Nidacon

Use of oil in IVF

Mineral oil has been a standard component of embryo culture since the early 1960s.

Following the initial success of culturing cleavage stage embryos to blastocyst in test tubes (Hammond et al 1949, Whitten et al 1957), two students in the laboratory of John Biggers at the university of Pennsylvania, Ralph Gwatkin and Ralph Brinster (1963), developed a method which allowed more frequent observation of embryos that entailed the use of mineral oil placed over the top of media in petri dishes (Biggers et al 1987).

Though culturing in test tubes is still a viable option, development of this micro drop method with oil overlay and variations of the approach in culture dishes, was a major step towards defining the culture requirements of early cleavage stage embryos and is the most common method of embryo culture in use today.

In addition, facilitating embryo assessment, the oil overlay approach allows culture in small volumes of culture medium while minimizing pH, temperature, and osmotic fluctuations (Biggers et al 1987, Bavister et al 1995).

The volume of medium used for culture can be very small, making it crucial to control water evaporation and fluctuations in the pH and temperature of the medium.

Evaporation increases medium salt concentrations and, consequently, osmolality, which can both independently impair embryo development and may lead to cellular damage.

Manufacturing of oil

The paraffin oil used in IVF is produced through a rigorous purification and refinement process to ensure it is safe for embryo culture. Here's an overview of how it is made:

1. Sourcing & Base Material

- Derived from highly refined mineral oil, typically a byproduct of petroleum.
- Only pharmaceutical or medical-grade raw materials are used.

2. Purification & Refinement

- Hydro processing (Hydrotreatment & Hydrocracking): Removes impurities, unsaturated hydrocarbons, and potential toxins.
- Distillation: Ensures consistency in molecular size and removes volatile compounds.
- Filtration: Eliminates any particulate contaminants.

3. Quality Testing & Certification

- Sterility Testing: Ensures the absence of microorganisms.
- Endotoxin Testing: Verifies low endotoxin levels to prevent embryo toxicity.
- Biocompatibility & Embryo Testing: The oil is tested on mouse embryos and human sperm to confirm it supports normal development.

4. Packaging & Storage

- Bottled in sterile, medical-grade amber bottle to protect against light degradation.
- Sealed under controlled conditions to maintain purity.

Paraffin- vs mineral oil

Paraffin oil and mineral oil are often used interchangeably, but there are some differences between them:

Source and Refinement:

Mineral Oil: Derived from petroleum through the distillation process. It is a by-product of refining crude oil to make gasoline and other petroleum products

Paraffin Oil: Also derived from crude oil but undergoes further refining to remove impurities, making it a purer substance compared to mineral oil.

Viscosity:

Mineral Oil: Has a lower viscosity, making it more suitable for applications where a thinner lubricant is required.

Paraffin Oil: Has a slightly higher viscosity, making it better suited for applications where a thicker lubricant is needed.

The choice between the two depends on the specific requirements of your application. If you need a thinner lubricant, mineral oil might be the better option. For a thicker, purer substance, paraffin oil would be more suitable.

Light paraffin oils, especially medicinal and cosmetic grades, are subject to stringent safety and regulatory standards set by health agencies (e.g., FDA, EU Cosmetic Regulations) to ensure they are free from harmful contaminants like polycyclic aromatic hydrocarbons (PAHs).

This process ensures that the oil is stable, safe, and highly pure for various applications.

Quality testing

Sterility and toxin analyses

Microbiological growth control

Performed after production of a batch and involve bacterial and fungal growth assays. The assays should be performed under a period of at least 3 weeks in order to be able to detect any growth. The tests conducted by Nidacon are carried out by an accredited testing laboratory.

Endotoxin detection

The method, often abbreviated to LAL (Limulus Amoebocyte Lysate), is based on a coagulation system in the lytic extract of the horseshoe crab's blood cells, which is activated by small amounts of endotoxin. The final enzyme in the coagulation system splits the chromogenic substrate, paranitro-aniline-pentapeptide, giving rise to a yellow substance that can be measured spectrophotometrically.

