

ESHRE Campus, Granada (Spain), 25-26 March 2010

## Quality Management Aspects of Sperm and Testicular Tissue Cryobanking

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### Commercial Conflicts of Interest Disclosure

David Mortimer has undertaken consulting work since 1986, and has been a full-time freelance consultant since October 1999.

He is currently President and co-owner of Oozoa Biomedical Inc, a Vancouver-based international consulting company providing services in the reproductive biomedicine field since March 2000.

He has performed work, on either commercial or a *pro bono* basis, for many clients and groups including: assisted conception clinics and sperm banks; biotechnology, pharmaceutical and ART products companies; academic institutions; researchers; government agencies; non-government organizations; professional associations and other bodies.

No commercial or financial interest has influenced the statements made in this presentation.

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### LEARNING OBJECTIVES

1. To understand the all-inclusive nature of a Quality Management System (“QMS”).
2. To know the essential elements of an effective QMS for a sperm and testicular tissue cryobank.
3. To be able to review operational systems for quality weaknesses.

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## QUALITY MANAGEMENT BASICS

- “Quality” is not synonymous with “luxury”.
- Manufacturing Quality = conformance to specifications set by the manufacturer, based on knowledge of what the customers want.
- Quality = fitness for use, with the focus on customers’ perceptions and opinions: the shift from being a “product out” company to a “market in” company.
- Quality = conformance to customer requirements.
- Quality management = all the systems and procedures needed to maintain and improve quality; together these constitute a Quality Management System (“QMS”).

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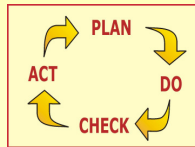
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## Levels of Quality Management

**Quality Control:** QC is the establishment of quality specifications for each quality characteristic, assessment of procedures used to determine conformance to these specifications, and taking the necessary corrective actions to bring them into conformance.

**Quality Assurance:** QA is the entirety of systematic activities implemented within a quality system (i.e. including QC) that are necessary to provide adequate confidence that a product or service will satisfy its required quality characteristics.

**Quality Improvement:** QI is that part of a quality systems focussed on continually increasing effectiveness and efficiency through actively seeking and pursuing QI opportunities.



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## Quality of Sperm Assessments

### EXPECTATIONS OF ACCURACY AND PRECISION

#### Traditional manual/visual methods (ESHRE, WHO)

- Establishment of method:  $\leq 5\%$  between replicates (precision)
- Training of new staff:  $\leq 5\%$  for 95% range of discrepancy
- Ongoing quality control:  $\leq 10\%$  for 95% range of discrepancy

#### CASA methodology

- Precision:  $\leq 5\%$  between replicates
- Accuracy:  $\leq 10\%$  for 95% range of discrepancy  
c.f. reference method

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## QMS Essentials

- **A Quality Manual**, including:
  - A Quality Statement
  - Policies and Procedures:
    - all technical procedures: pre-analytical, analytical & post-analytical
    - governance: organizational & administrative aspects, including clinical regulatory compliance
    - audits (internal and external)
- **Operational authority**: the Quality Manager, who may be supported by a Quality Committee.
- **Responsibility** – ultimately rests with the Sperm Bank Director.

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## Sperm Banking QMS Basics

- Establish and maintain all necessary procedures to ensure compliance with good tissue bank practice:
  - Ensure proper training and education of all personnel.
  - Evaluate the initial training and ongoing competency of personnel (via Internal QC, External QA and Proficiency Testing).
  - Establish & maintain monitoring systems to ensure good tissue bank practice (minimize procedural deviations).
  - Document control
  - Records maintenance
  - Labelling controls
  - Investigate (& document) any product deviations
  - Ensure appropriate corrective actions taken, including re-audits of deficiencies.

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## Routine Elements of a Sperm Bank QMS

- QC and QA are essential and must be routine.
- Environmental monitoring: temperature, ventilation, oxygen depletion, air filtration (particulates, micro-organisms, VOCs), infection control.
- Tolerance limits for quantitative technical procedures
- Monitoring of in-process controls.
- Monitoring of reagents and supplies.
- Monitoring of lab operational performance (e.g. via KPIs).
- Inspections and audits.
- Protocol qualifications, verifications and validations.
- Dealing with misconduct.

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## Internal Audits

- Purpose = regulatory compliance (procedural).
- Major components of operational system:
  - Donor evaluation and acceptance.
  - Donor and sperm testing.
  - Sperm collection, processing, preservation, packaging, labelling, storage, quarantining and distribution.
  - Records management, including computer system operations.
- Focussed audits on critical areas where problems have been identified.

**Note:** Audits must be performed by individuals with sufficient knowledge, training and experience relevant to the specific processes under review, but who do not have responsibility for the actual process(es) being audited.

