Cryopreservation: Key Performances Indicators

Optimizing Human Gamete and Embryo Freezing

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Sede dell'Evento
Sala Convegni
Ordine dei Medici e degli Odontoiatri della Provincia di Genova
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1991: First IVF Unit in Scotland
1992: First baby born in Scotland (frozen-thaw)
2010: First PGD center in Scotland
2013: 512 retrievals ICSI/ IVF
65 PGD Cycles
5 embryologists, 5 physicians
Success rate overall: 38% FH. Patient < 37: 54%
Top 10 IVF unit in UK
Outline

• Key Performance Indicators (KPIs)
• Benchmarks
• Embryology Process Indicators
• KPIs for Cryobanking

PLEASE ASK QUESTIONS!
1978 - 2014
Almost 5 million babies have been born after ART (Worldwide)

Research has resulted in the development of more physiological culture media capable of maintaining the viability of the developing embryo.
but still

IVF technique is still inefficient, as only 10–30% of all embryos replaced in the uterus implant and result in a live baby (Martin JR et al., 2010)
It is crucial to minimize perturbations in the atmosphere around the embryo.
The key to success in the IVF laboratory?
• Dedication and Skilled Staff

• Excellent attention to details

• Adequate Equipment and proper use

• Interaction with clinicians/communication

Quality control and quality management

Indicators and benchmarks
Indicators: You can’t control what you can’t measure

ISO terminology: Key Performance Indicators (KPIs)

RELIABLE: Measure something useful.
   Define the process to be monitored

ROBUST: Measure only the intended process
   Minimize extra effects

ROUTINE: Data collection must not be arduous
   or a lot of extra work (easy)

“WORK SMARTER NOT HARDER”
Benchmarks: Definition

The continuous process of measuring performance against a strongest competitors or a leader in the field.
Definitions

BENCHMARKS:
• What should be achieved if all goes well (Quality Assurance)
• Aspirational value (best practice goal)

KPIs:
• Must reflect your GOALS
• Not necessarily achievable by all Labs
• Aspirational targets for Quality Improvement
• Minimum performance level (basic competency)

KPIs must be comparable:
Require precise definitions and standardized methods for their determination
Key Performance Indicators (KPIs) are used:

- To evaluate a new technique or process
- As minimum standard for the program as a whole
- To monitor ongoing performance as part of a QM system
- For benchmarking and quality improvement

There are no published KPIs for oocyte and embryo cryopreservation
<table>
<thead>
<tr>
<th>Laboratory</th>
<th>IVF</th>
<th>ICSI</th>
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<tbody>
<tr>
<td>% mature COCs at OPU</td>
<td>% (mature) MIIs after stripping</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate (&gt;2PNs)</td>
<td>Damage rate during ICSI</td>
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<tr>
<td>Normal fertilization rate (2PNs)</td>
<td>Degeneration rate on Day 1</td>
<td></td>
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<tr>
<td>Low or failed fertilization rate</td>
<td>Normal fertilization rate (2PNs)</td>
<td></td>
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<tr>
<td>Zygote cleavage rate</td>
<td>Zygote cleavage rate</td>
<td></td>
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<tr>
<td>Day 2 embryo development rate (assess at 44 ± 1 h)</td>
<td></td>
<td></td>
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<tr>
<td>Day 3 embryo development rate (assess at 68 ± 1 h)</td>
<td></td>
<td></td>
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<tr>
<td>% good or better embryos on Day 3</td>
<td></td>
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<tr>
<td>Day 5 blastocyst development rate (assess at 116 ± 2 h)</td>
<td></td>
<td></td>
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<tr>
<td>Utilization rate (embryos suitable for ET or cryopreservation)</td>
<td></td>
<td></td>
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<tr>
<td>Clinic</td>
<td>Positive pregnancy test (+ve βhCG)</td>
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<tr>
<td>Implantation rate per embryo transferred</td>
<td></td>
<td></td>
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<tr>
<td>Clinical pregnancy rate (fetal heart per ET)</td>
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The Process Control ("Chat")

- Upper control limit
- Upper warning limit
- Target value ("benchmark")
- Control mean
- Lower warning limit
- Lower control limit

**Control limits** = mean ± 3SD

**Warning limits** = mean ± 2SD

**Graph**
- Y-axis: Indicator (0–100)
- X-axis: Monthly indicators from Jan to Dec

Points on the graph represent the indicator values for each month.
IVF Embryology KPI

Triggers for action:
- FR <60%
- CR <90%
- Blast Rate <40%
KPIs for Cryobanking

For gamete and embryo cryobanking: all commonly used KPIs related to cryosurvival

- Assessed immediately post-thawing or post-warming
- Evaluated through normal function or development after thawing or warming
Cryobanking: Terminology

