ALPHA - London April 27, 2012

Shady Grove Fertility
Reproductive Science Center

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IrvineScientific

Improving IVF Lab Efficiency with use of a True Single Step Medium

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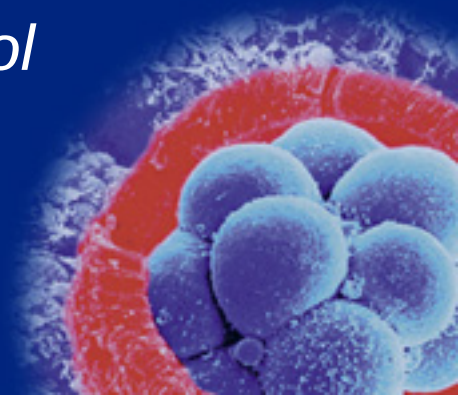
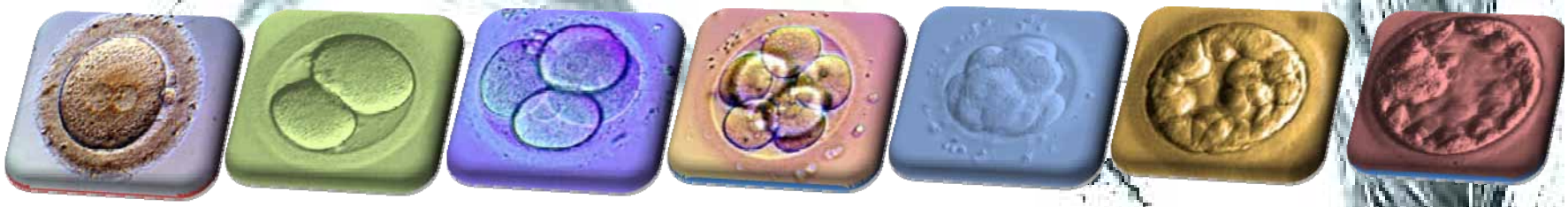


CURRENT PROTOCOLS FOR CULTURE OF HUMAN PRE-IMPLANTATION EMBRYOS



BASED ON TWO GENERAL PHILOSOPHIES:

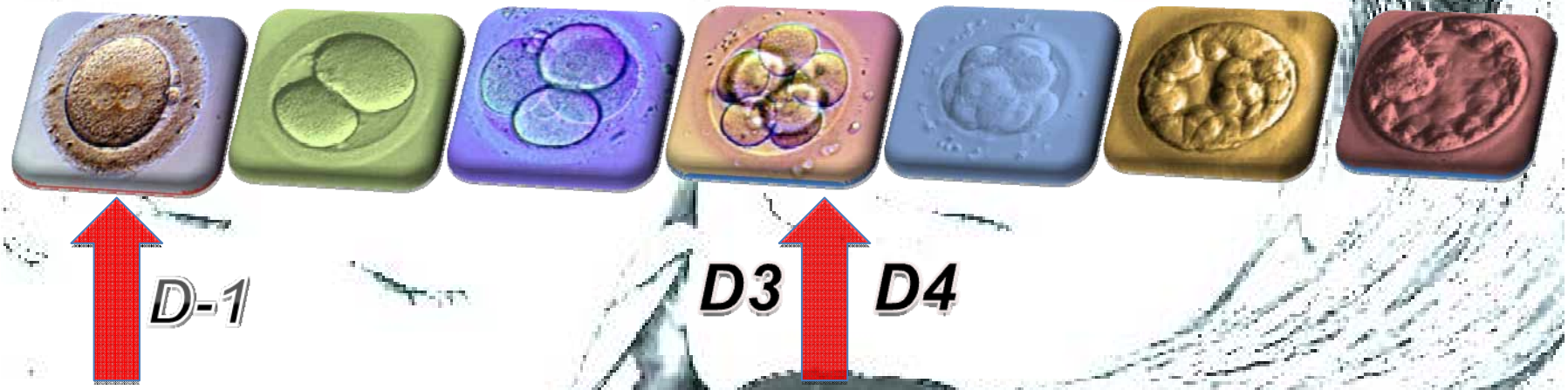
1) **'Back to Nature'** –
mimic composition of oviduct/uterine secretions + changes in metabolism during early embryo development

2) **'Let the Embryo Choose'** –
embrace *in vitro* state; 'start from scratch' with calculated balance of constituents pre-determined methodically by bioassay

Biggers, 2002; Biggers & Racowsky, 2002

1) 'Back to Nature'

- Protocol uses several media in sequence: insemination/cleavage/blastocyst *SEQUENTIAL* media
- Interrupted culture where media of different make-up are changed initially & midway



- Traditionally most actively promoted & accepted in IVF Labs

2) 'Let Embryo Choose'

- Protocols using one medium for culture of zygotes through blastocyst stage (insemination?)

  a) ***renewal single medium*** –
  Interrupted culture where one medium is used throughout but is renewed midway through culture

  b) ***non-renewal (TRUE) single medium***–
  Uninterrupted culture using one medium throughout 5-6 days of embryo culture

CONTINUOUS

- Few commercially available & not as widely used

Why are sequential media considered to be the norm for extended culture?