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## Laboratory QA Policies [1/2]

- **Personnel:** Training and Proficiency Testing.
- **Environment control:**
  - Temperature of rooms and workstations
  - Air quality: HVAC system and workstations
  - Cleaning and sanitization
  - Infectious disease monitoring
  - Orderliness of the physical space and workstations
- **Equipment:**
  - Calibration: e.g. thermometers, heated stages, incubators
  - Verification: Performance within pre-defined limits of accuracy & precision  
Adherence to maintenance schedules  
Performance of cryogenic storage and LN2 supply tanks  
Autoclave operation and maintenance logs  
Computer systems and software

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## Equipment Validation & Verification

**Installation Qualification:** Performed by certified engineers (manufacturer or installation contractor) when a piece of equipment or physical plant is first installed to ensure that it is operating in accordance with its design specifications.

**Operational Qualification:** Performed by certified engineers on a regular basis (usually annual, sometimes biannual or biennial) to establish that equipment or physical plant is operating in accordance with its design specifications.

**Operational Verification:** Performed by a member of staff, using appropriate calibrated instruments, to verify that equipment or physical plant continues to function within its required operational parameters. Testing is repeated as often as the user considers necessary in order to be confident that the unit continues to function as per defined requirements.

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## Laboratory QA Policies [2/2]

- **Corrective action:** includes the evaluation of any failure of equipment calibration or performance having an adverse effect on sperm or testicular tissue being cryobanked.
- **Reagents and Supplies:**
  - Storage under proper conditions
  - Observance of expiry dates
  - Prompt discarding of expired or deteriorated materials
  - Maintain adequate stock levels with stock rotation
  - Proper labelling
  - Proper use and handling (e.g. aseptic technique to prevent contamination)
  - Full bi-directional traceability
  - Up-to-date Supplies Log (may be electronic)
  - Certificates of Assay are kept on file (may be electronic)
  - Visual inspection of all containers for damage or evidence of contamination prior to use, and immediately after filling.

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## Sperm Processing QA [1/2]

1. All methods are validated, have SOPs, and all equipment qualified as suitable and fit for the intended purpose.  
Choice of cryomedium, packaging device, cooling method.
2. Procedures include monitoring the reliability, accuracy, precision and performance of lab test procedures and instruments; quantitative sperm assessments meet the allowable uncertainty of measurement.
3. All donor communicable disease test results are reviewed prior to releasing specimens from quarantine and for use.
4. Every specimen is monitored through its processing to ensure that release criteria for quality, integrity and safety are met.
5. Specimen processing is performed in accordance with the pre-defined schedule.

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## Which Cryomedium To Use?

- Different men's sperm freeze differently using different cryomedia.
- There is no "universal best" cryomedium.
  - For donor banks, donors are essentially selected who freeze well by whatever CPM has been chosen as standard.
  - For client depositors, should we run a trial of several CPMs on the first sample to identify the best?
- Egg yolk confers significant benefits, but is now omitted from almost all commercial CPM formulations due to its biological origin.
- Differences exist between seminal sperm, washed sperm, epididymal sperm, testicular sperm and testicular tissue that affect cryosurvival.

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## Sperm Processing QA [2/2]

6. After adding cryoprotectant, semen specimens are re-evaluated for sperm concentration and motility; acceptable values are:
  - Sperm concentration in accordance with the dilution ratio, &
  - Sperm motility reduced by no more than 15% (donors only).
7. Post-thaw examination of semen specimens:
  - Sperm concentration in accordance with the dilution ratio, &
  - Sperm motility reduced by no more than 50% (donors only).
8. Any specimen suspected to be of questionable quality must be immediately quarantined / stored separately, pending further investigation and resolution.
9. Deviations from written SOPs must be justified by unusual circumstances, logged, and approved (ideally in advance) by the Sperm Bank Director.

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## Cryosurvival Criteria: Semen or Washed Sperm

Criterion	Client Depositors	Donors
Fresh: Abstinence	Recommendation only	Likely requirement
Fresh: Ejaculate volume	n/a	Minimum requirement?
Fresh: Sperm concentration	n/a	Minimum requirement
Fresh: Sperm motility	n/a	Minimum requirement
Fresh: Sperm morphology	n/a	Minimum requirement
Fresh: Other criteria, e.g. WBCs	n/a	Minimum requirements
Post-mix: Sperm concentration	±10% of expected based on dilution rate*	
Post-mix: Sperm motility	Ideally ≥85% of fresh	Must be ≥85% of fresh
Post-thaw: Sperm concentration	±10% of expected based on dilution rate*	
Post-thaw: Sperm motility e.g. % progressive, "Motility Index", etc.	Ideally ≥50% of fresh	Must be ≥50% of fresh Other minima likely
Between straw variation in concentration and motility <sup>§</sup>	Ideally below ±10%	Must be below ±10%

