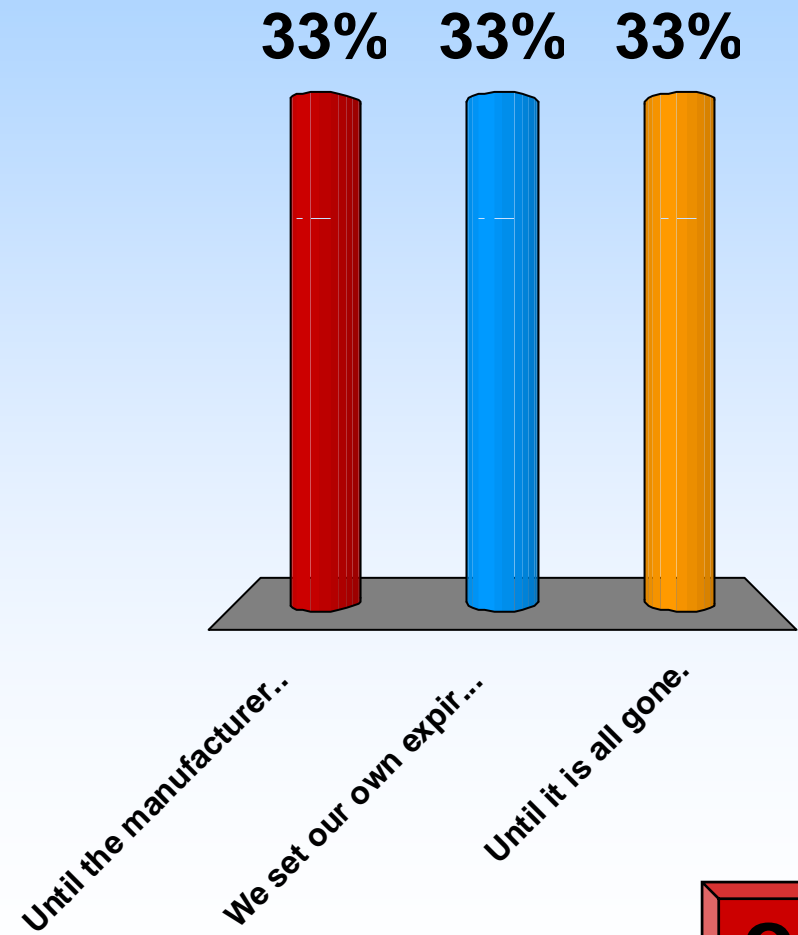
How do you store your oil?

1. Dark and cold – in the refrigerator
2. In the dark at room temperature
3. No special storage conditions



How long will you keep a specific lot of oil?

1. Until the manufacturer's expiration date.
2. We set our own expiration date less than the mfr's.
3. Until it is all gone.

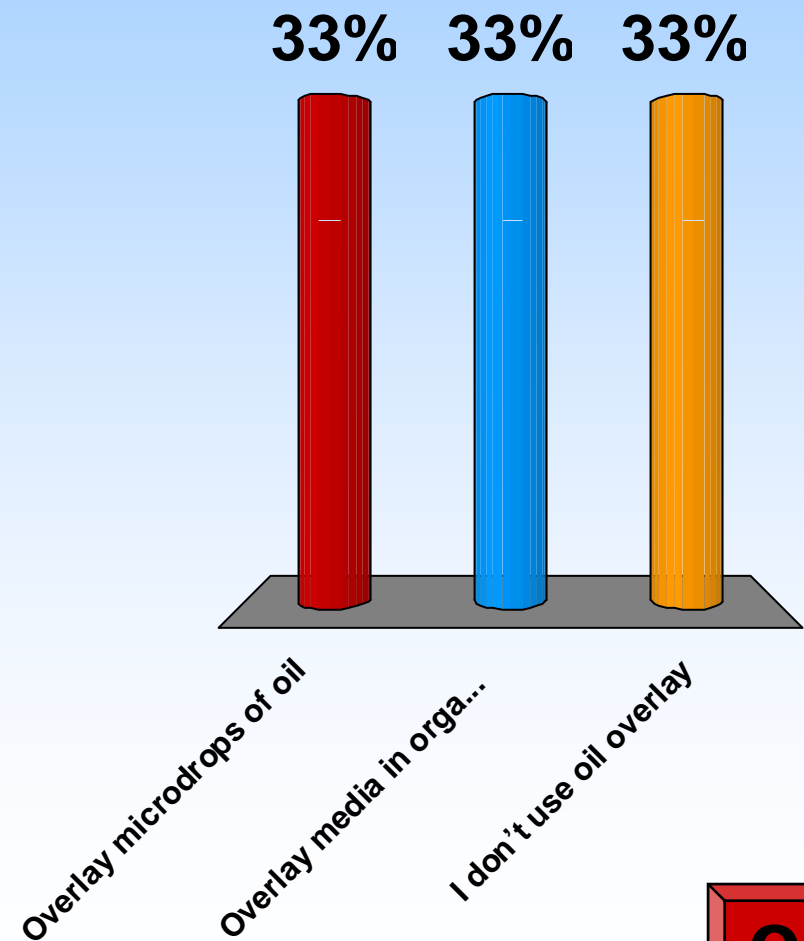


Factors that may affect oil toxicity.

- Type of oil
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How do you use oil for embryo culture?

1. Overlay microdrops of media
2. Overlay media in organ culture or 4-well dish
3. I don't use oil overlay

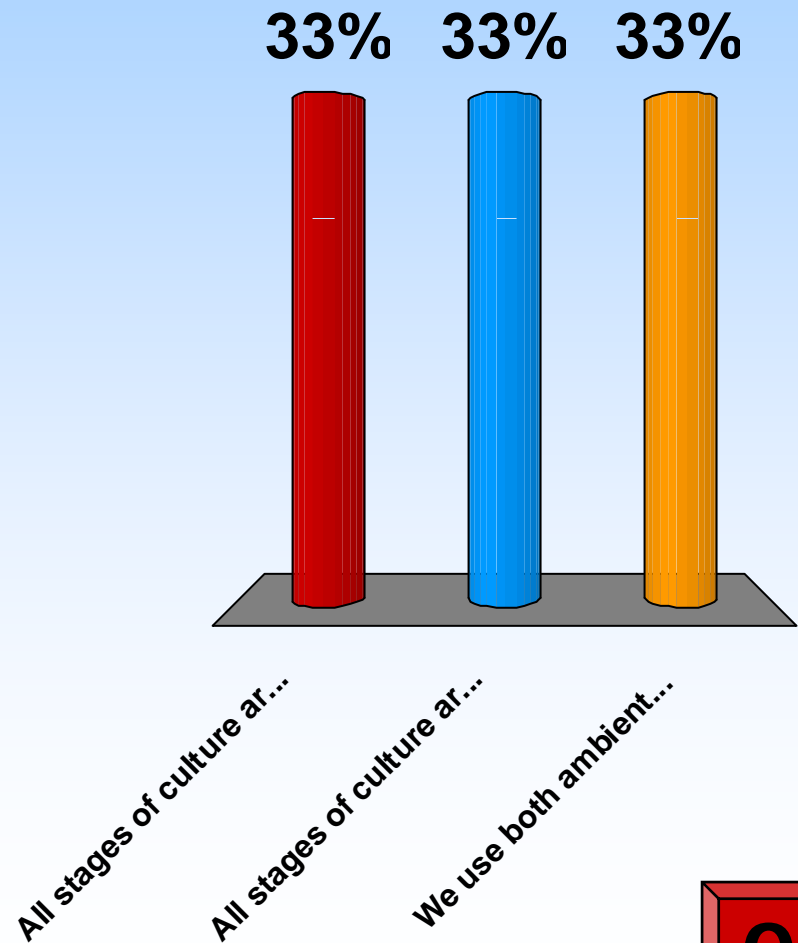


Factors that may affect oil toxicity.

