Identifying a high quality sperm…..

Subtitle : how close are we to accurate biomarkers?

Christopher LR Barratt

Reproductive and Developmental Biology,
Medical School,
Dundee,
Scotland

Members of Lab : Marios Stavridis, Senga Oxenham, Anne Duncan, Wardah Alasmari, Vanessa Kay, Erica Forster, Stephen Mansell, Anne Rice and Cathy Beaton.

Objectives of the lecture

- Current tools - semen analysis - is a blunt instrument [lower end of the scale] and is of [almost] no value when done under ‘uncontrolled‘ conditions.

- Sperm function testing (including DNA assessments) remains limited. Generally blighted by poor technical control, robust methods and/or low quality clinical studies.

- New tools (or more intelligent workings of old ones) are necessary to complement the above. Proteomics is an exciting example but is in it’s infancy. ? Patching. FUTURE

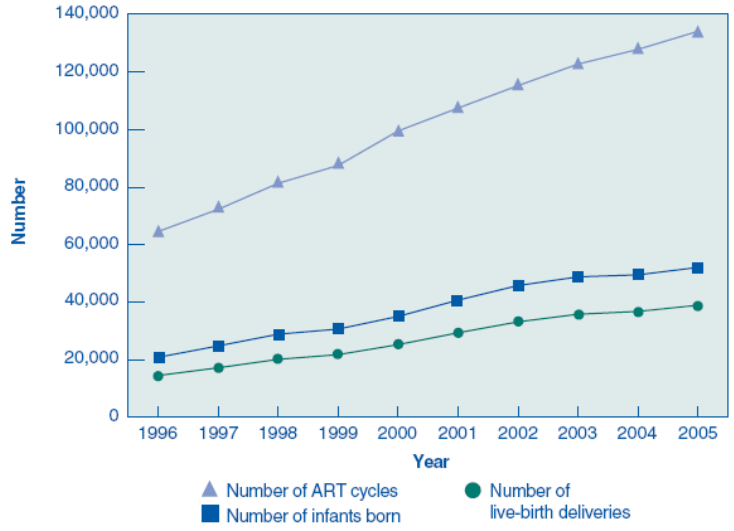
Where is male infertility at present?

- A significant problem : 1:6 couples in UK. 80 million couples worldwide.
- Epidemiological studies suggest sperm dysfunction is the single most common cause of infertility.
  [~30-60,000 new cases pa UK]
- Currently, almost no effective drug treatment therefore…..
- The only treatment is ART :

  IUI→IVF→ICSI [SFA]

- Possibly increasing as a problem?

Figure 49
Numbers of ART Cycles Performed, Live-Birth Deliveries, and Infants Born Using ART, 1996–2005

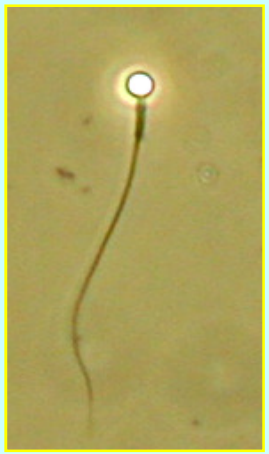


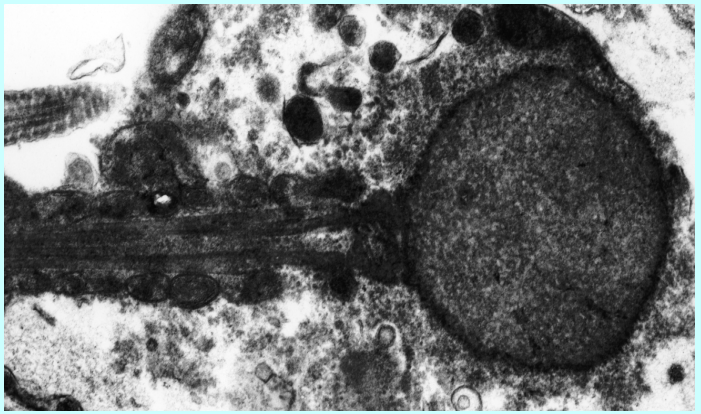
CDC report for 2005

Semen Analysis has significant clinical value for a number of conditions – for example -

