The work of a fertility specialist

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Scope of work

• Evaluation and diagnosis of the infertile couple

• Controlled ovarian hyper-stimulation and oocyte retrieval

• Sperm preparation and oocyte insemination

• Assessment of fertilisation and embryo culture

• Pre-implantation genetic screening and diagnosis

• Embryo transfer and cryopreservation
Diagnostic assignment and cause of infertility
Obesity and infertility in women
Median Age for mothers (Australia)
Aneuploidy and age

Hunt and Hassold (2001)
Nature Reviews Genetics,
2 280-291
Scale & origin of aneuploidy

Incidence of aneuploidy during development

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>Sperm</th>
<th>Oocytes</th>
<th>Pre-implantation embryos</th>
<th>Pre-clinical abortions</th>
<th>Spontaneous abortions</th>
<th>Stillbirths</th>
<th>Livebirths</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1–2%</td>
<td>~20%</td>
<td>~20%</td>
<td>?</td>
<td>35%</td>
<td>4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>6–8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Incidence of aneuploidy

Effects of paternal age on sperm quality

Male Reproductive Aging

Sexual function & activity
Age
Nutrition & lifestyle
Oxidative stress
Telomere shortening
Inflammatory & immune diseases
Genetics & genome stability
Decreased hormones

Epigenetic events during spermatogenesis

Fig. 1: Epigenetic events during spermatogenesis. In primordial germ cells (mitosis), DNA methylation occurs to set up the paternal specific imprints. Phosphorylation (in mitotic cell) occurs to assist in both recombination and XY body formation. Deacetylation, acetylation and incorporation of H2A.Z and H3.3 variants are all involved in XY body formation. Hyperacetylation occurs during spermatogenesis to assist in the Histon-Proline exchange. Spermatogenesis can also give rise to chromosome non-disjunction during its mitosis I and II along with double strand breaks, abnormal histone modification and alteration in the expression of miRNA and other non-coding RNAs. DNA fragmentation is the consequence of apoptosis following double strand breaks or abnormal proliferation during spermatogenesis.

Sperm DNA damage with age

$r = 0.56; P < 0.0001$
Genetic causes of male subfertility

- Chromosomal (eg. Klinefelter’s XXY)
- Translocations & Meiotic errors
- Genome instability
- Point mutations
- Duplications & Deletions
Azoospermia Factor (AZF) regions

Dada et al (2011) 
Open Reprod Sci J 
3: 42-56
Laboratory investigations: Female

- FSH (Day 2)
- AMH
- Oestradiol
- Progesterone
- Prolactin
- Testosterone
- AsAb
- Kremer test

- Ovarian reserve
- Ovarian reserve
- Folliculogenesis
- Ovulation
- Prolactinaemia
- PCOS
- Immunoinfertility
- Cervical hostility
Laboratory investigations: Male

- Semen volume
- Semen pH
- Sperm density
- Sperm motility
- Sperm shape
- Agglutination
- Sperm DNA
- FSH

- Retrograde ejac’n
- Occlusions
- Spermatogenesis
- Sperm transport
- Sperm binding
- Sperm function
- Sperm quality
- Spermatogenesis
## Functional evaluation of sperm populations – the semen analysis

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2.0</td>
<td>2.0</td>
<td>1.5 (1.4-1.7)</td>
</tr>
<tr>
<td>Concentration (x10^6 ml)</td>
<td>20</td>
<td>20</td>
<td>15 (12-16)</td>
</tr>
<tr>
<td>Total count (x10^6)</td>
<td>40</td>
<td>40</td>
<td>39 (33-46)</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>50</td>
<td>50</td>
<td>32 (31-34)</td>
</tr>
<tr>
<td>% Normal Morphology</td>
<td>30</td>
<td>-</td>
<td>4 (3.0-4.0)</td>
</tr>
<tr>
<td>% MAR</td>
<td>20</td>
<td>50</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>
Predictive factors

Only TOTAL MOTILE COUNT (TMC)


<table>
<thead>
<tr>
<th>Variable</th>
<th>Cumulative pregnancy rate, % (and standard error) [SE]</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count, × 10⁶/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 9 (n = 75)</td>
<td>30 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>10–19 (n = 44)</td>
<td>42 (8)</td>
<td></td>
</tr>
<tr>
<td>≥ 20 (n = 590)</td>
<td>50 (3)</td>
<td></td>
</tr>
<tr>
<td>Seminal volume, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 (n = 229)</td>
<td>46 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>3–9 (n = 480)</td>
<td>48 (3)</td>
<td></td>
</tr>
<tr>
<td>% of progressively motile sperm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 25 (n = 107)</td>
<td>36 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>26–50 (n = 175)</td>
<td>41 (5)</td>
<td></td>
</tr>
<tr>
<td>51–100 (n = 427)</td>
<td>52 (3)</td>
<td></td>
</tr>
<tr>
<td>% of sperm with abnormal morphologic features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 50 (n = 494)</td>
<td>51 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>51–100 (n = 215)</td>
<td>39 (5)</td>
<td></td>
</tr>
<tr>
<td>Total motile sperm count, × 10⁶/ejaculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 9 (n = 83)</td>
<td>20 (6)</td>
<td>0.001</td>
</tr>
<tr>
<td>10–19 (n = 43)</td>
<td>37 (9)</td>
<td></td>
</tr>
<tr>
<td>≥ 20 (n = 583)</td>
<td>52 (2)</td>
<td></td>
</tr>
</tbody>
</table>

