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

Stat-Ease 2021 Online DOE Summit

Steven F. Mullen, PhD
Cook Medical
Team Lead, Senior Scientist
Steven.Mullen@CookMedical.com

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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

Mullen SF. Toward a predictive theoretical model for osmolality rise with non-humidified incubation: a randomized, multivariate response-surface study. *Hum Reprod* 2021; **36**:1230-1241.

Background

- 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

Quick Facts About Infertility

Haga clic aquí para español

- Infertility is NOT an inconvenience; it is a disease of the reproductive system that impairs the body's ability to perform the basic function of reproduction.
- Impaired fecundity (the inability have a child) affects 6.7 million women in the U.S. -- about 11% of the reproductive-age population *(Source: National Survey of Family Growth, Centers for Disease Control and Prevention [CDC] 2006-2010).*
- In a survey of married women, the CDC found that 1.5 million women in the US (6%) are infertile *(Source: National Survey of Family Growth, Centers for Disease Control and Prevention [CDC] 2006-2010).*
- Infertility affects men and women equally.
- Twenty-five percent of infertile couples have more than one factor that contributes to their infertility.
- In approximately 40 percent of infertile couples, the male partner is either the sole cause or a contributing cause of infertility.

/www.reproductivefacts.org/faqs/quick-facts-about-infertility/

asrm ReproductiveFacts.org
From the American Society for Reproductive Medicine

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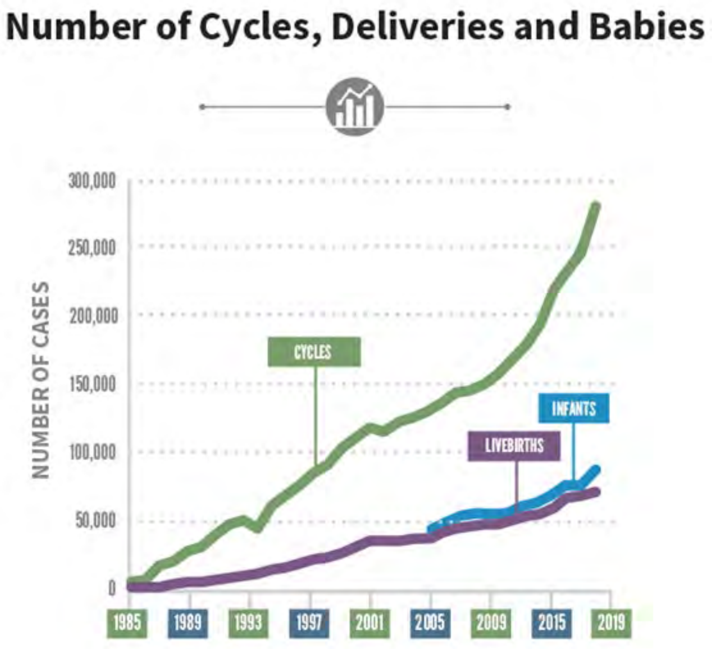
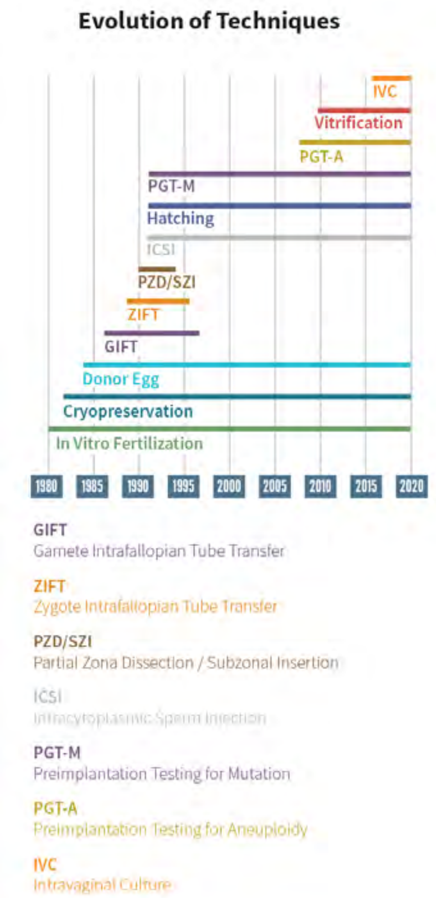
Background

