Quality Control Processes Within the Embryology Laboratory

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Introduction

- Thorough understanding of every process that occurs within the IVF lab is essential for efficient development of human embryos as well as maintaining high implantation rates.

- Understanding how variables and outside influences can impact outcomes is of paramount importance.
Challenges

• Trying to allow a highly regulated sequence of events to occur without external influences that a lab, its materials and environment might have on the processes required for an oocyte to become a competent embryo.

• The human embryo is a unique structure because of dynamic changes that occur during its course of development within the lab.

• Changes are not only morphological but functional as well.
Consequences of Preimplantation Development

- The fertilized oocyte changes from a metabolically quiescent entity under the control of maternal transcripts into a dynamic multicellular, rapidly developing embryo under its own functioning genome with complete homeostatic mechanisms.

- Perturbations can result in reduced embryo viability and impaired development post-transfer.
Basic Types of Quality Management Systems

**Proactive measures:** Identify potential sources of variables and establish standards for conditions. **Ex:** Temperatures within culture systems. Confirmation of standards prior to use.

**Reactive measures:** Allows one to react to a certain set of conditions. Helpful in determining when a variable entered the lab that is the suspected source of issue(s). **Ex:** Spreadsheet to log in plasticware use.

- Suggest using both! Both have their places in a management system.
Examples of Proactive Management Systems

- Meticulous attention to details.
- Testing of all materials that come in contact with gametes.
- Rinse all plastic ware used to store media.
- Effect of embryologist on outcomes.
- Maintenance of equipment:
  - Calibrated stages and hoods-temp in droplets
- Methods to confirm temperature, CO₂ and pH.
- Establish benchmarks: stressed embryos develop poorly.
Plasticware

- Major source of variation within the IVF lab.
- Source of debris contamination to chemical contamination.
- Effects on gametes range from overt to subtle. A major source of headaches!
- Human sperm assay. Has limitations when compared to the MEA.
- Easy to have sub sampling errors.
- Off gas for ≥ 48hrs.
- Rinse all plasteware used for media storage.
# Lab Staff Performance

<table>
<thead>
<tr>
<th>Measurement of Embryology Procedures</th>
<th>Threshold Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fertilization rates</td>
<td>&gt; 60%</td>
</tr>
<tr>
<td>Polyspermic rates</td>
<td>&lt; 15%</td>
</tr>
<tr>
<td>ICSI degeneration rates</td>
<td>&lt; 15%</td>
</tr>
<tr>
<td>Embryo cleavage rates</td>
<td>&gt; 90%</td>
</tr>
<tr>
<td>Blastocyst development rates</td>
<td>&gt; 50%</td>
</tr>
<tr>
<td>Cryopreservation rates</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Ongoing pregnancy rates</td>
<td>&gt; 50%</td>
</tr>
<tr>
<td>Implantation rates</td>
<td>&gt; 25%</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>± 10% of the mean</td>
</tr>
<tr>
<td>Sperm morphology</td>
<td>± 2% of the mean</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>±10% of the mean</td>
</tr>
</tbody>
</table>
Current Developmental Rates – Benchmarks

- Fertilization rates
  - Conventional: ≥ 60%
  - ICSI: ≥ 70%
- Early syngamy or cleavage: ≥ 50%
- Rate of 4-cell embryos: ≥ 68%
- Rate of 8-cell embryos: ≥ 60%
- Fragmentation rates < 20%: ≥ 60%
**eIVF Benchmark Queries**
Queries: Fertilization Rate (ICSI)
### Effect of Insemination Method on Blastocyst Development

<table>
<thead>
<tr>
<th>Insem Type</th>
<th>N</th>
<th>No. Blast (%)</th>
<th>No. HQ Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td>4622</td>
<td>3352 (73)</td>
<td>1342 (40)</td>
</tr>
<tr>
<td>ICSI</td>
<td>3514</td>
<td>2331 (66)</td>
<td>795 (34)</td>
</tr>
</tbody>
</table>
# Day 1 PM Characteristics Score and Blastocyst Development

