Metabolic and practical aspects of the interaction of culture media components and culture parameters on the developmental potential of mammalian embryos cultured in vitro

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DISCLOSURE

I am Vice President of Research and Development at Sage IVF.

We produce a range of commercial ART media products

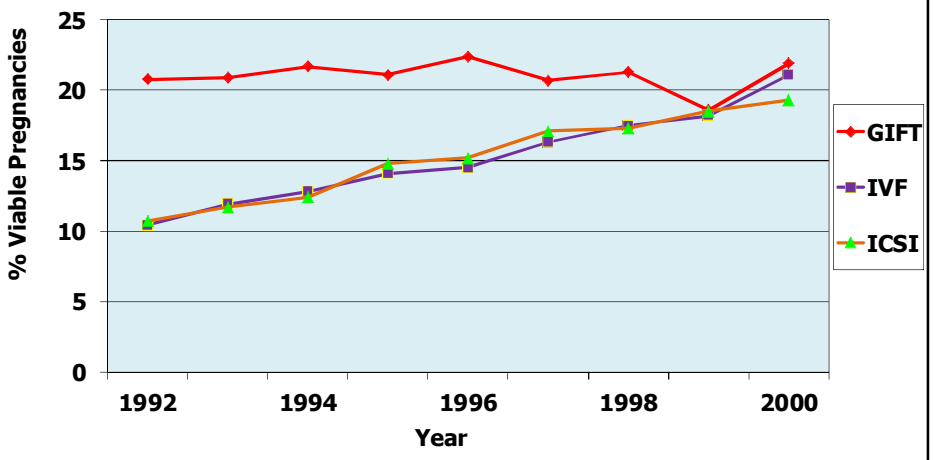
Or,

1. What is the effect of various components in ART media on the metabolic wellbeing of embryos during culture?, and

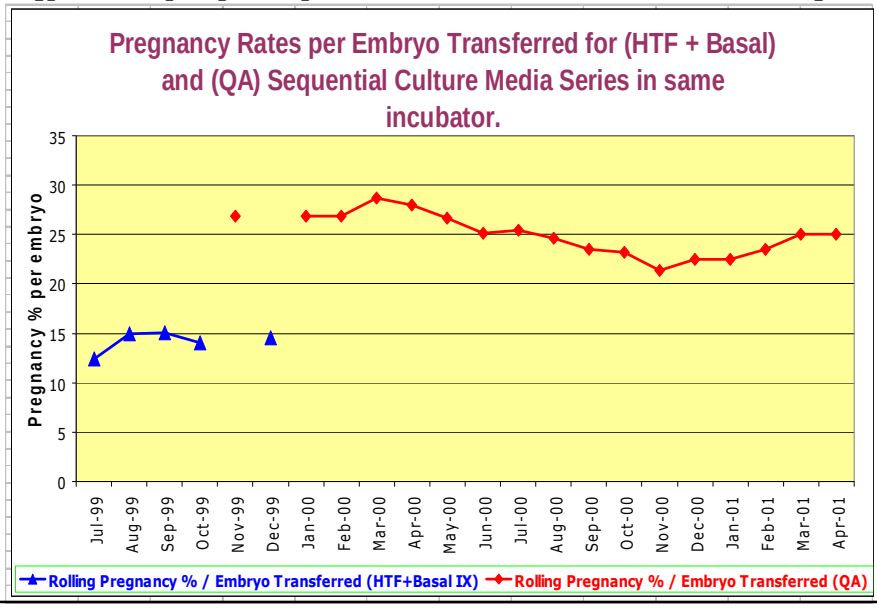
2. How do culture practices effect the wellbeing of cultured embryos?

Has culture media had an impact on pregnancy rates?

Viable Pregnancies per Transfer Australia & New Zealand



Results from CityWest IVF
(prize paper presenter: Simon Cooke)



Documentation for formulation and use of Quinn's culture media

FERTILITY AND STERILITY®
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Improvement in early human embryo development using new formulation sequential stage-specific culture media

Simon Cooke, B.Sc.Agr.,[a] Patrick Quinn, Ph.D.,[b] Lee Kime, B.Sc.,[a] Cheryl Ayres, M.Med.Sci.,[a] John P. P. Tyler, Ph.D.,[a] and Geoff L. Driscoll, M.D.[a]

CityWest IVF, Westmead, New South Wales, Australia

Objective: To determine whether altering selected components of sequential culture media can improve early development variables of human embryos.

Design: Prospective, randomized, sibling oocyte split trial.

Setting: Private ART center.

Patient(s): Two hundred eight undergoing treatment with in vitro fertilization or microinjection.

Intervention(s): Oocytes from each patient were randomly allocated to fertilization and cleavage media of a control and a trial culture medium formulation.

Main Outcome Measure(s): Rates of fertilization, cleavage, and uncontrolled division; average embryo morphology score; blastomeres per embryo; embryo score parameter (number of blastomeres × embryo morphology grade); and embryo utilization.

Result(s): The trial media resulted in a higher fertilization rate, higher cleavage rate, lower rate of uncontrolled division, higher number of blastomeres per embryo, higher average embryo morphology score, a higher embryo score parameter, and higher embryo utilization rate compared to the control media. All differences were statistically significant.

Conclusion(s): Improved sequential stage-specific culture media can reduce the occurrence of severe human embryo fragmentation and improve developmental variables in early IVF- and ICSI-generated embryos. (Fertil Steril® 2002;78:1254–60. ©2002 by American Society for Reproductive Medicine.)

Key Words: Sequential culture media, early embryo development, embryo morphology, in vitro development, uncontrolled division

OUTLINE

- Some of the history of Quinn's IVF media
- How do various media components work
- How can labs obtain the best results?
  i. pH
  ii. Aliquoting media
- Temperature
- Possible future trends

Part 1

Some of the background of Quinn's IVF media and how some components work

Documentation for formulation and use of Quinn's culture media

FERTILITY AND STERILITY
Vol. 44, No. 4, October 1985

Printed in U.S.A.

Improved pregnancy rate in human in vitro fertilization with the use of a medium based on the composition of human tubal fluid*

Patrick Quinn, Ph.D.†
John F. Kerin, M.D., F.R.A.C.O.G.
Graham M. Warnes, Ph.D.

Department of Obstetrics and Gynaecology, University of Adelaide, Queen Elizabeth Hospital, Woodville, South Australia, Australia

Significantly more mouse zygotes developed to blastocysts in culture in a medium formulated on the composition of human tubal fluid (HTF) than in modified Tyrode's medium (T6). In a randomized 2 × 2 factorial trial of human in vitro fertilization that compared the two media and culture under oil versus culture in loosely capped tubes, significantly more clinical pregnancies (30% of 60 transfers) were obtained with HTF medium than with T6 medium (11% of 53 transfers). Decreasing the K^+ content of HTF medium to that present in T6 medium significantly decreased the number of mouse zygotes that developed in culture. Modifying Ca^{++} levels had no effect. It is therefore likely that the higher K^+ content in HTF medium is primarily responsible for the superiority of HTF medium over T6 medium, but other differences in the composition of the two media could contribute to the results observed.
Fertil Steril 44:493, 1985

Documentation for formulation and use of Quinn's culture media

Table 1. *Composition of HTF and T6 Media*

Component	HTF	T6
	mM	
NaCl	101.6	99.4
KCl	4.69	1.42
$MgSO_4.7H_2O$	0.20	0.71
KH_2PO_4	0.37	—
Na_2HPO_4	—	0.36
$CaCl_2.2H_2O$	2.04	1.78
$NaHCO_3$	25	25
Glucose	2.78	5.56
Na pyruvate	0.33	0.47
Na lactate	21.4	24.9
Penicillin	100 U/ml	100 U/ml
Streptomycin SO_4	50 μg/ml	50 μg/ml
Phenol red	0.001% (wt/vol)	0.001% (wt/vol)

TABLE 1

Fertility and Sterility articles most frequently cited in the science citation index, 1975 through 2004.

