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Oil Uncovered: Why FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture is Right for Your Laboratory

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Where to Begin: Importance of Oil

Oil as an overlay is a necessary element in any assisted reproductive technology (ART) procedure that involves micro volumes of media and extended embryo culture. The overlay of oil prevents media evaporation, promotes media stabilization, and helps to reduce fluctuations of temperature, osmolality, and pH over time[1].

Because of oil's critical role in successful ART and *in vitro* fertilization (IVF) processes, it is crucial to discern its descriptive terms and understand its characteristics and fundamental properties. Heavy, light, mineral, paraffin, and a plethora of QC tests are all defining factors in selecting an oil product, but what do they actually mean? Is there truly any difference between these various types of products? How does FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture (Catalog # 90189) stack up against other types of oil?

Demystifying Viscosity: Heavy vs. Light

Oil plays a vital role in media stabilization, but it is more difficult to determine if the oil viscosity has any impact on media stabilization. While Mestres et al. found that higher viscosity oil maintains better pH stability and prevents more evaporation outside the incubator, Gaspard et al. found that regardless of microdrop volume and oil viscosity, the media remained stable and well within suitable pH and osmolality ranges from day 0 through day 7[2,3].

Equally as difficult is determining the comparable viscosity between oils from different manufacturers. When referencing the viscosity of commercially available ART oil products, "light," "low," "medium," "high," and "heavy" are all descriptors and differentiators. Although there are ways to measure the viscosity of the oil, there is not an industry standard that defines the values of these viscosity classifications, and most manufacturers do not publish viscosity specifications. Additionally, manufacturers may assign these classifications in relation to their own products as opposed to how they compare to other oil products on the market.

One thing is certain: The volume of oil used per dish is not dependent on the oil viscosity. As such, the decision of oil viscosity should not be based upon how much is used per plate.

While it may be difficult to determine which manufacturer makes the most viscous oil, and there is no definitive proof that viscosity matters in terms of media stabilization, another thing to consider is the effect of viscosity on ease of use. In many cases, as the viscosity of oil increases, the ease of handling in the many IVF laboratory processes involving oil increases. However, this is very subjective, and comes down to personal preference.

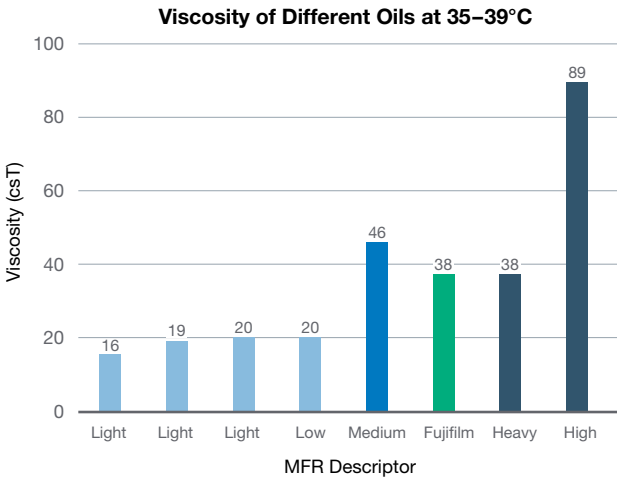


Figure 1. Comparing ART oil viscosity. The viscosity of eight different commercially available ART oil products was measured at 35–39°C. The graph above shows the measured viscosity of each oil along with the descriptor (light, low, medium, heavy, or high) given by the manufacturer. FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture is labeled as "Fujifilm."

What's in a Name: Paraffin vs. Mineral

It has been widely marketed that paraffin oil is superior to mineral oil for embryo culture. However, when looking at the available literature, there is no actual consensus as to performance variances. Sifer et al. found statistically higher "top-quality" embryos on day 3 in one company's paraffin oil than the other three oils tested. Yet, in the same study, another paraffin oil from a different ART supplier showed inferior performance[4]. Conversely, it was determined by Louanjli, et al. that there were no significant differences between the two groups cultured in either mineral oil or paraffin oil. In fact, this study even concluded that the two oils can be used together in the same protocol[5].

This finding is explained by looking at the definitions and substance identifiers assigned by various organizations and governing bodies. The US Pharmacopeia (USP), a governing body for medical quality standards worldwide, defines mineral oil as "a purified mixture of liquid hydrocarbons obtained from petroleum"[6]. The European Pharmacopeia (Ph. Eur.), a similar governing body for the European Union, defines both light liquid paraffin and liquid paraffin as "a purified mixture of liquid saturated hydrocarbons obtained from petroleum"[7]. The USP does not contain a definition for liquid paraffin, nor does the Ph. Eur. contain one for mineral oil.

Both organization's individual tests and parameters for each oil are similar, though not identical, and between the two they encompass the following:

- infrared spectroscopy
- acidity, or acidity and alkalinity
- viscosity
- relative density, or specific gravity
- readily carbonizable substances
- polycyclic aromatic hydrocarbons
- solid paraffin
- sulfur

Thus, any oil analyzed and found within specification for all of these tests for either the Ph. Eur. or the USP can be deemed compliant with both.