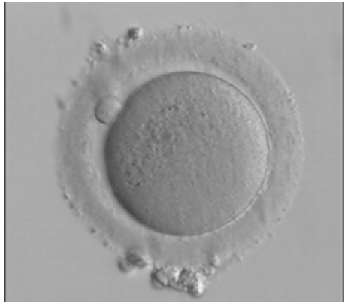




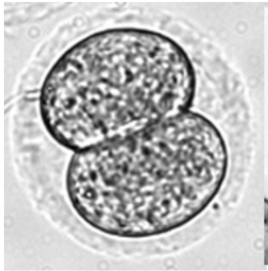
Information from the Society for Assisted Reproductive Technology (SART); www.sart.org

Background/introduction to the problem

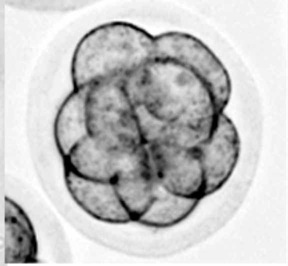
Embryo Culture and Development



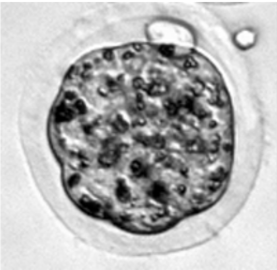
Unfertilized Human Oocyte
~125 µm diameter)



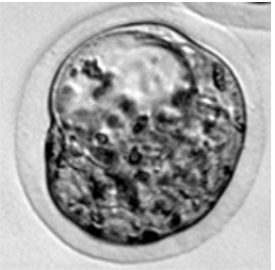
2-cell Embryo



8-cell Embryo



Morula

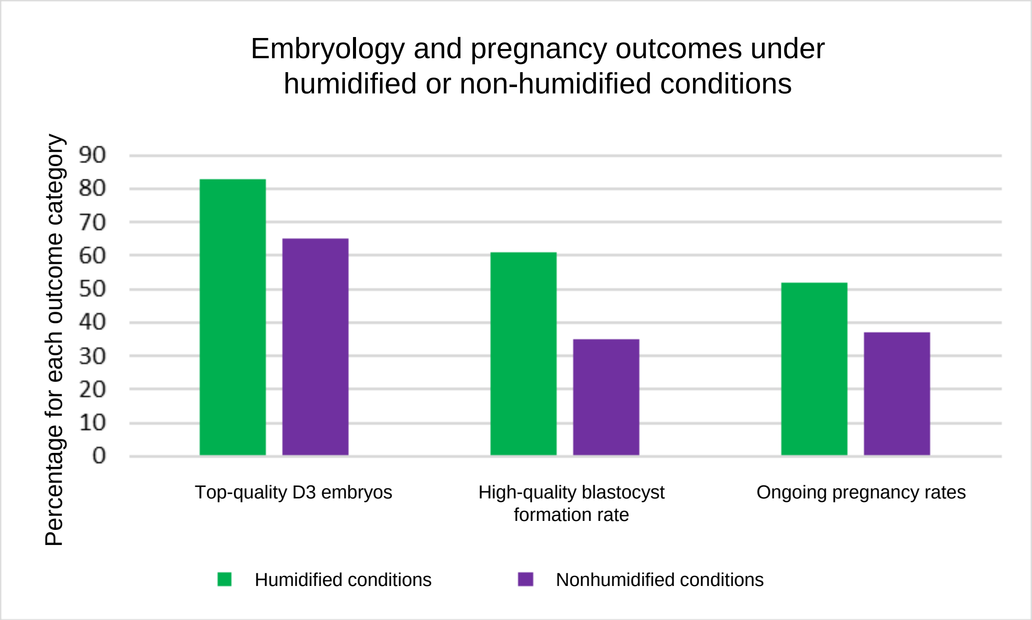


Early Blastocyst

Expanded
Blastocyst

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



Fawzy M, AbdelRahman MY, Zidan MH, et al. Humid versus dry incubator: a prospective, randomized, controlled trial. *Fertil Steril.* 2017;108(2):277-283. Initial osmolality ~ 265 mOsm.

