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TOPIC OF DISCUSSION

"Current Practices and Rationale for the Selection of Embryo Culture Dishes Among Clinical Embryologists: A Survey"

CHAT DISCUSSIONS COMPILED BY

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“Discussion on Current Practices and Rationale for the Selection of Embryo Culture Dishes Among Clinical Embryologists: A Survey”

INTRODUCTION

Culture dishes play a critical role in in vitro embryo development by directly influencing the microenvironment surrounding the embryo. Traditionally, standard flat sterile plastic Petri dishes with microdrops of culture medium under a mineral oil overlay have been widely used in embryo culture, enabling control of embryo density through adjustment of drop volume and embryo number [1] However, increasing evidence indicates that dish design itself can significantly influence embryo development by affecting embryo density, diffusion of metabolites, and the localized accumulation of embryotrophic factors. To address these considerations, newer culture dishes have been developed with conical or reduced-volume wells that allow embryos to settle at the lowest point of the well, thereby promoting localized concentration of autocrine and paracrine factors and optimizing the embryo microenvironment [2].

Embryo culture represents one of the most critical phases of assisted reproductive technology (ART), with direct implications for embryo viability, developmental competence, and clinical outcomes[3]. While substantial emphasis is placed on incubator systems, culture media composition, laboratory air quality, and temperature control, the selection of embryo culture dishes is often considered a routine laboratory choice. Nevertheless, dish selection plays a pivotal role in maintaining a stable and controlled microenvironment by influencing media volume, oil coverage, exposure time during handling, and overall workflow standardization [4]

The embryo culture dish constitutes the immediate physical environment for the developing embryo. Variations in dish size and design can alter surface-area-to-volume ratios, evaporation risk, temperature and pH stability, particularly during repeated microscopic assessments. In high-volume IVF laboratories, where embryos are frequently observed, the choice of culture dish may indirectly affect embryo safety through differences in handling time, ease of manipulation, and consistency of laboratory practices.[5]

This Embryo Chat Communication presents a structured summary of a WhatsApp group discussion involving both senior and junior embryologists from multiple ART centers. The discussion, supported by a poll, focused on real-world laboratory preferences, practical constraints, and SOP-driven decision-making rather than theoretical considerations. The objective was not to identify a single “best” culture dish, but to highlight prevailing trends, underlying rationale, and areas of consensus among practicing embryologists.

SUMMARY OF SURVEY RESULTS

Embryo Culture Dish Preferences (n = 208)

Preference	NumberofVotes	Percentage(%)
35 mm dish	133	63.9
60 mm dish	18	8.7
Centre-well dish	24	11.5
4-well dish	23	11.1
No fixed preference (availability-based)	10	4.8
Total	**208**	**100**

Discussion

The poll results reveal a **clear preference among embryologists for the use of 35 mm embryo culture dishes** in routine ART laboratory practice. This strong inclination reflects practical laboratory considerations rather than a belief in inherent superiority of any single dish type. The findings provide valuable insight into how workflow efficiency, embryo safety, and standard operating procedures influence daily embryology practices.

Detailed Breakdown

Categories

1. **Responses were categorized into five groups based on embryo culture dish preferences:**
   - Use of 35 mm culture dishes
   - Use of 60 mm culture dishes
   - Use of centre-well dishes
   - Use of 4-well dishes
   - No fixed preference, with dish selection based on availability

This categorization allowed for a comprehensive understanding of both standardized and flexible laboratory practices.

2. **Votes and Percentages**

The poll included 208 respondents, providing a robust dataset representative of embryologists working across diverse ART centers.

1. 35 mm Culture Dish

- **Votes**: 133
- **Percentage**: 63.9%

The majority of respondents favored the 35 mm dish, highlighting its widespread acceptance as a standard culture vessel. Embryologists cited ease of handling, reduced embryo exposure during observation, and compatibility with existing laboratory workflows as key reasons for this preference. The compact design facilitates faster assessment and minimizes environmental fluctuations, supporting embryo safety.

