Improving Process Understanding of an IVF Cell Culture Incubator via Response Surface Methodology

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Disclosure statement: I am employed by Cook Medical, a manufacturer and distributor of devices, including media and incubators, to the IVF industry.
Outline of the Presentation

- Background/introduction to the problem
- Brief overview of preliminary experiments to identify some key variables
- Approach to multifactor design
- Model development and analysis
- Accuracy of predictions and interpretation
- Summary/QA

In 2019, > 77 thousand babies born from assisted reproductive technologies (ART).

In vitro fertilization (IVF) and Embryo Culture (EC) are major components of ART.

Infertility is more common than many people realize.

Infertility is emotionally distressing for couples.
Background

Background/introduction to the problem

Information from the Society for Assisted Reproductive Technology (SART); www.sart.org
Embryo Culture and Development

- 2-cell Embryo
- 8-cell Embryo
- Morula
- Early Blastocyst
- Expanded Blastocyst

Unfertilized Human Oocyte (~125 µm diameter)
Defining the Problem?

- Evolution of Embryo Culture methods have included in recent years the use of non-humidified incubators

- It was assumed that the oil layer would buffer any significant changes to the culture medium resulting from this change

- It has been determined that this, in fact, is not true

- Changes in the medium osmolality due to water loss is significant

- Results in changes to the chemical concentration and pH

What factors have been suggested to be related to evaporative loss

- Humidity levels\(^1,2\)
- Oil type\(^3\)
  - Oil density
  - Oil viscosity
- Initial oil humidity\(^3\)
- Volume of drops and/or surface area of drops exposed to oil (confounded with drop volume in a microdrop setting)\(^4\)
  - Related to geometry of media in the dish
- Oil depth (volume) above drops\(^5\)
- Incubator type/manufacturer\(^5\)

Brief overview of preliminary experiments to identify some key variables

Experiment to measure osmolality rise with different culture drop volumes

Osmolality rise over 7 days; 3 drop volumes (35 mm dish, 3 mL oil)

Drop volume slope coefficients, combined data
Estimating surface area of microdrops

Brief overview of preliminary experiments to identify some key variables

Surface area $= \int 2\pi y \, ds$

where $ds = \sqrt{1 + \left(\frac{dy}{dx}\right)^2} \, dx$

https://tutorial.math.lamar.edu/Classes/CalcII/SurfaceArea.aspx

<table>
<thead>
<tr>
<th>Surface area (mm$^2$) estimates (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µL</td>
</tr>
<tr>
<td>Average</td>
</tr>
<tr>
<td>StDev</td>
</tr>
<tr>
<td>CV (%)</td>
</tr>
<tr>
<td>SA:vol</td>
</tr>
</tbody>
</table>
Brief overview of preliminary experiments to identify some key variables

Experiment examining the effect of oils from different manufacturers on the rate of osmolality change

**Conditions:**
- 7 brands, 9 oil types
- 30 µL medium drops
- 3 mL oil
- 35 mm dishes
- Culture in dry incubator (relative humidity at 37 °C ~ 10%)
- \( t_0 \) = Time dishes placed into incubator

**Osmolality rise with different oils**

**Rate of osmolality change per oil type**

Expected under humid conditions
Brief overview of preliminary experiments to identify some key variables

**Oil density and viscosity effects**

Viscometry performed at 37 °C by Shaun Tanner, Cook Research, Inc., using a TA Instruments AR 2000ex rheometer. Density measurements also performed at 37 °C.

*Viscosity is not a significant predictor of osmolality change independent of density*
Experiment examining the effect of oil volume on the rate of osmolality change

Brief overview of preliminary experiments to identify some key variables

Change in osmolality per hour

Hourly change in osmolality (mOsm/hr)

Oil volume (µL)

- 40 µL medium
- 50 µL medium
Experiment to examine culture medium SA:vol, oil density, and oil layer height

- Response surface methodology utilized; Design-Expert® software for the design and analysis.
- Examine media SA:vol (0.25 to 1.5 mm\(^{-1}\)), oil density (0.824 to 0.854 g/mL) and oil thickness (1 – 5 mm) on osmolality rise.
- Face-centered Central Composite Design (3x3) was utilized for the initial block of runs. Augmentation occurred to complete the total design size.
- Final experiment consisted of 5 blocks of data, 19 – 24 runs per block, 107 independent combinations of variables.
- Run order and well sampling order were randomized in each experimental block.
- Osmolality measured over 7 days, 1 well per measurement, with a Wescor Vapor Pressure Osmometer.
- Initial data analyzed by linear regression. Primary response was the change in osmolality per hour over 7 days.