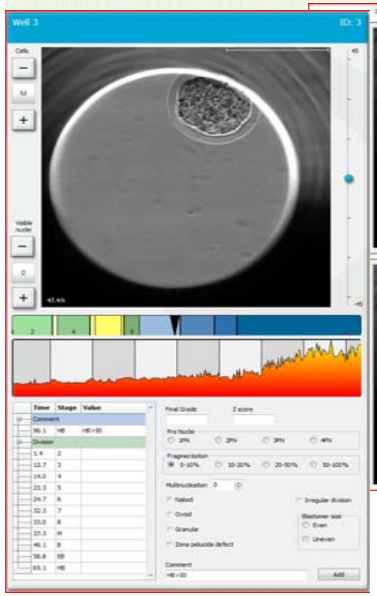
- 'Back to Nature' principle innately attractive
- Refreshing culture medium suggested for removal of ammonium build-up from AA breakdown, and/or to remove accrued VOCs
- Physical pipetting of embryos to fresh medium mimics movement experienced *in vivo*
- Favored by a number of clinical embryologists in the field (Gardner/Behr/Pool... 1994-98)
- Few studies comparing single vs. sequential media

Unisense EmbryoScope

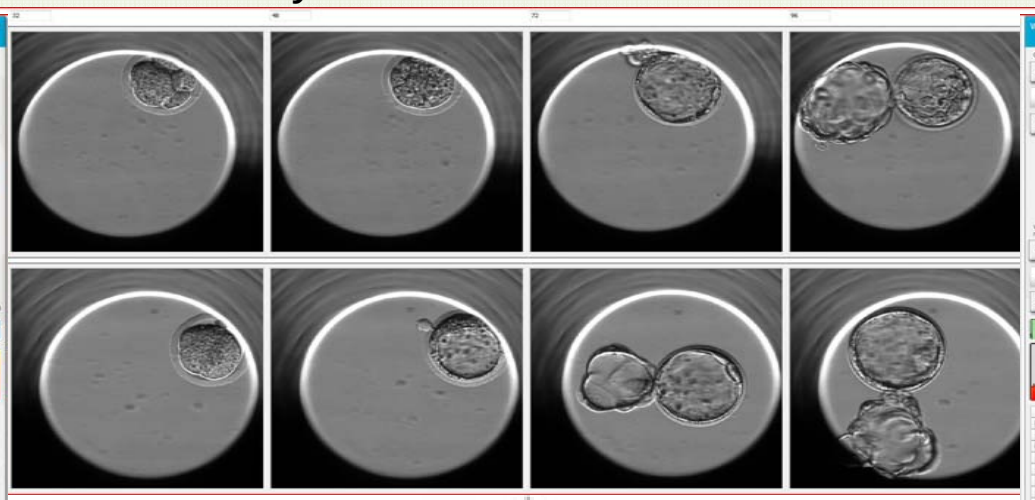
- 15min monochromatic time-lapse image capture of individual embryos
- Builds a record of embryonic activity related to 'health' of embryo

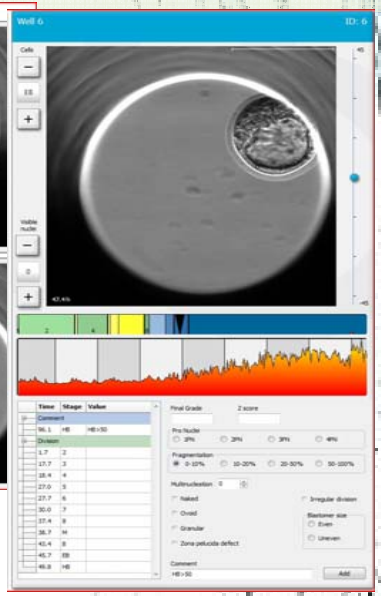
Redefining the MEA with continuous surveillance versus ‘snap-shot’ observations