No. of citations	Bibliographic data
512	Buttram VC, Reiter RC. Uterine leiomyomata—etiology, symptomatology, and management. Fertil Steril 1981;36:433–45 (Baylor Coll Med, Dept Obstet & Gynecol, Div Endocrinol Fertil, Houston)
508	Kruger TF, Menkveld R, Stander FSH, Lombard CJ, Vandermerwe JP, Vanzyl JA, Smith K. Sperm morphological features as a prognostic factor in in vitro fertilization. Fertil Steril 1986; 46:1118–22 (MRC, Inst Biostat, Tygerberg, South Africa)
504	Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta, JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 1988;49:112–7 (Eastern Virginia Med Sch, Dept Biol Sci, Androl Lab, Norfolk)

Yang & Pang 2006 Fertil Steril 86:795-7

Components of ART Culture Media

Ionic Composition
Energy Sources
Amino Acids
pH
Osmolality
Vitamins
Growth Factors

Components of ART Culture Media. Quinn's Media and most others

1. Inorganic Salts: NaCl, KCl, $MgSO_4$, KH_2PO_4, $NaHCO_3$, EDTA
   Variation in Ca/Mg during fertilization and embryo development.
2. Energy Sources: Sodium pyruvate, calcium-L+-lactate, glucose, sodium citrate.
   Only the bioactive L+ isomer of lactate present
3. Amino Acids: non-essential and essential, plus taurine, alanyl glutamine
4. pH: Specified under set CO_2 level – 7.3 for Fertilization and Blastocyst medium, 7.2 for Cleavage medium
5. Osmolarity: 265 mosmoles/Kg
6. Vitamins: in Blastocyst medium
7. Other: Phenol red
8. Antibiotic: Gentamicin

Quinn's media components

Inorganic salts

Energy sources

Non-essential amino acids

Essential amino acids

Vitamins

Ingredient mM	Quinn's Advantage Fertilization (HTF) Medium ART-1020	Quinn's Advantage Cleavage Medium ART-1026	Quinn's Advantage Blastocyst Medium ART-1029
Sodium Chloride	X	X	X
Potassium chloride	X	X	X
Magnesium sulfate, heptahydrate	X	X	X
Potassium phosphate, monobasic, anhyd.	X		X
Sodium bicarbonate	X	X	X
Glucose, D-(+)	X	X	X
Sodium pyruvate	X	X	X
Calcium lactate, (L+)	X	X	X
Alanyl-glutamine	X	X	X
Taurine	X	X	X
L-Asparagine	X	X	X
L-Aspartic acid	X	X	X
Glycine	X	X	X
L-Proline	X	X	X
L-Serine	X	X	X
L-Arginine.HCl			X
L-Cystine dihydrochloride			X
L-Histidine.HCl, monohydrate			X
L-Leucine			X
L-Isoleucine			X
L-Lysine.HCl			X
L-Methionine			X
L-Phenylalanine			X
L-Threonine			X
L-Tryptophan			X
L-Tyrosine			X
L-Valine			X
D-Calcium pantothenate			X
Pyridoxine.HCl			X
Riboflavin			X
Thiamine.HCl			X
EDTA, tetra-sodium salt	X	X	
Citric acid, tri sodium, dihydrate	X	X	X
Phenol red	X	X	X
Gentamicin	X	X	X
Osmolality mOs/Kg	265±8	265±8	265±8
pH	7.3±0.1	7.2±0.1	7.3±0.1

Specific Elements of Quinn's Media

1. **Why have EDTA?**
   It binds toxic heavy metals and also inhibits glycolytic enzyme phosphoglycerol kinase

2. **Why have sodium citrate?**
   It acts as a direct energy substrate, feeding into the TCA cycle. Originally found bound to albumin. Sage media already has citrate present, you do not have to add it with rHSA as in VL media.

Specific Elements of Quinn's Media

3. **Why vary the Mg^{2+} concentration?**
   High Mg2+ decreases uptake of exogenous Ca^{2+}. Therefore use with embryos to prevent damage to mitochondria and subsequent abnormal energy metabolism, but keep Mg^{2+} low in Fertilization Medium as sperm require a Ca^{2+} spike to undergo capacitation and acrosome reaction

4. **Why use gentamicin instead of other antibiotics?**
   Gentamicin is a broader antibiotic (eg, mycoplasmas), is much more stable than pen/strep and doesn't have patient interaction

Specific Elements of Quinn's Media

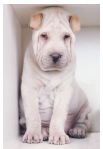
5. **Why use HEPES-buffered media?**
   It is an excellent way to maintain pH outside of a CO_2 environment. HEPES has <u>NOT</u> been shown to be embryotoxic. It has saved more embryos than it has killed.

6. **Why do we pregass our media?**
   This helps stabilize the media. Extreme variations in pH, as would occur with non-gassed media, do not occur. If media become too alkaline, calcium carbonate precipitates and remains as debris even if pH is lowered.

Specific Elements of Quinn's Media

7. Why do I use phenol red in my media?

It acts as an excellent pH indicator that can alert the user to deleterious pH changes. It does NOT have estrogenic properties; this was due to impurities that can be removed by extraction with ether. Vitrolife includes phenol red in media they sell in the USA. This is an example of DOGMA!!



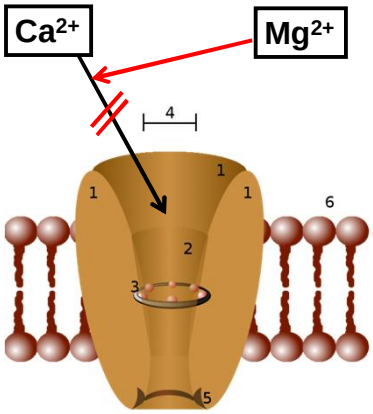
Specific Elements of Quinn's Media

8. Why should you use 5% CO_2 with our media?

It is more the pH that should be measured than the CO_2.
We have varied the $NaHCO_3$ concentration so that our media have the optimal pH under 5% CO_2 in most circumstances.

See Quinn & Cook, Fertil & Steril, 81: 1502-5, June, 2004
Poole, The Clinical Embryologist, Winter, 2004
www.embryologist.com

Calcium channels



Ligand ion channel blockade

Magnesium ions (Mg^{2+}) in cellular biology are usually in almost all senses opposite to Ca^{2+} ions, because they are bivalent too.

Mg^{2+} ions block Ca^{2+} channels and reverse decreased rates of development caused by increased levels of free Ca^{2+} during culture

BIOLOGY OF REPRODUCTION 59, 1000–1007 (1998)

Calcium Homeostasis in Early Hamster Preimplantation Embryos[1]

Michelle Lane[2] and Barry D. Bavister

Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706

Glucose needed in Fertilization Medium

Contains no glucose

Table II. Human Sperm Motility and Velocity in Basal and Basal XI Media

Parameter	Time	Basal	Basal XI	Significance
Motility (%)	30 min	74 ± 7[a]	79 ± 4	NS[b]
	18–24 h	52 ± 8	49 ± 9	NS
(μm/s)	18–24 h	45 ± 4	38 ± 6	NS

[a] Mean ± SEM of 10 matched pairs are given.
[b] NS = not significant.