<table>
<thead>
<tr>
<th>Day 1 PM</th>
<th>N</th>
<th>No. Blast (%)</th>
<th>No. HQ Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 PN</td>
<td>2409</td>
<td>1486 (62)</td>
<td>437 (29)</td>
</tr>
<tr>
<td>0 PN</td>
<td>2895</td>
<td>2073 (72)</td>
<td>809 (39)</td>
</tr>
<tr>
<td>2 Cell</td>
<td>2683</td>
<td>1982 (68)</td>
<td>845 (42)</td>
</tr>
</tbody>
</table>
## Day 2 Stage and proportion that develop to the Blastocyst Stage*

<table>
<thead>
<tr>
<th>Cell Stage</th>
<th>N</th>
<th>No. Blast (%)</th>
<th>No. HQ Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>317</td>
<td>6%</td>
<td>81 (26)</td>
</tr>
<tr>
<td>3</td>
<td>380</td>
<td>7%</td>
<td>80 (21)</td>
</tr>
<tr>
<td>4</td>
<td>4033</td>
<td>71%</td>
<td>1782 (44)</td>
</tr>
<tr>
<td>5-6</td>
<td>856</td>
<td>15%</td>
<td>177 (21)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>56</td>
<td>&lt;1%</td>
<td>10 (18)</td>
</tr>
</tbody>
</table>

*5642 embryos in culture on Day 5/6
## Day 3 Cell stage and its effect on Blastocyst Development*

<table>
<thead>
<tr>
<th>Cell Stage</th>
<th>N</th>
<th>No. Blast (%)</th>
<th>No. HQ Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>148</td>
<td>3%</td>
<td>15 (10)</td>
</tr>
<tr>
<td>6-7</td>
<td>1027</td>
<td>18%</td>
<td>249 (24)</td>
</tr>
<tr>
<td>8-10</td>
<td>4074</td>
<td>72%</td>
<td>1699 (30)</td>
</tr>
<tr>
<td>11-12</td>
<td>274</td>
<td>5%</td>
<td>103 (38)</td>
</tr>
<tr>
<td>&gt;12</td>
<td>120</td>
<td>2%</td>
<td>62 (52)</td>
</tr>
</tbody>
</table>

*5643 embryos in culture Day5/6
### Day 3 Morphology Score and Proportion that develop to the Blastocyst Stage

<table>
<thead>
<tr>
<th>Morph Score D3</th>
<th>No. Viable D5</th>
<th>No. Blast (%)</th>
<th>No. HQ Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>701</td>
<td>338 (48)</td>
<td>44 (13)</td>
</tr>
<tr>
<td>2</td>
<td>800</td>
<td>523 (65)</td>
<td>123 (24)</td>
</tr>
<tr>
<td>3</td>
<td>968</td>
<td>742 (77)</td>
<td>272 (37)</td>
</tr>
<tr>
<td>4</td>
<td>835</td>
<td>731 (88)</td>
<td>360 (49)</td>
</tr>
<tr>
<td>5</td>
<td>271</td>
<td>248 (92)</td>
<td>141 (52)</td>
</tr>
<tr>
<td>≥ 3</td>
<td><strong>2074</strong></td>
<td><strong>1721 (83)</strong></td>
<td><strong>773 (45)</strong></td>
</tr>
</tbody>
</table>

*Data based upon 3,575 embryos*
Heated Sources of Variation

- Microscope stages
- Block heaters
- Work stations
- Culture dishes: droplet size and oil interactions
Microscope Stages

- Major source of heat variation during micromanipulation and embryo evaluation.
- Stages lack temperature consistency.
- Heat in a pulsating fashion.
- Surface has “hot” and “cold” areas. Use a thermo couple to find these areas.
- Check temperatures in drops for both MM/culture dishes. Establish polices based upon results.
Block/Tube Heaters

- Notorious for wide temperature fluctuations.
- Lack of consistency. Must check daily.
- Use an appropriate water filled tube with thermometer. Provided a more stable reading. Reflects temperature of liquids.
- Establish policy for number of tubes containing follicular aspirates.
Work Stations