- Azoospermia
- 'significantly above normal'
- Specific abnormalities e.g. globozoospermia, very large sperm, no tails…
- Antibodies
- OAT correlated with :
  - higher degree of aneuploidy

- *But* : Clearly different populations with similar parameters e.g. severe oligozoospermia ($5x10^6$/ml)





Semen analysis has limited value - overlap of semen values **[not a new discovery]**

Fertile, Indeterminate and sub fertile ranges and corresponding odds ratio for infertility

Variable	Concentration x10^6/ml	Motility %	Morphology %
Fertile	>48	>63	>12
Indeterminate	13.5 - 48.0 1.5 (1.2-1.8)	32 - 63 1.7 (1.5-2.2)	9 -12 1.8 (1.4-2.4)
Sub fertile	<13.5 5.3 (3.3-8.3)	<32 5.6 (3.5-8.3)	<9 3.8 (3.0-5.0)

- 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 Gold in* **1951** *'the real difference [n=1000 in each group] between the two groups lies in the relative frequency distributions and only at the lower count levels'*
- *Almost 60 years ago……'*

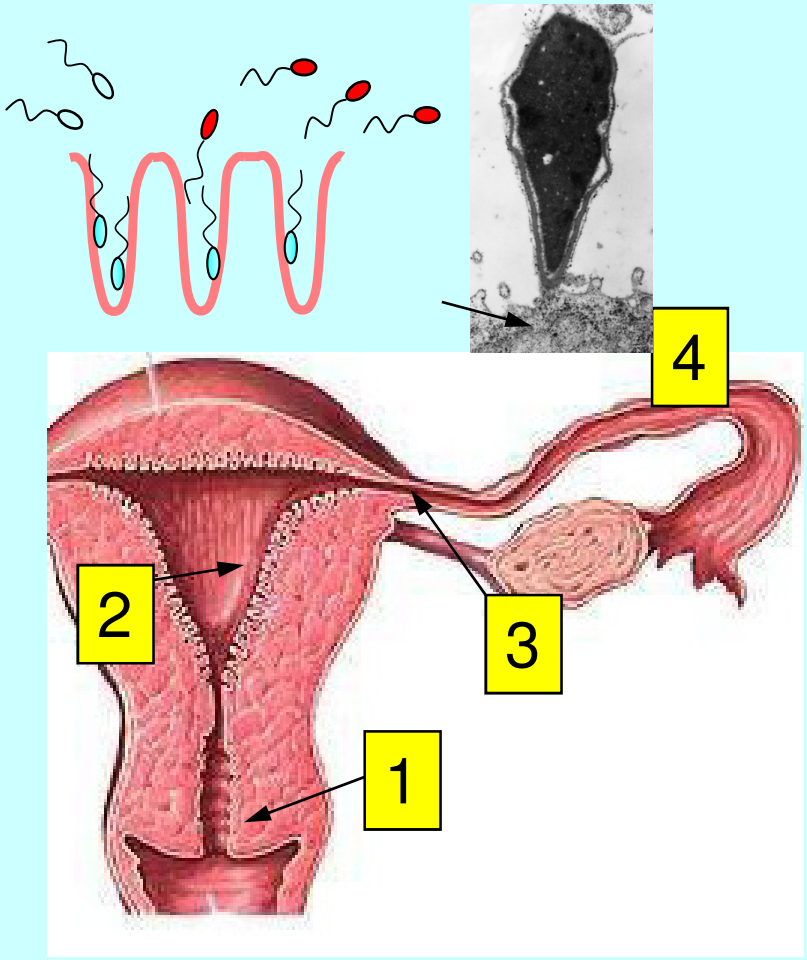
- *Remarkably : Data similar to new WHO 2009*

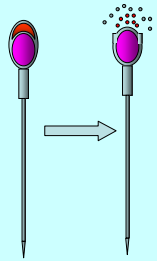
Guzick *et al.,* (2001) NEJM 345, 1388-1399