Table III. Fertilization Rate of Mature Human Oocytes Using Different *Sperm* Numbers

Phase	Number of sperm inseminated	Basal	Basal XI	Significance
II	25K and 40K	126/147 (86%)	111/129 (86%)	NS[b]

[a] First figure is the sperm number (×10³) used in Basal medium, and second figure is the number used in Basal XI medium.
[b] NS = not significant.

Changes between cleavage and blastocyst phases

Concentration of Pyruvate, Lactate & Glucose in Human Reproductive Tract Fluids

Gardner et al 1996

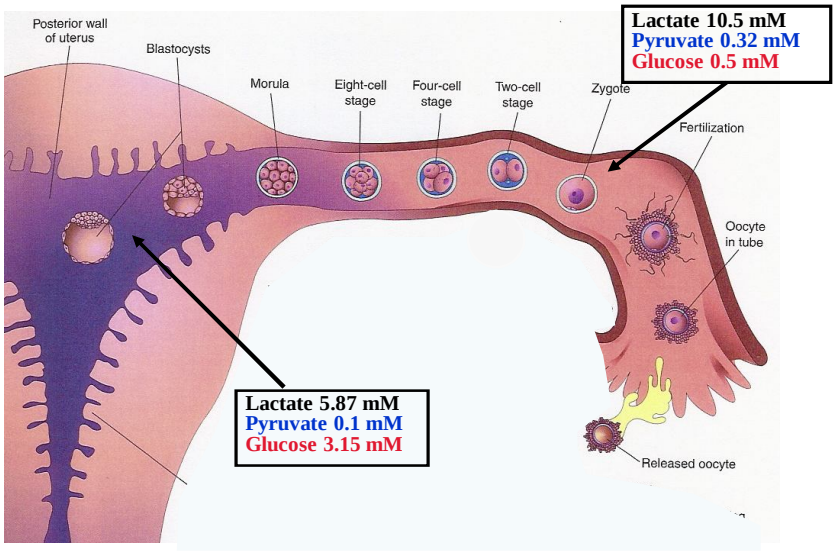
	Site	Cycle phase	Pyruvate	L (+) Lactate	Glucose
G1	Oviduct	Mid-cycle	0.32	10.5	0.5
G2	Uterus	All phases	0.10	5.9	3.2

Table II. Concentration of carbohydrates in the human oviduct and uterus (from Gardner *et al.*, 1996a)

	Pyruvate (mM)	Lactate (mM)[a]	Glucose (mM)
Oviduct (midcycle)	0.32	10.5	0.50
Uterus	0.10	5.87	3.15

[a]Lactate measured as the biologically active L-isoform.

Concentration of Metabolites in Oviduct & Uterine Fluids

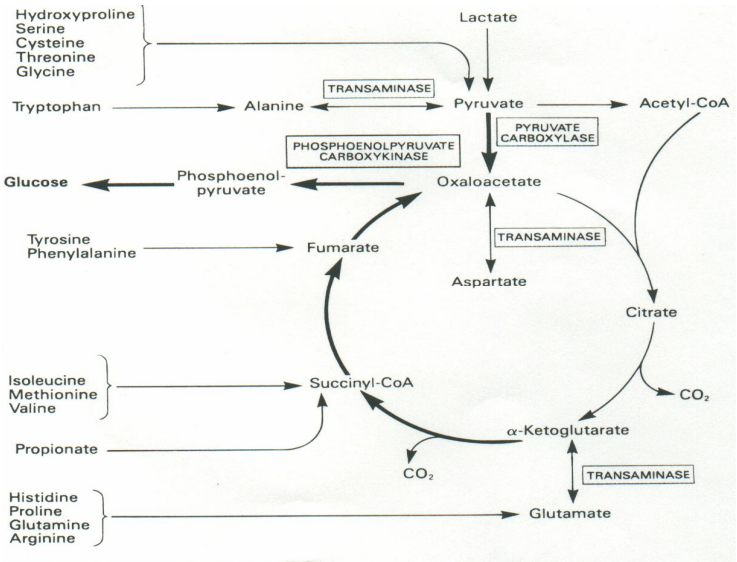


Gardner et al Fertil Steril 1996;65:349-53

Amino Acids: Role in ART Media

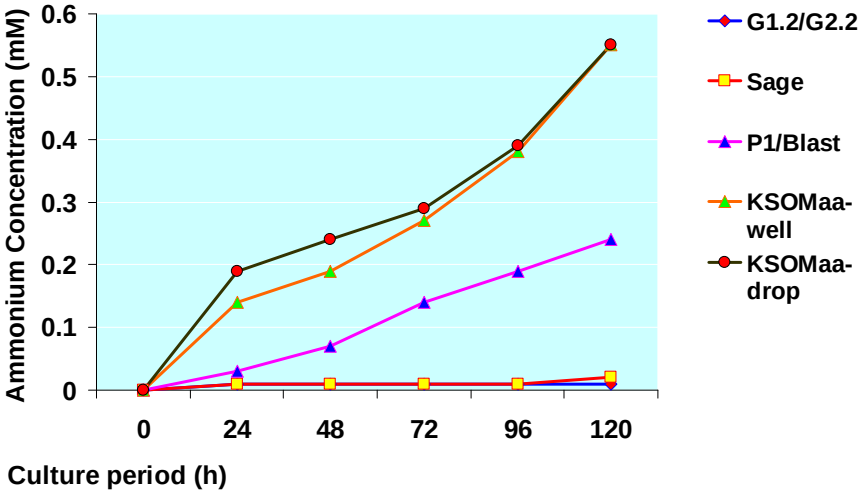
- Present in reproductive tract fluids and in embryos
- Some are consumed by the embryo
- Used for protein synthesis and as energy sources
- Act as internal buffers, osmolytes, antioxidants, chelators and regulators of development

Tricarboxylic Acid (TCA) Cycle
Krebs Cycle



Ammonium production from different culture media

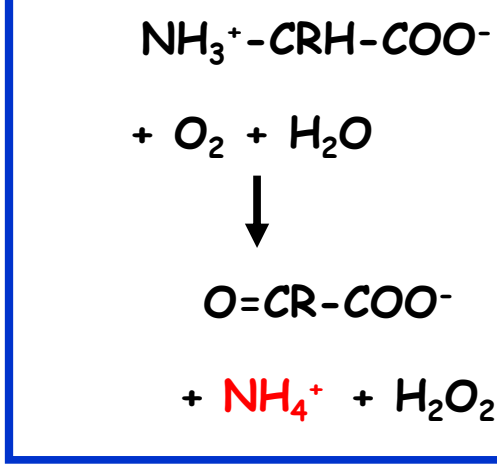
Lane & Gardner, BOR 69:1109-17, 2003



Amino Acids and Ammonium in Culture Media

- Ammonium is embryotoxic (Lane & Gardner, 2003)
- Toxic levels accumulate by 48-72 h of incubation
- Most of the ammonium comes from the spontaneous deamination of the amino group

Spontaneous deamination of the amino group



Ways to minimize toxic effects of ammonium

- Do not incubate medium for longer than 72 h
- Use stable form of glutamine: alanyl-glutamine
- Optimize conversion of pyruvate to alanine by omitting alanine from culture medium