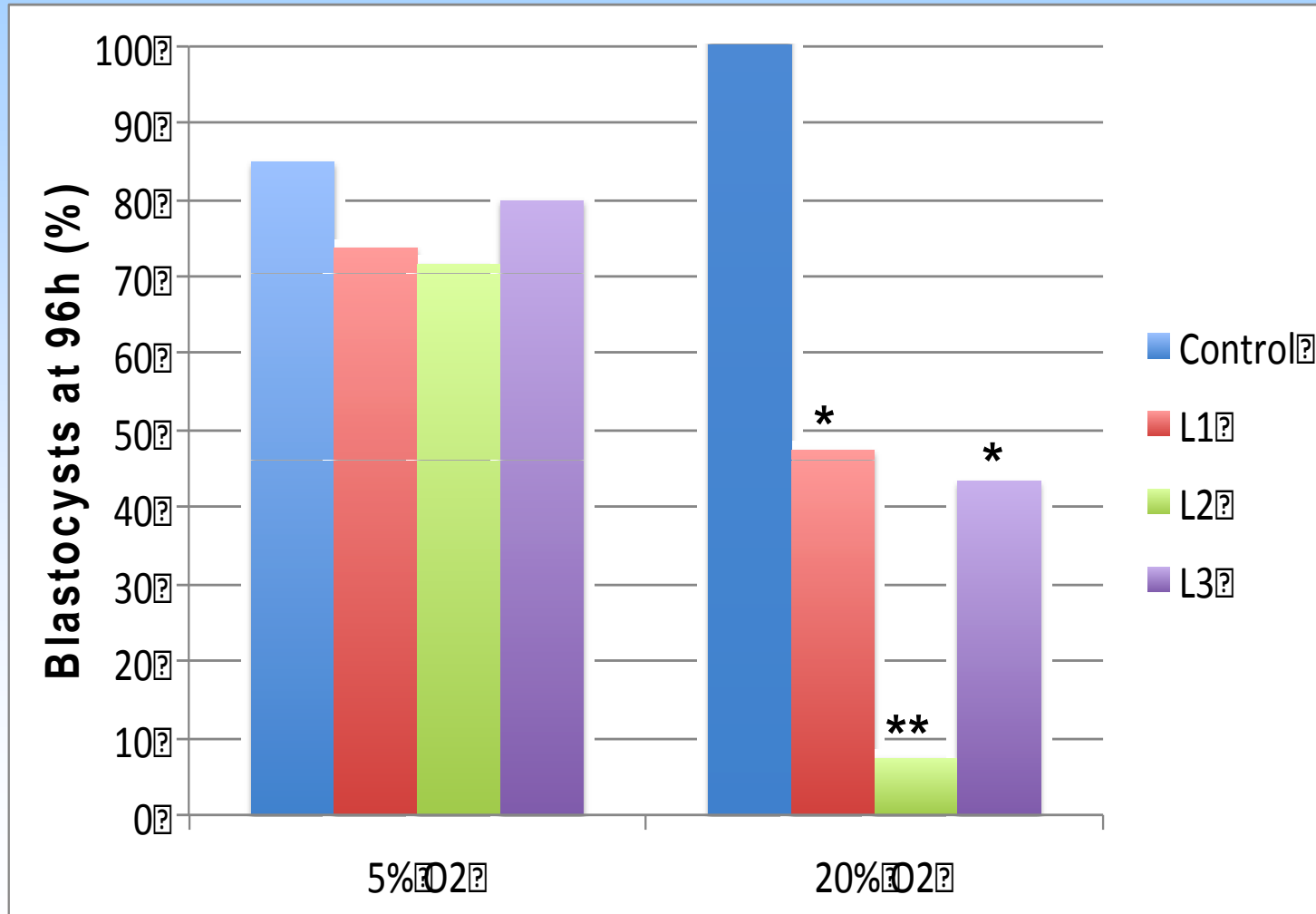
- Type of oil
- Storage conditions
- Culture system – media:oil
- Oxygen tension
- Protein
- Air quality

Which statement describes your culture system:

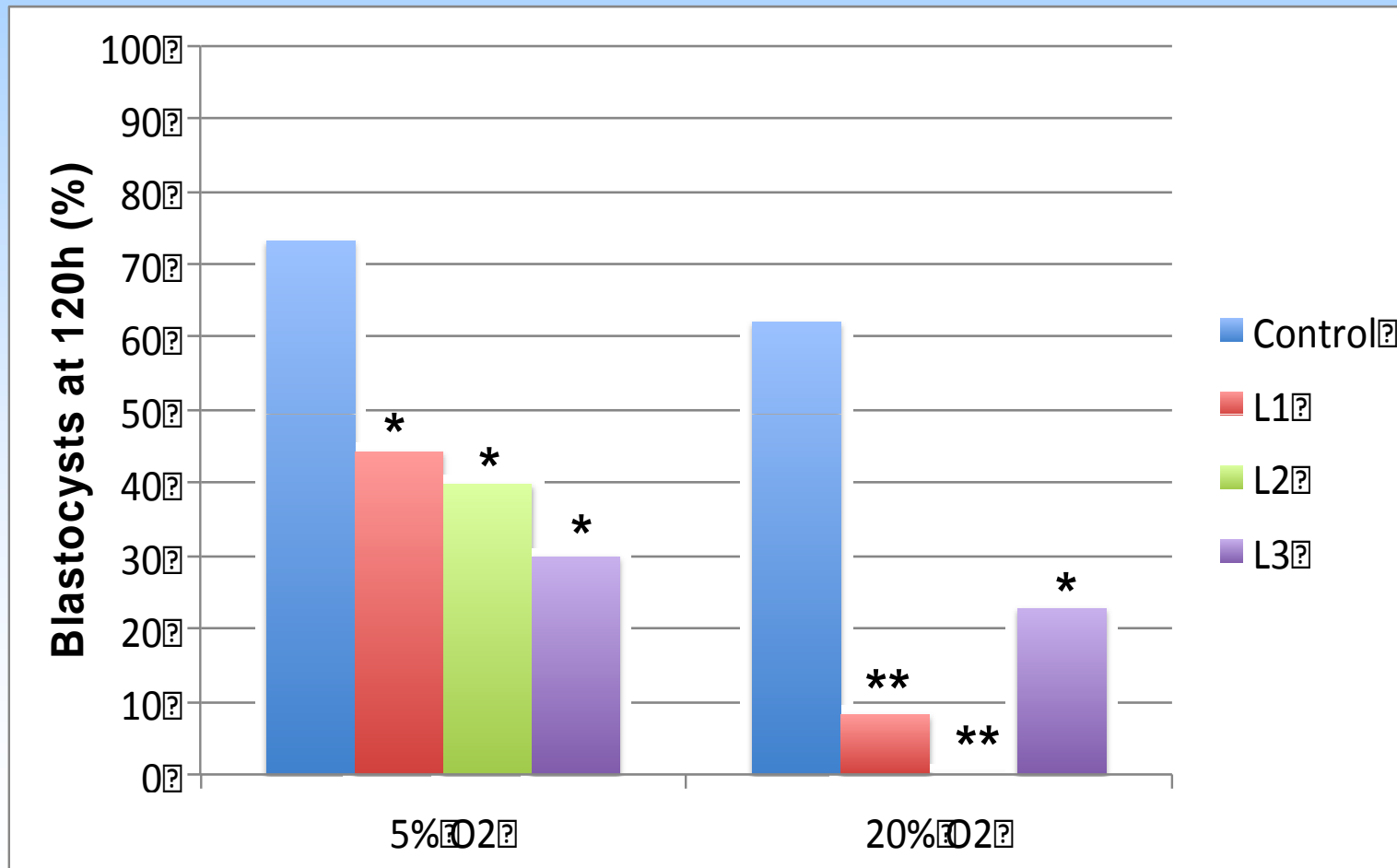
1. All stages of culture are at ambient O₂ (20%)
2. All stages of culture are at reduced O₂ (<10%)
3. We use both ambient and reduced O₂.



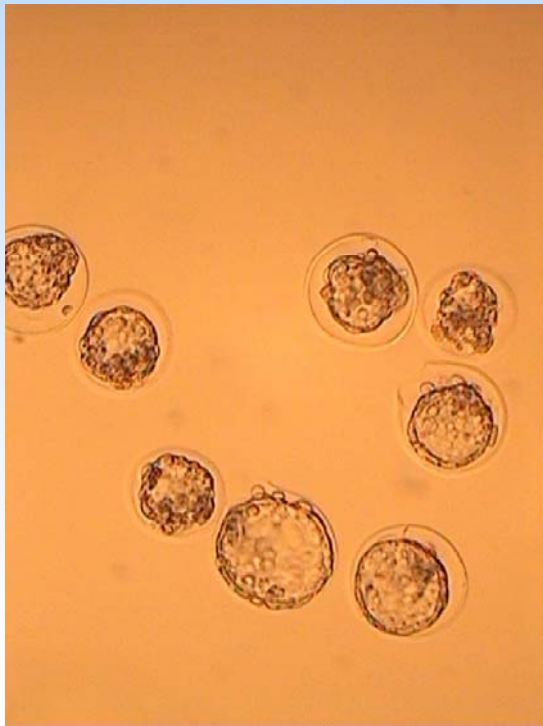
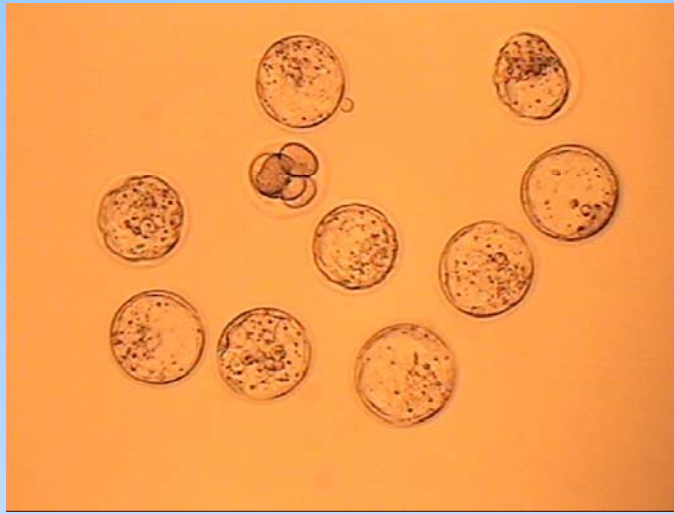
Oxygen vs Oil 96 Hours



