Sperm function : an alternative [very bias] lecture

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Summary

- Poor sperm function is a clinical problem –where are we now?
- Semen analysis has limitations.
- Tools do exist but generally are not robust or not rigorously tested [too much hype and no joined up writing].
- High quality [repeatable and reliable] clinical data missing or in it's infancy [old].
- Objective [for ART]– at least clinically – to determine significant chances of failure [>10% of norm] in addition to semen assessment.
- New tools [simple, cheap, reliable, repeatable, effective] combined with more robust assessments urgently required.

Where is sperm function at present?

- A significant problem : 1:6 [1:7] couples in UK.
- Epidemiological studies suggest sperm dysfunction is the single most common cause of infertility.
  [~30,000 new cases pa UK –based on # ART cycles]
- Currently no 'recognised/effective' drug treatment.
- The only treatment is ART : IUI→IVF→ICSI
- Increasing as a problem??

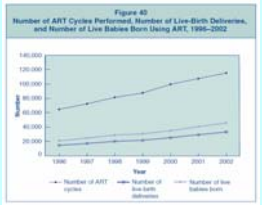
HFEA : 39000 IVF/ICSI cycles in 2005 :12% increase compared with 2004

Expected increase for 2006 of 6%

50:50 IVF ICSI

1.3-4.2% births In EU; e.g. Denmark 4.2% new born

EIM/ESHRE (2006) Hum Reprod 21, 1680-97

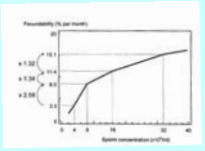


Semen analysis has limited value..

Overlap of semen values

Guzick *et al.*, (2001) NEJM 345, 1388-1399

•696 fertile couples, 765 infertile couples
•Considerable overlap between the groups
•'none of the measures are diagnostic of infertility'
•Minimal values similar to MacLeod and Gould in 1951



Comhaire (2000) Hum Reprod 15, 2067-71.

TABLE 2. FERTILE, INDETERMINATE, AND SUBFERTILE RANGES FOR SPERM MEASUREMENTS FROM CLASSIFICATION-AND-REGRESSION-TREE ANALYSIS AND CORRESPONDING ODDS RATIOS FOR INFERTILITY.*

VARIABLE	SEMEN MEASUREMENT		
	CONCENTRATION	MOTILITY	MORPHOLOGY
	×10⁶/ml	%	% normal
Fertile range	>48.0	>63	>12
Indeterminate range	13.5–48.0	32–63	9–12
Univariate odds ratio for infertility (95% CI)	1.5 (1.2–1.8)	1.7 (1.5–2.2)	1.8 (1.4–2.4)
Subfertile range	<13.5	<32	<9
Univariate odds ratio for infertility (95% CI)	5.3 (3.3–8.3)	5.6 (3.5–8.3)	3.8 (3.0–5.0)

*CI denotes confidence interval.

TABLE 3. ODDS RATIOS FOR INFERTILITY FOR COMBINATIONS OF SPERM MEASUREMENTS.*

SPERM MEASUREMENT RANGE			ODDS RATIO (95% CI)
MORPHOLOGIC FEATURES	MOTILITY	CONCENTRATION	
Fertile	Fertile	Fertile	1.0
Subfertile	Fertile	Fertile	2.9 (2.2–3.7)
Fertile	Subfertile	Fertile	2.5 (1.6–4.2)
Fertile	Fertile	Subfertile	2.2 (1.3–3.6)
Subfertile	Subfertile	Fertile	7.2 (4.3–12.2)
Subfertile	Fertile	Subfertile	6.3 (3.8–10.3)
Fertile	Subfertile	Subfertile	5.5 (3.0–10.2)
Subfertile	Subfertile	Subfertile	15.8 (8.7–29.0)

WHO new guidelines

[possible, unpublished and confidential …..]