Pyruvate acting as a sink for ammonium production during in vitro culture

Quinn's media contain no alanine

Part 2

How can labs obtain the best results?

i. pH

ii. Aliquoting media

Culture Strategies That Affect Outcomes

* pH & CO_2
* Temperature
* O_2 concentration
* Oil

pH and CO_2

Swain RBM Online 2010 21:6-16

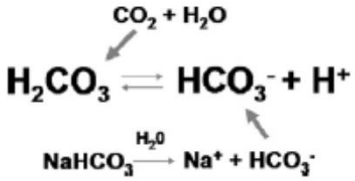
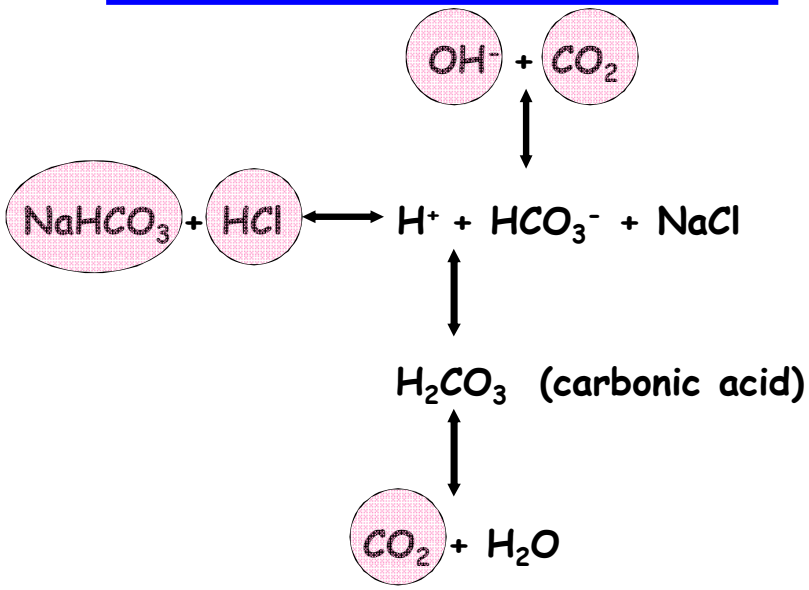


Figure 2 pH of culture media is primarily regulated by a balance of CO_2 concentration, supplied by the incubator, and by concentration of bicarbonate in the media. Because bicarbonate concentrations are set by commercial suppliers of media, it is easiest to adjust CO_2 concentration in the incubator to adjust pH. Raising CO_2 lowers media pH, while lowering CO_2 raises the pH.

pH and CO_2



Ways to adjust pH_o

- HCl / NaOH
- CO_2
- $NaHCO_3$

I have chosen to vary pH of the media by changing the concentration of $NaHCO_3$. Then, media have different pHs under the same CO_2 concentration.

pH of Media

Historical Observations

"7.2 with P1 medium gives more 8-cells
7.4 is better for blastocyst medium"

Council Advancement Ovulation Induction & ART, Ferring Pharmaceuticals, 1999

Intracellular pH Regulation in Human Preimplantation Embryos

Phillips et al., Human Reprod 15:896, 2000

- Mean pH_i of cleavage stage embryos (2 - 8-cells) = 7.0 - 7.3 (mean 7.12 ± 0.01)
- Two mechanisms to relieve changes
  - * HCO_3^-/Cl^- exchanger to relieve alkalosis
  - * Na^+/H^+ antiporter to relieve acidosis
- Further system that is both Na^+ and HCO_3^- dependent mediates further recovery from acidosis
- These three systems maintain pH_i within a narrow range

- Medium with a pH similar to pH_i is probably best

Recommended pH of Media

Fertilization medium: pH 7.3 ± 0.1

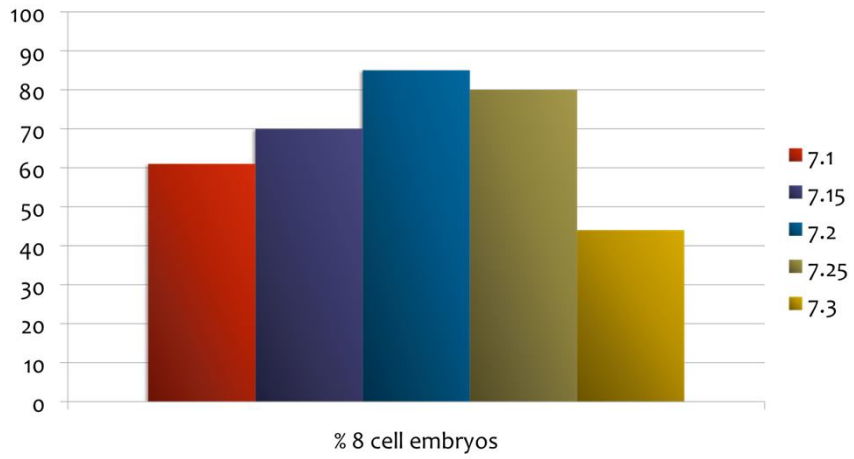
Cleavage medium: pH 7.2 ± 0.1

Blastocyst medium: pH 7.3 ± 0.1

All of the above values can be obtained with Quinn's IVF media under 5% CO_2 (on average), because we have adjusted $NaHCO_3$