- Most embryology tasks performed on modified heated laminar flow hoods or converted infant isolates.
- Establish temperature ranges and characteristics by diagramming surface. Establish hot and cold areas and adjust accordingly.
- Establish temperature ranges with culture and micromanipulation dishes. Check temperatures within micro droplets a thermocouple.
- Mimic actual procedures and measure temperature before, during after processes.
Impact of Heated Surfaces on Culture Systems

• Thermodynamics of culture system influenced by:
  • Culture dish
  • Oil: volume and viscosity.
  • Micro droplet size and location.
• In general, culture droplets have greater temperature fluctuations than the oil.
  • pH properties will be influenced by droplet size and oil type.
  • Establish time policies for culture and micromanipulation processes.
Incubators

- Single most important equipment within the IVF Lab yet is often the most misunderstood.
- Most designed for dynamic cell culture systems with high developmental tolerances.
- Performance and tolerance levels not applicable to embryo development.
- Establish temperature within culture system. Ex: 37.0° C incubator temp →36.8°C in droplet.
- Poor ability to regulate pH. ? Effect of pH fluctuation of embryo development?
- Establish strict incubator policies.
Incubators Characteristics

- Designed for tissue culture
- Cell culture systems are metabolically very active
- pH and temperature stress causes:
  - Poor blastomere morphological characteristics
  - Slow cleavage rates
  - Excessive fragmentation
- Reduced oxygen
  - More physiological
  - Better overall development
How long does it take for media to return to 37°C?

Temperature recovery of MINC-1000 vs 100L CO2 water-jacket incubator. Remove culture media, place on 37°C stage for 5 mins then return at time 0.
How long does it take for the pH to return to an acceptable value – post procedure (100L Incubator)

100L CO2 incubator, remove media for 5 mins then return to incubator. K-SICM-20, Lot 433520, 6% CO2, testing for pH recovery time.
How long does it take for the pH to return to an acceptable value – post procedure? (MINC Incubator)

6% CO2, K-SICM Lot 433520, Remove culture well from Minc 1000 for 5mins then return at time 0.
QC Testing of Mouse Embryos

Compare cell counts in Blastocysts
QC Testing of Mouse Embryos

- Compared cell counts in Blastocysts
- Two by two comparisons in two different culture media systems comparing Minc and Forma
- Start point of culture with frozen 2-cells
- Counts on day 6 with BX41 and DAPI
QC Test – Mouse Blast Count

Cell Count
Day 6

Forma
Minc

Medium A
Medium B

P<0.01
P<0.01
Culture Conditions

- Well maintained incubators:
  - %CO₂
  - Ability to maintain pH at 7.2→7.3
  - Better developmental rates pH <7.28
  - Minimize incubator openings: stresses embryos.
  - Impact of incubator openings on intracellular pH.
  - Intracellular pH fluctuation have largest impact prior to genetic activation.
- CODA units if no HVAC.
- Gas phase of 6-7% CO₂, 5% O₂ and 88-89% N₂ is optimum.
Quality Issues Associated with Culture Media/ Embryo Culture

- 1 cell MEA all HSA lots (90% > Ex blasts).
  - Oil free systems.
- HSA products containing alpha and beta immuno globulins.
- Endotoxin testing of new HSA.
- Split oocytes when receive new media lots.
- Spilt oocytes when testing new oil lots.
- Wash and equilibrate oil; protect from light → Peroxidase.
- Size of micro droplets (20-40 µl): group culture?
- Off gas plastic ware > 48 hours.
- Rinse all plastic ware used to store media/protein.
Embryology Processes

- Following many of the quality management processes described; many embryology procedures were modified. Techniques were modified to reflect how one’s technique can introduce variables. **Ex:** reduce time oocytes out during retrieval and reduce number of oocytes out during ICSI.

- Reduce variability to that inherent to the initial oocyte quality.
Acknowledgements

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