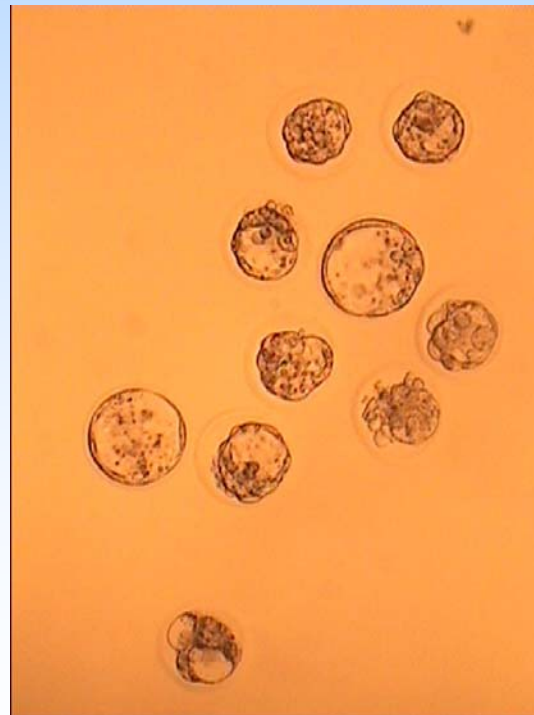
Oxygen vs Oil 120 Hours



96 hours in
ambient O₂



L1



L2



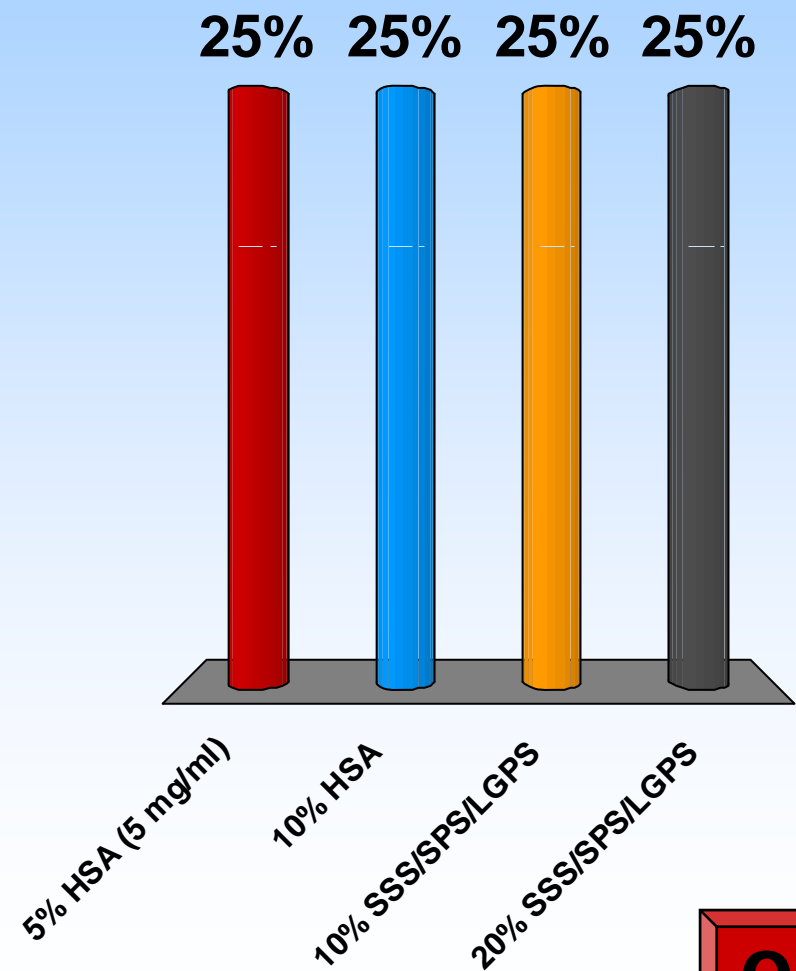
L3

Factors that may affect oil toxicity.

- Type of oil
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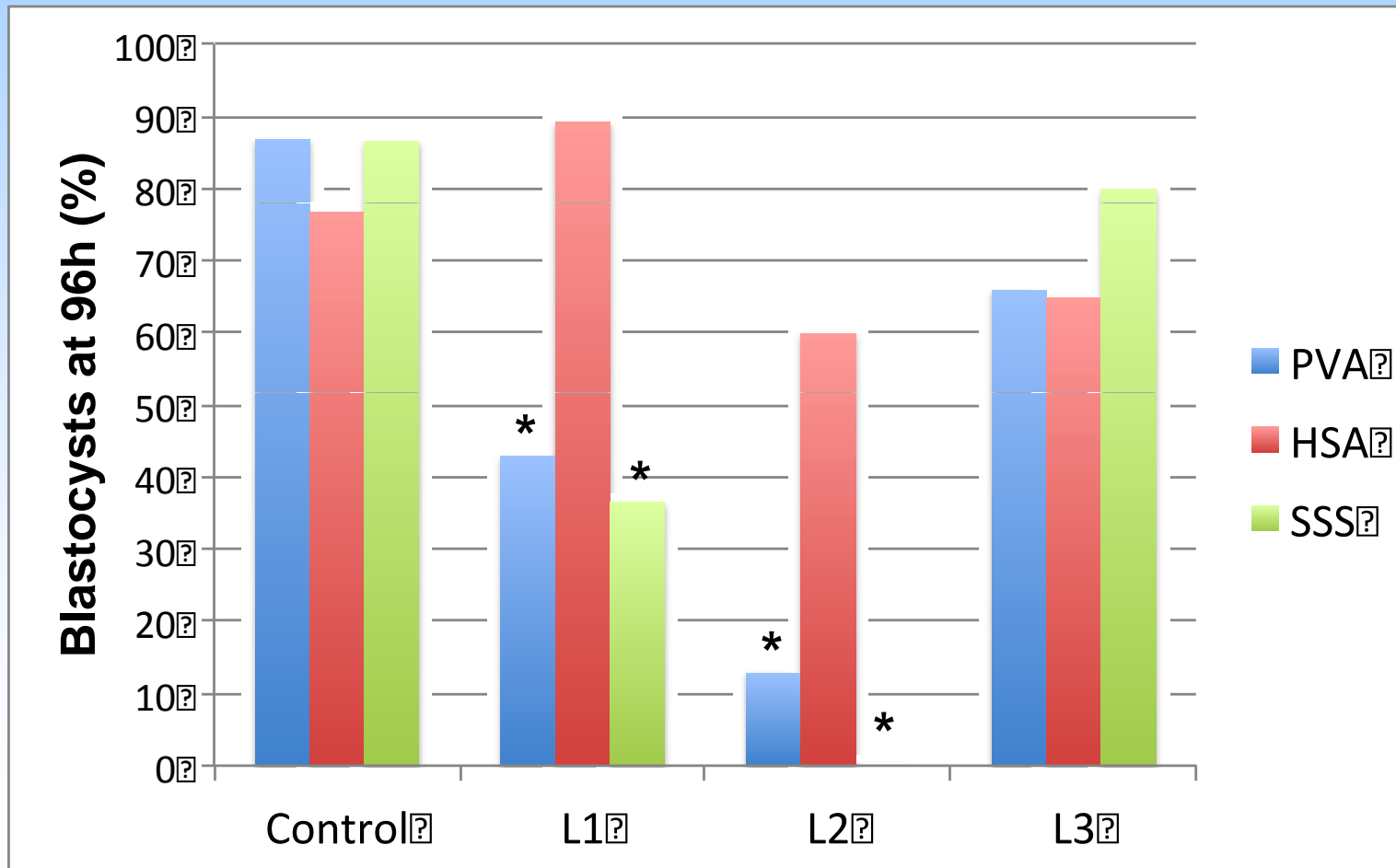
What do you use for protein supplementation?