The Chemical Abstract Services (CAS) is an organization that is relied upon by government agencies for regulatory applications due to their unique and internationally recognized status. This organization assigns substances an individual identifier and continually maintains available data and information regarding chemical compounds[8]. Although CAS numbers for commercially available ART oil products can be considered trade secrets and therefore not universally provided on the product's Safety Data Sheet (SDS), Morbeck publicized the verified CAS numbers for the USP mineral oil and the Ph. Eur. paraffin oil as being the same (8042-47-5)[9].

Additionally, in review of two Safety Data Sheets, one for an available mineral oil and one for an available paraffin oil, it was found that the CAS numbers are the same for both.

When considering the USP and Ph. Eur. definitions for mineral oil and paraffin oil, as well as the fact that the internationally recognized CAS numbers for "paraffin" and "mineral" oil are the same, one can confidently discern that the nomenclature is simply interchangeable.

So, the type of oil has no appreciable bearing on which oil to choose. What else should be considered?

What About Safety? Aspects of Quality Testing

The 96-hour mouse embryo assay (MEA) has long been the standard for quality testing for all ART products, media, and disposables. As of 2021, the United States Food and Drug Administration has issued specific guidance as to the MEA testing parameters for ART purposes[10]. In order to provide the safest possible product, select commercial suppliers also perform supplemental quality control testing along with the standard MEA.

In 2016, FUJIFILM Irvine Scientific developed a genetic mouse embryo assay, called MEGA, to test the quality of every barrel of raw material oil that goes into the finished product[11]. This specialized quality assay uses a functional molecular biomarker to detect toxicity or environmental stress via the level and location of fluorescence produced by green fluorescent protein (GFP). GFP is expressed under the control of the Oct4 promoter, also known as Pou5f1. Regulation of Oct4 expression is one of the most critical early indicators of normal embryonic development. While MEA relies solely on traditional morphological evaluations, MEGA considers both morphological and fluorescent assessments to monitor both the early and late stages of embryo development, and has increased sensitivity to toxic materials and suboptimal culture conditions.

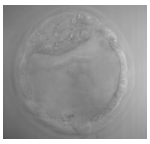
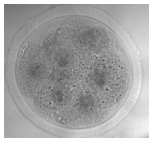
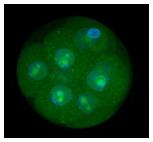
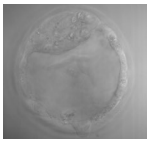
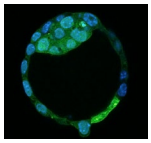
	48 hrs. Morphology	48 hrs. Gene Expression	96 hrs. Morphology	96 hrs. Gene Expression
MEA	Sometimes Observed	–	Graded	–
MEGA	Observed	Graded	Graded	Observed
MEA				
MEGA				

Figure 2. MEA vs. MEGA. This table shows the differences between MEA and MEGA observations and grading at 48 hrs. and 96 hrs.

In addition to MEA and MEGA evaluations, FUJIFILM Irvine Scientific performs stringent, gold standard peroxide value testing (POV) on all oil products. The presence of peroxides in embryo culture media can have extremely deleterious and detrimental effects on the growth and development of the embryos. Therefore, POV levels are strictly and fastidiously tested and monitored for all raw materials and finished product.

So far, we have established that user preference plays the biggest role when determining which oil viscosity to use, there is no discernable difference between mineral oil and paraffin oil, and rigorous quality control testing is important when choosing the oil that is right for your lab. With that in mind, let's a take a look at what went into the development and testing of FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture to ensure it has all the qualities that matter the most.

Dependability: Maintaining pH and Osmolality

Maintaining pH and osmolality stability for the embryo is key in embryo culture environments. Ideal osmolality for pre-implantation embryos is 255–295 mOsm/kg, and ideal pH in the embryo culture medium is 7.20–7.50.

In a recently released abstract for European Society of Human Reproduction and Embryology (ESHRE) 2024, Gaspard et. al. measured the pH and osmolality in both humidified and dry incubators over eight days of continuous embryo culture conditions. This study used Continuous Single Culture - NX Complete (CSCM-NXC) in both 25 µL and 100 µL drops under either Light Oil for Embryo Culture or Heavy Oil for Embryo Culture (Irvine Scientific). Their results showed that regardless of microdrop volume and oil viscosity, the media remained stable and well within suitable pH and osmolality ranges from day 0 through day 7. This supports the conclusion that heavy oil is just as safe and effective for use in embryo culture as light oil.

Conclusion

FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture is a high-performing, sterile mineral oil designed and optimized for use as an overlay in embryo culture environments. It has the ability to maintain a safe and secure pH and osmolality over the course of embryo culture in both dry and humid incubators. Additionally, the documented increase in viscosity not only retains an ideal *in vitro* environment, but it also serves to improve the handling and workability for embryologists.