This quantitative method is called kinetic chromogenic test. Using a microprocessor and a microplate reader, the time it takes for the reaction to produce a measurable amount colour in the basin could be measured. The reaction time for a certain absorbance is inversely correlated to the amount of endotoxin in the sample. Endotoxin in samples is measured by comparison with a standard curve.

Biological anlysis

Human Sperm Survival test

Prepared sperm are covered by oil and incubated overnight in 37°C, 5-6% CO_2. Reading in the morning next day (after 18 hours). The percentage of motile sperm is checked.

Mouse Embryo Assay (MEA)

Mouse embryos are extremely sensitive to toxic agents and suboptimal conditions, making them an effective biological indicator for detecting contaminants in IVF products such as culture media, oils, and disposables.

The assay evaluates the actual biological effect of a product on embryo development. This is more relevant than purely chemical or physical testing because it directly mirrors the conditions under which human embryos are cultured during IVF.

Despite species differences, the findings from MEAs are generally predictive of how products will perform with human embryos. If a product supports mouse embryo development to the blastocyst stage, it is highly likely to be safe and effective for human embryo culture. Many regulatory and professional guidelines in assisted reproduction endorse MEAs as a critical component of quality assurance for IVF products. Compliance with these standards ensures that laboratories meet high safety and performance benchmarks.

In the MEA, mouse embryos are exposed to oil and cultured in vitro for 5-6 days. At least 80% of the embryos must reach the expanded blastocyst stage and pass a strict daily visual examination of morphological quality. The MEA tests are performed at an external and independent laboratory with long experience that provides quality control (QC) services specifically designed to detect toxicity in all types of materials, culture media, products or equipment used routinely in IVF laboratories.

Day 6 (eMEA)

Additional information regarding Mouse Embryo Assay (MEA) results on day 6 is provided on the Quality Assurance certificates for Nidoil. Day 6 (eMEA) results offer an extra layer of safety assessment, enhancing our ability to detect potential toxicity and ensuring the highest product standards.

Peroxide analyses

Peroxide (e.g. hydrogen peroxide H_2O_2) is one of the key reactive oxygen species formed under oxidative stress conditions and have been shown in several publications to affect embryo culture. All Nidoil batches are measured using a QuantiChrom™ Peroxide Assay. A kit designed to measure peroxide concentration using the chromogenic $Fe3^+$-xylenol orange reaction.

Visual control

Constant visual control during production, filling, labelling and final control of chosen ready packages.

Packaging

PET is chosen for NidOil due to its beneficial properties for our intended use. PET is short for polyethylene terephthalate, the chemical name for polyester. PET is a clear, strong, and lightweight plastic that is widely used for packaging foods and beverages, especially convenience-sized soft drinks, juices and water.

Global Safety Approval
PET is approved as safe for contact with foods and beverages. The safety of PET for food, beverage, pharmaceutical and medical applications has been repeatedly demonstrated through extensive studies, regulatory approvals, testing, and its widespread acceptance for more than 30 years. PET is biocompatible and is FDA approved for the repair of large blood vessels and other soft tissues. PET does not contain bisphenol-A (BPA) or phthalates (plasticizers). Because of PET's unique properties, it is rapidly becoming the world's preferred packaging material for foods and beverages.

Like glass, it is a very strong and inert material that does not react with foods, is resistant to attack by micro-organisms, and will not biologically degrade. But unlike glass, PET is extremely lightweight, easy and efficient to transport, and shatterproof.

Fully recyclable
PET is completely recyclable and is the most recycled plastic worldwide.

PET can be commercially recycled by thorough washing and re-melting, or by chemically breaking it down to its component materials to make new PET resin. Almost every municipal recycling program in North America and Europe accepts PET containers.