1. 5% HSA (5 mg/ml)
2. 10% HSA
3. 10% SSS/SPS/LGPS
4. 20% SSS/SPS/LGPS

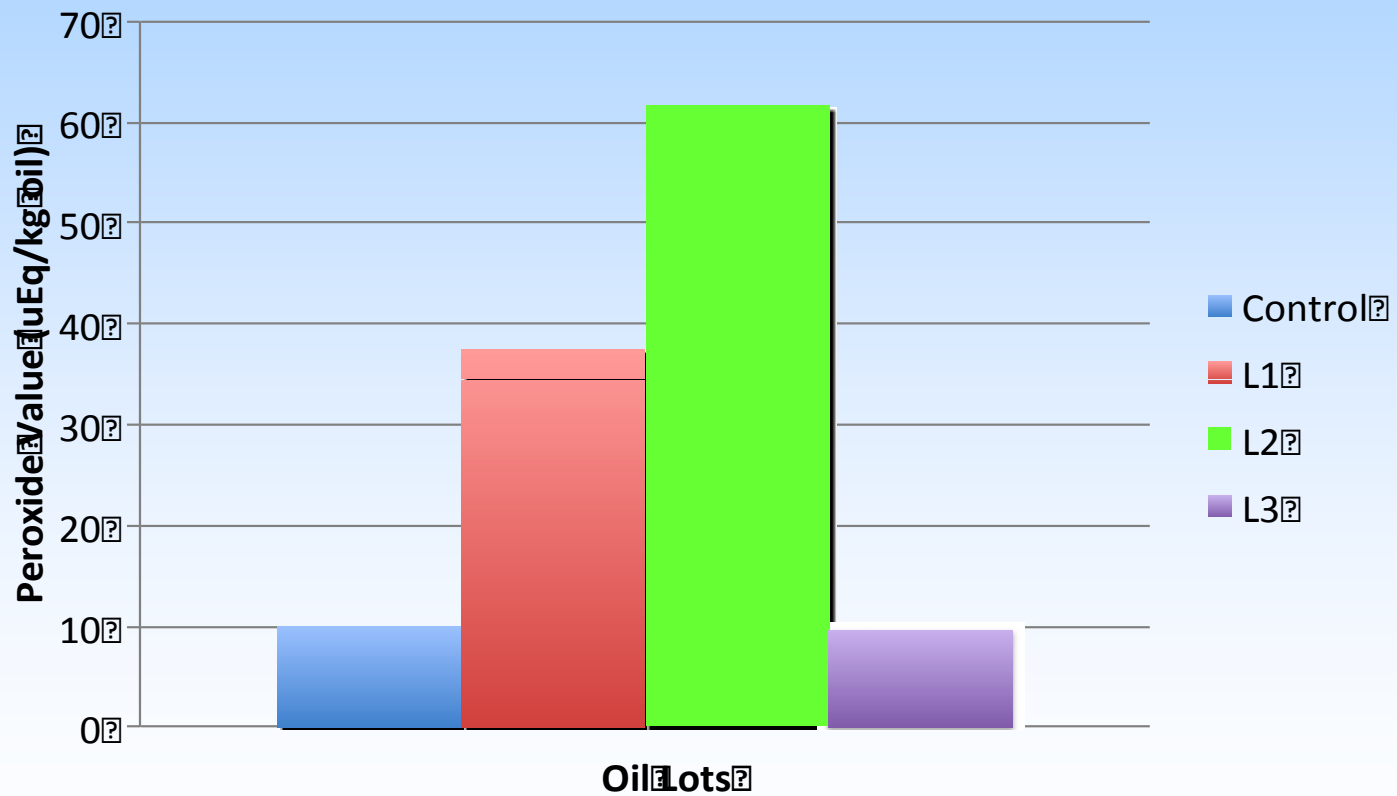


Protein vs Oil

96 hours, ambient O2



Peroxide in Oil



What Can Manufacturers Do?

- **Define oil**
- **Develop better analytical tools**
- **Use time-lapse MEA or other more sensitive bioassays**



Atomic Fingerprinting Ensures Purity, Consistency and Reliability of SAGE® Oil for Tissue Culture

Written by:
Charles S. Smith, Ph.D.
Director of Operations – Sage IVF

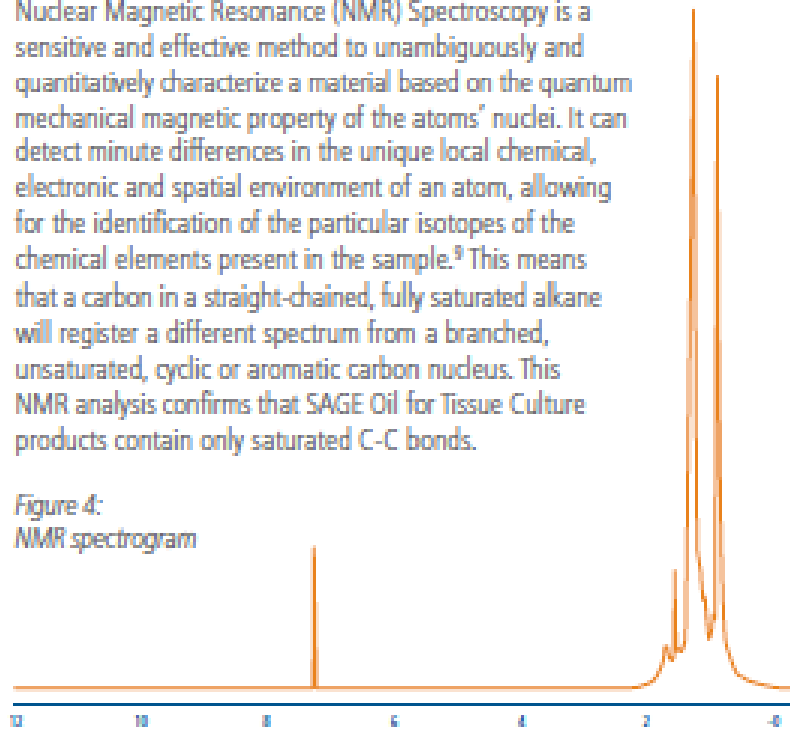
CooperSurgical



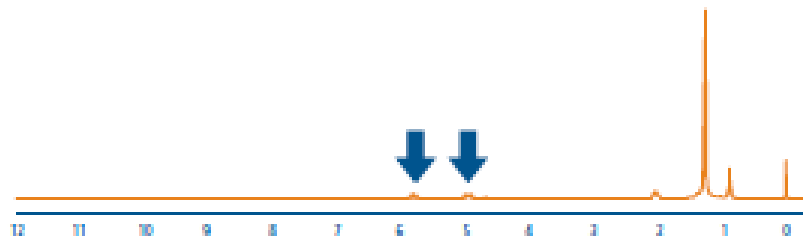
NMR – Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is a sensitive and effective method to unambiguously and quantitatively characterize a material based on the quantum mechanical magnetic property of the atoms' nuclei. It can detect minute differences in the unique local chemical, electronic and spatial environment of an atom, allowing for the identification of the particular isotopes of the chemical elements present in the sample.⁹ This means that a carbon in a straight-chained, fully saturated alkane will register a different spectrum from a branched, unsaturated, cyclic or aromatic carbon nucleus. This NMR analysis confirms that SAGE Oil for Tissue Culture products contain only saturated C-C bonds.

