Troubleshooting Malfunction in the IVF Laboratory

Maintaining consistent and reliably high success rates is a daily challenge for every IVF laboratory. The IVF lab culture system is a complex web of physical, chemical, biological, and logistic parameters for example: temperature, pH, osmolality, gas supplies, air quality, light exposure, infections, managing supplies, personnel, as well as overall quality control.

When an IVF practice experiences a dip in rates, whether they are pregnancy, implantation, blastulation, or even mature egg rate or number of eggs recovered, an analysis should be performed. Root cause analysis was performed to identify clinical and laboratory and patient specific factors. An additional concern regarding low blastocyst rate on day five of embryo culture was noted. The IVF lab noted a concern about the rate of abnormal fertilization. The program has a robust quality monitoring, management, and continuous improvement plan that triggered an outside / expert evaluation to identify, propose, consider, and correct possible outcomes. The current staff are well trained, highly experienced, and highly competent, and appropriate for cycle volume.

by Fertility Guidance Technologies

Review Embryology Key Performance Indicators (KPIs)

1

Fertilization Rates

Monitor 2PN, 1PN, 3PN, 0PN rates

2

Embryo Cleavage Rates

Track progression of embryo development

3

Blastulation Rates

Measure percentage of embryos reaching blastocyst stage

4

Embryo Grading

Assess morphology of developing embryos

5

Aneuploidy Rates

If performing PGT, monitor chromosomal abnormality rates

6

Cycle-specific Trends

Determine if issues are batch-related or ongoing trends

Patient-Specific Factors

Check Patient-Specific Factors

- Ovarian stimulation protocol: Did patients receive appropriate medications? Were there signs of over/under-stimulation (poor egg quality, empty follicle syndrome, etc.)?
- Sperm parameters: Were there abnormal sperm characteristics (low motility, high DNA fragmentation, poor morphology)?
- Patient medical history: Any underlying genetic, metabolic, or autoimmune conditions affecting embryo development?

Incubator Evaluation

Temperature Monitoring

Review temperature logs for stability and accuracy

Gas Levels

Check CO_2 and O_2 monitoring logs for consistency

Humidity Levels

Verify appropriate humidity is maintained

Maintenance History

Was there any recent calibration, maintenance, or breakdown?

Capacity

Were the incubators overloaded?

Gas Supply and Culture Media

Gas Supply Quality

- Any contamination or pressure fluctuations?

Culture Media and Oil Overlay

- Were these from a new lot?
- Were they pre-equilibrated correctly?
- Any reports of supplier contamination?

Laboratory Air Quality

VOC Levels

Monitor Volatile Organic Compounds that can affect embryo development

Particulate Counts

Assess HEPA filter performance and air cleanliness

Formaldehyde Exposure

Check for potential exposure from renovations or new furniture

Temperature and pH Monitoring

Temperature Exposure

Were oocytes, sperm, or embryos exposed to non-physiologic temperatures during handling or micromanipulation?

pH Monitoring

Any changes in pH of media due to CO_2 fluctuations?

Embryologist Techniques: ICSI and Oocyte Collection

ICSI Technique

- Was there excessive oocyte damage?
- Was sperm selection performed optimally?

Aspiration Pressure

- Were oocytes damaged due to excessive suction pressure during collection?

Biopsy and Vitrification

- Was there excessive laser damage?
- Were embryos overexposed to cryoprotectants?

Consumables and Supplies

Culture Media, Oil, and Plasticware

Were they tested for toxicity (MEGA or mouse embryo assay, endotoxin testing)?

Were all products within expiration dates and stored correctly?

Lot Numbers Tracking

Was this issue associated with a specific batch of media or oil?

Sterility

Any reports of microbial contamination?

Lab Workflow and Procedural Deviations

Examine Lab Workflow and Procedural Deviations

- Double witnessing system (RI Witness) logs: Any mismatches or procedural errors?
- Staff shifts and workload: Were embryologists overworked or fatigued?
- Recent staff changes: Were new team members trained adequately?

Retrospective Data Analysis

Comparative Analysis

Compare these cases with previous successful cycles under similar conditions.

Trend Identification

Identify trends or anomalies related to patient demographics, batch processing, or environmental factors.

Next Steps if Lab Parameters Are Ruled Out

If all lab parameters are within acceptable limits, the focus should shift to patient biology (egg/sperm quality, genetics, or underlying conditions).

Consider consulting with clinicians to reassess stimulation protocols, sperm preparation methods, or alternative treatment strategies (e.g., modified culture conditions, IVM, PICSI).

Troubleshooting Checklist: Equipment and Facilities

Category	Subcategory	Inspection
Equipment	Validation	X
Equipment	Calibration	X
Equipment	Maintenance	X
Equipment	Replacement or Growth Needs	X
Facilities	Safe	X
Facilities	Secure	X
Facilities	Arrangement	X
Facilities	Space	X

X = Inspected, X = Highlighted area of concern, - = Not inspected

Troubleshooting Checklist: Personnel and Infection

Category	Subcategory	Inspection
Personnel	Training	X
Personnel	Skills	X
Personnel	Experience	X
Infection	Yeast	X
Infection	Mold	X
Infection	Bacteria	X
Infection	Mycoplasma	X
Infection	Virus	X

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Troubleshooting Checklist: VOCs and Power

Category	Subcategory	Inspection
VOCs	Plastics	X
VOCs	Building Materials	X
VOCs	Furniture	X
VOCs	External Environment	X
Power Failure	Power Supply	X
Power Failure	UPS	X