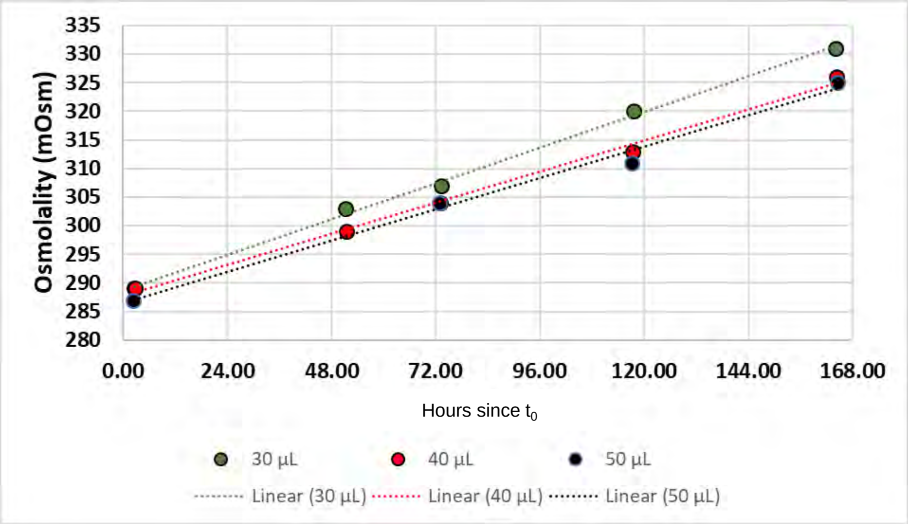
What factors have been suggested to be related to evaporative loss

- 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]

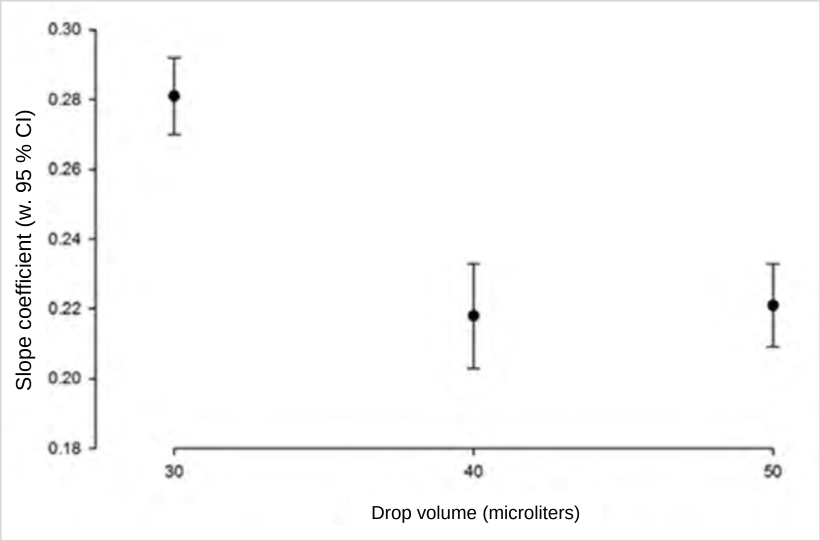
1. Del Gallego et al., 2018; 2. Holmes and Swain 2018, Swain 2018 & 2019; 3. Yumoto et al., 2018; 4. Iwata et al., 2016; 5. Carpenter et al., 2018.

Experiment to measure osmolality rise with different culture drop volumes

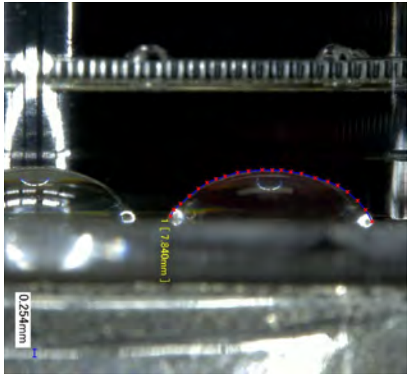
Osmolality rise over 7 days; 3 drop volumes (35 mm dish, 3 mL oil)



Drop volume slope coefficients, combined data



Estimating surface area of microdrops



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

$$\text{Surface area} = \int 2\pi y \, ds$$

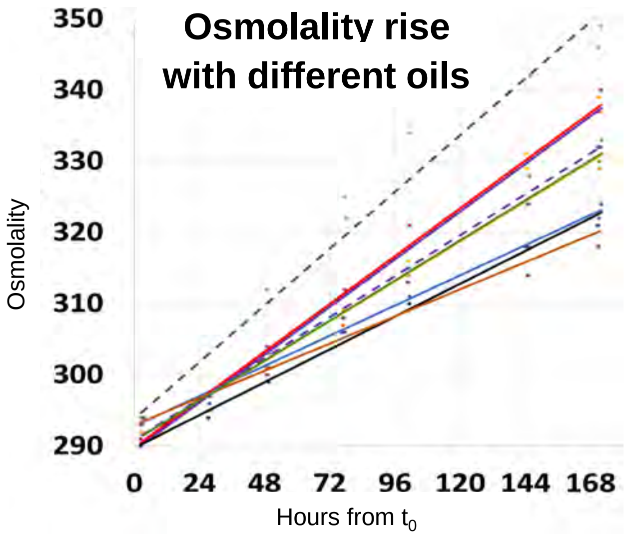
$$where\ ds = \sqrt{1 + \left(\frac{dy}{dx}\right)^2}\ \mathrm{dx}$$

