Maximizing successful implantation: TLI, PGS and Annexin A5 M2 haplotype

Simon Fishel
CARE Fertility Group
Ovarian Club
Sept 15th 2017
Maximizing the most efficient route – SAFELY!

Multiple Embryo Transfer
\downarrow
Multiple Pregnancy
High Risk

\downarrow

Single Embryo Transfer
\downarrow
High Rates of Live Birth

EMBRYO SELECTION: ENDOOMETRIAL RECEPTIVITY

\downarrow
OHSS

UK HFEA Directive!
The embryo & the female reproductive system

- Ovary
- Fimbria
- Uterine cavity
- Fallopian tube
- Ovarian artery
- Arcuate artery
- Radial artery
- Basal artery
- Spiral artery
- Endometrium
- Myometrium
- Uterine artery
- Endocervical canal
- Cervix
- Endocervix
- External os
- Ectocervix
- Vagina

Hormones:
The Embryo – what we know, and don’t

Percentage of Monosomies and Trisomies

*Aneuploidy testing
• Time-Lapse Imaging
  • Morphogenic anomalies
  • Morphokinetic algorithms
• Metabolic (‘quiet embryo’) assessment
• Gene anomalies

Data provided by Genesis Genetics
Selecting embryos – Time Lapse Imaging

• There’s Time-Lapse Imaging......
  (using it as a closed microscope)

• Predicting blastulation

• Live Birth Algorithms
– Meta-analysis of RCT of morphokinetic algorithms vs single time point morphology for embryo selection

– Outcome measures:
  • Clinical pregnancy, Live birth, EPL, Stillbirth

– 5 RCTs (n=1637)
  • LB: 44.2% vs 31.3% (OR= 1.668; P<0.009)
  • EPL: 15.3% vs 21.3% (OR= 0.662; P<0.019)
  • Stillbirth: NS

“time-lapse is shown to significantly improve overall clinical outcome”

Live births after embryo selection using morphokinetics versus conventional morphology: a retrospective analysis.

Fishel S¹, Campbell A², Montgomery S³, Smith R⁴, Nice L⁵, Duffy S³, Jenner L⁶, Berrisford K⁶, Kellam L⁶, Smith R⁷, D'Cruz J⁸, Beccles A².
TLI – computing morphokinetics

- Time to cell stages
- Durations
- Dynamics e.g.
  - Pronuclei
  - Reverse cleavage
  - Compaction
  - Blastulation
  - Strings
‘CARE TLI’ – Evolution of the algorithms

VP = t1 - tPNf
CC1 = t2 - t2PB
CC2 = t4 - t2
CC3 = t8 - t4
CC4 = t16 - t8
S2 = t4 - t3
S3 = t8 - t5

Compaction = tM - tSC
Blastulation = tHN - tSB
Collapse = tBCend(n) - tBCi(n)
Time-Lapse Imaging – constant visual of morphodynamics

- Intracytoplasmic
  - Granulation
  - Refractile bodies
  - Vacuolation
  - Smooth Endoplasmic Reticulum Clusters
  - Multi Nuclei
  - Pronuclei

- Extracytoplasmic
  - Polar body
  - Perivitelline space
  - Zona Pellucida
  - Fragments

Imaging - Single v 300/day
Using Time-Lapse data only when outcome known (using Live Birth data only)

SET

- KID positive
- KID negative

Det

- KID negative
- (KID Positive)

No KID

- One Live Birth
- No Live Birth

Pregnancy loss

- Two Live Birth
- No LB KID

Using Time-Lapse data only when outcome known

>2,000 LIVE BIRTHS!
Study Data: TLI v Standard Incubation (SI)

>14,000 Patients, >23,000 Cycles: Live Births (=delivery events)

TLI Live Births n= 1,007; ‘SI’ – n= 6,874

Mean ♀ Age:

TLI = 36.9

v

SI = 35.3
Inclusive Confounding Variables

- Embryoscope (y/n)
- Patient age (<38/38+)
- Day of embryo transfer
- No. embryos transferred
- Patient type
- Donor age
- Total previous cycles
- Total no. previous live births
- ICSI (y/n)
- Total no. miscarriages
- Intralipid (y/n)
- Duration of infertility
- Total # ectopic
- BMI
- AMH
- AFC
- Gonadotropin type
- Gonadotropin dosing days
- Gonadotropin total dose
- # Eggs collected
- # M2 eggs
- Ratio M2 eggs to total
- # M2 eggs fertilised
Confounding variables included in the model

- Embryoscope (y/n)
- Patient age (<38/38+)
- Day of embryo transfer
- No. embryos transferred
- Patient type
- Donor age
- Total previous cycles
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- Ratio M2 eggs to total
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24,000 records of treatment