<sup>\*</sup>Combination of dilution, mixing & counting errors

<sup>§</sup>Determined post-mix or post-thaw as a QC exercise

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## Variable Cryosurvival Between Straws

- Poor mixing of semen and cryoprotectant medium, leads to localized inadequate or excessive (toxic) permeating cryoprotectant.
- Non-uniform cooling of straws; common causes:
  - Freezing in bundles (sometimes even inside a visotube).
  - Freezing vertically in a temperature gradient.
- Improper handling of straws while frozen and/or while thawing:
  - Differences in accumulated cryodamage (ice re-crystallization) due to variable warming events above -132°C.
  - Variable – or even different – thawing protocols.
- Counting errors, within or between observers (i.e. variability may not be real).

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### Cryosurvival Criteria: Testicular Tissue / Sperm

- No general criteria defined.
- What is expected?
- Are they necessary?

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### Sperm Donor QA

- Proper technical procedures and processes; all carried out in accordance with the cryobank's *Donor Policies Manual, Laboratory Procedures Manual* and *Quality Manual*.
- Donor screening must be performed in strict accordance with current SOPs (compliant with applicable regulations); external testing is under contract, and performed only by certified laboratories (with documentation reviewed / approved by the Sperm Bank Director).
- Quarantine of donor sperm is compliant with applicable regulations, and monitored by the Sperm Bank Director.
- Specimens are only released under the authority of a written release disposition statement from the Sperm Bank Director.
- All the above are subject to regular audit.

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### Client Depositor QA

- Proper technical procedures and processes; all carried out in accordance with the cryobank's *Donor Policies Manual, Laboratory Procedures Manual* and *Quality Manual*.
- Pre-freeze screening shall be performed in strict accordance with current SOPs (compliant with applicable regulations); external testing is under contract, and performed only by certified laboratories (with documentation reviewed / approved by the Sperm Bank Director).
- Storage of client depositor sperm complies with applicable regulations, including necessary separate storage of "known positive" specimens or specimens from "known positive" men.
- All the above shall be subject to regular audit.

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## Sperm Labelling QA

- **Nomenclature:** Must be consistent throughout (incl. measurement units).
- **Labels:**
  - Must adhere to the packaging under all conditions.
  - Must be clean, legible, indelible and robust.
  - Cannot be moved, removed, altered or obscured.
- **Currency of labels:**
  - Must be described (with example) in the SOP
  - Must be controlled (including archiving old label formats).
- **Checking labels:** By 2 members of staff prior to use to avoid errors.
- **Re-labelling:** *Only due to label error (or loss):*
  - Must be done in accordance with an SOP.
  - Requires per-event authorization by the Sperm Bank Director.
- **Controls:** Reviews to ensure accuracy and policy compliance.

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## Traceability QA

- **In-process:** All specimens are identifiable and traceable at any phase of their collection, processing or storage.
- **Client depositors:** Every unit is traceable to the client for whom it is being stored, in terms of provenance and use.
- **Donor-to-recipient:** Every specimen is be traceable in terms of the donor from whom it came, to the recipient who was either inseminated using the sperm, or who received embryos created using the sperm.
- **Recipient-to-donor:** Every specimen is traceable in terms of the recipient who was either inseminated using the sperm, or who received embryos created using the sperm, back to the donor from whom it came.

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## Sperm Storage QA

- Take all appropriate steps to protect specimens from being contaminated by the cryogenic storage environment, and from their contaminating that environment (effective biocontainment is the ideal).
- During storage, including handling during cryobank access and audit, movement between cryotanks, and prior to thawing, specimens must remain within the pre-defined storage temperature range; i.e. at a minimum, below -132°C.
- Unscreened specimens must held "in quarantine".
- Use of quarantine tanks must be rational.
- Known infectious specimens must be held "in isolation".
- If packaged in devices that are known to achieve effective biocontainment, quarantine and isolation are, in reality, unnecessary.

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## Cryotank QA



### Monitoring cryotanks

- Measure LN2 levels at regular (e.g. weekly) intervals *before* re-filling.
- Record and plot the values.

### Low level / temperature alarms

- Connect to a dial-out alarm or real-time monitoring system.