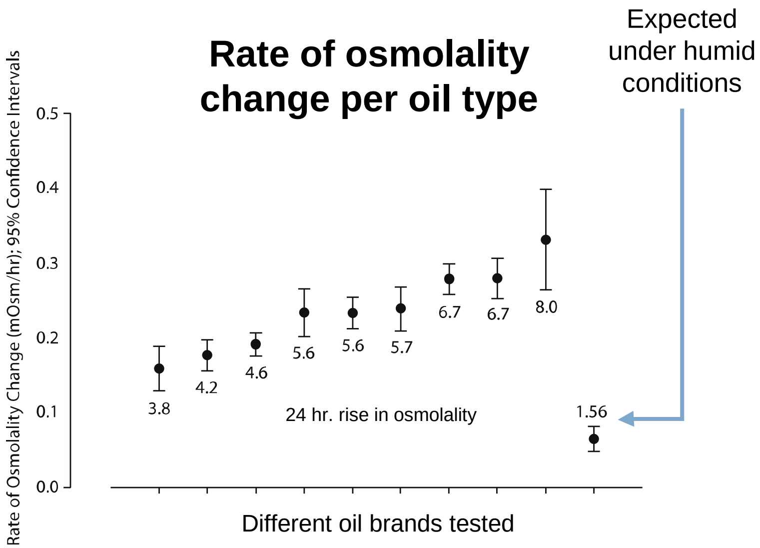
Surface area (mm^2) estimates (n = 3)	20 µL	30 µL	40 µL	50 µL
Average	23.4	32.0	41.1	51.5
StDev	2.3	1.5	3.3	5.8
CV (%)	10.0	4.6	8.1	11.2
SA:vol	1.17	1.07	1.03	1.03

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

Conditions:

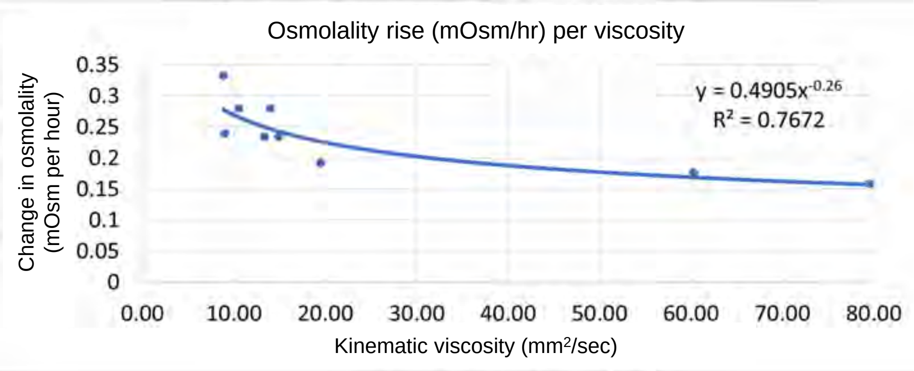
- 7 brands, 9 oil types
- 30 µL medium drops
- 3 mL oil
- 35 mm dishes
- Culture in dry incubator (relative humidity at 37 °C ~ 10%)
- t_0 = Time dishes placed into incubator