- 21,379 Standard incubation treatments
- 2,527 Embryoscope treatments
- 14,878 unique patients
Live Births (delivery events age <38)

TLI

SI

* P<0.0001

0.0
10.0
20.0
30.0
40.0
50.0
60.0

% # pos Beta

CAREfertility
Live Births (delivery events age <38)

CAREfertility
Live Births (delivery events age < 38)

TLI     SI

* P<0.0001

* P<0.0001

* P<0.0001

%                   

# pos Beta  # with >=1FH  Biochem Loss  # Implantations
Live Births (delivery events age < 38)

<table>
<thead>
<tr>
<th>Event</th>
<th>Percentages</th>
<th>P-Value</th>
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<tbody>
<tr>
<td># pos Beta</td>
<td>TLI: 50.0%</td>
<td>* P&lt;0.0001</td>
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<tr>
<td># with &gt;=1FH</td>
<td>SI: 45.0%</td>
<td>* P&lt;0.0001</td>
</tr>
<tr>
<td>Biochem Loss</td>
<td>TLI: 35.0%</td>
<td>* P&lt;0.0001</td>
</tr>
<tr>
<td>Implantations</td>
<td>SI: 30.0%</td>
<td>* P&lt;0.0001</td>
</tr>
<tr>
<td># Clin miscarriage</td>
<td>TLI: 30.0%</td>
<td>* P&lt;0.0001</td>
</tr>
<tr>
<td># Live Birth Event</td>
<td>SI: 25.0%</td>
<td>* P&lt;0.0001</td>
</tr>
</tbody>
</table>

* P<0.0001 indicates a statistically significant difference.
Live Births (delivery events age <38)

TLI SI

* P<0.0001

* P<0.0001

* P<0.0001

* P<0.0001

* P<0.001

* P<0.001

* P<0.001

# pos Beta # with >=1FH Biochem Loss # Implantations # Clin miscarriage # Live Birth Event babies/EmbTrd

%
LIVE BIRTH OUTCOME – all ages (CAREmaps, Deliveries v Standard, 6,874 Deliveries)

Live Births (delivery events) – All Ages

Overall

Single Blast

* P<0.0001
Live Births (delivery events) – All Ages

**Overall**
- TLI: 40%
- SI: 42%
- Uplift: 20%
- *P<0.0001

**Single Blast**
- TLI: 45%
- SI: 47%
- Uplift: 25%
- *P<0.0001
Hierarchical selection of embryos:

<table>
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<tr>
<th></th>
<th>tSB or relSBIVF ≤ 93.1 h</th>
<th>tSB or relSBIVF &gt;93.1 h dB ≤ 12.5 h</th>
<th>tSB or relSBIVF &gt;93.1 h dB &gt;12.5 h</th>
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<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
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</tr>
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</table>

**# ET**

- A: 373
- B: 297
- C: 93
- D: 80

**% LBR**

- A: 51.7
- B: 35.0
- C: 31.2
- D: 13.8

**Miscarriage (%)**

- A: 24.0
- B: 32.5
- C: 32.6
- D: 35.3

**Miscarriage (%)**

- A: 47.7%
- B: 26.2%
Strong evidence of an effect of embryo rank on the odds of live births.

- D << A (OR = 0.3046; P<0.010)
- D << B (OR = 0.428; P<0.01)
- B < A  (OR = 0.7114; P<0.01)
- C <A   (OR = 0.6501; P< 0.01)
- B > C  (OR = 1.09; P<0.01)
- C >> D (OR=2.135; p<0.01)

A has a 233% chance of LB compared to D.
TLI v morphology:

Grade 2:2 highest LB!

[Graph showing odds ratio compared to transfer grade 2:2]
Chromosome screening: PGS/PGT-A

- Largest single cause of IVF failure
- Main cause of miscarriage
### US CDC –SART data 2014 - Embryo ‘Success’

- **# Embryos Transferred:** 139,832
- **# babies born from those embryos:** 35,631

#### Age Group Distribution

- **<35:**
  - Non-PGS %: 12%
  - PGS %: 16%
- **35-37:**
  - Non-PGS %: 35%
  - PGS %: 37%
- **38-40:**
  - Non-PGS %: 26%
  - PGS %: 35%
- **41-42:**
  - Non-PGS %: 20%
  - PGS %: 20%
- **43-44:**
  - Non-PGS %: 15%
  - PGS %: 13%
- **>44:**
  - Non-PGS %: 10%
  - PGS %: 12%
- **TOTAL:**
  - Non-PGS %: 21%
  - PGS %: 12%