VerMilyea et al, P-410 ASRM 2010



SSM

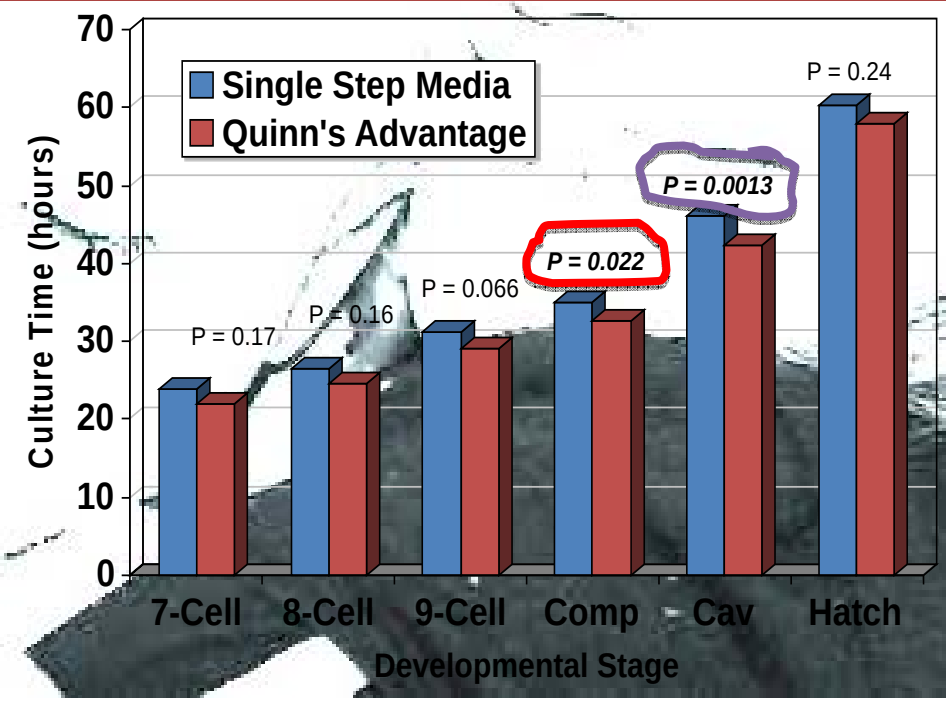




QA

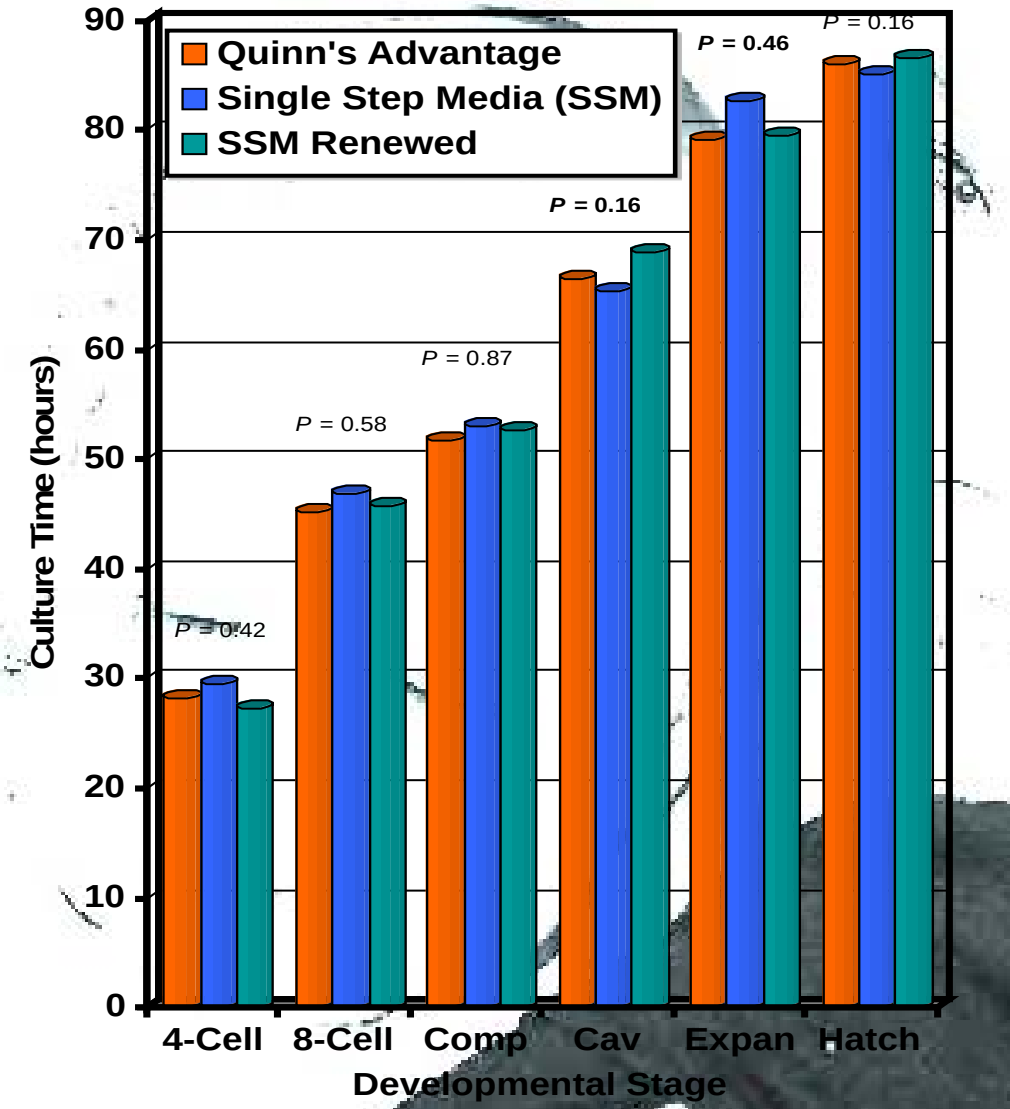
- Traditional Scoring @ 96hrs & >50% hatched