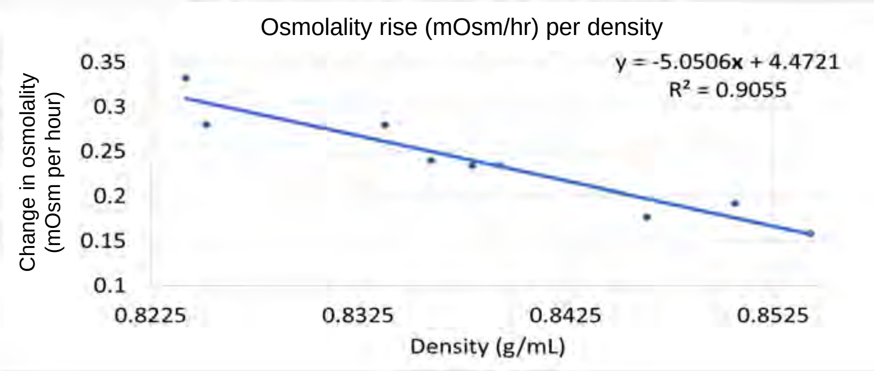




Brief overview of preliminary experiments to identify some key variables

Oil density and viscosity effects

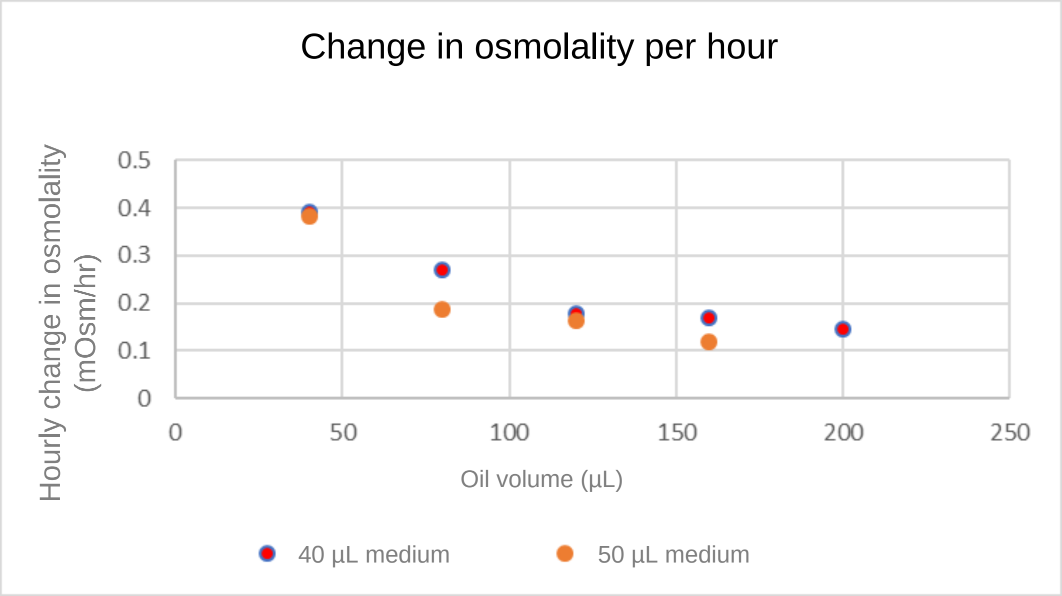




*Viscosity is not a significant predictor of osmolality change independent of density

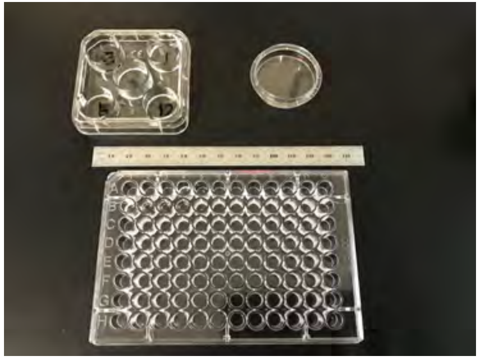
Viscometry performed at 37 °C by Shaun Tanner, Cook Research, Inc., using a TA Instruments AR 2000ex rheometer. Density measurements also performed at 37 °C.

Experiment examining the effect of oil volume on the rate of osmolality change



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 osmolaliy per hour over 7 days.

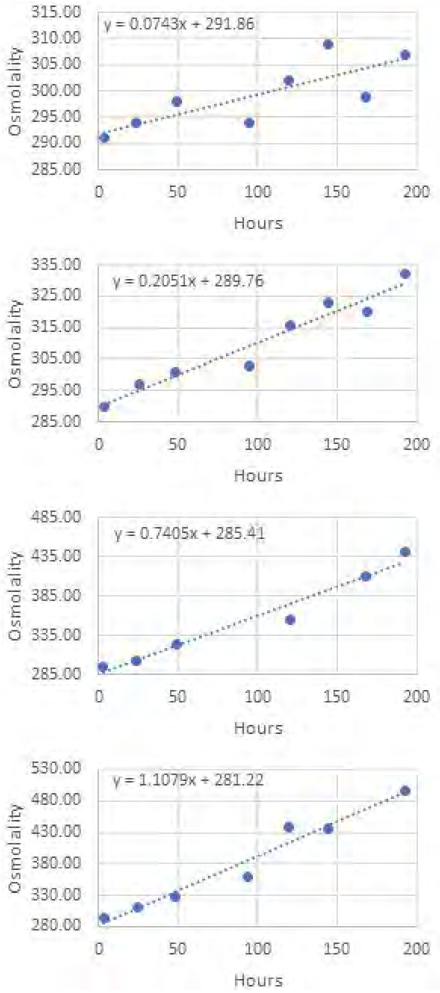


Note: 1 mL oil in a 35 mm dish is approximately 1.1 mm thick.