3. **60 mm Culture Dish**

- **Votes:** 18
- **Percentage:** 8.7%

A smaller proportion of embryologists reported using 60 mm dishes, suggesting that larger dishes are reserved for specific laboratory protocols or personal preferences. These dishes may offer greater working space but are less commonly used in routine embryo culture due to increased surface area and handling considerations.

4. **Centre-Well Dish**

- **Votes:** 24
- **Percentage:** 11.5%

Centre-well dishes were preferred by embryologists practicing group culture or uninterrupted incubation protocols. Respondents highlighted their ability to maintain embryos within a centralized microenvironment, which may support culture stability, particularly in time-lapse or low-handling systems.

5. **4-Well Dish**

- **Votes:** 23
- **Percentage:** 11.1%

The use of 4-well dishes reflects laboratory priorities focused on individual embryo segregation and traceability. Embryologists using this approach emphasized improved identification and reduced risk of mix-ups, especially in high-volume ART centers.

6. **No Fixed Preference**

- **Votes:** 10
- **Percentage:** 4.8%

A small percentage of respondents indicated no fixed dish preference, selecting culture dishes based on availability. This highlights practical operational realities such as consumable supply and procurement policies without compromising embryo safety.

Key Insights

The findings demonstrate that while a majority of embryologists (**63.9%**) favor the **35 mm culture dish**, no single dish type is universally adopted. Dish selection is influenced by laboratory SOPs, embryologist comfort, workload, and practical constraints rather than direct effects on embryo quality. The preference for smaller dishes underscores the importance placed on minimizing embryo exposure and maintaining a stable culture environment.

Overall, the results reinforce the understanding that **consistency in practice and controlled laboratory conditions** are more critical to successful embryo culture than the specific type of dish used.

DISCUSSION SUMMARY

In the Embryo Chat discussion group, a practical and frequently debated question was raised concerning routine embryo culture practices in IVF laboratories.

The question posed to the group was:

Which embryo culture dish do you prefer in routine practice, and why?
This question initiated an extensive discussion among senior and junior embryologists from different ART centers, focusing on daily laboratory workflow, embryo safety, exposure control, and SOP-driven practices rather than theoretical advantages.

Identification of Culture Dish Options

Embryologists participating in the discussion identified **multiple embryo culture dish formats** currently in use:

1. 35 mm culture dish
2. 60 mm culture dish
3. Centre-well culture dish
4. 4-well culture dish
5. No fixed preference (availability-based use)

Each dish type was discussed based on laboratory experience, handling convenience, and perceived impact on embryo safety.

Figure 1: 35 mm culture dish

35mm culture dishes are standard sterile labware used for handling, washing, and culturing eggs (oocytes) and early embryos, providing a stable, gas-exchange-friendlyenvironment with culture medium, often featuring air vents and clear bottoms for microscopic observation.

Figure 2: 60mm culture dish

60mm culture dish in IVF is a specialized, sterile, medical-grade plastic dish used for various stages of assisted reproduction, including oocyte collection, fertilization (IVF/ICSI), and early embryo culture, offering specific features like optical clarity, thermal consistency, and improved handling for microscopy and manipulation of gametes and embryos, with certified quality.

Figure 3: Center well dish

Figure 4: 4-well culture dish

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A Center Well Dish in IVF is a specialized petri dish with a central depression (well) and often outer compartments, designed for microscopic manipulation of eggs, sperm, and embryos during fertilization, culture, and transfer, offering better visibility, temperature stability, and controlled environments for critical steps like insemination .

4-well dish in IVF is a specialized, sterile plastic plate with four separate compartments (wells) used in embryology labs for culturing, handling, and manipulating human eggs (oocytes) and embryos, allowing for simultaneous assessment or culture of multiple samples while maintaining stable conditions like pH and temperature, crucial for successful IVF procedures.

Poll Results and Initial Observations

The poll included **208 embryologists**, providing a diverse and representative dataset. The results demonstrated a clear dominance of the **35 mm culture dish**, followed by centre-well and 4-well dishes.
Senior embryologists immediately pointed out that this preference reflects **practical laboratory realities**, not necessarily a belief that one dish type improves embryo quality.