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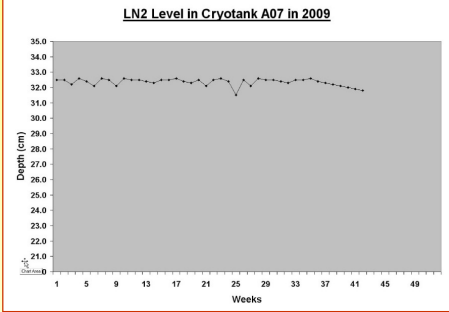
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## Monitoring Cryotank LN2 Levels

WEEK	Depth (cm)
1	32.5
2	32.2
3	32.6
4	32.6
5	32.4
6	32.1
7	32.6
8	32.5
9	32.1
10	32.6
11	32.5
12	32.4
13	32.4
14	32.3
15	32.5
16	32.5
17	32.6
18	32.4
19	32.3
20	32.5
21	32.1
22	32.5
23	32.6
24	32.4
25	31.9
26	32.5
27	32.1
28	32.6
29	32.5
30	32.5
31	32.4
32	32.3
33	32.5
34	32.5
35	32.6
36	32.4
37	32.3
38	32.2
39	32.1
40	32.0
41	31.9
42	31.8



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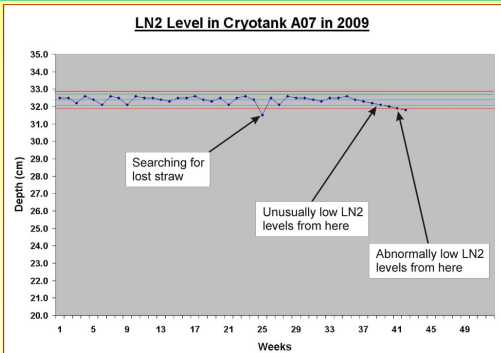
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## Monitoring Cryotank LN2 Levels



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### Sperm Distribution QA

- Shipments are prepared and transported only as per SOPs.
- Sperm are provided only in accordance with applicable regulations (e.g. only to medical practitioners, or upon their written request).
- Transport containers:
  - Must be validated to maintain the required refrigeration conditions.
  - Packaging must maintain the integrity of the shipping container, sperm survival, and prevent (cross-)contamination.
  - Dry shipper tanks must be maintained cold between uses for maximum life expectancy and to reduce the risk of failure.
- Inspection, before shipping and upon receipt:
  - Containers are intact and secure.
  - Specimen labelling is accurate.
  - Package inserts and other required documentation are present.
  - Addressing and package labelling are correct.
  - Verify temperature loggers or tell-tales.

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### Specimen Return QA

- All returns must be pre-authorized (by the Sperm Bank Director).
- Donor specimens must not be returned into "open stock".
- Donor specimens may be returned for the recipient's own later use.
- Client depositor specimens may be returned for their own later use.
- Relevant parties are notified of any problems with return shipments; when appropriate, specimens are discarded.
- All returns are documented.
- The "Returns Log" is audited regularly.

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### Donor Specimen Recall QA

- Must be initiated in accordance with a formal policy.
- Must be performed as per an SOP.
- Must be logged and documented.
- Records must show the completeness, or otherwise, of a recall.
- The documentation of a recall must be reviewed by the Sperm Bank Director.
- Recall events must be subject to audit.

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## Records QA

- All records must be confidential, complete, legible and indelible (e.g. handwritten records are in ink, *not* in pencil).
- All entries must be signed and dated by the person performing the activity being documented.
- Each specimen must be identified by at least two unique identifiers, one of which shall be the donor identifier, and one of which shall be numeric.
- Records must be held secure from unauthorized access, and protected as far as possible from accidental destruction.
- Records must comply with applicable regulations for content and format.
- Records must be maintained for a period of time at least compliant with applicable regulation.

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## Computer and Data QA

- Access to computers and data:
  - Only authorized persons have access to computers where data and records can be accessed.
  - Each staff member must keep their log-on password secret, and change them if they suspect that another person might know.
  - Do not leave a computer unattended when someone is logged-on.
  - Only authorized software can be installed.
  - Violations of the above should be considered disciplinary offences.
- Custom software and data analysis spreadsheets must be validated, and documented, prior to use.
- Data entry: Must be verified for accuracy to reduce errors.
- Data backup: Up-to-date and secure backups of all data must be maintained, including provision for off-site, secure (encrypted) backups.

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## Reporting QA

### External Reports: e.g.

- Adverse reactions
- Adverse events / incidents
- Governance matters (e.g. changes in ownership / direction, fiscal / taxation matters, etc)

### Internal Reports: e.g.

- Adverse events / incidents (= ISO "non-conformities")
- "Near-misses"
- Regular QMS reports
- Audits

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## TAKE-HOME MESSAGES

**Sperm banking is no different to any other biomedical laboratory service:**

- Quality must be inherent in every step of every process; it must be integral, it cannot be “added-on”: hence the importance of process control and systems management.
- Quality is everyone’s business, but leadership is vital.
- Risk management is an essential principle in any QMS.
- Adequate resources must be provided for “quality” activities.
- A QMS can only operate effectively within a “culture of quality” – i.e. a permissive, enabling environment, where errors are seen as opportunities for improvement.

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