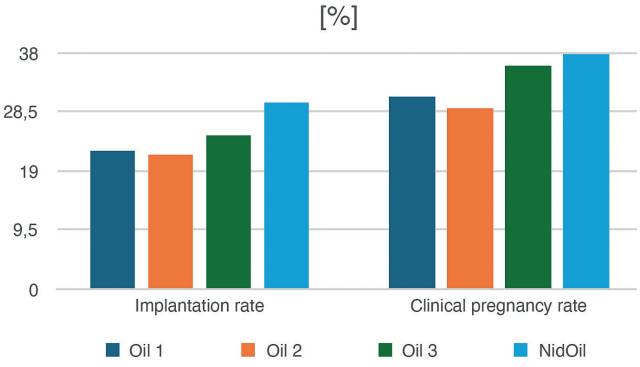
Why amber bottle?
There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the l aboratory bench. As a precaution against any light-induced changes, NidOil™ is supplied in amber, screw-top bottles.

Clinical data

A prospective randomized study to compare four different mineral oils used to culture human embryos in IVF/ICSI treatments (Sifer et al)

This study evaluated and compared the embryo quality using different oils. A total of 500 IVF/ICSI treatments were analysed in a prospective randomized study. Oocytes/embryos were treated into microdroplets of appropriate medium overlaid with the four different oils.

Results:



Conclusion,
The results indicate that using NidOil is a safe and well-performing option. The results are for both implantation and pregnancy rates slightly higher compared to similar products avalable on the market.

Any need for washing paraffin oil?
Studies performed at Nidacon comparing washed and un-washed oil have shown that blastocyst development is comparable or better when using a non-washed NidOil compared to washed NidOil.

Aliquots of washed and unwashed oil were used as an overlay in a culture system for mouse embryos (Embryotech, Wilmington, US). The number of embryos developing to the blastocyst stage within 72 hours was compared.

This has also been demonstrated in other studies. Swain et al showed that washing of oil had no protective impact on stabilizing media osmolality compared to unwashed oil when utilized for up to 7 days in a non-humidified incubator environment.

How to best use NidOil

In an IVF laboratory, culture dishes must be prepared in advance to allow their equilibration, prior to be used. pH is the factor that requires a longer incubation time to change and stabilize, as CO_2 must diffuse through the oil overlay to reach the medium and react with its bicarbonate buffer.

The preparation should be performed in a timely manner since evaporation of media during dish preparation can impact concentration of other components in the culture media. This may in itself also impact embryo development and is another consideration for preparing culture dishes in an appropriate fashion to avoid media evaporation.

The amount of mineral oil used as an overlay when culturing human embryos is critical to maintaining optimal conditions. The correct volume ensures proper temperature and pH stability while preventing evaporation of the culture medium.

Here are general guidelines:

Oil-to-Medium Ratio: A layer of mineral oil should be sufficient to fully cover the culture drops or wells, creating an overlay of about 1-2 mm thickness above the medium. Ensure each droplet is adequately covered.

Practical Considerations:

- Ensure the oil is pre-equilibrated to the culture conditions (temperature, gas composition) before use.
- Too much oil can make handling droplets challenging, while too little might allow medium evaporation or pH instability.
- The number of drops per dish and number of dishes prepared before addition of oil should be limited. Could lead to alterations in media characteristics if too many.
- Be aware of airflow, working-surface temperature and adjust protocol accordingly.

Using more mineral oil can help reduce evaporation and preserving osmolarity and pH to a certain extent. A very thick layer may slow down the exchange and make it difficult to observe and manipulate the culture drops, increasing the risk of contamination or technical errors during embryo handling.

Why use NidOil

- Easy to use and handle in the laboratory
- Long shelf-life of two years
- Same shelf- life after opening
- Storage and transportation in room temperature
- Packaging in amber bottles for light protection

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Order info:

NO-100 NIDOIL™ 100 mL
NO-400K NIDOIL™ 4 x 100 mL

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