Why Do Most Embryologists Prefer the 35 mm Dish?
In the discussion, embryologists expressed their views on the preference for 35 mm dishes in routine IVF work. They mentioned that the smaller dish size helps in faster identification of embryos during observation, thereby reducing the duration of embryo exposure outside the incubator. Participants felt that this shorter exposure time contributes to better maintenance of temperature and pH stability. They also shared that handling is quicker with 35 mm dishes, allowing embryos to be returned to the incubator promptly, which is particularly beneficial in high-workload laboratories. Several embryologists pointed out that the compact surface area enables rapid scanning under the microscope with minimal movement. In addition, better control of microdrops under oil and long-standing familiarity with 35 mm dishes in laboratory SOPs were highlighted as reasons for consistent handling and reduced variability. Overall, the discussion reflected a common opinion that the 35 mm dish supports efficient workflow while maintaining a stable embryo culture condition.

Discussion on Embryo Exposure and Microenvironment
A major theme that emerged was **embryo exposure**, rather than dish size alone.
Senior embryologists clarified that:

- The **culture dish itself does not improve embryo quality**
- The dish influences **how embryos are handled**, not how they develop biologically

The Embryo Chat discussion consistently emphasized that embryo exposure to suboptimal external conditions, rather than the intrinsic characteristics of the culture dish, is the primary concern influencing embryo safety during in-vitro culture. Senior embryologists unanimously agreed that no culture dish, by itself, confers a biological advantage capable of enhancing embryo developmental potential.
Participants clarified that embryo quality is fundamentally determined by the stability of the culture microenvironment, including temperature regulation, pH balance, osmolarity, and gas composition. The culture dish functions as a handling platform rather than a biological modifier. Consequently, variations in dish size or design influence embryo outcomes only indirectly, through their effect on handling efficiency and exposure duration.
Smaller culture dishes, particularly 35 mm formats, were frequently favored because they facilitate rapid microscopic assessment and prompt return of embryos to the incubator. This operational advantage was repeatedly highlighted as a key factor in minimizing transient environmental fluctuations encountered during

routine observation. Importantly, embryologists stressed that the preference for smaller dishes does not stem from any inherent developmental benefit but from their role in supporting exposure control.

The discussion further reinforced that even brief deviations from optimal culture conditions—such as temperature drops or pH shifts occurring during prolonged observation—can adversely affect embryo physiology. As such, minimizing the time embryos spend outside the incubator was regarded as far more critical than the specific dish configuration employed.

Centre-Well Dish – When and Why It Is Preferred

During the discussion, embryologists who routinely use centre-well dishes explained that their preference is primarily driven by **culture philosophy and incubator systems** rather than by perceived differences in embryo quality. It was shared that centre-well dishes are commonly used for **group culture**, where embryos remain centralized, thereby reducing unnecessary movement during observation. Several participants mentioned that these dishes are particularly preferred in **time-lapse incubation systems** and in laboratories following **uninterrupted incubation protocols**. The discussion clarified that centre-well dishes are **not considered inferior**, but are selected based on **validated laboratory protocols and established workflow practices**.

4-Well Dish – Focus on Traceability and Segregation

Those embryologists using 4-well dishes highlighted that this format supports **individual embryo identification and segregation**. Participants shared that culturing embryos in separate wells reduces the **risk of mix-ups** and simplifies **documentation**, particularly in high-volume ART centers. Some embryologists acknowledged that well-based systems may require slightly longer handling time; however, they felt that the **benefits of traceability and clarity in embryo management** outweigh this limitation.

60 mm Dish – Limited but Purposeful Use

Only a small proportion of embryologists reported routine use of **60 mm culture dishes**. During the discussion, it was agreed that larger dishes are generally used for **specific workflows** rather than routine embryo culture. Participants noted that the increased surface area may lead to **longer handling time**, making them less preferred for routine cleavage or blastocyst culture. Their use was described as **center-specific**, driven by established laboratory practices rather than individual preference.