Day 3 embryo cohort quality based on culture pH value

Courtesy Kathy Miller

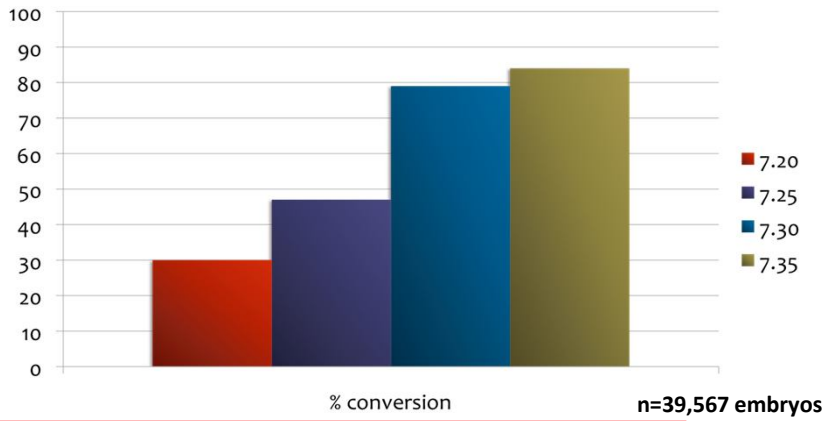


Optimum pH for Cleavage Medium 7.18-7.25

n = 96,431 embryos

Conversion of Day 3 embryos to blastocyst embryos based on culture pH value

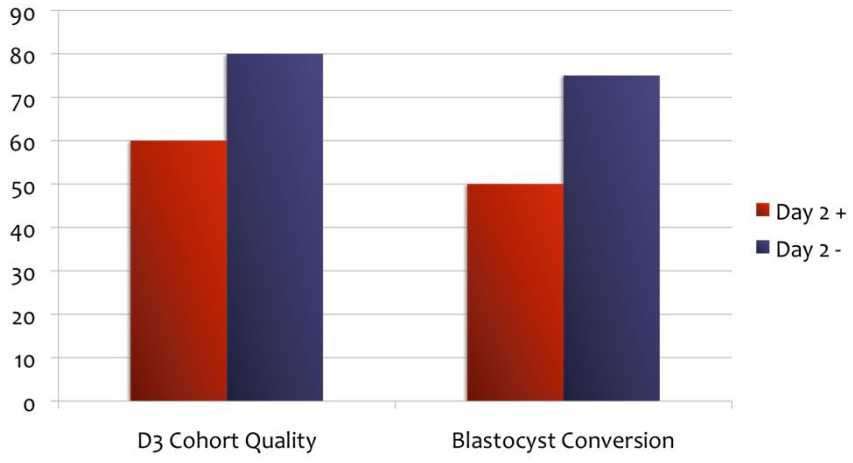
Courtesy Kathy Miller



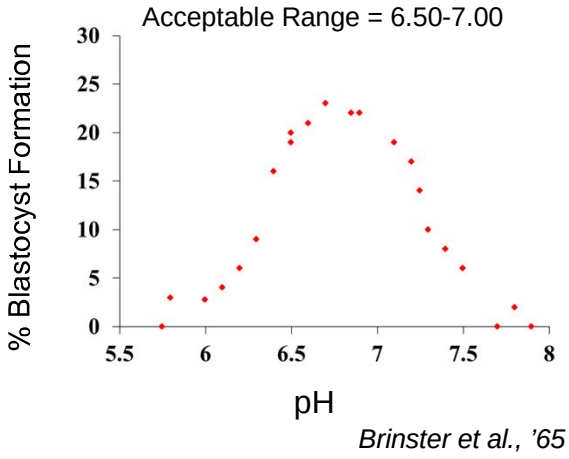
n=39,567 embryos

Optimum pH for Blastocyst Medium 7.28-7.35

Deletion of Day 2 Observations on Embryo Development



The Relationship Between Medium pH and Mouse Blastocyst Formation Rate



Brinster et al., '65

Recommended pH of Commercial Media

Swain RBM Online 2010 21:6-16

Table 2 Recommended pH ranges of various commercially available media used in clinical IVF.

Supplier	*Medium*	*Recommended pH range*
Irvine	P1	7.27–7.32
	ECM	7.2–7.25
	Single-step (SSM)	7.28–7.32
	Multi-blast	7.3–7.4
	HTF	7.2–7.3
	[redacted]	
	IVM	7.2 ± 0.1
Vitrolife	G5 Series Media	7.27 ± 0.07
Life Global	Global	7.2–7.4[a]
	Global Fert	7.2–7.4[a]
	Blastocyst	7.2–7.4[a]
	HTF	7.2–7.4[a]
	HTFxtra	7.2–7.4[a]
Medicult	Universal IVF	7.3–7.4
	ISM1	7.2–7.3
	ISM2	7.35–7.45
	EmbryoAssist	7.2–7.3
	BlastAssist	7.35–7.45
Cook	Sydney IVF Cleavage	7.3–7.5
	Sydney IVF Blastocyst	7.3–7.5
	Sydney IVF Fert	7.3–7.5

Recommended pH of Media

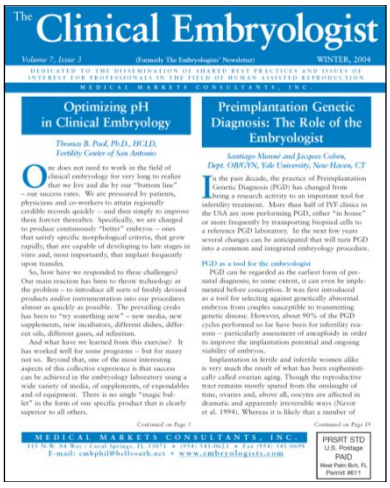
MEASURE YOUR pH!!!!

- for every new lot of medium
- when gas tanks are changed
- at least every week

Measuring pH

Rusty Pool,

The Clinical Embryologist, Winter, 2004
www.embryologists.com



The Clinical Embryologist

Volume 7, Issue 3 (Formerly The Embryologists' Newsletter) WINTER, 2004

DEDICATED TO THE DISSEMINATION OF SHARED BEST PRACTICES AND ISSUES OF INTEREST FOR PROFESSIONALS IN THE FIELD OF HUMAN ASSISTED REPRODUCTION

MEDICAL MARKETS CONSULTANTS, INC.

Optimizing pH in Clinical Embryology

Thomas B. Pool, Ph.D., HCLD, Fertility Center of San Antonio

Preimplantation Genetic Diagnosis: The Role of the Embryologist

Santiago Munné and Jacques Cohen, Dept. OB/GYN, Yale University, New Haven, CT

The Clinical Embryologist,
Now,…

The Journal of Clinical Embryology

www.embryologists.com

Not only is selecting the optimal pH important, but also maintaining it

The most important thing is to prevent CO_2 out-gassing by keeping culture vessels under the CO_2 atmosphere as much as possible…

..and renewing the desired CO_2 concentration as quickly as possible after culture vessels are returned to the incubator.

Rapid change in pH

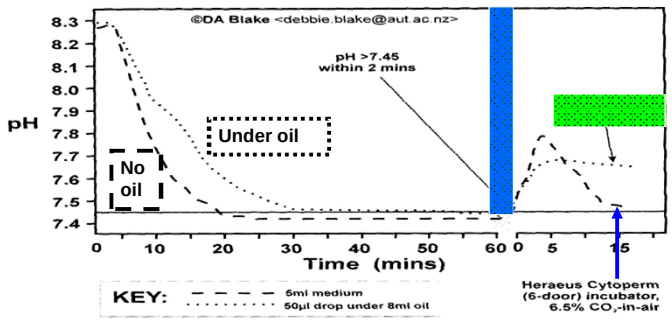
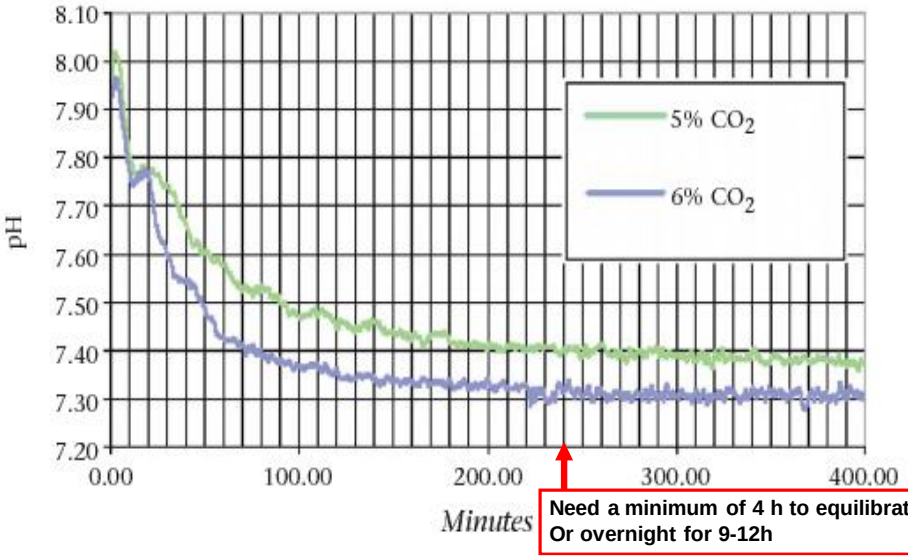
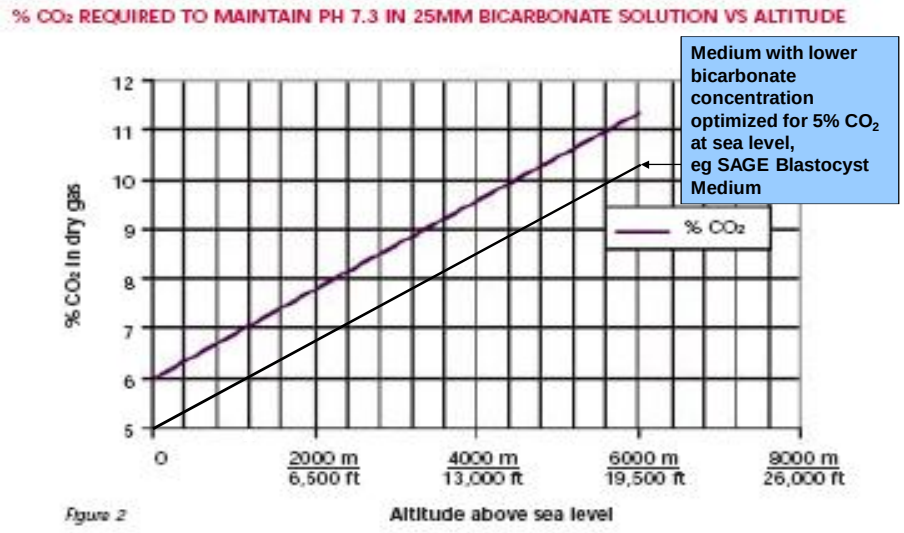


