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EMBRYO CHAT

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EMBRYO-CHAT

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TOPIC OF DISCUSSION

"Embryo Fragmentation"

CHAT DISCUSSIONS COMPILED BY

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SUMMARY OF SURVEY RESULTS

" EMBRYO FRAGMENTATION "

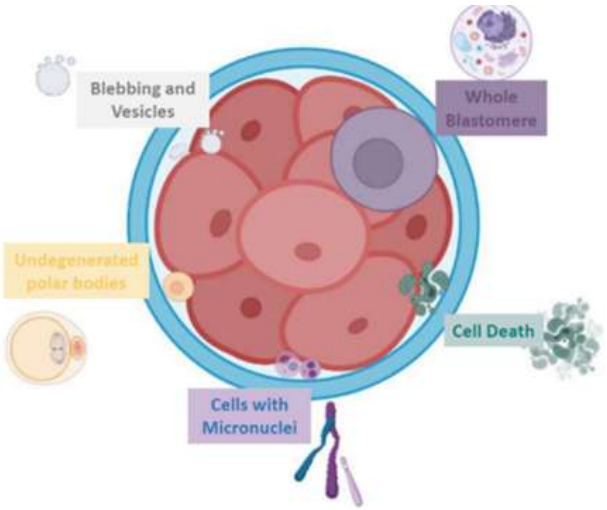
By Team iHera

Introduction

Embryo fragmentation represents a phenomenon generally characterized by the presence of membrane-bound extracellular cytoplasm into the perivitelline space. In vitro, human embryo fragmentation has been reported since the 1980s , but it has been described also in human embryos conceived in vivo , indicating that it is not an artifact of the in vitro culture. Many diverse terms have been used to refer to these cytoplasmic fragments, including corpse, cytoplasmic pinching, micronuclei, debris, and shedding macrovesicles. The cellular and molecular heterogeneity of embryo fragments: they can vary in size, kinetics, and organelle and molecular content . Importantly, during assisted reproduction technology (ART) procedures, fragmentation and cell debris are considered important prognostic factors in the static morphologic assessment of human embryo quality, along with cell number, size, and symmetry, the presence of cytoplasmic fragments is suggestive of a poor prognosis embryo development and poor ART outcomes.

Fragment size

Embryo fragments are heterogeneous in size: they can vary from normal-size blastomeres to simple cellular debris. Johansson et al. classified 44 cleavage embryos according to fragment size: entities smaller than 45 µm in day 2 and smaller than 40 µm in day 3,respectively, have been considered as anucleated cytoplasmic fragments, while larger structures as blastomeres . In addition, human embryo can naturally release extracellular vesicles (EVs)that, on the basis of cellular origin, size, and release mechanism, can becategorized into exosomes (30–150 nm in diameter) , microvesicles (50–1000 nm), andapoptotic bodies (50 nm–5 µm)[1].



Causes of Embryo Fragmentation

1. **Cellular Processes:**

   Chromosomal abnormalities.
   Mitochondrial dysfunction.

2. **Extrinsic Factors:**

   Inadequate culture conditions (Temperature, PH and Oxygen levels).
   Oxidative stress.

3. **Intrinsic Embryo factors:**

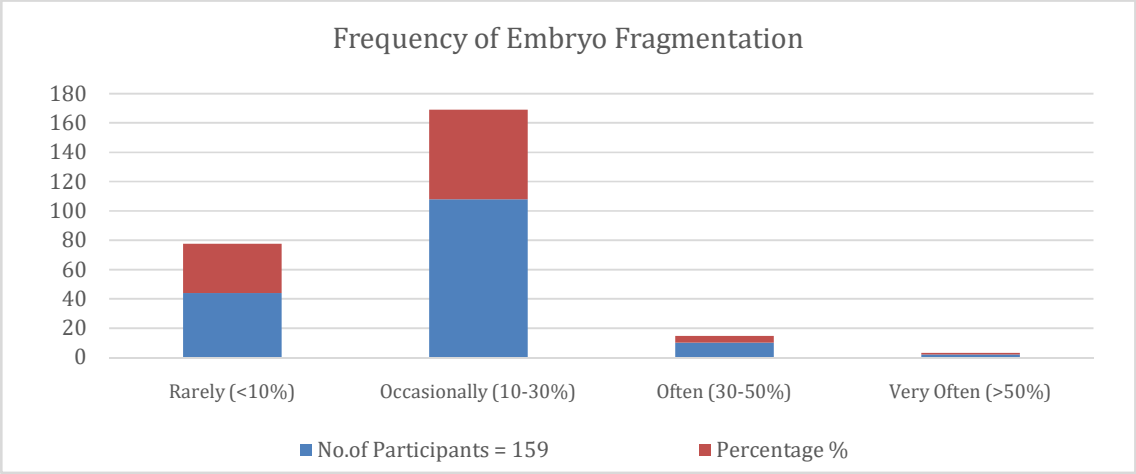
   Poor oocyte quality.
   Sperm DNA fragmentation.