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

Model type	PRESS statistic	-2 log likelihood	Pred. R-squared	Adjusted R-squared	BIC	AICc
Full quadratic	4.84	-62.93	0.9182	0.9355	2.49	-30.37
Modified quadratic	4.76	-62.82	0.9196	0.9361	-2.07	-32.9
Full cubic	0.18	-443.46	0.9541	0.9681	-331.31	-380.82
Modified cubic	0.16	-436.77	0.959	0.9687	-357.33	-395.89
Full quartic	0.13	-515.95	0.9659	0.9802	-333.71	-391.38
Modified quartic	0.12	-502.88	0.9692	0.98	-358.03	-414.43

Note: Response variable transformations performed: Log for quadratic, and SqRt for cubic and quartic.

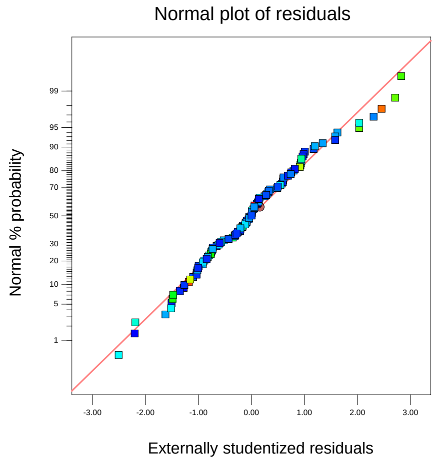
Cubic model analysis after backward selection

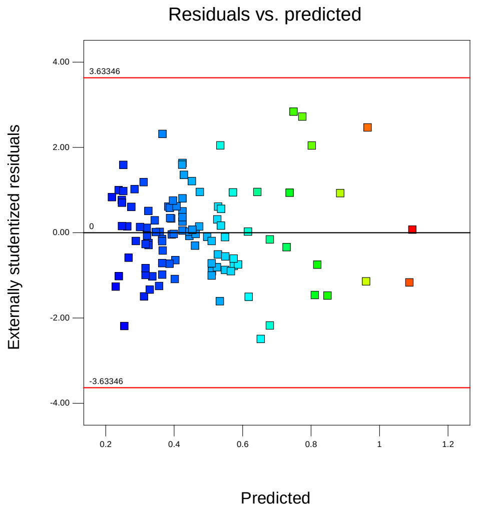
- Seven of the terms were eliminated and none had to be returned to maintain model hierarchy. All remaining terms have a very low *P* value in the ANOVA analysis.

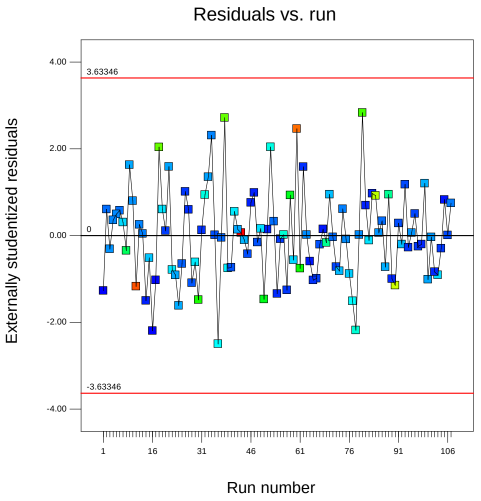
Analysis of variance table [Partial sum of squares – Type III]

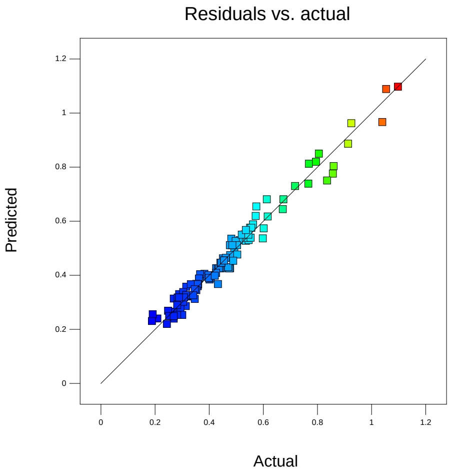
Source	Sum of squares	df	Mean square	F-values	*P* value (Prob > F)
Block	0.028	4	7.070E-003		
Model	3.72	12	0.31	263.70	< 0.0001
A-SA:vol	*0.41*	*1*	*0.41*	*344.99*	*< 0.0001*
B-Oil_Height	*6.475E-003*	*1*	*6.475E-003*	*5.51*	*0.0211*
C-Oil_Density	*0.066*	*1*	*0.066*	*56.32*	*< 0.0001*
AB	*0.25*	*1*	*0.25*	*210.30*	*< 0.0001*
AC	*0.040*	*1*	*0.040*	*33.83*	*< 0.0001*
BC	*0.034*	*1*	*0.034*	*29.04*	*< 0.0001*
A^2	*0.038*	*1*	*0.038*	*32.21*	*< 0.0001*
B^2	*0.31*	*1*	*0.31*	*261.86*	*< 0.0001*
ABC	*0.036*	*1*	*0.036*	*30.46*	*< 0.0001*
A^2B	*0.024*	*1*	*0.024*	*20.81*	*< 0.0001*
AB^2	*0.068*	*1*	*0.068*	*57.48*	*< 0.0001*
B^3	*0.062*	*1*	*0.062*	*53.09*	*< 0.0001*