X = Inspected, X = Highlighted area of concern, - = Not inspected

Troubleshooting Checklist: Osmolality and Gas

Category	Subcategory	Inspection
Osmolality	Temperature	X
Osmolality	Airflow	X
Osmolality	Oil Layer	X
Osmolality	Evaporation	X
Gas (CO2 O2)	Tubing	X
Gas (CO2 O2)	Pressure	X
Gas (CO2 O2)	Quality	X

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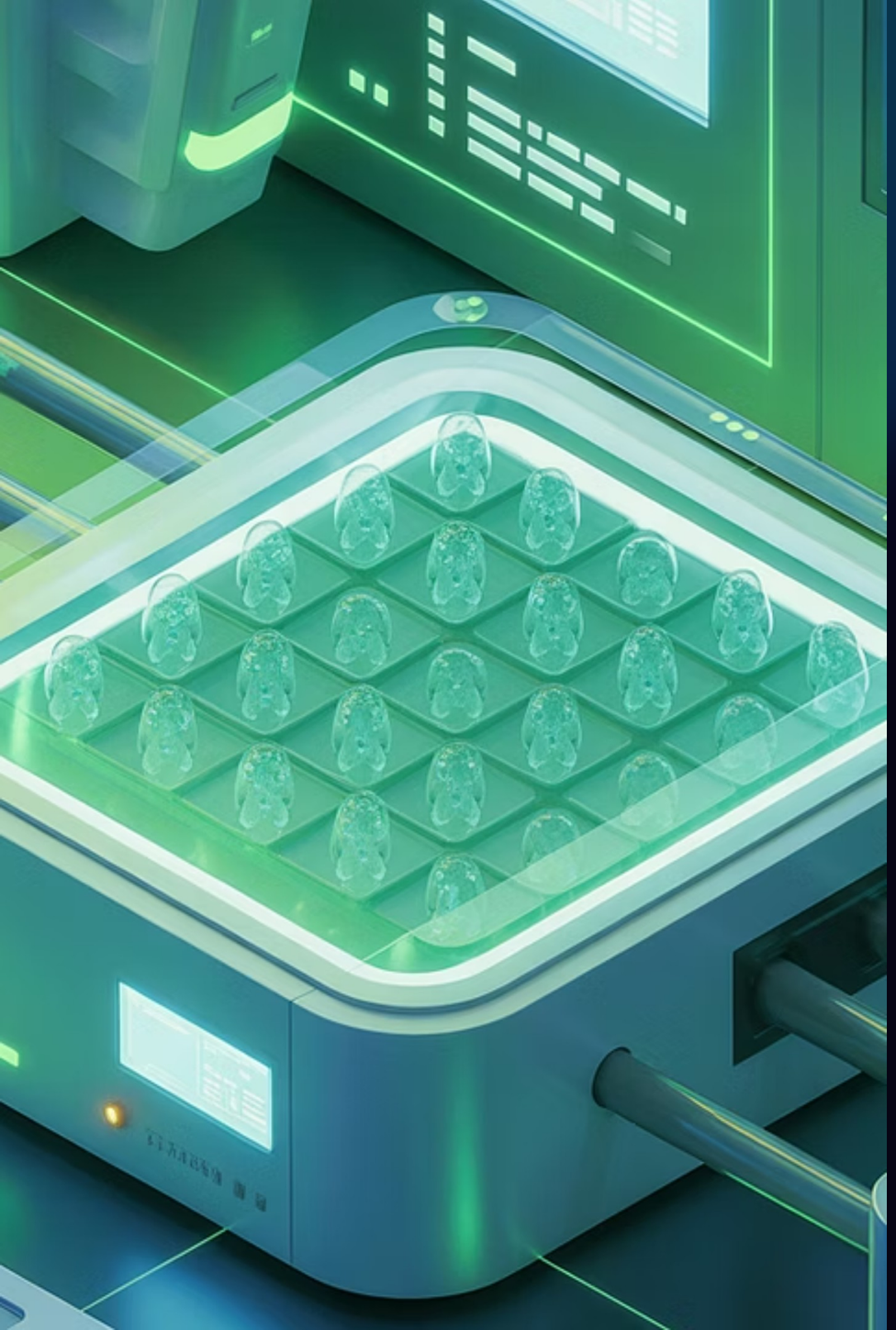
Troubleshooting Checklist: Cell Toxicity and Medium

Category	Subcategory	Inspection
Cell Toxicity	MEA	X
Cell Toxicity	SSA	X
Cell Toxicity	Testing	X
Medium	Type/Quality	X
Medium	Composition	X
Medium	Delivery Temperature	X
Medium	Stability	X
Medium	Expiration	X

X = Inspected, X = Highlighted area of concern, - = Not inspected



The Complexity of IVF Laboratory Systems

The IVF laboratory represents the crossroads of multiple complex systems, and the symptoms of a problem can have many possible causes. Any element in the culture system might cause physiological stress to embryos, reducing their potential viability.

Importance of Record Keeping

Consistent Records

In order to make a valid assessment of a potential problem, it is essential to keep consistent and reliable records, so that parameters can be compared during different time periods.

Baseline Metrics

Rates of fertilization, cleavage, blastocyst development, implantation, biochemical and clinical pregnancy should form a baseline for comparison.

Factors Affecting IVF Outcomes

Patient Demographics

Age, fertility diagnoses, and medical history

Stimulation Protocols

Drug batches, doses, and patient response

Culture Conditions

Incubators, temperature, pH, gases, dishes, and culture media

Gamete Quality

Sperm characteristics and oocyte quality

Any changes in patient demographics, including age, fertility diagnoses, stimulation protocols, drug batches and doses, response to stimulation, sperm characteristics, and oocyte quality for embryos cultured can have downstream effects on results. Any changes in culture conditions can reduce viability, including: incubator(s), measured temperature and pH, gases, dishes, and the culture media (basic constituents and supplemented protein.)