Types of Embryo Fragmentation

1. **Low Fragmentation (Grade 1-2):** Minimal effect on embryo quality.
2. **Medium Fragmentation (Grade 3):** May reduce viability and implantation.
3. **High Fragmentation (Grade 4-5):** Poor prognosis, usually non-viable.

Poll Results:

1) **How frequently do you observe high fragmentation in embryos in your lab?**



Fragmentation Frequency	No. of Participants = 159	Percentage %
Rarely (<10%)	44	33.65
Occasionally (10-30%)	108	61.13
Often (30-50%)	10	4.73
Very Often (>50%)	2	1

KEY INSIGHTS:

Prevalence of Fragmentation:

- The majority of participants (108 out of 159, or approximately 61.13%) report seeing fragmentation occasionally (10-30%). This suggests that a moderate level of fragmentation is the most common observation among the participants.

Low Frequency of High Fragmentation:

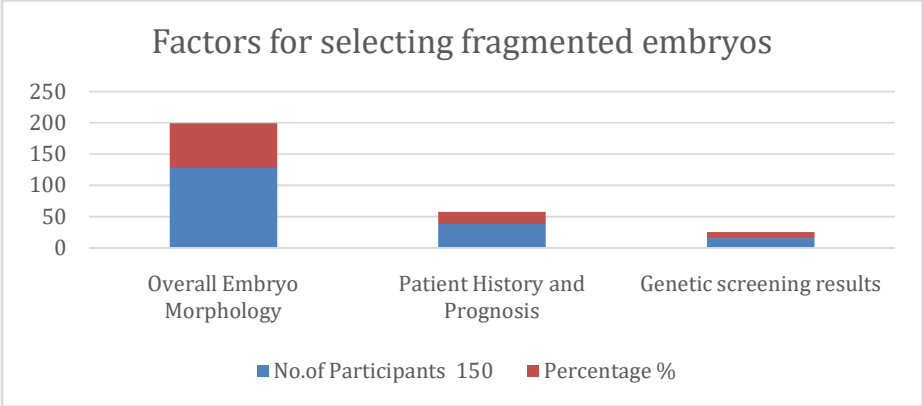
- A smaller group (44 out of 159, or about 33.65%) reports seeing fragmentation rarely (<10%), indicating that for a significant portion of respondents, fragmentation is not a frequent concern.
- Only 2 participants (1.3%) observe fragmentation very often (>50%), suggesting that severe fragmentation is a rare occurrence in their practice.

Moderate to High Fragmentation:

- A small proportion (10 out of 159, or 6.3%) see fragmentation often (30-50%), indicating that for a few, a higher level of fragmentation is seen more regularly, though this is still much less common than the "occasionally" group.

2) **What other factors do you consider when selecting fragmented embryos for transfer?**

The poll was conducted to understand the factors behind embryologist's preference for selection of fragmented embryos for transfer. Participants were asked to select the primary reason while choosing fragmented embryos. The results are as follows:



KEY INSIGHTS:

Factors	No. of Participants =150	Percentage %
Overall Embryo Morphology	128	71
Patient History and Prognosis	38	19.5
Genetic screening results	16	9.5

- 71% of participants prioritize overall embryo morphology, making it the most important factor in embryo evaluation.
- 19.5% focus on patient history and prognosis, indicating that medical factors are considered secondary.
- 9.5% rely on genetic screening results, suggesting that genetic information is the least influential factor.
- Embryo morphology is clearly the dominant criterion in embryo selection.
- Patient history and genetic screening play smaller, but still relevant, roles in decision-making.

DISCUSSION SUMMARY:

By Enitha

In the Embryo Chat Group discussion, following questions was raised: How frequently do you observe high fragmentation in embryos in your lab? Additionally, What factors do you consider when selecting fragmented embryos for transfer?

Initially discussion begins with exploring the multifactorial causes of embryo fragmentation, including intrinsic factors like Poor genome of gamete and extrinsic influences such as culture conditions and mechanical stress, Followed by Strategies to minimize fragmentation including optimized culture media, advanced micromanipulation techniques. Also Insights were shared on the potential of Day 3 fragmented embryos to develop into high-quality blastocysts under favourable conditions , Later navigating through Advanced embryo selection methods, including time-lapse imaging, AI-based morphological assessments.