Note: 1 mL oil in a 35 mm dish is approximately 1.1 mm thick.
Initial Analysis

- For every factor combination, linear regression was conducted to determine the rate of osmolality change over the 7-day period.
- The slope parameter was used as the input variable in DE.
## Model selection

<table>
<thead>
<tr>
<th>Model type</th>
<th>PRESS statistic</th>
<th>-2 log likelihood</th>
<th>Pred. R-squared</th>
<th>Adjusted R-squared</th>
<th>BIC</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full quadratic</td>
<td>4.84</td>
<td>-62.93</td>
<td>0.9182</td>
<td>0.9355</td>
<td>2.49</td>
<td>-30.37</td>
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<tr>
<td>Modified quadratic</td>
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<td>-62.82</td>
<td>0.9196</td>
<td>0.9361</td>
<td>-2.07</td>
<td>-32.9</td>
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<tr>
<td>Full cubic</td>
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<td>-443.46</td>
<td>0.9541</td>
<td>0.9681</td>
<td>-331.31</td>
<td>-380.82</td>
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<tr>
<td>Modified cubic</td>
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<td>0.9687</td>
<td>-357.33</td>
<td>-395.89</td>
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<tr>
<td>Full quartic</td>
<td>0.13</td>
<td>-515.95</td>
<td>0.9659</td>
<td>0.9802</td>
<td>-333.71</td>
<td>-391.38</td>
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<tr>
<td>Modified quartic</td>
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<td>-502.88</td>
<td>0.9692</td>
<td>0.98</td>
<td>-358.03</td>
<td>-414.43</td>
</tr>
</tbody>
</table>

Note: Response variable transformations performed: Log for quadratic, and SqRt for cubic and quartic.
Cubic model analysis after backward selection

- Seven of the terms were eliminated and none had to be returned to maintain model hierarchy. All remaining terms have a very low $P$ value in the ANOVA analysis.

### Analysis of variance table [Partial sum of squares – Type III]

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F-values</th>
<th>$P$ value (Prob &gt; F)</th>
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</thead>
<tbody>
<tr>
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<td>0.028</td>
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<td></td>
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<tr>
<td>A-SA:vol</td>
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<td>0.41</td>
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<tr>
<td>B-Oil_Height</td>
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<td>6.475E-003</td>
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<td>0.0211</td>
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<tr>
<td>C-Oil_Density</td>
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<tr>
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<tr>
<td>AC</td>
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<tr>
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<tr>
<td>$A^2$</td>
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<tr>
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<tr>
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<td>0.068</td>
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<td>$B^3$</td>
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<td>1</td>
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<td>53.09</td>
<td>&lt; 0.0001</td>
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</tbody>
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Model development and analysis

Cubic model analysis after backward selection

Residuals vs. predicted

Residuals vs. run

Residuals vs. actual

Normal plot of residuals

Externally studentized residuals

Normal % probability

Predicted

Run number

Actual
Model development and analysis

Cubic equation to model osmolality change

$$\frac{\Delta \text{osmolality}}{\Delta \text{time (hour)}} = (-2.095 + 9.562x + 0.204y + 2.926z - 2.099xy - 9.865xz - 0.713yz - 0.317x^2 + 0.157y^2 + 2.056xyz + 0.066x^2y + 0.032xy^2 - 0.017y^3)^2$$

Where $x = \text{SA:vol (mm}^{-1})$, $y = \text{Oil height (in mm)}$, and $z = \text{Oil density (in g/mL)}$
Model development and analysis

Experimental data and model predictions

X1 = A: SA:vol
X2 = B: Oil_Height
Actual Factor
C: Oil_Density = -1

X1 = A: SA:vol
X2 = B: Oil_Height
Actual Factor
C: Oil_Density = 1

X1 = A: SA:vol
X2 = B: Oil_Height
Actual Factor
C: Oil_Density = 0
Accuracy of predictions and interpretation

Model predictions and Actual Measurements

Model prediction confidence interval range and experimental confirmation values

- Model predicted rate of change with confidence intervals
- Measured rate of change

Actual and model predicted rate of osmolality change (mOsm/hr, with 95% prediction intervals)

Treatment numbers
Comparison of dry and humid conditions during continuous culture

Actual data, replicating the conditions in the report by Fawzy et al, discussed earlier.

Actual Osmolality change and model predictions:

0.208 mOsm/hr (actual)

0.223 (0.162 – 0.292) (Model predictions, (95% CI)

Percent Osmolality Change over 7 Days:
Dry: 13.3 %
Humid: 3.5 %
Questions?

Thank you!

- Kassi Shelton (BS, TS (ABB); Christina Dann, PhD; Brianna McSwain, BS. (All from Cook Medical IVF Research Team.)
- James Benson, PhD, Assistant Professor, U. of Saskatchewan
- A.J. McKechnie, Statistician, Cook Medical
- Bryan Woodard, Director, Global Product Development for Reproductive Health, Cook Medical
- Elizabeth Brown, Director, Global R&D, Cook Medical