Figure 11.4 Graph showing the equilibration of either 50 µl drops of medium under oil (dotted line) or 5 ml of medium (broken line) in 60 mm diameter Falcon 3004 dishes. Dishes were then taken out of the incubator (a Heraeus Cytoperm fitted with a 6-section inner door and running at 6.5% CO_2) and placed under an air atmosphere for 3 minutes before being replaced in the incubator. In both cases the pH of the medium had exceeded 7.45 within 2 minutes of exposure to air, and re-equilibration took about 15 minutes for the 5 ml of medium in a dish and 35 minutes for the 50 µl droplets. (Blake *et al.*, 1999; data generously provided by Debbie Blake).

Time to equilibrate medium



Effect of Altitude on CO_2 Requirement



Relationship between CO_2 and pH

trations. Yet another way to alter the pH of medium is to vary the CO_2 concentration. By applying the Henderson-Hasselbach equation:

$$pH = pKa + \log_{10} ([HCO_3^-] / [CO_2])$$

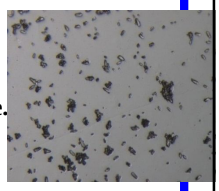
it can be calculated that in a solution containing 25 mM $NaHCO_3$ at 37°C at sea level, the following pHs would be obtained at different CO_2 concentrations.

% CO_2	*pH*
4.6	7.5
5.8	7.4
7.3	7.3
9.3	7.2

This is with 25 mM $NaHCO_3$. Sage media have a lower NaHCO3 than this and therefore would have a lower pH than in the example above. As a general rule, for every 1% increase in CO_2, the pH will decrease by 0.05 - 0.1 units.

Aliquoting Media

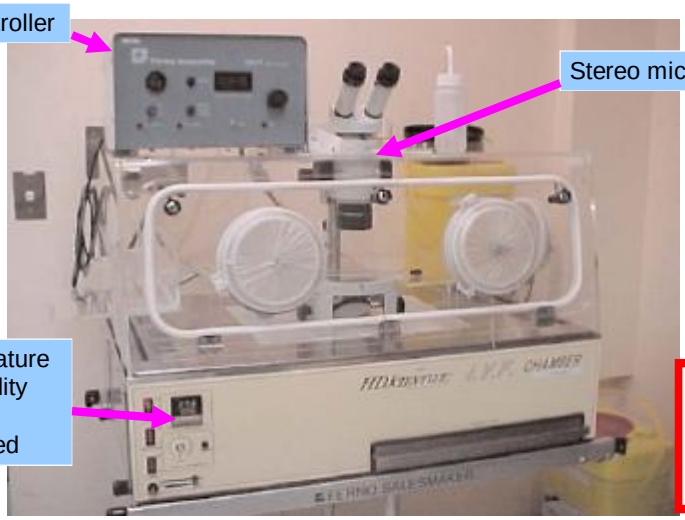
1. Crystalline deposits of calcium carbonate appear when pH goes too high, eg when bottle is opened too often.
   Once precipitated it is impossible to get CaCO3 back into solution even if the pH is lowered to a more acceptable pH range.



2. I do not recommend aliquoting.
   A. Many labs aliquot medium to extend use, eg 3 x 7-8 mL.
   B. Do you store these under 5% CO2 at 2-8 C before use (except for the first aliquot that you would use straight away)?
   C. Are the crystals immediately present when the dishes are set up or do they appear over time? If immediately present, this means the pH has been too high in the shipped bottle. If they appear over time, this means that the pH of the aliquot is gradually increasing during prolonged storage.

3. If you must aliquot, quickly remove 2 x 7 mL portions from a 20 mL bottle and place them into 14 mL tubes, tightly cap the tubes and place them in a sealed container that has been gassed with 5% CO2 in air at 2-8°C. Use one portion entirely on one day. The other 2 portions should be used within one week of opening the bottle.

IVF Chamber for microscopic viewing culture dishes in a CO_2 environment

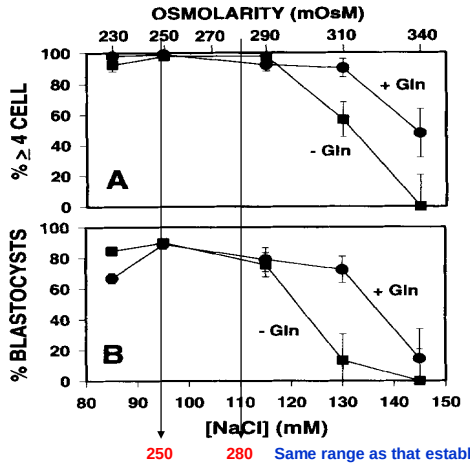


pH SUMMARY

- Keep in mind that different media have different compositions and will probably have different pH under same CO_2 concentration.
- The way media are used has an important impact
- pH control probably has one of the most important impacts on the success of embryo culture

Effect of Osmolality on Development of Mouse Zygotes to Blastocysts

Dawson & Baltz, BOR 59:225-32, 1998



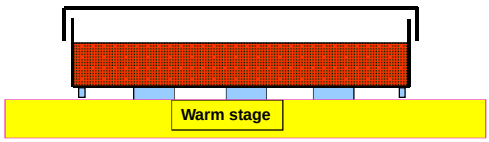
Same range as that established by Whitten, 1971

Part 3

Temperature

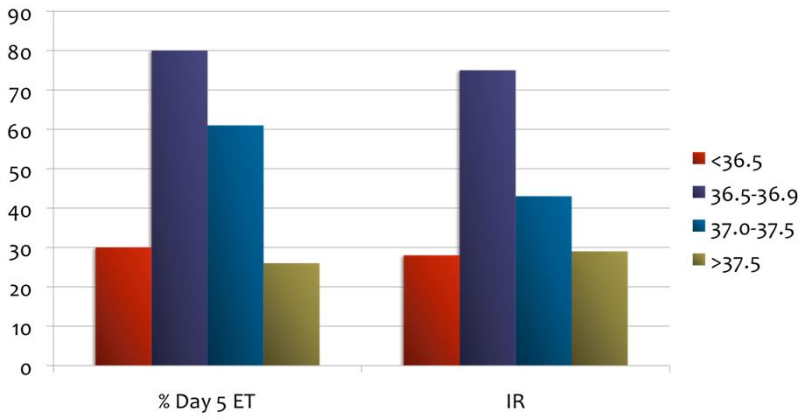
Hand in hand with maintaining pH is maintaining temperature