Figure 4:
NMR spectrogram

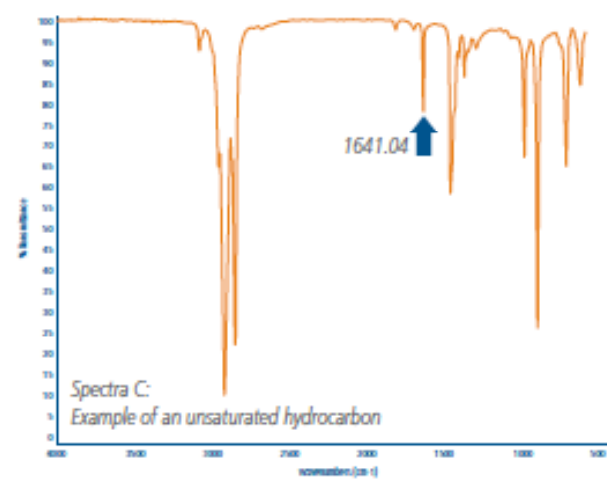
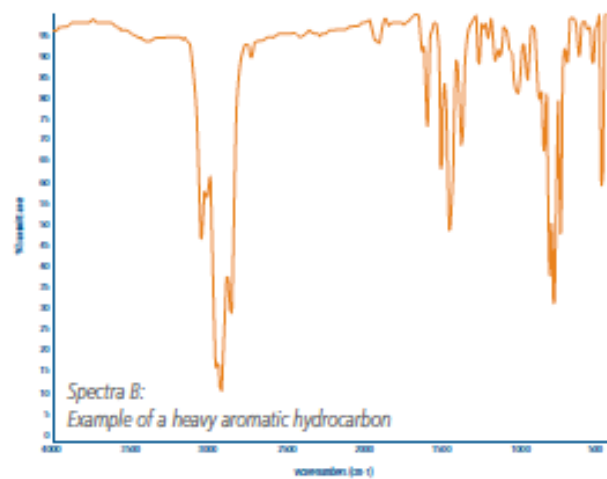
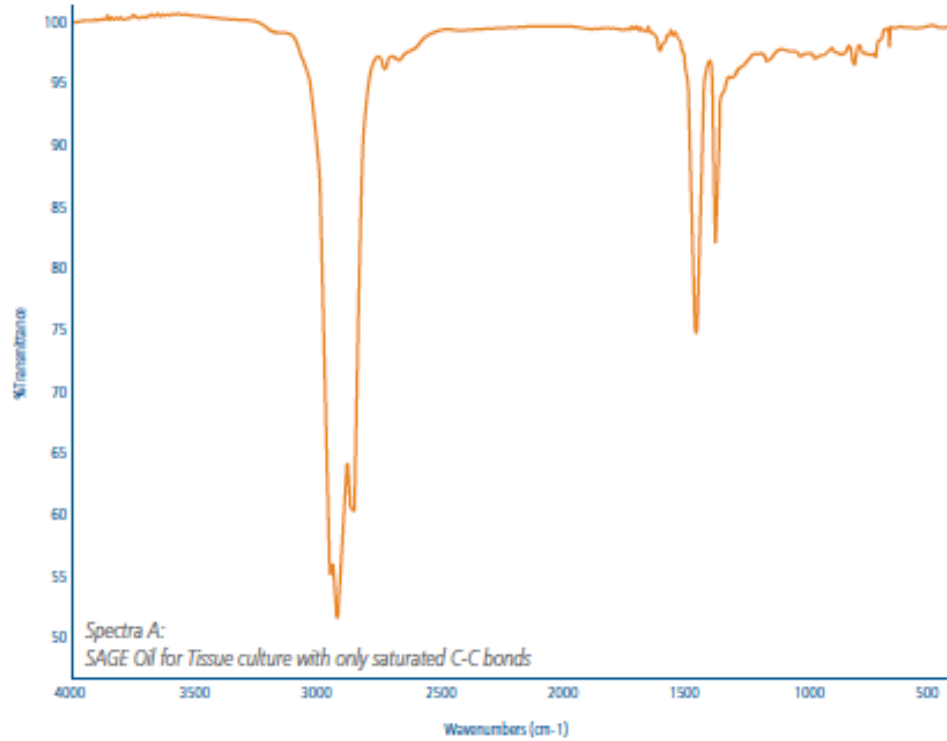


Spectra A: SAGE Oil for Tissue Culture with only saturated C-C bonds

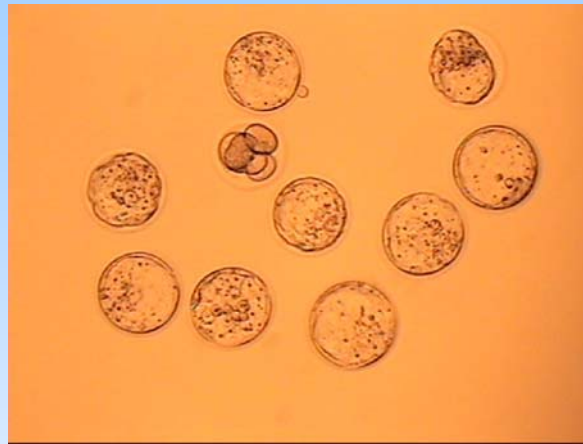


Spectra B: Example with two double-bonded alkenes present

Figure 3: FTIR spectrograms



QC using Time-Lapse?



VS.