**Total babies born with PGS:** ≈16,780
US CDC – SART data 2014 Publication - Embryo ‘Wastage’?

- # Embryos Transferred: 139,832
- # babies born from those embryos: 35,631
Meta-analyses

Matched Cohort Studies of PGS in AMA Women
Clinical Pregnancy Rate (IR)

PGS vs. Routine Care
Clinical IR (Gestational Sacs/#ET)

Observational Studies

Clinical IR 1.78x more likely with PGS in observational studies
STAR Trial - Illumina

ASRM - 2107
The implantaing embryo and placenta

- Embryo and Endometrium forming the Placenta
- Impaired blood flow can cause
  - Implantation failure
  - Early or late miscarriage
  - Small for gestational age babies
  - Obstetric complications
Human placental anticoagulant protein: isolation and characterization.

Funakoshi T', Heimark RL, Hendrickson LE, McMullen BA, Fujikawa K.

Kaolin in the presence of cephalin and Ca²⁺. It also prolonged the factor Xa induced clotting time of platelet-rich plasma but did not affect thrombin-induced conversion of fibrinogen to fibrin. The purified placental protein completely inhibited the prothrombin activation by reconstituted prothrombinase in the absence of factor Xa, factor Va, phospholipid, and Ca²⁺. The placenta inhibitor had no effect on prothrombin.

<table>
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<tr>
<th>Approved Symbol</th>
<th>Approved Name</th>
<th>Previous Symbols</th>
<th>Synonyms</th>
<th>Chromosome</th>
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<tbody>
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<td>ANXA1</td>
<td>annexin A1</td>
<td>ANX1, LPC1</td>
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<td>annexin A13</td>
<td>ANX13</td>
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</table>

Protein Annexin A5
CONCLUSIONS: Antiphospholipid antibodies reduce the levels of annexin V and accelerate the coagulation of plasma on cultured trophoblasts and endothelial cells. The reduction of annexin V levels on vascular cells may be an important mechanism of thrombosis and pregnancy loss in the antiphospholipid-antibody syndrome. Levels of annexin V, a phospholipid-binding protein with potent anticoagulant activity, are markedly reduced on placental villi from women with this syndrome. Hypercoagulability in such women may therefore be due to the reduction of surface-bound annexin V by antiphospholipid antibodies.
Relationship between Cause and Effect

- Causes of IVF failure are heterogeneous
- ‘Treatment’ given according to outcome e.g. pregnancy loss
- Single largest cause is aneuploidy, but...
- Placenta-Mediated Pregnancy Complications - PMPC (e.g. RPL, FGR, SGA and PE) share features e.g. implantation impairment, placental infarction and microvascular thrombosis
- Suggests coagulation activation may be implicated in some losses
Need to Stratify by Mechanism

- Failure to stratify by common mechanism leads to:
  - Potential treatment discarded incorrectly for lack of apparent effect
  - Dilution of positive effect in a patient subset
  - Having an effect – for the wrong reason!
Targeting use of LMWH

Identification of a biomarker(s) for those patients sharing a common underlying disease process permits targeted, appropriate treatment.
Annexin A5 gene variants
Initially found through systematic sequence analysis of the ANXA5 gene in 70 patients in Northern German origin with RPL (>2 foetal losses), non-carriers of P'Tm and/or FVL mutations.

To Date: * >4500 patients of * Different ethnicities and various obstetric complications have been genotyped

Associated risk in Anxa5 M2 Haplotype carriers is 2.4-4.0 for the various thrombophilia-related phenotypes
Studies showing ANXA5 M2 Linked to Significant Reduction in Annexin A5 Production

- Reporter gene assay in M2 women w. RPL, showed in vitro reduction of the ANXA5 promoter to 37-42% of normal levels i.e. 60% reduction. (Bogdanova et al 2007)

- Expression of ANXA5 mRNA in placentas from M2 carriers with PE and FGR significantly lower (three fold) than normal non carriers. (Chinni et al 2009)

- M2 resulted in reduction of placental mRNA levels in heterozygous carriers to 42% of normal non carriers. (Markoff et al 2010)

- Associated with transcriptional ANXA5 down regulation in FGR pregnancies. (Sifakis et al 2010)

- ANXA5 mRNA expression in PE samples lower than 1% of its expression in normal samples (p<0.001), ANXA5 protein levels in PE samples were approximately 65% of the average normal expression. ANXA5 down regulation could be part of the pathophysiology of PE. (Gourvas et al 2014)
CARE Pragmatic Observational Study

Tested and treated patient couples cohort  N=103
696 patients (369 couples) screened prospectively
All M2 positive couples female treated w LMWH