Cubic model analysis after backward selection









Cubic equation to model osmolality change

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

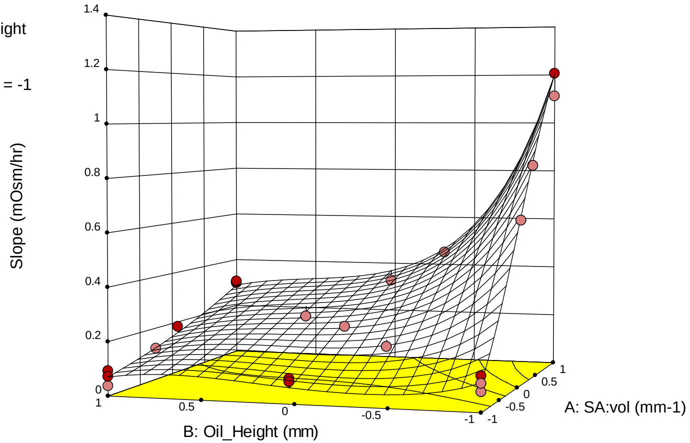
Where x = SA:vol (mm^{-1}), y = Oil height (in mm), and z = Oil density (in g/mL)

Model development and analysis

Experimental data and model predictions

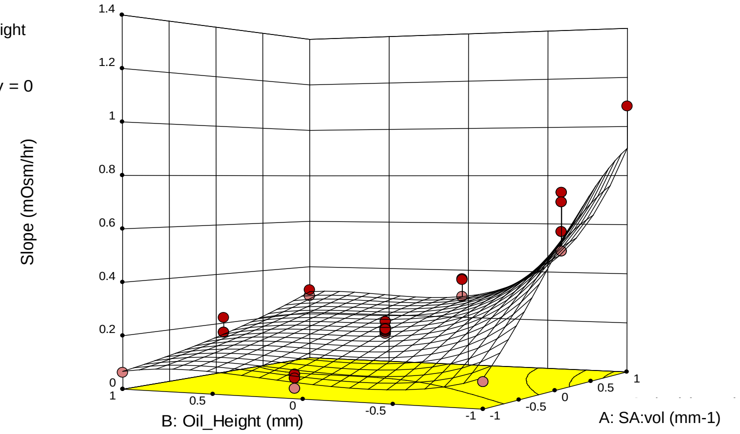
X1 = A: SA:vol
X2 = B: Oil_Height

Actual Factor