**Thaw Time
(0 hours)**



t2



t3



t4

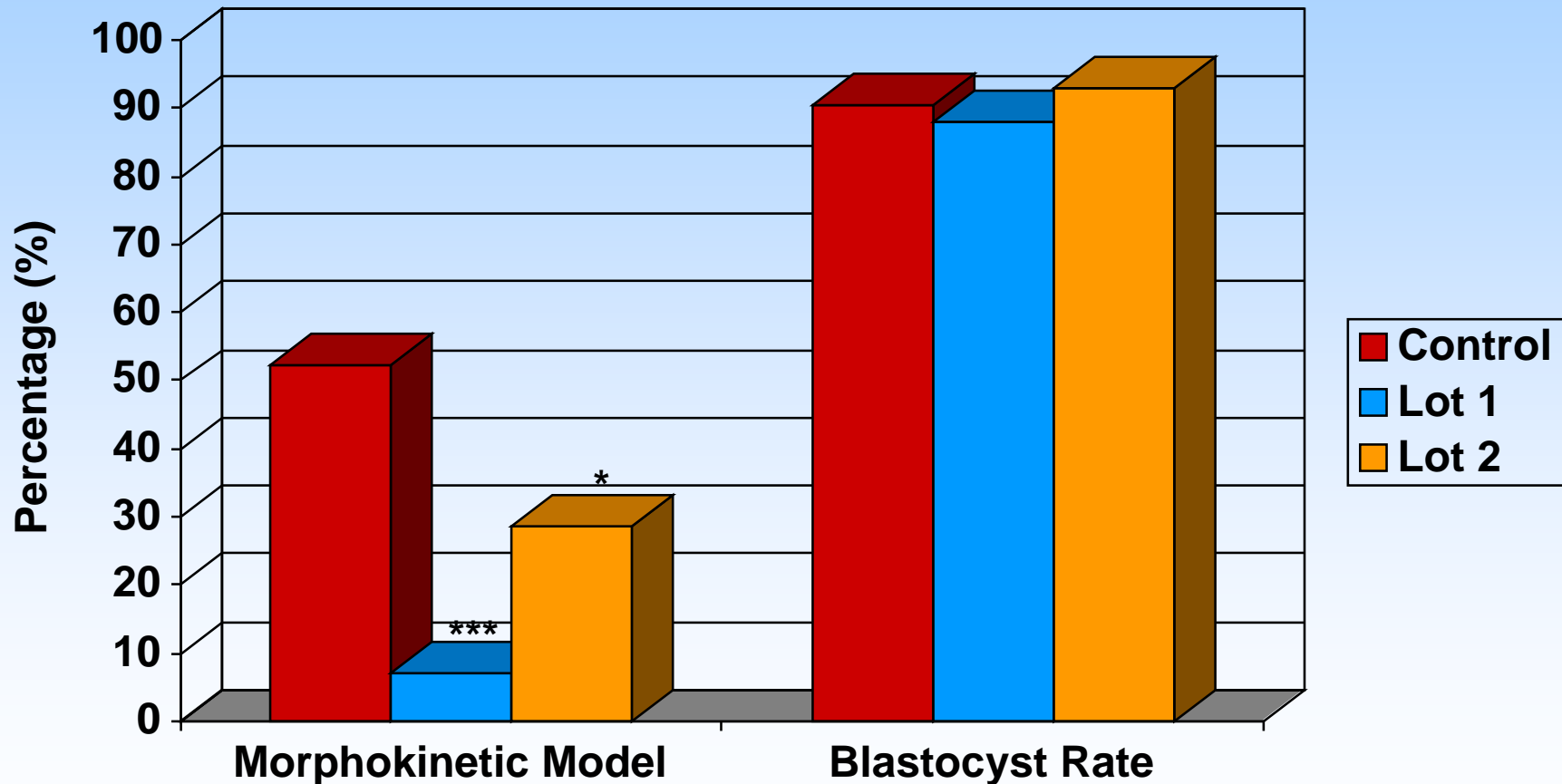


t5



t8

Toxic Oils and Morphokinetics Model

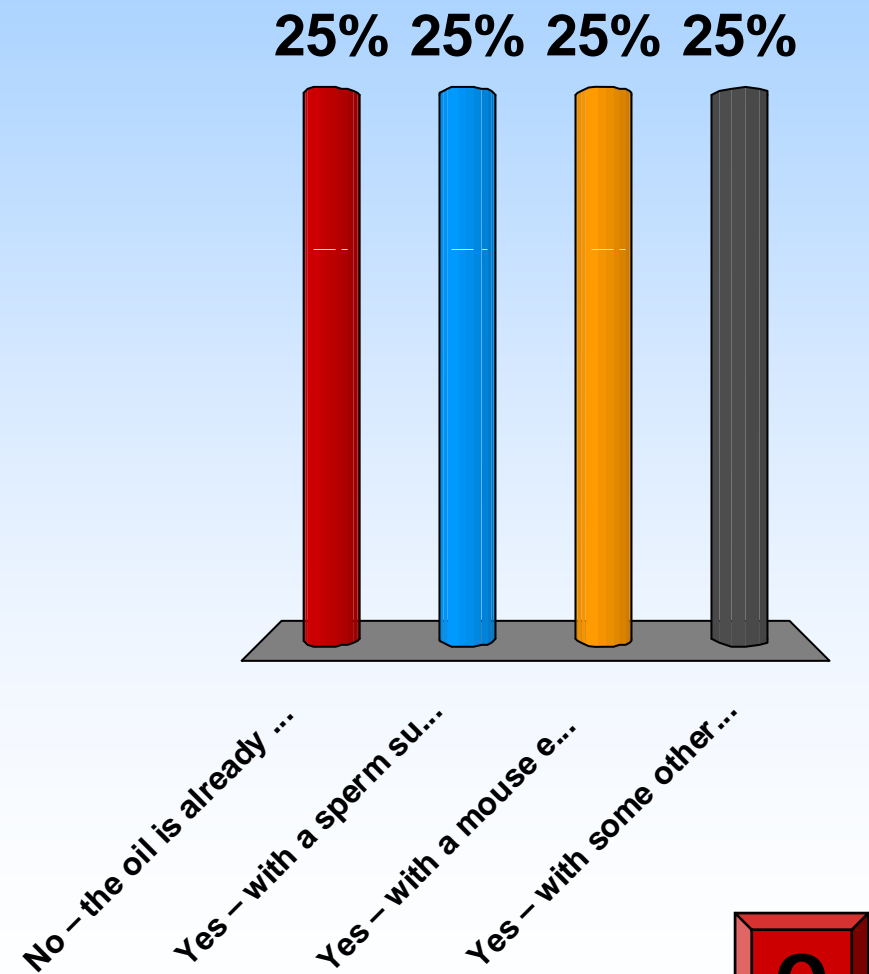


What can YOU do about it?

- **Good culture conditions!**
- **Proper handling**
- **Two-step QC**
 - **120h mouse assay or human sperm testing**
 - **Split cases – 1/2 old lot + 1/2 new lot**
- **Wash oil**

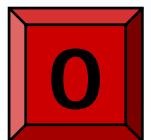
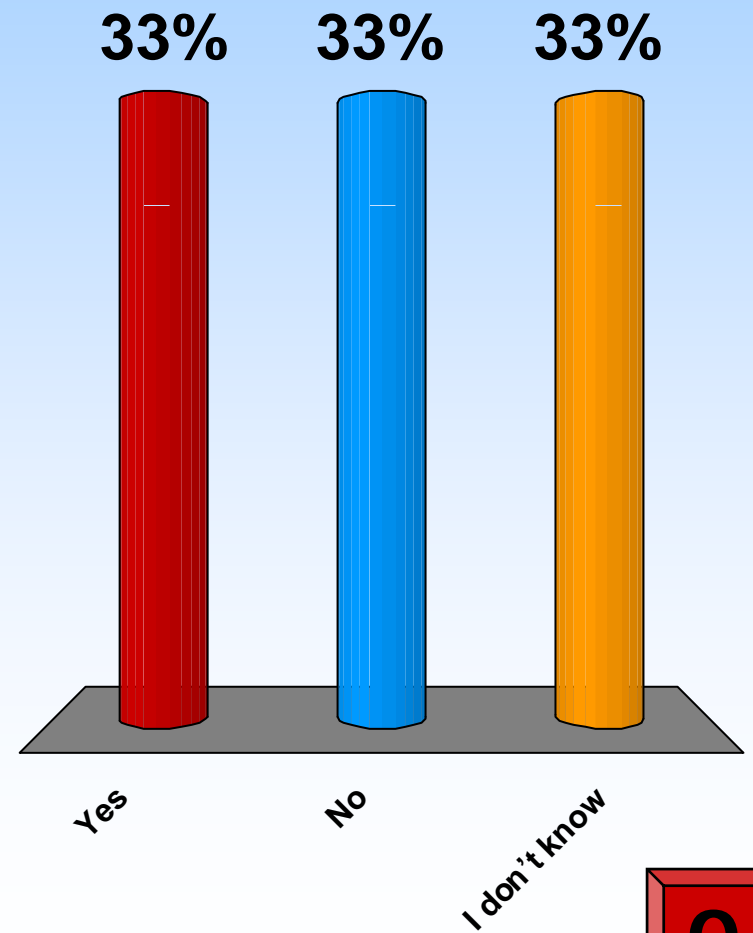
Do you perform a QC test on a new lot of oil?

1. No – the oil is already tested.
2. Yes – with a sperm survival/motility assay.
3. Yes – with a mouse embryo assay.
4. Yes – with some other test.