*NS = not significant.
Menstrual Cycle
Ovarian Stimulation - regimens

FSH = follicle stimulating hormone
GnRHa = gonadotrophin releasing hormone antagonist
hCG = human chorionic gonadotrophin
Ovarian Stimulation - regimens

More oocytes does not always mean better results

Possibly optimised results with low numbers after mild stimulation

Potentially detrimental effects of supra-physiological levels of estrogens

Oocyte Retrieval – Egg Collection

Heated & Adjustable Operating Table

Digital Suction Pump System

Single & Dual Lumen Needle Sets & Tube Heater
Oocyte recovery procedure
Cumulus-Oocyte-Complexes in follicular fluid
Oocyte Maturational Stages

1. Germinal vesicle.  
   *Immature.*

2. Oocyte in metaphase I (MI), the polar body (PB) not extruded.  
   *Might mature in Culture!*

3. Oocyte in metaphase II (MII), the first PB is extruded.  
   *Mature!*
Sperm Preparation & Selection

• remove all seminal fluid

• remove all non-sperm cells

• isolate normal, motile sperm

• support capacitation

• reduce %DNA frag
Sperm Preparation & Selection

- vary time, speed (g) and/or gradient
  - (%, volume, number)
- reduced DNA fragmentation
- effects of temperature
  - pre-incubation
- sperm concentration
  - depends on use (IUI v IVF v ICSI)
  - 5 M/ml motile
Retrograde ejaculates

Development and in vitro testing of a new method of urine preparation for retrograde ejaculation; the Liverpool solution

Thomas R. Aust, M.B., Ch.B., a Stephanie Brookes, B.Sc., a Stephen A. Troup, Ph.D., a William D. Fraser, M.D., b and D. Iwan Lewis-Jones, M.D. a

Liverpool Solution
Dilution required: 1 in 8. 8.4% (wt/vol) sodium bicarbonate in sterile distilled water. This solution then is diluted 50:50 with normal saline.

APPENDIX TABLE 1

<table>
<thead>
<tr>
<th>Time from start (min)</th>
<th>Liverpool solution drink volume (mL)</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>250</td>
<td>Urinate to empty bladder completely</td>
</tr>
<tr>
<td>90</td>
<td>250</td>
<td>Ejaculate and void mixture</td>
</tr>
<tr>
<td>120</td>
<td>Nil</td>
<td></td>
</tr>
</tbody>
</table>


FIGURE 1
Change in urinary osmolarity in 10 subjects ingesting Liverpool solution.

FIGURE 2
Change in urinary pH in 10 subjects ingesting Liverpool solution.

FIGURE 4
Change in progressive motility (grade A and B) with time in sperm exposed to prepared urine (n = 6).
Surgical sperm retrieval
Mechanical dissection
Sterilising sperm defects

Globozoospermia
‘round head defect’

Stump tail defect
Specialised fertilisation media

Higher glucose supports

- sperm maturation
- sperm function:
  - capacitation
  - binding
  - acrosome reaction
- fertilisation

Mahadevan 1997
PHASE 1
Embryo genome is inactive
Low metabolism, development controlled by maternal gene transcripts

PHASE 2
Embryo genome is activated
Metabolism rises. Proteins, many growth factors and receptors are produced.
Intrauterine insemination (IUI)

- often the first step in ART
- prepared sperm injected into the uterine cavity
- 3-6 cycles of IUI often attempted before other more invasive fertility treatment
- with or without ovarian stimulation
IVF - Insemination

[Image of egg cell with sperm cells on the surface]

Denudation of Oocytes

*Natural in vivo function:*

Hyaluronidase enzyme breaks the HA bonds connecting cumulus cells

Sperm uses hyaluronidase enzyme to pass through oocyte cumulus

- N-Acetyl Glucosamine
- Glucuronic Acid
- PH20 Hyaluronidase
ICSI-Cumulase® (rHuPH20) smoothly removes the cumulus matrix

+ Cumulase 80 U/ml

2.5 Minutes
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No Vacuolation</td>
</tr>
<tr>
<td>II</td>
<td>$\leq 2$ Small Vacuoles</td>
</tr>
<tr>
<td>III</td>
<td>$&gt;2$ small or $\geq 1$ large Vacuoles</td>
</tr>
<tr>
<td>IV</td>
<td>Large Vacuole and other head abnormalities</td>
</tr>
</tbody>
</table>

