Time lapse & embryo aneuploidy - an indirect method to replace PGS?

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Overview

- CARE Fertility background and approach
- The EmbryoScope & PGS
- Published aneuploidy risk classification model
- Blastocyst Selection model V 2+3
- The evolution of CARE’s early cleavage model V3
- Concluding remarks
Who is CARE Fertility?
Dynamic versus static methodology

More information
Single v >100/day
EmbryoScope at CARE

- First ES installation May 2011
- 10 instruments in 6 units
- Global medium / low oxygen
- Nearly 2000 ES cycles
- >7500 fully annotated embryos
- >1000 ‘KID’ embryos
What is our approach?

- Standardised, consistent & complete annotation
- Open minded ethos
- Regular review of data
- PGS/MK studies
- Evolving models
Key questions...

• Is there a correlation between morphokinetics and blastocyst ploidy?

and if so,

• Can EmbryoScope™ help to classify the risk of aneuploidy in embryos?

CARE fertility
98 blastocysts, we studied 20 variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPNfaded</td>
<td>Time for PN fading (hours)</td>
</tr>
<tr>
<td>t2</td>
<td>Time from insemination to divisions to two cells complete (hours)</td>
</tr>
<tr>
<td>t3</td>
<td>Time from insemination to divisions to three cells complete (hours)</td>
</tr>
<tr>
<td>t5</td>
<td>Time from insemination to divisions to five cells complete (hours)</td>
</tr>
<tr>
<td>t8</td>
<td>Time from insemination to divisions to eight cells complete (hours)</td>
</tr>
<tr>
<td>tSC</td>
<td>Time from insemination to start of compaction (hours)</td>
</tr>
<tr>
<td>tM</td>
<td>Time from insemination to morula (hours)</td>
</tr>
<tr>
<td>tSB</td>
<td>Time from insemination to start of blastulation (hours)</td>
</tr>
<tr>
<td>tB</td>
<td>Time from insemination to Blastocyst formation complete (hours)</td>
</tr>
<tr>
<td>tEB</td>
<td>Time from insemination to expanded blastocyst (hours)</td>
</tr>
<tr>
<td>tHB</td>
<td>Time from insemination to hatched blastocyst (hours)</td>
</tr>
<tr>
<td>cc2</td>
<td>The time period of the second cell cycle (t3-t2)</td>
</tr>
<tr>
<td>cc3</td>
<td>The time period of the third cell cycle (t5-t3)</td>
</tr>
<tr>
<td>s2</td>
<td>The time period of the synchrony of the first cell cycle (t4-t3)</td>
</tr>
<tr>
<td>s3</td>
<td>The time period of the synchrony of the first cell cycle (t8-t5)</td>
</tr>
<tr>
<td>Blastulation</td>
<td>The time period of blastulation (tB-tSB)</td>
</tr>
<tr>
<td>MN2</td>
<td>Multinucularity at the two cell stage (TRUE/FALSE)</td>
</tr>
<tr>
<td>MN4</td>
<td>Multinucularity at the four cell stage (TRUE/FALSE)</td>
</tr>
<tr>
<td>1-&gt;3</td>
<td>Direct cleavage from one to three cells</td>
</tr>
<tr>
<td>2-&gt;5</td>
<td>Direct cleavage from two to five cells</td>
</tr>
</tbody>
</table>
Data collection

- 98 ICSI EmbryoScope blastocysts screened
- **Aneuploid** –
  - Single chromosome (30)
  - Multiple (30) chromosomes
  - Euploid (38)
- Mean age 38 years
- Varied indications for PGS
Specific delays in aneuploid v euploid

* P<0.05 ** P<0.01 (MWW test), n=98

- tSB: time from insemination to start of blastulation (h)
- tB: time from insemination to reach ‘full’ blastocyst (h)
Are the MK data reliable?

- Annotations blind to ploidy
- Strict annotation policy
- Average of ~6h difference between euploid and aneuploid blastocyst groups.
  =38 EmbryoScope™ frames

CAREfertility
Two model candidates - tSB & tB
1. DEVELOPING THE ANEUPLOIDY RISK CLASSIFICATION MODEL

And

2. VALIDATING THE MODEL
1. Modelling – partition into 3 risk groups.

- Risk classification model used only these two MK variables.
- Embryos partitioned according to tSB & tB values.
- High, Medium and Low risk of aneuploidy
- Predictive power assessed - AUC of ROC
Overview of the model

Aneuploidy incidence per risk class:
- 69% overall
- 36% in low risk
- 61% in medium risk
- 100% in high risk

AUC of the ROC curve was 0.72, indicating predictive power.
2. Testing the Model

- Retrospective analysis of the MK data from transferred non-PGS blastocysts.

- Plotting each previously transferred blastocyst by tSB and tB (i.e. risk class) - ploidy unknown.

We need to look specifically and retrospectively at Known Implantation Data blastocysts ‘KID’.
Definition of ‘KID’

Single ET

- KID positive
- KID negative

Pregnancy loss

One Live Birth KID positive
No Live Birth KID negative

Double ET

- KID negative
- (KID Positive)
- No KID**

1 x implantations lost
2 x implantations lost

Two Live Birth KID positive
No LB KID
2 x LB KID negative

Compare rates of implantation / live birth by MK variables
Retrospective analysis of blastocysts selected (without PGS) – clinical pregnancy
Retrospective analysis of blastocysts selected (without PGS) – live birth!