TEMPERATURE

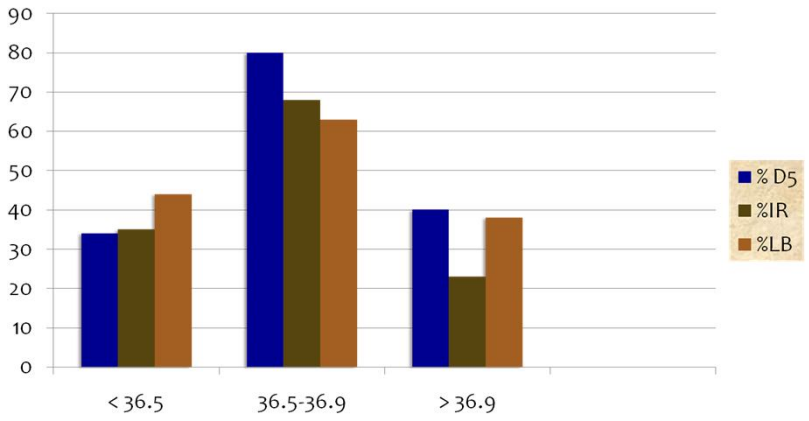


Gap = heat loss
Temperature of drop measured
Stage temp adjusted

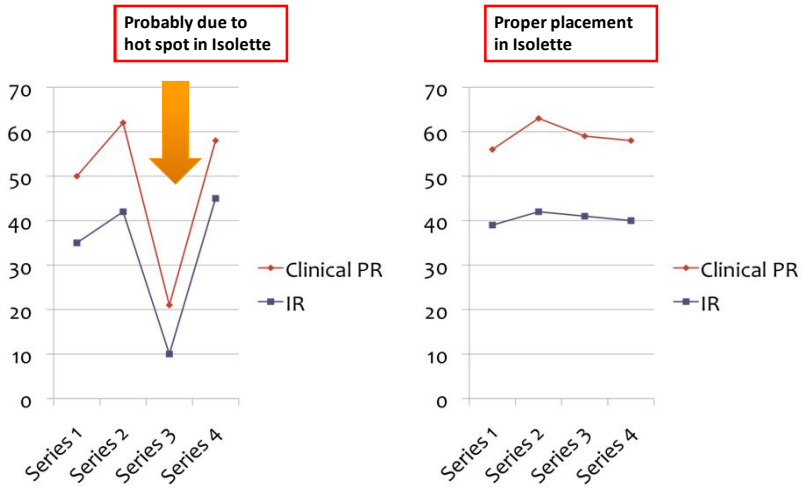
The Effect of Aspirate Temperature on Day 5 ET % and Implantation Rate



Effect of Temperature



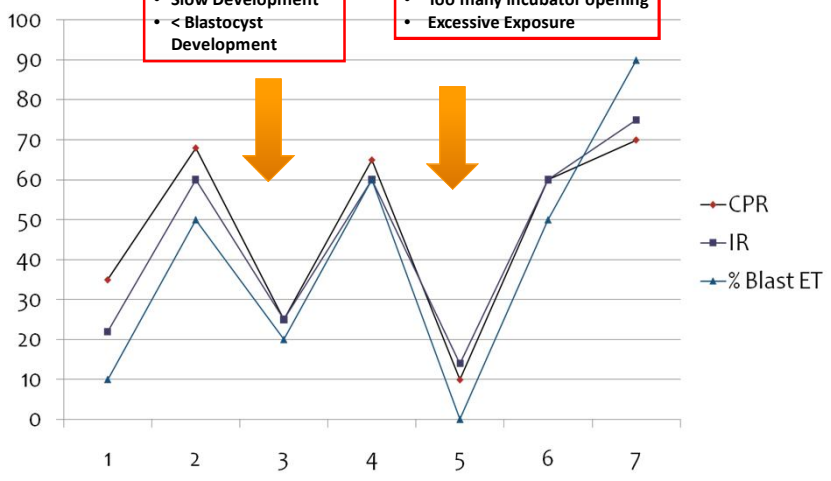
Placement of Embryo Transfer Dish in Isolette



January 2010-May 2010

What happened
- Cleavage Arrest
- Fragmentation
- Slow Development
- < Blastocyst Development

Probably Why
- Improper Dish Preparation
- Aggressive Pipetting
- Too many incubator opening
- Excessive Exposure



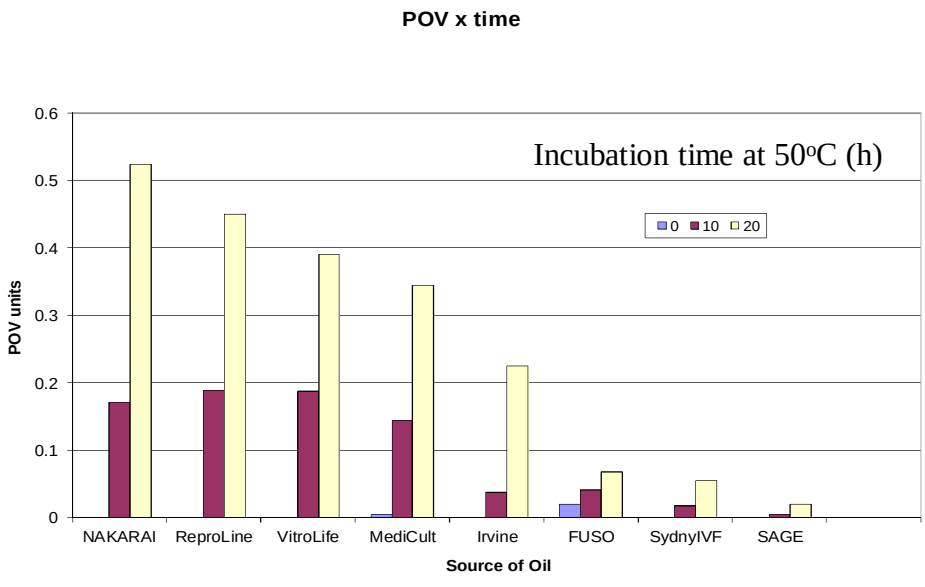
Bench top Incubator

Incubator Environment

Understand the operation of your laboratory's incubator to have the most efficient recovery of temperature, humidity and gaseous state.

Limit embryo observations as a way to decrease incubator door openings and decrease embryo exposure to unnecessary changes in temperature or pH.

Peroxidative Value of Different Sources of Oil



Part 4

Future Trends?

Sequential v. 1-Step Medium

Sequential Media

Separate media for
D1 ⟶ D3 = zygote to 8-cell: Cleavage medium, and
D3 ⟶ D5/6 = 8-cell to blastocyst: Blastocyst medium

Based on the "Back to Nature" concept

- **Monoculture Medium (aka 1-Step medium)**

Same medium for D1 D5/6: Global,
Continuous Single Culture =
Irvine's Embryo Choice

Based on the "Let the Embryo Choose" concept

Culture Media for Human IVF

Strategies for Development

"Back to nature" = add selected compounds probably present at a specific location when the embryo is there.