Not Significant



Renewing of medium in SSM provides no advantage for mouse embryo development when observed by time-lapse

VerMilyea et al, P-488 ASRM 2011



Clinical Study June-Sept 2011:
Sibling Embryo Culture - Sage Quinn's Advantage (QA) vs. Irvine Continuous Single Culture (CSC)

- 923 embryos cultured (QA n=448 & CSC n=475)
- Best quality embryos, regardless of treatment were transferred (Day-3/-5) OR vitrified on Day-5/-6
- Clinical pregnancy determined by Fetal Cardiac Activity (FCA)
- 61 eggs / 8 patients: conventionally inseminated in CSC™ to determine support of normal fertilization

Results of Split Culture

Blastocyst Utilization Rate (BUR) =

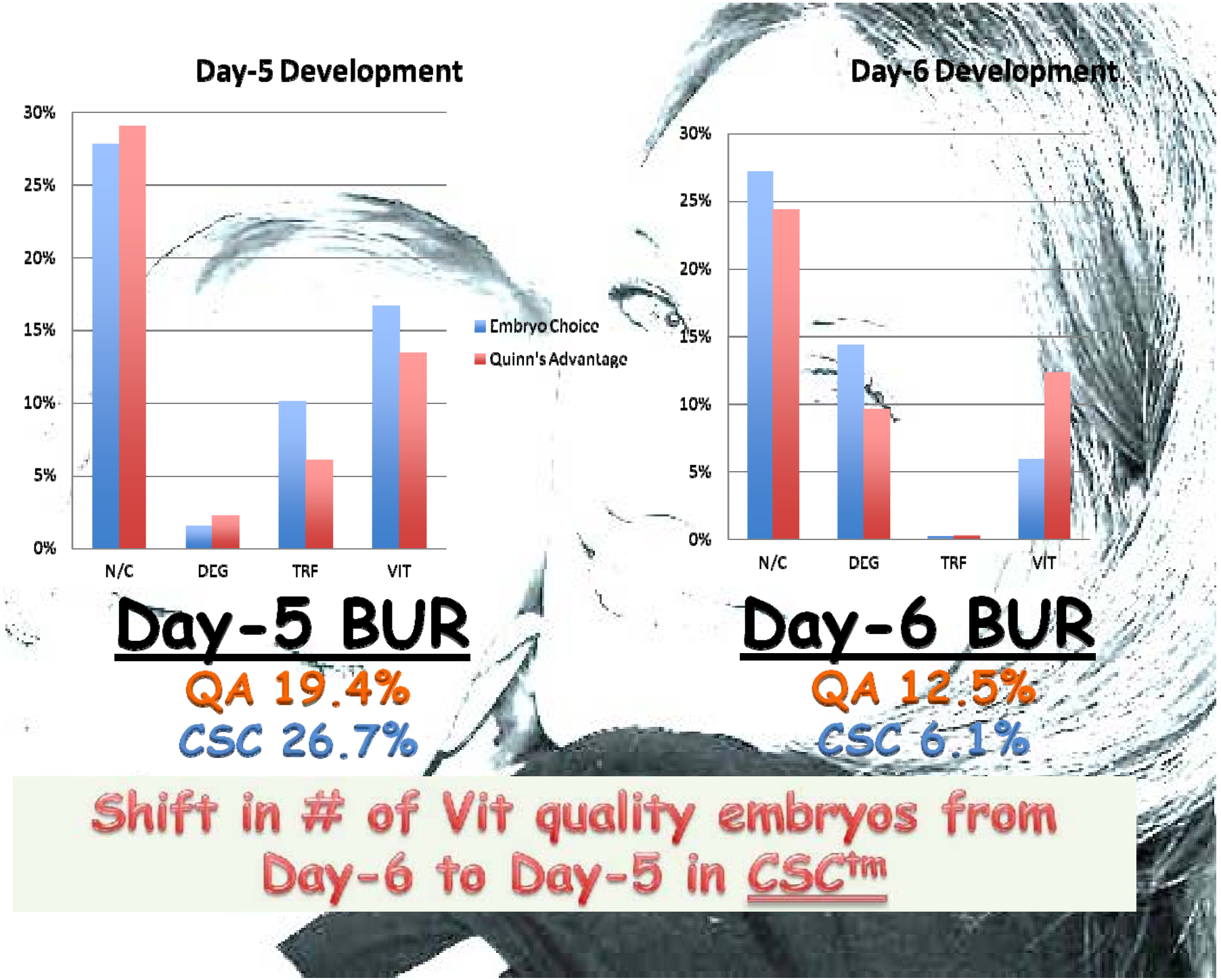
- Measure of blastocyst QUALITY, not quantity
- # Blastocysts of Transfer or Vitrification 'Quality' on Day-5/-6