5th centile fertile men (conception within 12 months)
Remarkably similar to NEJM study…

	n	Value	CL
Progressive motility (%)	**1599**	**33**	**29-37**
Concentration ($x10^6$/ml)	**1679**	**14**	**10-19**
Morphology (%)	**564**	**3**	**2-4**

•Semen analysis is a blunt instrument [lower end of scale]
•No value when done under 'uncontrolled conditions'.

Sub optimal sperm function is a clinical issue….

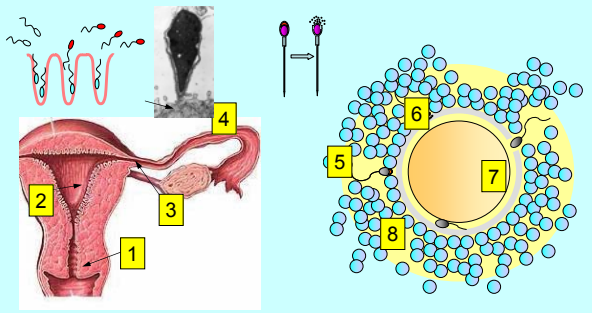
TABLE 2

Comparison of fertilization and cleavage from sibling oocytes subjected to two protocols of conventional insemination (IVF) and ICSI in 73 cycles.

	Protocol A (5,000 sperm/oocyte; 35 cycles)		Protocol B (20,000 sperm/oocyte; 38 cycles)	
Parameter	IVF	ICSI	IVF	ICSI
Oocytes inseminated or injected				
n	235	235	248	250
Mean ± SD	6.7 ± 2.2	6.7 ± 2.3	6.5 ± 2.1	6.6 ± 2.1
Fertilization				
no. of two-pronuclei (%)*	88 (37.4)	151 (64.3)	148 (59.6)	169 (67.6)
average polyspermy rate (%)	4.5	2.1	5.1	2.3
Cycles with no fertilization				
no. (% of cycles)*	9 (25.7)	0 (0.0)	2 (5.3)	0 (0.0)
Cycles with fertilization				
no. (% of cycles)	26 (74.3)	35 (100)	36 (94.7)	38 (100)
average fertilization rate (%)*	49.6	66.0	61.2	69.0
Cleavage				
Average % embryos with ≤20% fragmentation	72.4	80.1	64.3	71.6
Average % embryos with ≥50% fragmentation	10.6	9.1	18.6	18.7

Note: Differences not significant unless stated otherwise.
* $P<.0001$ by χ^2 test.
† $P<.0001$ by χ^2 test for protocol A.
‡ $P<.007$ by Wilcoxon signed rank test for protocol A.
Tournaye. IVF vs. ICSI for male infertility. Fertil Steril 2002.

What tools are available to assess the function of the cell ?
How do they cover the journey?

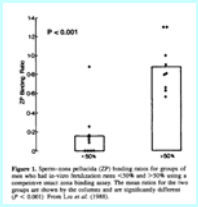


What are sperm function tests trying to achieve ?

1. Some subtle changes in function for a particular test/assay e.g. research or toxicology.
2. ***But*** : primary – clinical to direct therapy : which treatment is most appropriate and what are the chances of failure?

Consensus workshop on advanced diagnostic Andrology
Fraser & Mortimer Hum Reprod 1996 11, 1463-1479

- CASA
- Acrosome reaction
- *HPOT*
- Zona binding



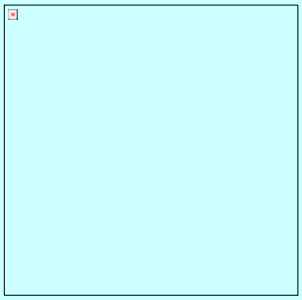
Conclusion : Some impressive data and there is a need for targeted sperm function testing but to who and which one(s) is unclear.

Tools do exists to determine 'function of the cell'….

