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Review Article

Mineral Oil for *in vitro* Embryo Production: What We Should Know?

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Abstract

Mineral oil as a barrier can minimize temperature, osmolality, and pH fluctuation of the media in the *in vitro* embryo production system (IVP). Regardless of these advantages, mineral oil quality is varied and may deteriorate during storage or transport conditions. So, it can affect the IVP outcome by absorbing the essentials factors or realizing the toxic components into the media. Although, some methods have already been developed to reduce these side effects, still there is a big concern about the safety and use of mineral oil in the IVP system. In this review, we provided an overview of the advantages and disadvantages of using mineral oil in the IVP system. We also reviewed available methods for its quality control and finally, we introduced some methods for reducing the side effects of mineral oil.

Keywords: Toxicity, Embryo production, Oocyte membrane, *in vitro* culture

1. Context

The IVP consists several parts and each part can influence embryonic development. Mineral oil (MO), as one of the major parts of the IVP culture system, is a byproduct of crude oil and contains mixtures of complex hydrocarbons and undefined compounds. Due to this, it is the most problematic hurdle to warrant safety for gametes and embryos (1). It is commonly used to overlay the media in order to reduce temperature and osmolality fluctuation throughout the embryonic culture (2). Furthermore, it can prevent air-born contamination and reducing the accumulation of lipophilic toxic substances from the media (3). Despite these advantages, the MO can negatively influence the *in vitro* embryo development in bovine (4), mouse (5), porcine (6), sheep (7), and human (8). Also, they can reduce the survival rates of the cryopreserved embryo by altering the composition and distribution of membrane lipids (9). However, the embryo uses different defense mechanisms for facing the *in vitro* chemical threats, but it tacks amounts of cell energy to hinder the development. Considering the importance of MO in the IVP system, this review discussed the using of MO, quality control assays and reducing the side effects.

2. Evidence Acquisition

The objectives of this article were to explore the use of MO in the IVP system, available methods for quality control assays and reducing the side effects. In this regard, a detailed search was carried out on Google Scholar and PubMed databases to find out the relevant

research studies. The search process was performed using the following keywords: "mineral oil", "toxicity and oocyte", and "oocyte culture system". Only published studies were included in this review, and all other articles and studies were excluded.

3. Results

3.1. Effects of Mineral Oil on IVP

Mineral oil contains unsaturated hydrocarbons and several embryo-toxic elements, such as peroxides (1, 10) or volatile organic compounds (6). The amount of these toxic elements depend on production procedure, storage time and conditions, like temperature and UV light exposure (11). So, they can increase over time and get worse by inadequate storage conditions (12).

Also, MO has several toxic substances (Triton X, alkenes, and aldehydes) which can transfer to the media (Figure 1) and influence oocyte viability and embryo development both in animals and human (1, 6).

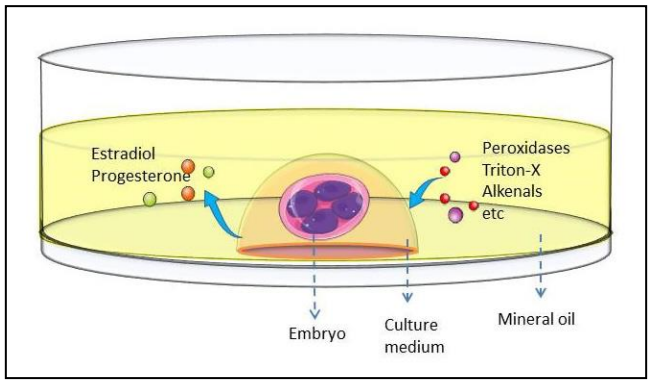

Figure 1. Effect of mineral oil on embryo in the micro droplet culture system
Mineral oil can add some toxic substances to the *in vitro* culture media or absorb some lipid soluble substances from the media, such as hormones (Estradiol and Progesterone). Low volume of culture media and high contact surface with the MO can accelerate this exchange between media and MO.