>900 embryos from >100 patients

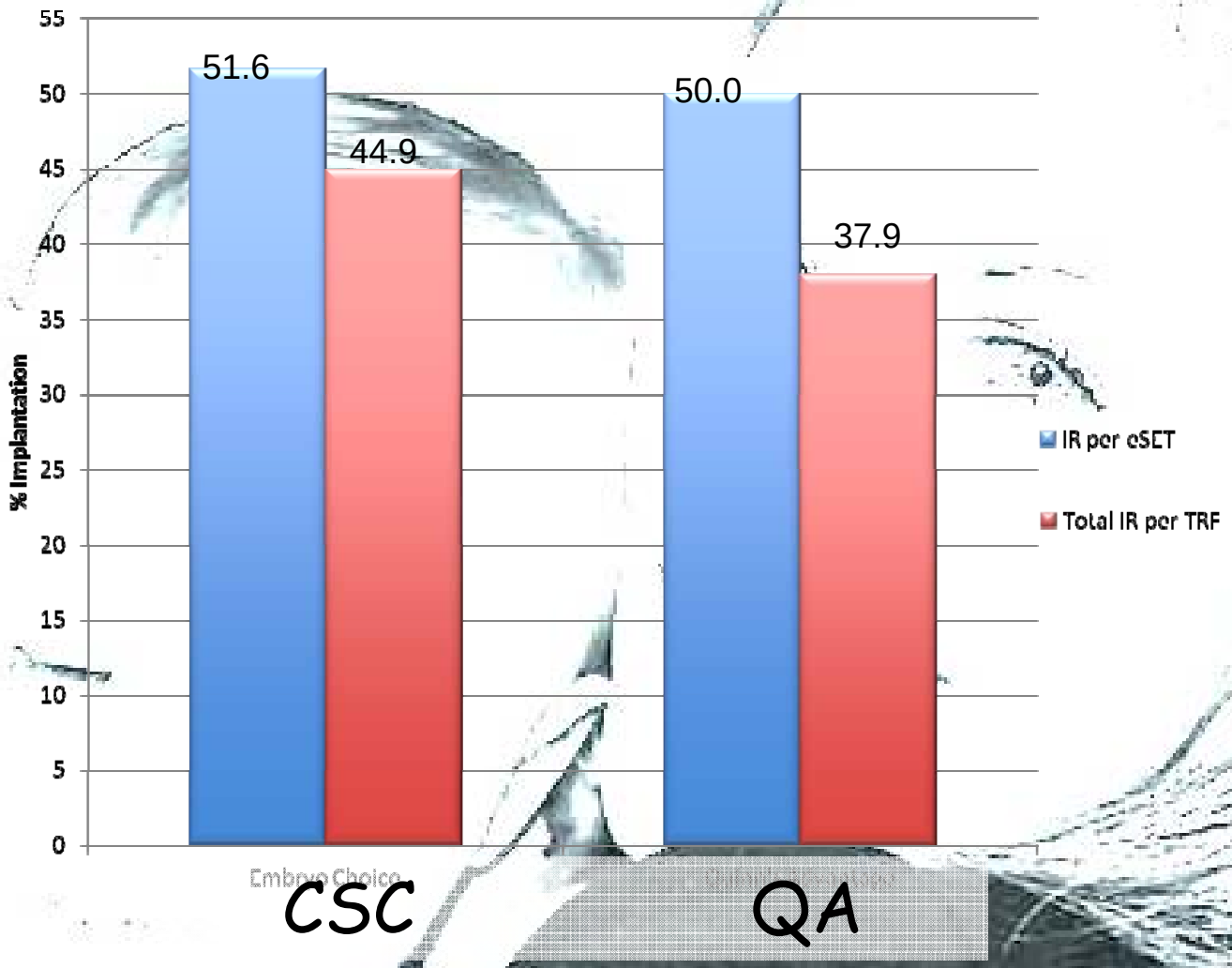
Overall BUR

Quinn's Advantage 36%

Continuous Single Culture 36%

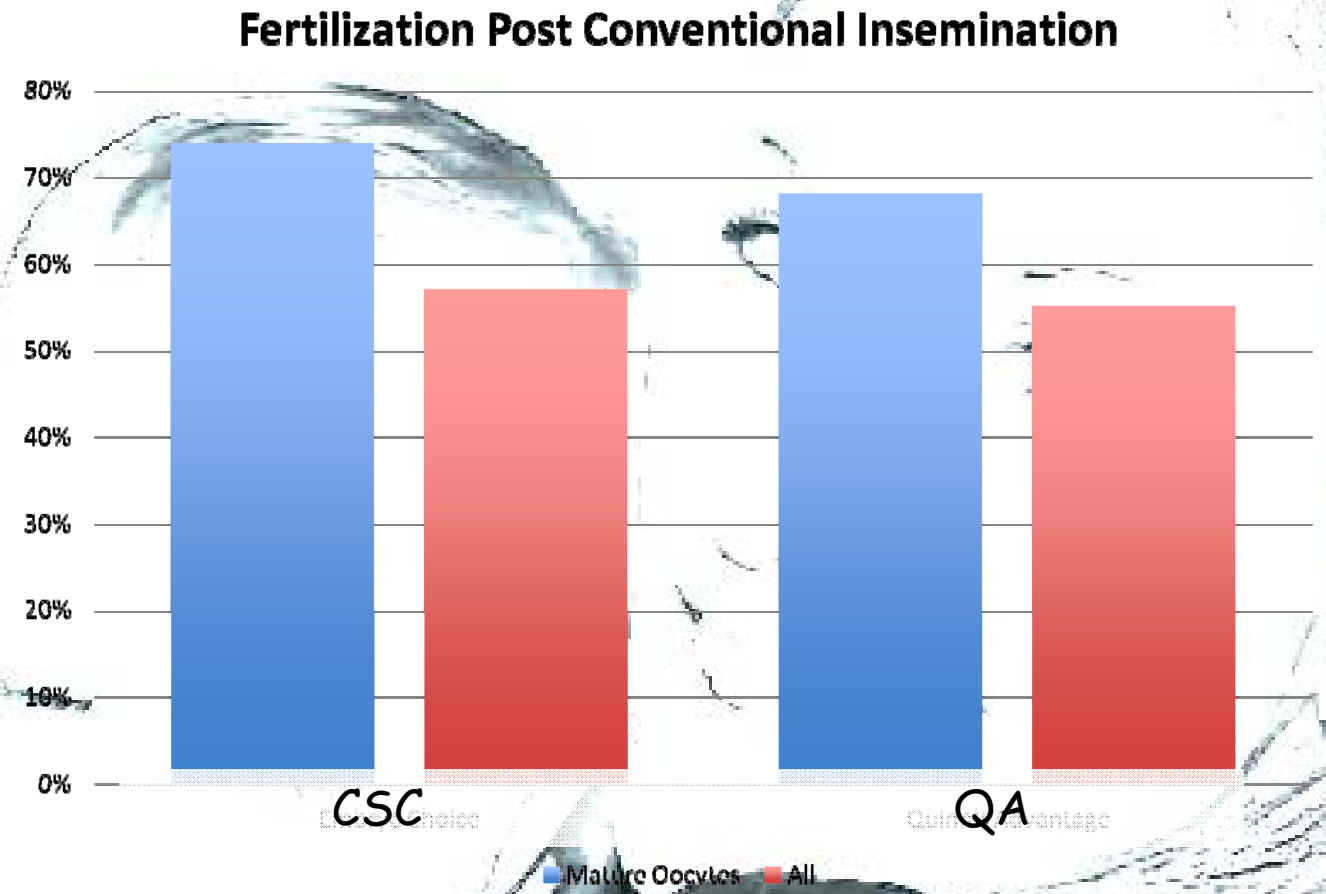

Day-5 Development
30%
25%
20%
15%
10%
5%
0%
N/C
DEG
TRF
VIT
Embryo Choice
Quinn's Advantage
Day-6 Development
30%
25%
20%
15%
10%
5%
0%
N/C
DEG
TRF
VIT
Day-5 BUR
QA 19.4%
CSC 26.7%
Day-6 BUR
QA 12.5%
CSC 6.1%
Shift in # of Vit quality embryos from
Day-6 to Day-5 in CSC™