- Quantitative motility/power.
- Zona binding.
- Calcium influx.
- Acrosome reaction.
- *Reactive oxygen assessments/detection.*

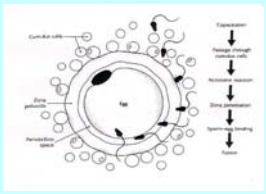
None are universal and come with 'challenges'

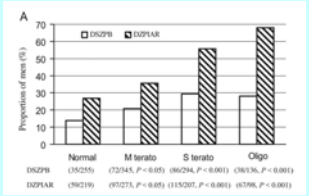
Penetration into cervical mucus can indicate defective cells.



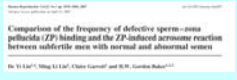
Barratt *et al.*, (1989) Hum. Reprod. 4, 430-434

Use of zona binding/zona induced AR



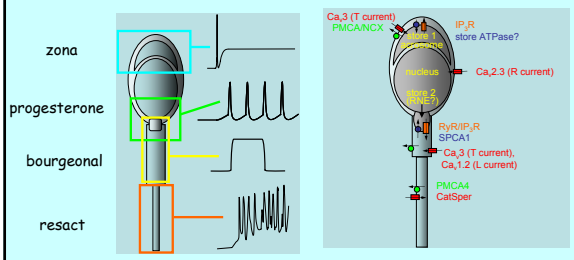


Significant problem : 35% of 'normal' sub fertile men.

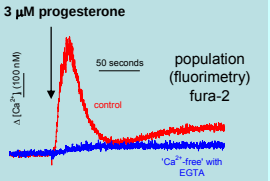

Comparison of the frequency of defective sperm–zona pellucida (ZP) binding and the ZP-induced acrosome reaction between subfertile men with normal and abnormal semen

Calcium Regulation in Sperm

Publicover, Harper & Barratt (2007) Nature Cell Biology 9, 235-42.



Oocyte-Derived Activation of Sperm $[Ca^{2+}]_i$ Signalling - Rapid Response of Human Cells to Progesterone



population (fluorimetry) fura-2

single cell (oregon green BAPTA 1)

- **Defective response associated with reduced fertilization rate and sub-fertile males**
- **Processes involved still unknown – ? receptor**

Defective calcium response in men with reduced fertilisation success

Krause *et al* (1995) Hum. Reprod. 10, 120-124

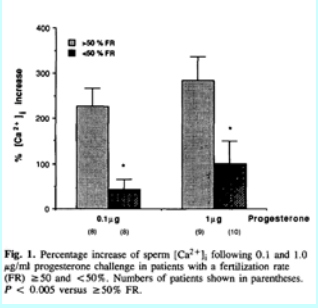
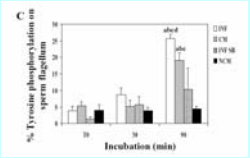


Fig. 1. Percentage increase of sperm $[Ca^{2+}]_i$ following 0.1 and 1.0 µg/ml progesterone challenge in patients with a fertilization rate (FR) ≥50 and <50%. Numbers of patients shown in parentheses. $P < 0.005$ versus ≥50% FR.

Problems/challenges

- Methodology – a very significant problem.
- Must have repeatability and reliability [recombinant ZP a good/bad example]
- Relatively poor tools (how measure ROS??? [wbc. Vs. sperm, marker]).
- High quality clinical data. Is the old data relevant today?
- Currently - no perceived need – thus research [in last 15 years] has been minimal. [no one I contacted in UK uses sperm function prior to IUI, IVF]



Moseley *et al.*, (2005) Mol. Hum. Reprod. 11, 523-9.

However ….useful to indicate chances of failure but not regularly performed prior to ART treatment.

- If no zona binding, failed calcium response, no 'power' or acrosome reaction significant chances of failure.
- If we could perform these with good R&R and at minimal cost would they be used/useful?
- So…[worse case scenario –usual question] [ignoring IUI]:
  - Assume at IVF FF rate 1.5% (<10% FR in 3%) and test cost €30 to perform.
    - Identify 3% patients = €3000 for 100 patients.
    - If test pick out 2 in 100 (at €3000).
    - Average IVF clinic in UK approx 450 cycles thus < €7000 pa (50 : 50 IVF/ICSI).