C: Oil_Density = -1



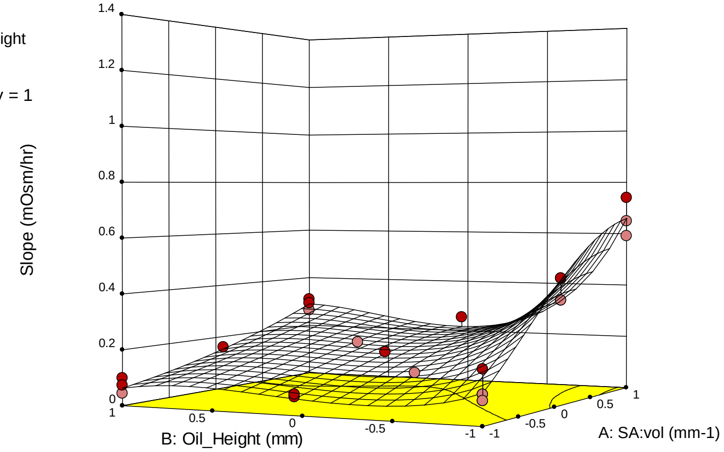
X1 = A: SA:vol
X2 = B: Oil_Height

Actual Factor
C: Oil_Density = 0

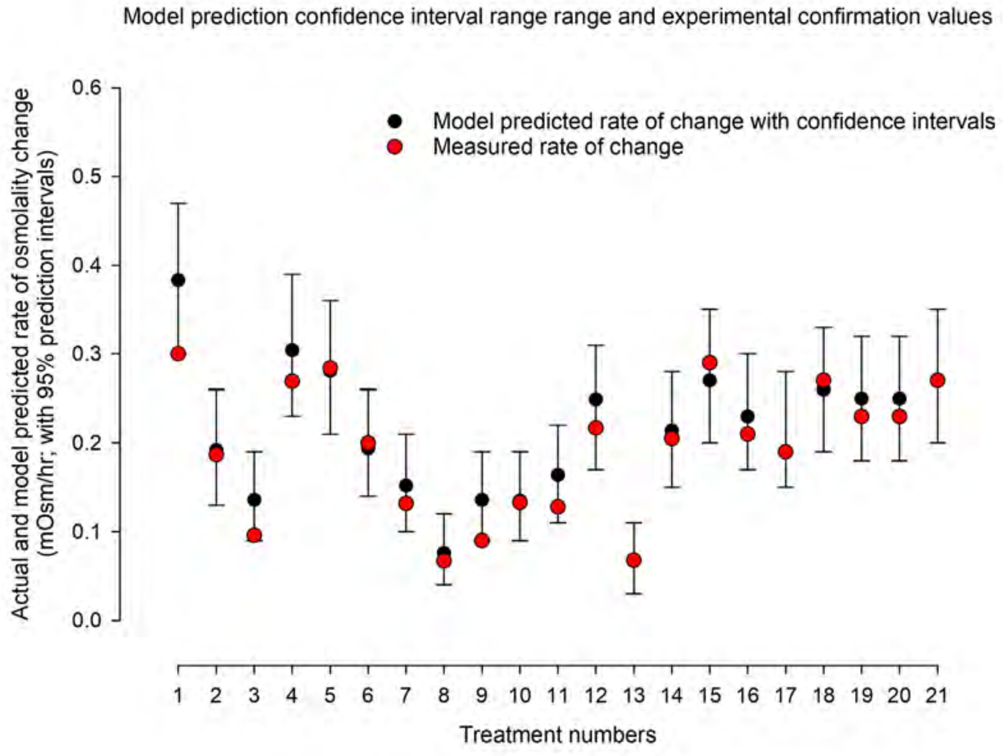


X1 = A: SA:vol
X2 = B: Oil_Height

Actual Factor
C: Oil_Density = 1



Model predictions and Actual Measurements



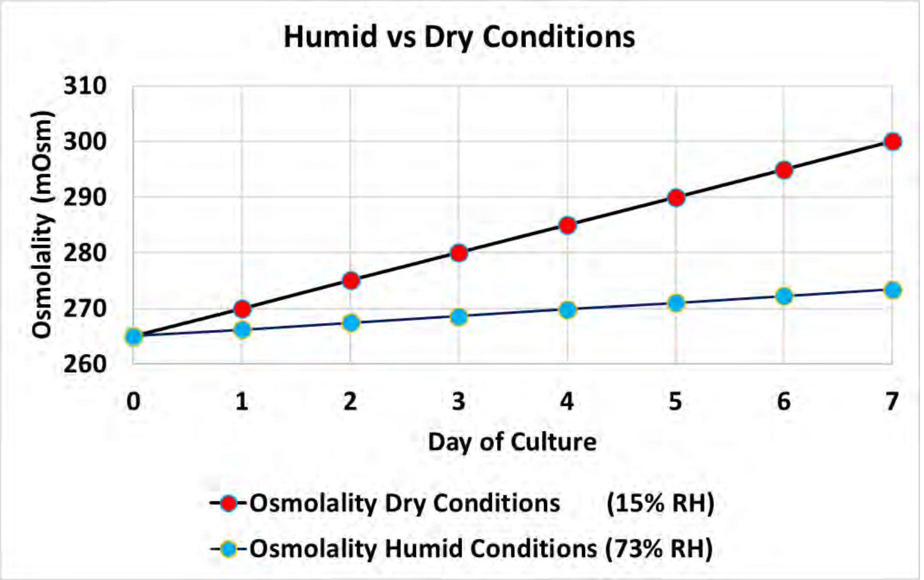
Comparison of dry and humid conditions during continuous culture

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