Study Implantation Rates



Implantation Rates: eSET or All ETs

Fertilization in CSC™

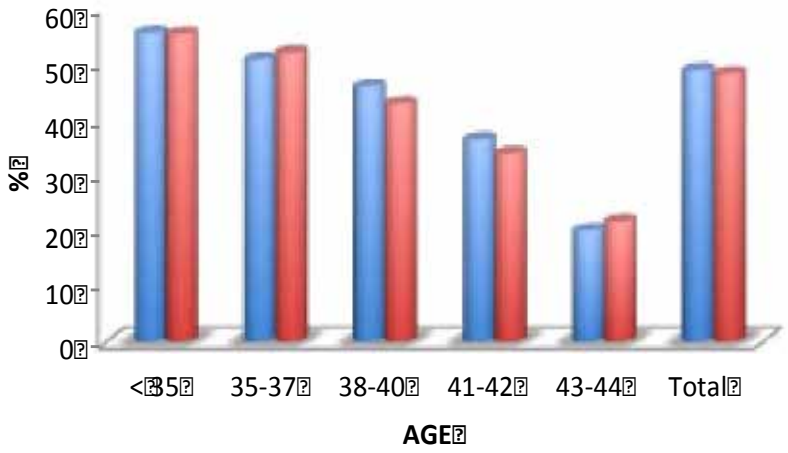


CSC™ appears to support normal fertzn events compared to HTF

Single Step Medium Use – Initial Lessons Learned Sept 2011

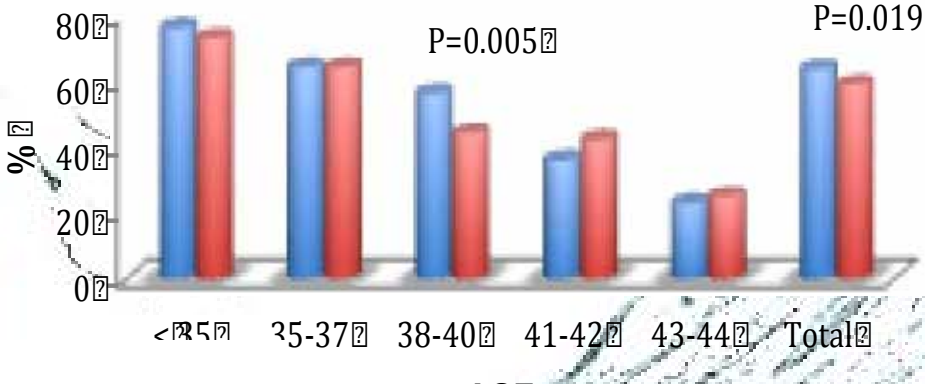
- Continuous Single Culture™ can be used as TRUE single step medium for uninterrupted culture -egg & embryo-
- Supports normal fertilization after conventional insemination & ICSI
- Implantation Rates at least comparable with sequential culture media
- Higher Blastocyst Utilization Rate (BUR) – overall 'faster development'

CSC™: SGF Oct-Dec 2011

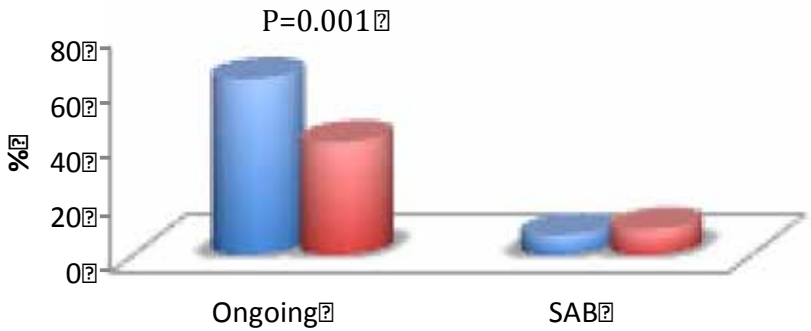


Clinical Pregnancies

892 CSC cf. 2423 QA



%Blastocyst ETs



FET Outcomes

SGF Jan 2011(QA/SAGE) vs. Jan 2012 (CSC/Irvine)

	2011Rock	2012Rock	2012Balt
Rertrievals	454	447	44
Eggs	6010	6331	650
Avg #/Patient	13.2	14.2	14.8
# MII	4805	4980	546
%MII	80.0%	78.7%	84.0%
Transfers	444	423	43
% Day-5 ET	25.7%	56.2%	58.1%
# Emb. Trans.	961	848	77
# eSET Trans.	56	98	15
% of Trans. eSET	12.6%	23.2%	34.9%
Avg # Emb Trans	2.2	2.0	1.8
Pregnant	227	233	24
% Pregnant/Retrieval	50.0%	52.1%	54.5%
% Pregnant/ET	51.1%	55.1%	55.8%
Multiple Preg Rate	40.5%	30.5%	33.3%
+ve FCA	323	303	32
Implant. Rate	34%	36%	42%