KID (LB)

- Negative
- Positive

Risk levels:
- Low risk
- Medium risk
- High risk
Larger data - Aneuploidy risk model v2

Aneuploidy incidence per risk class (n=195)

- 45.8% in low risk
- 65.9% in medium risk
- 95.8% in high risk

118.1h

96.6h
Is this confounded by age?

- More aneuploidy in >40 group
- Increasing proportions of aneuploid blastocysts as theoretical risk increases both age groups.
- Risk classification valid
- Useful tool to rank embryos according to their implantation potential.
Modelling embryo implantation (n = 536)

![Graph showing embryo implantation rates](image)

**Figure 1:** Embryos from 27 clinics as they distribute into the three risk classification groups from Campbell et al. (2013a). Implanted embryos are shown with green circles and non-implanted with red crosses.

The grey area indicates that though the criteria seem universal, the actual timing intervals may be different between clinics.
Q: ‘An indirect method to replace PGS?’

- Model correlates to clinical outcome well.
- Blastocysts in low risk aneuploidy class – high clinical potential
- Blastocysts in high risk class – low clinical potential
- Widely applicable.
- Non invasive/indirect
- ?Cost effective

- Not as reliable as PGS
- Could compliment or be an alternative choice.

- Prioritise embryos for PGS
- Ethical/legal alternative to PGS
- Where no PGS service available

Answer: NO
So can we develop further?

- Aneuploidy risk ranking effective but can we go further?

- We considered outcome data to make a predictive selection model.

  **Blastocyst Selection Model version 3**

  - Outcome measure: Clinical pregnancy
CARE model ‘Blast3’\hspace{1cm} n=300

Definitions

t_{pnf} - Time to PN fade
t_{M} - Time to Morula
t_{SB} - Time to start Blastulation
rt_{SB} - (t_{SB}-t_{pnf})
rt_{M} - (t_{M}-t_{pnf})
KID – Known implantation data
FH – Foetal heart

Simple Model\hspace{1cm} t_{SB} + t_{M}

rt_{SB} < 75.8hrs

rt_{M} between 52.6-61.4hrs

Score 2
KID FH 63%
High implantation potential
Grade A

Score 1
KID FH 36%
Medium implantation potential
Grade B

rt_{M} <52.6hrs or >=61.4hrs

Score 0
KID FH 11%
Low implantation potential
Grade C

Exclusion Event
rt_{SB} >75.8hrs
EARLY CLEAVAGE EMBRYO SELECTION MODELS

Clinical pregnancy & live birth
Evolution of our early cleavage embryo selection models

V1: t2, CC2, t2, CC2

V2: t2, CC2, t4, CC2, t4

V3: t2, CC2, CC3, RELCC2

t3-t2
Significant variables in EC3 model

- CC2 \((t3-t2)\)
- CC3 \((t5-t3)\)
- REL CC2 \(\frac{(t3-t2)}{(t5-t2)} \times 100\)

2h IVF adjustment
What is ‘RELCC C2’

REL CC 2 \( \frac{(t3-t2)}{(t5-t2)} \times 100 \)

Optimum 44-47%
CARE multiplicative model ‘EC 3’ \( n=600 \)

**Definitions**
- **t2** - time to 2cell
- **CC2** - 2nd cell cycle \((t3-t2)\)
- **CC3** - 3rd Cell cycle \((t5-t3)\)
- **RelCC2** - \(\frac{(t3-t2)}{(t5-t2)} \times 100\)
- **KID** – Known implantation data
- **FH** – Foetal heart
- **LB** – Live birth

**Model EC3**
\( n=600 \)

**Exclusion Event**
- **CC2 <= 2.0hrs or CC3 <= 5.0hrs**

**Score 2**
- **RelCC2** 44-47%
- **KID FH** 31.0%
- **KID LB** 36.0%

- High implantation potential
  - Grade A

**Score 1**
- **RelCC2** 0-43.9%
- **KID FH** 17.3%
- **KID LB** 16.1%

- Medium implantation potential
  - Grade B

**Score 1**
- **RelCC2** 47.1-100%
- **KID FH** 22.1%
- **KID LB** 10.2%

- Medium implantation potential
  - Grade B

**Score 0.5**
- **RelCC2** 0-43.9% or 47.1-100%
- **KID FH** 9.9%
- **KID LB** 2.0%

- Low implantation potential
  - Grade C

**Score 0.25**
- **KID FH** 2.9%
- **KID LB** 1.6%

- Low implantation potential
  - Grade C

**Score 0**
- **KID FH** 0.5%

- Low implantation potential
  - Grade C

**Overall KID FH** 15.1%
Predictability is improving

AUC FOR LB
EC 1: N/A
EC 2: 0.77
EC 3: 0.80
• Strict annotation of time lapse images results in effective selection model building.

• The aneuploidy risk classification model cannot replace PGS but may compliment it.

• Embryo selection models should be based on LB outcome, ideally.

• Further work to fine tune models and search for other measures of embryo competence.

• Models may be clinic specific – timings need to be calculated from own data.
What next?

- Collaboration
- Consensus
- Best practice
- Better models
- Improving outcomes

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