Opinions on Gamete Quality and Its Relationship with Embryo Fragmentation:

- **Clinical factors**: The discussion mentioned clinical contributors to embryo fragmentation, particularly focusing on stimulation protocol, Endometriosis, Infection
- **SpermQuality:**
  Embryologists strongly agree that sperm with abnormal parameters, such as severe oligoasthenoteratozoospermia (OATS) and a high DNA fragmentation index (DFI), can directly cause embryo fragmentation. In one case discussed, a patient with high DFI, who wished to conceive using their own gametes, had an embryo that developed into a good-quality blastocyst but unfortunately ended in miscarriage. This scenario highlights the complex role of sperm quality, particularly DNA integrity, in embryo development and pregnancy outcomes. Software tools are also being used to help select non-fragmented sperm for ICSI, improving the chances of successful fertilization.
- **OocyteQuality:**
  The quality of the oocyte is also a crucial factor. Mature MII oocytes with delayed meiotic division, fragmented polar bodies, debris in the peri-vitelline space, and vacuolated oocytes are associated with reduced developmental potential. Advanced maternal age is linked to increased fragmentation and a decline in oocyte quality, further complicating the chances of successful embryo development.
- **Biochemical Factors:**.
  Mitochondrial dysfunction results in lower ATP levels and impaired embryo mitosis, adversely affecting embryo viability Were discussed.

DISCUSSIONON EXTRINSIC FACTORS: CULTURE CONDITIONS:

Participants of discussion suggest that Optimal in vitro culture conditions are critical for embryo development. Deviations in the following parameters can lead to fragmentation:

- **Temperature:** Fluctuations disrupt embryo metabolism.
- **pH:** Variations affect cytoskeletal structures.

- **Gas Levels:** Imbalances in CO_2, O_2, and N_2 disturb cellular function.
- **Lab Quality:** Inadequate quality control of culture medium and non-compliance with ideal lab conditions contribute significantly to high DNA fragmentation rates.
- **Cleaning agents:** few embryologist have came across reduction in embryo fragmentation when they stopped using Oosafe for cleaning laminar airflow chamber in IVF lab.
- **Documentation :**

Embryologists pointed out that many external factors causing fragmentation can be reduced with careful attention to key steps. For example, keeping track of gamete quality, making sure ICSI is done at the right time, and limiting how long the oocyte is outside the incubator during ICSI are important for reducing fragmentation. Other factors, like the pH and temperature of the culture medium and consistent grading, also play a big role in maintaining embryo quality. They also mentioned that regular checks after fertilization and grading embryos on Day 3 and Day 5 are important parts of ensuring quality control in the process.

DISCUSSION ON TECHNICAL FACTORS:

A question was raised about how many embryos can be taken for ICSI at once and the ideal timing for the injection.some experienced embryologists perform ICSI with maximum number of oocytes at a time. However, there are some disadvantages to handling a larger number of oocytes outside the incubator. For instance, if an junior embryologist attempts to perform ICSI on maximum number of oocytes at once, the last oocyte's exposure time outside the incubator before injection is prolonged, which can negatively impact its developmental potential. Ideally minimum number of oocyte can be taken for ICSI.And harsh or rush icsi,& improper setting of microinjectors also increase degeneration.

Also excessive amount of PVP during insemination leads to fragmentation

Evidence: Studies also supports the above statement suggesting higher concentration of PVP >10% causing embryo fragmentation.[2].

Initially, MOPS and HEPES buffers were commonly used during the ICSI procedure to maintain pH stability. However, some embryologists now prefer bicarbonate-based medium with an oil overlay, as it helps maintain a stable pH without drastic changes. They have reported improved fertilization and blastulation rates with bicarbonate medium compared to HEPES and MOPS. Additionally, the higher NaCl concentration in HEPES buffers has been associated with increased aneuploidy rates, making bicarbonate-based systems a potentially better choice.

Evidence: Selecting physiological bicarbonate buffer may reduce stress on oocyte resulting in improved embryo development and clinical results because intracellular MOPS and especially HEPES may negatively impact intrinsic biological mechanism in oocyte[3].

FACTORS FOR SELECTING FRAGMENTED EMBRYOS FOR TRANSFER

Most of participants prioritize overall embryo morphology for selecting embryos for transfer.

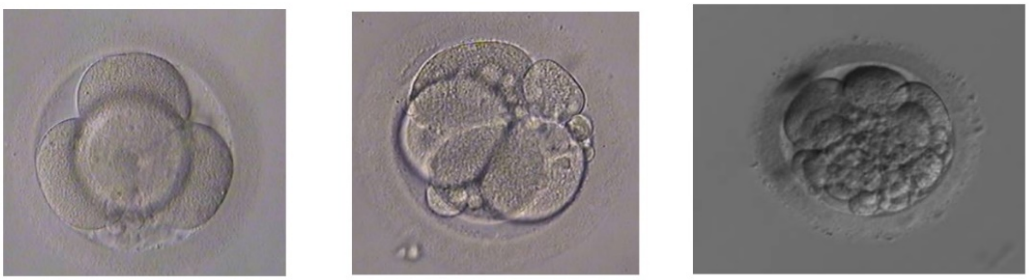
Fragmentation Patterns:

- **Scattered:** Fragments dispersed throughout the embryo.
- **Concentrated:** Fragments localized in specific areas.