Based on analysis of constituents *in vivo*

Menezo et al 1984
Quinn et al 1985
Leese 1991,1998
Gardner et al 1996;1998;2002;2003;2004

****

"Let the embryo choose" = add all compounds likely to be throughout the reproductive tract

Based on systematic assessment of responses of mouse embryos to controlled changes in medium composition

Lawitts & Biggers 1992; 1993
Biggers et al 1997; 1998; 2003

Time Line for Human Embryo Evaluation

Author	Date	Observation
Edwards	1980s	Cell # & Size, fragmentation
Mohr & Trounson	1985	Morphological scoring
Steer et al	**1992**	**Cumulative Embryo Score**
Pickering	1995	Multinucleation
Sakkas	1997	Early cleavage
Scott, L	1998-2007	Zygote scoring & gated Embryo Score
Gardner	1999	Blastocyst scoring
Fisch et al	2001, 2003	Graduated Embryo Score
Angle	2006	Modified GES
Scott R, Emri et al	2006 →	Metabolomics?
various	present	Morphokinetics by time lapse videography

Day of ET & Embryo Selection

1. **If sufficient embryos, eg ≥3 good quality 8-cell embryos on D3, then culture to blastocyst stage.**
2. **If not pregnant after 3 IVF cycles of D3 ET, then go to D5 ET.**
3. **However, if few embryos, eg < 5 oocytes, then D2 (or even D1) ET may be better.**

Day 2 transfer improves pregnancy outcome in in vitro fertilization cycles with few available embryos

Shehua Shen, M.D.,[a] *Mitchell P. Rosen, M.D.,*[a] *Anthony T. Dobson, M.D., Ph.D.,*[a] *Victor Y. Fujimoto, M.D.,*[a] *Charles E. McCulloch, Ph.D.,*[b] *and Marcelle I. Cedars, M.D.*[a]

[a] Department of Obstetrics, Gynecology, and Reproductive Sciences and [b] Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California

Objective: Delaying ET to day 3 to optimize embryo selection is well accepted. However, in cases where there are not enough embryos to perform selection, it is not clear whether there is a difference in clinical outcomes with the day of ET.
Design: Cohort study.
Setting: Academic medical center.
Patient(s): Two hundred forty-two fresh IVF/intracytoplasmic sperm injection (ICSI) cycles from 2002–2004, where all generated embryos were transferred irrespective of quality because of an extremely low number of available embryos.
Intervention(s): In time period 1, ET was on day 3. In time period 2, ET was on day 2.
Main Outcome Measure(s): Patient response to stimulation was analyzed along with pregnancy outcome and implantation rate.
Result(s): Miscarriage rates were decreased, and ongoing pregnancy rates were increased with a day 2 ET in patients <40 years of age.
Conclusion(s): In women <40 years of age, the day of transfer is a significant predictor of clinical outcome in cases in which a low number of embryos are available for transfer. The evidence suggests that limiting embryo culture to only 2 days reduces the incidence of miscarriage and increases ongoing pregnancy rates. (Fertil Steril® 2006;86:44–50. ©2006 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, pregnancy rates, implantation rates, spontaneous abortion

Efficiency of changing the embryo transfer time from day 3 to day 2 among women with poor ovarian response: A prospective randomized trial

Mustafa Bahceci, M.D., Ulun Ulug, M.D., H. Nadir Ciray, M.D., Ph.D., Mehmet Ali Akman, M.D., and Halit Firat Erden, M.D.

Bahceci Women Health Care Center and German Hospital in Istanbul, Istanbul, Turkey

Objective: To compare the outcome of day 2 and day 3 embryo transfers in women demonstrating poor ovarian response.
Design: Prospective randomized clinical trial.
Setting: Private assisted reproductive technology center.
Patient(s): Two hundred eighty-one women demonstrating poor ovarian response to controlled ovarian hyperstimulation.
Intervention(s): Women who were poor responders were randomly allocated to day 2 or day 3 embryo transfer following oocyte retrieval.
Main Outcome Measure(s): Implantation rates and pregnancy rates per oocyte retrieval and embryo transfer.
Result(s): The clinical pregnancy rates per oocyte retrieval (37.2% vs. 21.4%, respectively; $P<.05$) and per embryo transfer (38.9% vs. 24.1%, respectively; $P<.05$) were significantly higher in the day 2 embryo transfer group compared with day 3. On the other hand, implantation rates were not different between groups (23.9% vs. 17.2%, respectively; $P=.08$).
Conclusion(s): Our results demonstrated that transfering embryos on day 2 could provide an alternative to the management of poor responder patients. (Fertil Steril® 2006;86:81–5. ©2006 by American Society for Reproductive Medicine.)

Key Words: Poor responder, embryo transfer, day 2, day 3

Incubator Environment

Understand the operation of your laboratory's incubator to have the most efficient recovery of temperature, humidity and gaseous state.

Limit embryo observations as a way to decrease incubator door openings and decrease embryo exposure to unnecessary changes in temperature or pH.

This has lead to the use of time-lapse videography.

This technology still has to be validated in prospective randomized trials where embryos for ET are selected based on morphokinetic parameters.

Other Techniques that may be useful

- Well of the Well culture
- Vibration during culture
- Low oxygen concentration

Well of the Well Culture

Figure 1. Preparation of the Well–of-the-Well system. Nunc four-well dishes were filled with 400 μl medium, and covered with 400 μl oil. Microwells were made manually with a strong mechanical force using BLS aggregation needles. Bar indicates 2 mm. (Unlike the well shown on the figure, for the present experiment only five WOW per well were prepared.)

RBM Online - Vol 17 No 1. 2008 73-81 Reproductive BioMedicine Online; www.rbmonline.com/Article/3282 on web 28 May 2008

Article

The Well-of-the-Well system: an efficient approach to improve embryo development

Dr Gábor Vajta

Vibration during culture

Clin. Lab. 2010;56:569-576

ORIGINAL ARTICLE

Mechanical Agitation During the *in vitro* Culture of Human Pre-Implantation Embryos Drastically Increases the Pregnancy Rate

EVGENIA ISACHENKO [1], ROBERT MAETTNER [2], VLADIMIR ISACHENKO [1], STEFFEN ROTH [3], ROLF KREIENBERG [1], KARL STERZIK [2]

[1] Section of Gynaecological Endocrinology and Reproductive Medicine, University of Ulm, Germany
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[3] Xcaltis GmbH, Germany

Figure 1. The NSSB-300 device (Nepa Gene, Ichikawa, Chiba, Japan) used for the mechanical agitation of the *in vitro* culture system. Petri dishes contain the fertilized oocytes, and embryos are placed in the machine's CO_2 incubator. Scale bar = 8 cm.

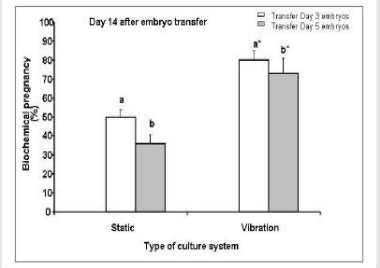

Figure 3. Biochemical and clinical pregnancy rates depending on the type of culture system. Values with different subscripts differ significantly ($p < 0.05$).

"Good" media used badly → poor results

Thank you!

Jack our grandson!
Conceived and cultured in Quinn's medium!