Some thoughts…

CSC™ ADVANTAGES

- ↑ Implantation/Pregnancy Rates?
- TRUE Single Step Medium
- Ideal for non-interrupted Time-Lapse applications
- Convenience (Ordering, Stock & Storage) Less waste
- Reduction in Cost - ~9% / IVF case
- Labor Reduction - ~15%/case (e.g. dishes 4hrs/day @ SGF)
- Reduced embryo manipulation/loss/contamination
- Reduction in '<u>embryo stress</u>': same medium/osmotic concentrations/pH/stable temperature
  - *'quiet embryo culture system'*

Less Labor = More Time to Play!

Preliminary Clinical Trial of "SSM3" (= CSC™) at Shady Grove Fertility

- Study conducted in three phases:

1) 25 patients — ⅛ of embryo cohort — SSM3(CSC)/SAGE QA
2) 15 patients — ⅜
3) 75 patients — ½

- Participant Criteria:
  - ≤ 36 years of age
  - ICSI or CONV insemination
  - Unexplained diagnosis excluded
  - ≥16 follicles
  - ≥10 zygotes (2PN)

- Fert^{zn} check Day-1: zygotes randomly selected

- Max 5 embryos / ½mL medium under oil in 5-well dish

- Cultured in MINC benchtop (Low O_2) @ pH 7.25 - 7.35

- Sibling embryos 'changed' to blastocyst medium early D-4

Clinical Observations: CSC™ at SGF

- CSC supplemented with 10% SSS (Irvine) – used within 24hrs of opening
- Complete transition to culture in CSC beginning Day-0 September 2011

-Oocytes retrieved & placed into pre-equilibrated 100µl CSC droplets

Post retrieval - oocytes 'changed-over' into fresh drop of CSC conventionally inseminated & cultured Overnight

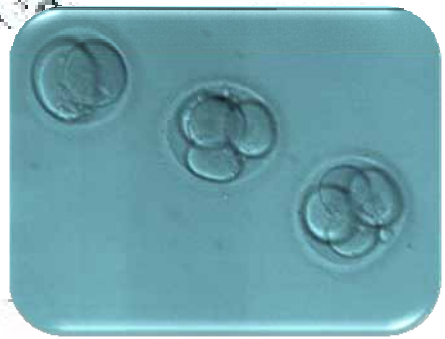
-OR-

ICSI then into fresh drop of CSC & cultured Overnight

- Day-1 Fertilization Assessments: embryos placed into fresh well 0.5ml CSC w/oil overlay ('4-well' Biogenics culture dish)

- Day-2 Cleavage Assessments: Embryos sorted by stage

- Maximum 5 embryos per well
  e.g. Well #1 has 5 x 4-cell
  Well #2 has 3 x 3-cell
  Well #3 has 1 x 2-cell



- After sorting on Day-2, Embryos stay in same well for additional 4 days or until chosen for Embryo Transfer or Vitrification

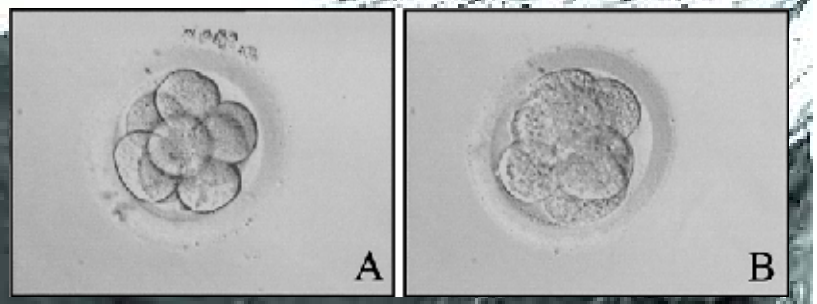
- No renewal of CSC

- No physical embryo Day-3/4 'change-over'

Observations with Embryo Choice™

- More embryos available for Day-5 vitrification
- No longer having to 'push' embryo transfer to Day-6 for slower developing embryos
- Eliminated Day-7 vitrification and culture
- Assessments based on morphology much more 'clear-cut' (either 'Hot or Not!')
- Some embryos noted to have cytoplasmic pitting & granularity*

*Significance unknown – observed in KSOM based single-step medium. EDTA? (Biggers, JD RBMonline 2002)



Observations of CSC™ at SGF

- **Embryos develop quicker in CSC™**

Similar to *in-vivo* development ?!?

Cell cycle appears to be 12-16hr earlier than with Sage QA

-Observe more compaction on Day-3

QA CSC

-Less Cellular and more Cavitating embryos & greater # of Early Blastocysts on Day-4

QA CSC