Is it worth doing?

So how can we develop new clinically useful tools ?

New Biomarkers : the Use of Proteomics

clinical/basic research

Rifai *et al*. (2006) Nat Biotec 24, 971-983

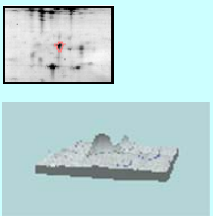
Sperm are ideal for proteomic analysis - basis of sperm dysfunction

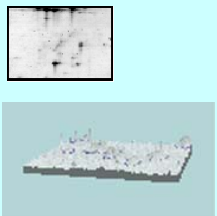
Three strategies :

1. Dynamic studies i.e. capacitation related changes.
2. The sperm proteome [or compartments].
3. Unbiased comprehensive [global] comparison of normal with pathology**.**

Lefievre *et al.*, 2007 Reproduction 133, 675-84.

Differences *can* be easy to identify.





Patient

Normozoospermic donor

Case Study: 6 patients (A-F) who failed to fertilise at IVF

normal man, normal female [2003-2007]

Pixton *et al.* 2004 Hum Reprod 19, 1438-1447.
Conner *et al.* 2007 Soc Reprod. Fertil. Suppl. 63, 237-55.

Phosphoprotein phosphatase 1-gamma catalytic chain	Reduced in patients B, C and F
Isocitrate Dehydrogenase (NAD) alpha chain precursor	Reduced in patients C, E and F
Glutathione-S-transferase Mu 3	educed in patients A, C, E and F
Secretory actin binding protein	Increased in patients A, B, E and F
Lysozyme-like acrosomal sperm-specific secretory protein	Reduced in patients D and E
Clusterin	Reduced in patients C, E and F
Lactate dehydrogenase (testis-specific)	Reduced in patients B,C,E and F
Voltage dependent anion channel 2	Reduced in patients D and F
Semenogelin I	Increased in patients C, D and E
Semenogelin II	Reduced in patients C and D

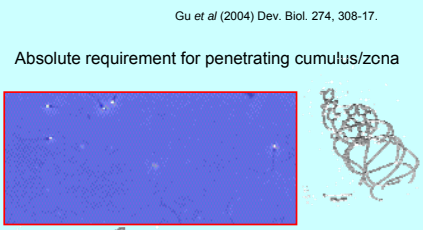
Proteins showing at least a four-fold change in expression levels, as determined by 2D-electrophoresis compared to control.
But 1. methodology 2. select patients.

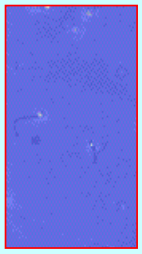
Any other [simple] gems??

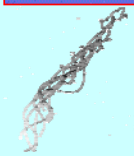
New tools [drugs] to address hyperactivation

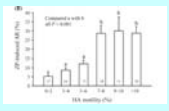
Gu *et al* (2004) Dev. Biol. 274, 308-17.

Absolute requirement for penetrating cumulus/zona









Liu *et al., (2007) Hum. Reprod.* 10, 2632-2638.

Summary

- Poor sperm function is a clinical problem.
- Tools do exist but generally are not robust or not rigorously tested [too much hype and no joined up writing].
- High quality [repeatable and reliable] clinical data missing or in it's infancy [old].
- Objective [for ART]– at least clinically – to determine significant chances of failure [>10% of norm] in addition to semen assessment.
- New tools [simple, cheap, reliable, repeatable, effective] combined with more robust assessments urgently required.

Non clinical lecturer in Reproductive/developmental/cell biology

- Collaborative post between IVF, Medical School and College of Life Sciences.
- CLS in Dundee houses the greatest concentration of the highest cited scientists in biological sciences/biochemistry in Europe [over last 10 years - Science magazine].