- **Cryopreservation** ("slow freezing"):
  - Freezing "frozen"
  - Thawing "post-thaw"

- **Vitrification**:
  - Cooling "vitrified"
  - Warming [not devitrification*] "post-warming"
Sperm Freezing KPIs

Process measures:

• Sperm concentration in accordance with the dilution ratio

• Timing of process steps

• Average number of units frozen per ejaculate

Cryosurvival:

Post-thaw assessment of semen / sperm specimens; generally-accepted values are:

• Sperm motility reduced by no more than 50%
No generally-accepted benchmarks are available even within the basic cryopreservation approaches, exist numerous sources of variability

- Biological: patients, stimulations, workstations, lab and culture systems
- Cryo / vitrification media formulations
- Processing protocols
- Packaging devices
- Cooling / vitrification methods / protocols
- Thawing / warming methods / protocols
The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting
% of oocytes that appear intact post-cryo: ≥ 75%

Fertilization rate per post-cryooocyte, using ICSI: ≥ 65% (same as for fresh oocytes)

Cleavage rate per fertilized post-cryooocyte: ≥ 95% (same as for fresh oocyte zygotes)
Embryo Cryo KPIs: Zygotes

• % of zygotes that appear intact post-cryo:
  \[ \geq 90\% \]

• % of post-cryo zygotes that cleave:
  \[ \geq 95\% \text{ (same as for fresh zygotes)} \]
Main factors that predict cryosurvival:

- Fragmentation
- Uneven cell number
- Dark, granular cytoplasm
- No / minimal fragmentation
- Even cell number
- “Good” cytoplasm, “shiny” membranes

But what about embryo developmental competence?
Embryo Cryo KPIs: Cleavage Stages

• % of embryos with at least 50% cells intact post-cryo: ≥ 80%

• % of embryos with all blastomeres intact post-cryo: ≥ 50%

• % of post-cryo embryos that cleave during overnight culture pre-ET: ≥ 75%

• Implantation rate per post-cryo embryo women < 38 years: ≥ 20%
“GOOD” blastocysts show better cryosurvival and subsequent developmental competence

- Always Day 5 blastocysts? Or Day 6?
- Optimum criteria to select blastocysts for freezing?
- Early Blast? Expanding blastocysts?
- Expanded blastocysts?
- Laser to Collapse?

IVF Labs use different methods and procedures for assessing embryos
Embryo Cryo KPIs: Blastocysts

- % of blastocysts that are more-or-less intact post-cryo: ≥ 80%
- % of blastocysts that re-expand within 3 h post-cryo: ≥ 50%
- % of early blastocysts that expand during overnight culture pre-ET: ≥ 75%
- Implantation rate per post-cryo embryo women < 38 years): ≥ 30%
CONSIDERATIONS

• The full exact cryo protocol, including denudation timing and method (for oocytes) should be considered.

• Insufficient general experience with Day 4 cryo to support KPI development.

• Endometrial preparation issues were not analyzed.

• All of the recommended KPIs are for cases that do not include manipulation for PGD.
CONCLUSION

- Cryo KPIs must reference Centre-specific fresh KPIs, as there are no international KPIs for fresh IVF/ICSI

- KPIs must always be considered within each Centre’s own Quality Management system

- Standardization of protocol details and training are essential for meaningful KPIs
In Memoriam: Professor Stanley Leibo

- Stanley Leibo passed away on March 25th at the age of 77 following a battle with melanoma cancer.

- Leibo has been a pioneering researcher in cryobiology.

- In 1972, working with D. Whittingham and P. Mazur, Leibo freeze successfully for the first time mouse embryos. Millions of children and live animals have since been produced from cryopreserved embryos.

In 2009 Leibo was honored with the Pioneer Award from the International Embryo Transfer Society: was described as "indisputably one of the best, and best known, cryobiologists."
Thanks for your attention

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Which Embryos to Freeze or Transfer

**Figure 2.** Comparison of the development of slow- and fast-cleaving embryos from fertilisation to the 4-cell stage. Pronuclear breakdown and first cleavage occur earlier in faster-developing embryos than in those with slow development. The time-span between the development of faster- and slower-developing embryos can be up to 8–12 hours. It can easily occur that faster- and slower-developing embryos have the same number of blastomeres at the time of morphological assessment performed before embryo transfer.

0PN = no pronucleus; 2PN = two pronuclei; 1PB = one polar body; 2PB = two polar bodies; 1C, 2C, 4C = 1-, 2- and 4-cell stage of the embryo; *Too early assessment of pronuclear breakdown does not help to distinguish early- and late-developing embryos. **Morphological assessment in the indicated period does not allow differentiation of early- and late-developing embryos. Assessment of early pronuclear breakdown or early cleavage can help to determine viability in case of embryos with similar morphological characteristics. [Author permission granted by P. Fancsovits]