Sperm Selection – HA binding (PICS1)
Sperm immobilisation for ICSI
’Normal’ ICSI
Embryo development

from: Chason, Rebecca J. et al.  
Incubators – with inbuilt optics
Assessing fertilisation

Two polar bodies (PB) = excess genetic material discarded by oocyte

Two pronuclei (2PN)
- one female (from oocyte)
- one male (from sperm)

3PN
More than 1 sperm entered egg (polyspermy)
OR
Retained PB (digyny)

1PN
Usually an activated egg — so female PN forms in absence of fertilisation or sperm nucleus decondensation
Embryo assessment – day 1

10.3%  22.4%  34.1%
zygote  Syngamy  2 cell embryo
Embryo assessment – day 2/3

2-cell

4-cell

8-cell

Grade 4

Grade 3

Grade 2

Grade 1

BLASTOMERE SIZE

FRAGMENTATION
Cell division and genetic health

from Hardarson et al, 2001
Embryo assessment – day 5/6

TROPHOCTDERM

Grade a
Grade b
Grade c

Inner cell mass

Fluid pumped across cells causes expansion

ICM

Grade B
Grade A
Grade E
Grade C
Grade D

Grade 4
Grade 5

Grade 2
Grade 6

Grade 1

Expansion status
Genetic testing

Preimplantation genetic diagnosis
PGD for detection of specific inherited genetic abnormalities

Preimplantation genetic screening
PGS for detection of spontaneously arising abnormalities
Polar body biopsy
Blastomere biopsy

- laser
- simple aspiration – bevelled pipette

video courtesy of A Chatziparasidou & M Nijs
Preimplantation genetic screening

- Chromosomal mosaicism in early screening embryos,
- Where embryo biopsy is not representative of the other blastomeres.
Trophectoderm Biopsy
First-time IVF patients with a good prognosis (age <35, no prior miscarriage) and normal karyotype.

**Pilot study, prospective randomised**
**Transfer on day 5**

44.9% aneuploidy rate in Blastocysts

Morphology + aCGH: 69.1% OPR
Morphology: 41.7% OPR  p<009
Future applications
MtDNA

Information on chromosomal status, amount of mtDNA, and presence of mutations in the mitochondrial genome

379 embryos analysed with aCGH, quantitative PCR and NGS

123 were determined to be aneuploid

- Abnormal mtDNA levels are present in 30% of non-implanting euploid embryos, but are not seen in embryos forming a viable pregnancy
- The quantity of mtDNA was significantly higher in embryos from older women (P=0.003).
- MtDNA levels were elevated in aneuploid embryos, independent of age (P=0.025).
- Blastocysts that successfully implanted tended to contain lower mtDNA quantities than those failing to implant (P=0.007).

A novel biomarker?
Embryo transfer

- precise placement of the embryo inside the uterus is important
  - touching the fundus may cause the uterus to contract and harm chances of implantation
  - if the catheter is not inserted far enough the embryo may be unintentionally placed in the cervix

- difficult transfers:
  - trial transfer (normal catheter but no opening)
  - stylet (mandril; inner that helps form shape of catheter)
  - pre-formed (shaped before use)
CONCLUSIONS: Available evidence suggests that vitrification is the current method of choice when cryopreserving MII oocytes. Early cleavage stage embryos can be cryopreserved with equal success using slow cooling and vitrification. Successful blastocyst cryopreservation may be more consistently achieved with vitrification but optimal slow cooling can produce similar results.
Choice of cryoprotectant

- no clear evidence to favour any one system
- optimise system within each laboratory
- ORIGIO: PROH/EG; Sage: DMSO/EG
- anecdotally, DMSO favoured for oocytes?
Time required for equilibration

- stage-dependent
- 5 – 15 minutes
- options
  - observe and wait to see full (90%) re-expansion
  - establish median (fixed) time for your laboratory
- blastocysts – effect of collapse
  - collapsed: use 5 minutes
  - non-collapsed: check for re-expansion of cells NOT blastocoel
Collapsing blastocysts

• some clinics doing well without collapsing
• preferred options
  - puncture with ICSI needle
  - laser
  - small tip (micro-pipetting)
• generally advised
• wait for 50% shrinkage and then move straight to VM
Choice of carrier: open v closed

- simple device
- loading
  - minimal volume
  - numbers
  - maximal cooling
  - timing (not too soon as evaporation significant)
Summary

To optimise any lab, one needs...
- the right people with...
- the right skills, provided with
- the right equipment in
- the right facilities.

To get the best outcomes, one must also ...
- optimise egg quality with good stimulation regimens
- provide good culture conditions
- be able to choose the right gametes and/or embryo(s) for treatment and transfer
- be skilled at replacing embryos