Fragmentation Grading (ESHRE):[4]

- **Mild (<10%):** No significant impact on blastulation or pregnancy outcomes.
- **Moderate (10-25%):** Reduced developmental potential.
- **Severe (>25%):** Associated with aneuploidy and poor outcomes, significantly affecting blastulation and implantation rates.

Some embryologists suggest removing fragmentation to improve embryo quality, but they are cautious about disturbing the embryo, as minimizing manipulation is important for maintaining its development potential.



Mild Moderate Severe

The discussion shifted to the cell stage and the degree of fragmentation ,and importance of day 3 and day 5 embryo grading, Time lapse and AI selection of embryo. Key questions and insights are summarized below:

1.How important is it to check the Day 3 embryos ?

Majority of Embryologists focus heavily on Day 3 embryo grading because it marks embryonic genomic activation, when the embryo starts controlling its own development. This helps in deciding whether to transfer the embryo on Day 3, culture it to Day 5, freeze, or discard it. Key factors assessed include cell number, symmetry, fragmentation, and multinucleation. Some embryologists also check on Day 2 to spot early sign of fragmentation and multinucleation

Very few embryologists believe that Day 3 grading loses importance once the embryo develops into a blastocyst.

2. Cell stage and Abnormal division ?

More than 6-7 cells at 45 hours seems abnormal division, Ideally there should be 4 equally sized mononucleated blastomere with < 10% fragmentation on day 2 and day 3 embryo should be 8 equally sized mononucleated blastomere with <10% fragmentation are considered to be euploidy

Embryologist do not prefer choose abnormal division day 3 embryo even if turns into good quality blastocyst in discussion.

Evidence: Embryo dividing too slow or too fast may have metabolic or chromosomal errors.[5].

3. Will Highly fragmented Day 3 embryo turns into good quality blastocyst?

Yes, moderate to highly fragmented embryos on Day 3 can develop into good-quality blastocysts. An important milestone, “zygotic genome activation,” occurs on Day 3, allowing the embryo to acquire self-corrective mechanisms. The degree and pattern of fragmentation play a crucial role in selecting embryos for transfer. Many

embryologists have reported cases where highly fragmented embryos>30 % fragmentation on Day 3 developed into Day 5 high-quality blastocysts (e.g., 5AA) and achieved live birth rates similar to non-fragmented embryos. This highlights the potential for fragmented embryos to still result in successful outcomes.

4 . What is the Role of AI in Embryo selection ?

AI-based embryo selection tools aim to standardize assessment, reduce subjectivity, and improve IVF success rates. These tools analyze embryo characteristics to identify the most viable ones for transfer. Some widely used AI tools include STORK AI, Eeva Test (Early Embryo Viability Assessment), and iDAScore. While AI has shown potential in enhancing consistency and precision, some embryologists report limited benefits in their practical application, suggesting the need for further refinement and validation of these technologies.

5.How do time-lapse incubators enhance embryo assessment in IVF?

Time-lapse imaging enhances embryo selection by reducing environmental disturbances and allowing continuous monitoring of development, minimizing the need for manual handling. However, the high cost remains a significant drawback from the perspective of embryologists.

6. Is PGT is necessary for All fragmented Embryos?

9.5% of the poll respondents advocated for the application of preimplantation genetic testing for aneuploidy (PGT-A) in fragmented embryos to assess chromosomal integrity, emphasizing the value of genetic screening in optimizing transfer outcomes.

The majority of poll respondents do not recommend performing PGT on all fragmented embryos unless specifically indicated. Instead, most fragmented embryos are transferred without additional testing.

CONCLUSION:

This discussion highlighted the critical importance of understanding embryo fragmentation in assisted reproductive technologies (ART), drawing valuable insights from both clinical and technical experts. Fragmentation, influenced by various intrinsic and extrinsic factors, plays a key role in embryo development, impacting blastulation, implantation, and overall pregnancy outcomes.

The poll results revealed that 61.13% of participants observe occasional high embryo fragmentation (10-30%) in their labs. Various multifactorial reasons for fragmentation were discussed, along with tips to help prevent it. When it comes to selecting fragmented embryos, 71% of participants prioritize morphology, acknowledging that live birth rates have been achieved even with highly fragmented embryos, meaning such embryos are not necessarily discarded.

The discussion also emphasized advancements in embryo selection, including the use of AI and time-lapse technology.

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