No Fixed Preference – Practical Laboratory Realities

A small group of embryologists reported having **no fixed dish preference**, explaining that dish selection is often influenced by **consumable availability, procurement policies, and supply chain limitations**. Participants emphasized that embryo safety is **not compromised** as long as **standard operating procedures are followed** and embryo handling remains consistent.

Drop Size and Oil Layering

Figure 5: culture droplet

During the Embryo Chat discussion, embryologists also addressed the role of **culture drop size and oil layering** and how these factors influence embryo handling and microenvironment stability. Participants emphasized that, similar to culture dish selection, drop size and oil overlay do not directly determine embryo developmental potential but significantly affect **exposure control and culture stability**.

Embryologists shared that **smaller culture drops** are often preferred because they allow **better control of the microenvironment** and facilitate faster handling during routine observation. However, it was noted that excessively small drops may be more susceptible to **pH shifts and osmolarity changes** if oil coverage is inadequate. The discussion highlighted that drop size must therefore be balanced to ensure both stability and ease of handling.

The role of the **oil overlay** was repeatedly emphasized as critical in maintaining culture conditions. Participants agreed that adequate oil layering helps prevent **evaporation**, stabilizes **pH and osmolarity**, and provides a protective barrier against temperature fluctuations during observation. Embryologists stressed that uneven or insufficient oil coverage can compromise the culture environment, regardless of dish type or drop size.

Several embryologists pointed out that variations in drop size are often guided by **center-specific SOPs**, workload, and incubator systems. Consistency within the laboratory was considered more important than the absolute volume of the culture drop. Similarly, oil type and layering technique were described as standardized practices that should remain unchanged once validated.

The consensus from the discussion was that **drop size and oil layering influence embryo safety indirectly by supporting microenvironment stability and minimizing exposure**, rather than by exerting any direct biological effect on embryo development. As with culture dish selection, embryologists agreed that **validated protocols, consistent handling, and efficient workflow** are the key factors in maintaining optimal culture conditions.

Key Question Raised in the Embryo Chat Discussion: Does Oil Viscosity Influence Embryo Culture Conditions?

Discussion Summary

During the Embryo Chat discussion, embryologists raised the question of whether the **viscosity of culture oil** plays a role in maintaining the embryo culture microenvironment. Participants shared observations based on routine laboratory use of different mineral oils and handling experiences rather than differences in embryo developmental outcomes.

Embryologists discussed that **higher-viscosity oils** are generally perceived to provide **better coverage and stability over culture drops**, particularly during prolonged incubation. Such oils were noted to reduce the risk of oil displacement during dish handling and microscopic observation, thereby supporting consistent protection of the culture medium from evaporation and environmental exposure.

Conversely, embryologists noted that **lower-viscosity oils** may spread more easily but can be more prone to movement during dish manipulation, especially in smaller dishes or during frequent observations. This movement may indirectly increase the risk of exposure-related fluctuations if oil coverage becomes uneven.

Importantly, senior embryologists emphasized that **oil viscosity does not directly influence embryo developmental competence**. Instead, its role is supportive—helping to maintain **pH stability, osmolarity, and temperature buffering** by ensuring uniform and adequate oil overlay. The discussion reinforced that the effectiveness of oil viscosity depends largely on **consistent application, validated SOPs, and embryologist handling technique**.

The consensus from the discussion was that oil viscosity should be selected based on **laboratory workflow, dish type, and validated protocols**, rather than expectations of improved embryo quality. As long as oil coverage is adequate and handling is consistent, embryos can be safely cultured under different oil viscosities without compromising outcomes.