Understanding the interchangeability of terminology that surrounds available embryo culture oil is an important step in having confidence in your embryo culture system. In addition, the importance of tangible and reported quality testing that not only meets, but exceeds industry standards cannot be disregarded. Based on these qualifications, FUJIFILM Irvine Scientific Heavy Oil for Embryo Culture is conducive to a safe, efficacious *in vitro* environment for embryo growth and development, and may be a perfect fit for your lab.

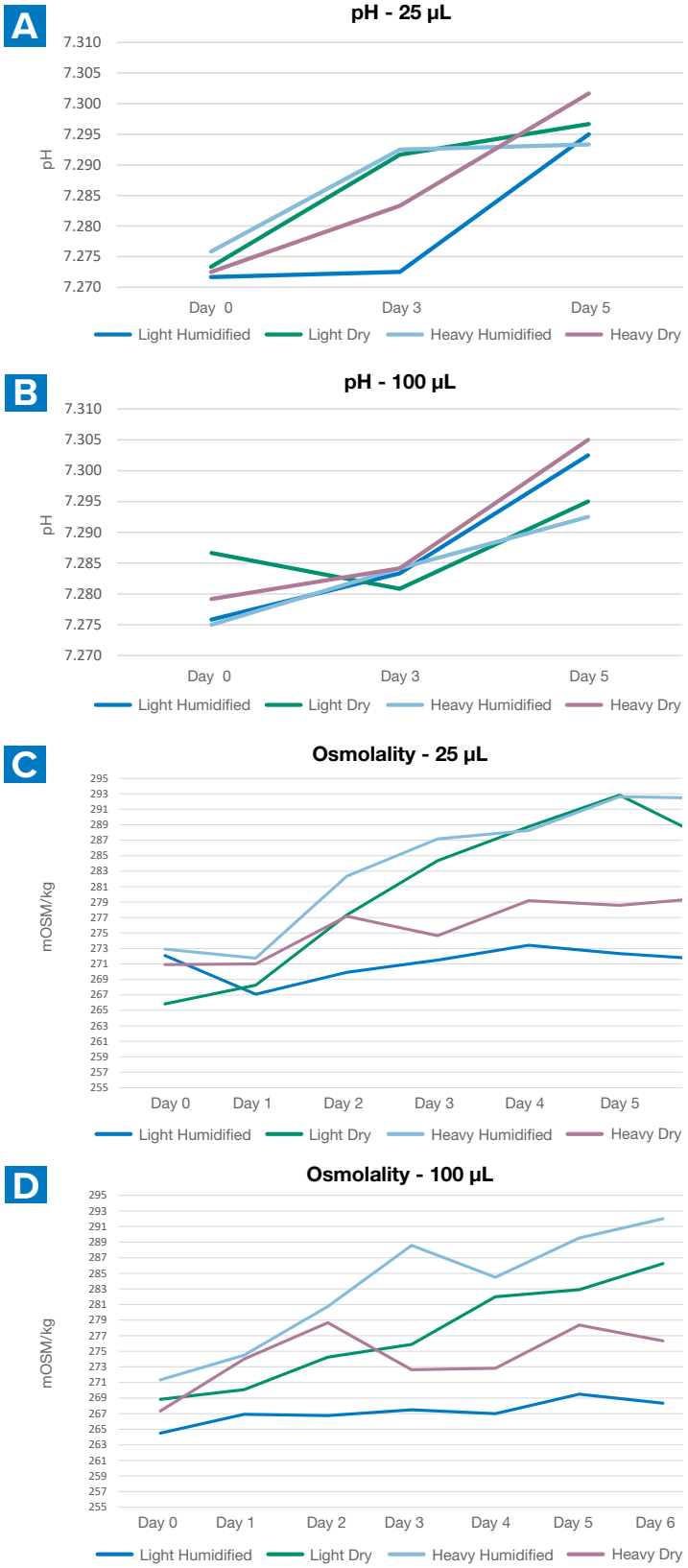


Figure 3. Graphs **(A)** and **(B)** show the pH measurements for the 25 µL and 100 µL samples, respectively. The average aggregate pH overall was 7.290 (σ=0.012). Graphs **(C)** and **(D)** show the osmolality measurements for the 25 µL and 100 µL samples, respectively. Aggregate osmolality, measured as milliosmoles per kilogram (mOsm/kg), over the course of incubation in all conditions remained well within acceptable safety range (M=277 mOsm/kg, σ=5). Dry incubator incubation exhibits a 7% increase from day 0 to day 6 (M=270 mOsm/kg, σ=4 and M=289 mOsm/kg, σ=5) compared to a 2% increase in humidified incubation (M=269 mOsm/kg, σ=5 and M=274 mOsm/kg, σ=5) regardless of oil type or microdrop size, both remaining within accepted limits.

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