Female given LMWH (40 mg daily) from day of oocyte retrieval for fresh embryo transfer and on day of embryo transfer if frozen embryo transfer for minimum of 12 weeks but advised to delivery

Pregnancy outcomes analysed

Untreated couples (Yardstick cohort, N=77) screened retrospectively after failure (screened 171 female/154 male partners) for M2 after IVF failure

Not treated previously w LMWH

Pregnancy outcomes analysed

Retrospective contemporary control group N=1000
103 couples selected randomly from this group as paired untreated control group

Unscreened and untreated w LMWH

Pregnancy outcomes analysed

CAREfertility
Controls were:
0-4 years infertile, first time IVF and 5% RPL

Treated Group:
5-9 years infertile, 2-3 failed cycles and 17.5% RPL
Multicentre study of the clinical relevance of screening IVF patients for carrier status of the annexin A5 M2 haplotype

Simon Fishel a,*, Rashmi Patel b, Alison Lytollis c, Jeanette Robinson c, Mary Smedley c, Paula Smith d, Craig Cameron e, Simon Thornton e, Ken Dowell e, Glenn Atkinson e, Adel Shaker e, Philip Lowe e, Rahnuma Kazem c, Sandra Brett f, Anna Fox f

Research Paper

Precision Medicine in Assisted Conception: A Multicenter Observational Treatment Cohort Study of the Annexin A5 M2 Haplotype as a Biomarker for Antithrombotic Treatment to Improve Pregnancy Outcome

Simon Fishel a, Deborah Baker b, Janine Elson a, Maha Ragunath c, Glenn Atkinson d, Adel Shaker e, Ahmed Omar f, Rahnuma Kazem g, Ashley Beccles a, Ian A. Greer h
CARE Published Studies on the Incidence in our IVF Population

- Male 26%
- Female 24%
- Both partners carriers ~10%
- Couples 44%
- Couples w. unexplained infertility 37%
- Co-exists w. male and female infertility patients (27%)
- Co exists w PCOS (35%)
Risk is embryonal – passaged equally from ♂ or ♀
Does searching for the viable embryo matter?
<table>
<thead>
<tr>
<th>Clinic</th>
<th>CS</th>
<th>Total Embryos</th>
<th>ET</th>
<th>OR</th>
<th>Clinical Preg (%)</th>
<th>% per Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARE Nottingham</td>
<td>313</td>
<td>327</td>
<td>279</td>
<td>297</td>
<td>169</td>
<td>51.7%</td>
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<tr>
<td>CARE Northampton</td>
<td>276</td>
<td>310</td>
<td>252</td>
<td>272</td>
<td>151</td>
<td>48.7%</td>
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</tbody>
</table>

### CLINICAL PREGNANCY RATES

<table>
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<tr>
<th>Clinic</th>
<th>CS</th>
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<th>ET</th>
<th>OR</th>
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<th>% per Embryos</th>
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<tbody>
<tr>
<td>CARE Nottingham</td>
<td>529</td>
<td>670</td>
<td>483</td>
<td>520</td>
<td>249</td>
<td>37.2%</td>
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<tr>
<td>CARE Northampton</td>
<td>656</td>
<td>755</td>
<td>577</td>
<td>621</td>
<td>280</td>
<td>37.1%</td>
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</table>

### CLINICAL PREGNANCY RATES

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<tbody>
<tr>
<td>Nat Ave</td>
<td>33.4%</td>
<td>24.5%</td>
<td>18%</td>
<td>15%</td>
<td>12%</td>
<td>37.9%</td>
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### US CDC SART

<table>
<thead>
<tr>
<th>All Ages</th>
<th>Nat Ave</th>
<th>US CDC SART</th>
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<tbody>
<tr>
<td>33.4%</td>
<td>24.5%</td>
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<tr>
<td>All Ages</td>
<td>33.4%</td>
<td>24.5%</td>
<td>18%</td>
<td>15%</td>
<td>12%</td>
</tr>
</tbody>
</table>
Conclusions

- Conventional morphologic & kinetic observations are inadequate to maximise live birth rates
- Time-Lapse Imaging (TLI) significantly improves live birth rates above conventional embryology for patients aged <38
- Women >37 benefit less from TLI, probably due to aneuploidy – (although more cleavage anomalies: deselection may be an advantage)
- Hierarchical Selection (Ranking) critical to maximising success
  - Evidence for Grade A diminishing the effect of age
- Does PGS reduce TTLB - - - - -?
- PGS testing and TLI are not necessarily mutually exclusive due to TLI visible morphological anomalies
- Important to continue search for genetic markers of viability