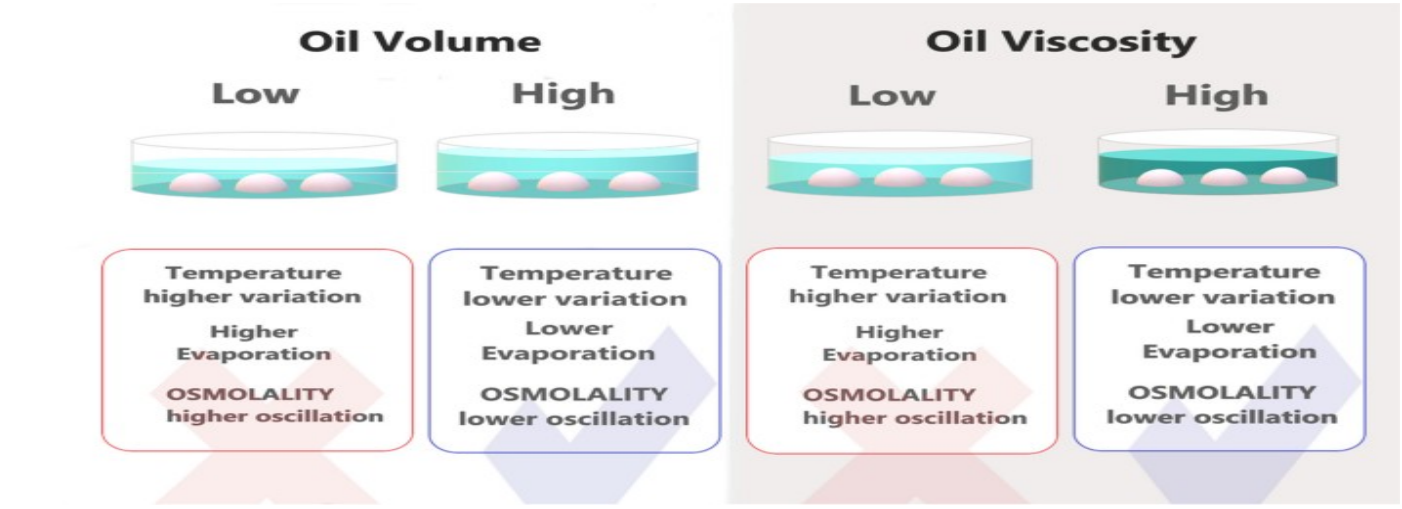

Figure 6-culture oil

Key Question Raised by Embryologists: Does Culture Dish Type Directly Affect Embryo Quality?

In response to this question, senior embryologists agreed that **no single culture dish directly determines embryo quality**. The discussion emphasized that embryo development depends primarily on **incubator stability, culture media, laboratory air quality, embryologist technique, and consistency of protocols**. Culture dish selection was described as a **supportive tool**, influencing handling efficiency rather than biological development.

CONCLUSION

This Embryo Chat discussion highlights that routine embryo culture practices—such as culture dish selection, drop size, oil layering, and oil viscosity—play a supportive role in maintaining a stable culture microenvironment, rather than directly determining embryo developmental potential. Across the discussion, embryologists consistently emphasized that embryo exposure control and handling efficiency are the central factors influencing embryo safety during in-vitro culture.

The preference for the 35 mm culture dish emerged primarily due to its convenience and ability to facilitate rapid embryo assessment with minimal exposure outside the incubator. Centre-well and 4-well dishes were equally valued when used within validated laboratory protocols, reflecting differences in culture philosophy, incubator systems, and traceability requirements rather than differences in embryo quality outcomes. Larger dishes and availability-based choices were recognized as practical adaptations within real-world laboratory settings.

Discussions on drop size, oil layering, and oil viscosity further reinforced that consistency and protocol validation are more important than absolute volumes or oil characteristics. Adequate oil coverage and appropriate drop size were viewed as essential for minimizing evaporation and maintaining pH and osmolarity stability, while oil viscosity was regarded as a handling-support parameter rather than a biological determinant.

Overall, the collective consensus from the Embryo Chat discussion was that no single culture dish, drop size, or oil type is universally superior. Optimal embryo culture outcomes depend on stable incubator conditions, standardized SOPs, embryologist expertise, and minimized exposure, underscoring the importance of consistency and controlled laboratory practices in assisted reproductive technology.

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