It has been shown that peroxides produced by the oxidation of reactive double-bond carbons in the mineral oil, could be the cause of DNA fragmentation (13) and loss of membrane fluidity and integrity (14), especially, in the micro-droplet culture system (11) (Figure 2). In this culture system, a high ratio of mineral oil to media along with high contact surface in the micro-droplet system (Figure 1), sink the embryos in a pool of contamination which may be responsible for the membrane damages and differences in the cleavage and blastocyst outcomes (7). The severity of damage could be varying according to the type of mineral oil, concentration, and exposure time of the cells to the toxic mineral oil.

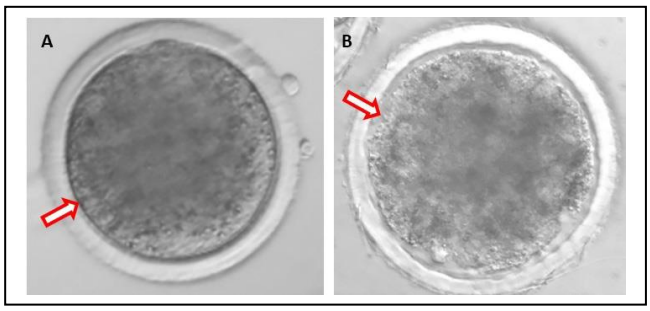

Figure 2. Effect of toxic mineral oil on sheep oocyte membrane.
A) The membrane integrity after IVM seems healthy without any detectable signs. B) Blastomer membrane disappeared and cells shape are not recognizable at 72h after *in vitro* fertilization (arrows shows the membrane line) (7).

Although most of MO passed the mouse embryo assay test (MEA), it seems that MEA does not have enough sensitivity to detect suboptimal toxicity or could be toxic during storage in unsuitable conditions (8).

Another negative point of mineral oil is its different potential in the absorbing of soluble substances (Figure 1). They can absorb and reduce the bioavailability of liposoluble substances in the media such as progesterone and estradiol (15, 16), which have a crucial role in the resumption of meiosis, increasing the maturation, fertilization and cleavage rate (17). However, it has been shown that some types of them such as silicon oil take up more estradiol (E2) than paraffin oil (18). So, they can be significantly reduce or delay nuclear maturation of oocytes in the presence of an oil cover (15, 19).

3.2. Quality Test Assays for Mineral Oil

Currently, MEA is the main test used for assessing the quality of mineral oil, which involves culturing one-cell or two-cell stage mouse embryos up to the blastocyst stage (more than 80% of embryos should develop to blastocyst upon completion of the assay-

FDA). Also, it has been shown that mouse embryos cultured *in vitro* from the 1-cell stage are more sensitive to suboptimal culture conditions than those cultured *in vitro* from the 2-cell stage. For example, using 2-cell stage mouse embryos completely failed to demonstrate an effect on embryo development when there was a significant increase in osmolarity and trace amounts of Cidex added to culture media (20). This study, among the other reports, proves that in the absence of a gold standard quality control technique, the 1-cell MEA is a more sensitive and useful assay to test culture media for toxicity and suboptimal culture characteristics as compared to the 2-cell MEA (21). Furthermore, the variability in the MEA protocols such as the strain of the mouse used for the assay (10) or other cultural characteristics can unintentionally affect the sensitivity of the MEA (21).

Human sperm survival assay (HSSA) is an alternative bioassay (22, 23) as a fast, easy and cheap assay compare to MEA (21). This test is based on measuring the survival or motility rate of sperm after exposure to the mineral oil and comparing it with the control group. The result expresses as the sperm motility index (SMI) which is calculated by the ratio of the sperm motility of the sample tested divided by the motility of the control. Although the sensitivity of the HSSA could be improved by assessing both the motility and the quality of the motility of the sperm (23), the sensitivity of the 1-cell MEA is more than the HSSA for certain toxins present in mineral oil and cannot be more than the 2-cell MEA (24). In addition, some biological differences between sperm and embryo raise the question that HSSA may not be a good choice for quality control purposes.