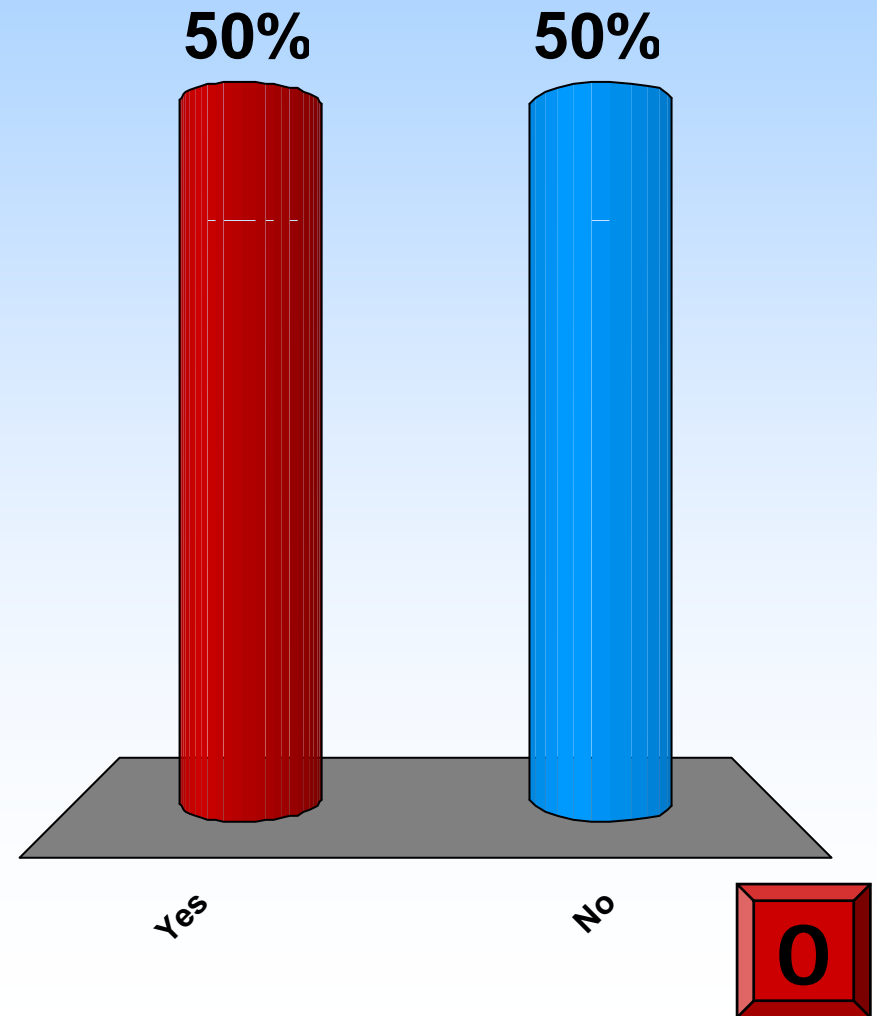
Is the oil you purchase washed by the supplier?

1. Yes
2. No
3. I don't know



Do you wash oil in your lab?

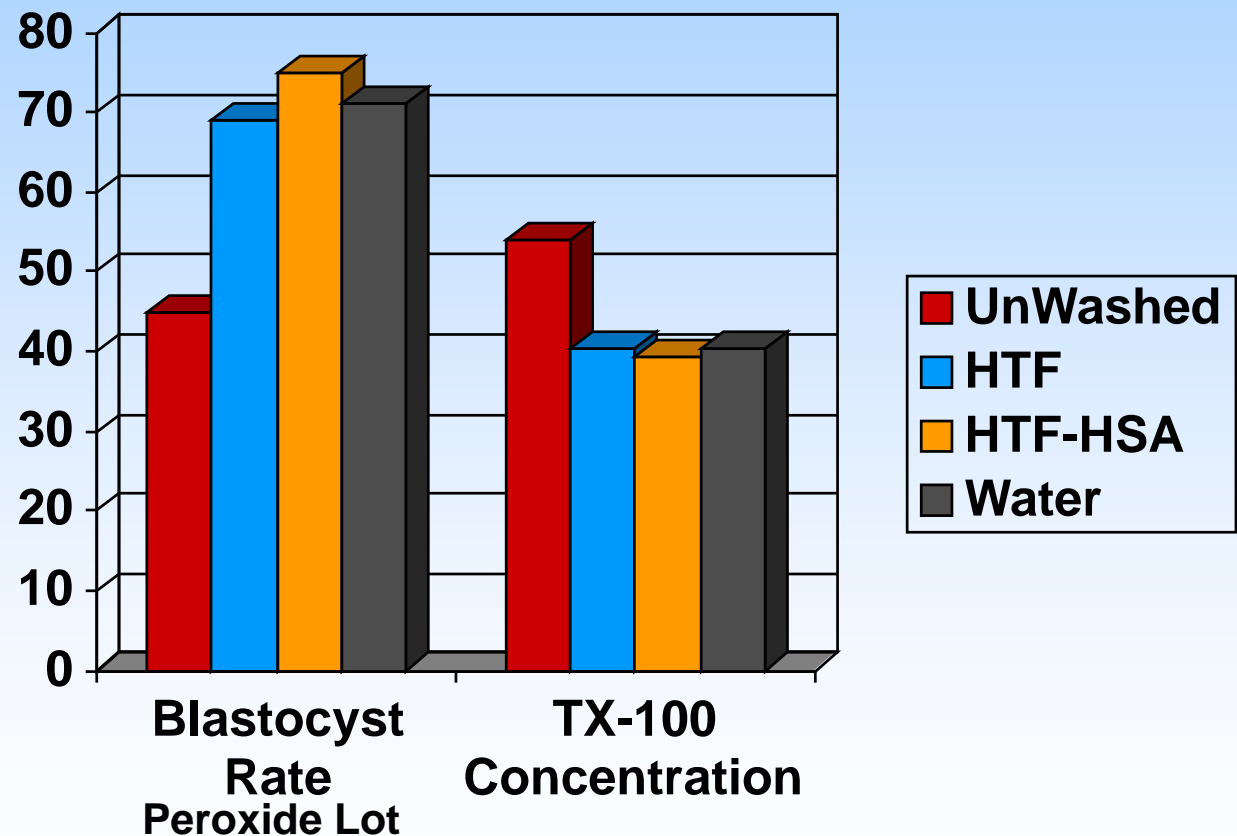
1. Yes
2. No



Washing Oil

Quantitative and qualitative improvements documented.

Most manufacturers wash oil.



Summary

- **Oil is often toxic and testing is inadequate**
 - **Mouse embryos are not as sensitive as human embryos!**
- **Toxicity appears to be condition dependent**
- **Quality of oil will continue to be in question. Labs need to be diligent and suppliers need to be responsive.**

Special Thanks

- **David Barnidge, Ph.D.**
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Mayo Proteomics Core
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**Department of Civil
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