Recently, a simple somatic cell assay has been reported for screening mineral oil quality which is a simple and cost-effective assay. Briefly, somatic cells were cultured for 24h in a 4-well dish contained culture media and cell attachment rate and proliferation are monitored. The attachment statutes and membrane morphology are used as a quality index (25). Although this type of quality control in the laboratories could be time-consuming, the development of these types of simple and cheap assays is necessary to avoid mineral oil toxicity.

Collectively, it seems that the different quality control programs employed for MEA and HSSA testing in the main manufacturers of IVF consumables are highly variable and no regulations or standardization exist on this issue. The variations are in the mouse strain (outbred, inbred, or hybrid), culture media, the number and the origin (fresh or thawed) of the embryos as well as their stage (one-cell or two cells) which highly could influence the sensitivity of the test (26). So, it is reasonable to see the high variation in the quality of the mineral oil by the different companies or even different batch numbers, although they have successfully passed the MEA quality test. Also, most of the quality assessments focused on the blastocysts rate or morphological parameters; while it is necessary to assess the impact of mineral oil on gene expression patterns as well.

3.3. Reducing the Side Effects of Mineral Oil

Due to the toxicity of mineral oil, it is necessary to reduce its toxicity before use. Therefore, scientists have been tried to reduce the toxic effects of mineral oil on embryo production systems by washing the mineral oil, reducing the volume, or using a large amount of media. It has been reported that washing the oil with 0.9% physiological saline, synthetic human tubal fluid (HTF), HTF with 5 mg/mL human serum albumin, or distilled water, effectively reduce the transfer of toxic compounds from the oil to the aqueous phase and subsequently improve embryo quality (1, 14). However, in our lab, we did not see any significant difference between unwashed and washed mineral oil by using H-TCM199 + 0.4%BSA, ration 1:1 (4). So, it may not be useful for reducing the harmful effects of high toxic mineral oil.

Reducing the volume of mineral oil is the other option that had a positive result in oocyte cleavage and embryo production in cow and sheep IVP system (7,

27). In this method, the volume of mineral oil could be decreased by using the multi-well culture system, in which, a high volume of culture media could dilute or deactivate the toxic substances by albumin which can chelating toxins in the culture media and mask their effects (28, 29). Conversely, the high volume of the media can dilute paracrine-autocrine factors which are essential for the growth and quality of the embryo (30, 31).

Another option for reducing the negative effect of mineral oil is using an oil-free culture system. In this method, osmolality can be stabilized by surrounding the media with water (32). There are some successful reports in the cow (15) and pig (30) using an oil-free culture system without affecting maturation and development rates. However, Van Soom, Mahmoudzadeh (9) reported a significant decrease in the development of cow embryos using the oil-free culture system.

4. Conclusions

Mineral oil is still an undefined product with different compositions, purity, and high reactive components. Although all of the mineral oils presumably passed the QC testing by the manufacturers, because of variation in the QC methods using by manufacturers, we do not know if the testing is sensitive enough to detect all of the contaminations. Also, because of unknown storage conditions and transportation, we cannot be sure about its quality, especially if it will be used in the human IVP system. So, it seems that another QC control is needed before using MO in the laboratories. However, because of time-consuming and costs, it may not be possible for most of the laboratories to handle this test. Therefore, a simple and reliable QC test is highly needed.

Finally, Due to the shortage of other alternative substances or methods, mineral oil should be used with caution by respecting the storage recommendation of the manufacturer (storage in a cool and dark place) to limit heat and UV-oxidation and preventing long-term conservation even for unused bottles. Also, it seems that washing the mineral oil could be helpful in some cases. More studies on embryo gene expression patterns are required to confirm oil safety in the IVP system, and developing a new oil-free culture system is highly recommended.

Authors' Contribution

Study concept, acquisition of data, interpretation of data and drafting the manuscript: M. E.
Administrative, technical, and material support: M. D.
Interpretation of data and critical revision of the manuscript: L. M. and A. P

Conflict of Interest

The authors declare that they have no conflict of interest.

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