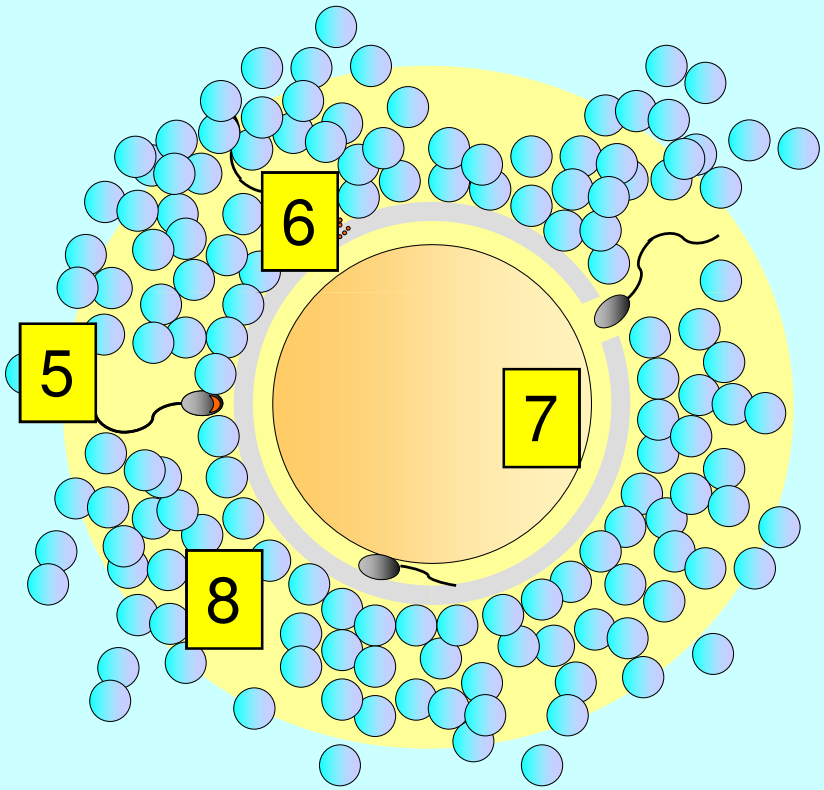
Why so ‘ineffective’?

1. We are asking the impossible

The spectrum to cover is too large *plus* baby to term….







Adapted from Publicover, Harper & Barratt 2007 Nature Cell Biology

What about sperm function testing?

Consensus workshop on advanced diagnostic Andrology

Fraser & Mortimer Hum Reprod ***1996*** 11, 1463-1479

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

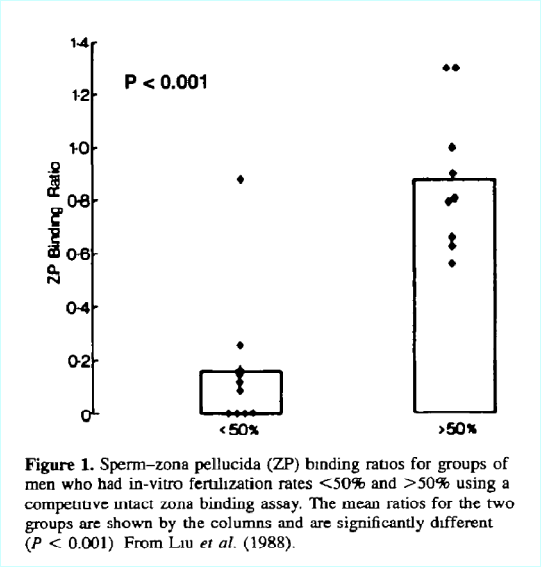
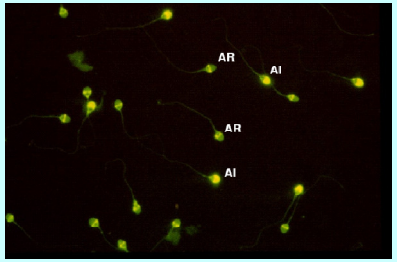


Figure 1. Sperm–zona pellucida (ZP) binding ratios for groups of men who had in-vitro fertilization rates <50% and >50% using a competitive intact zona binding assay. The mean ratios for the two groups are shown by the columns and are significantly different ($P < 0.001$) From Liu *et al.* (1988).



WHO 2009

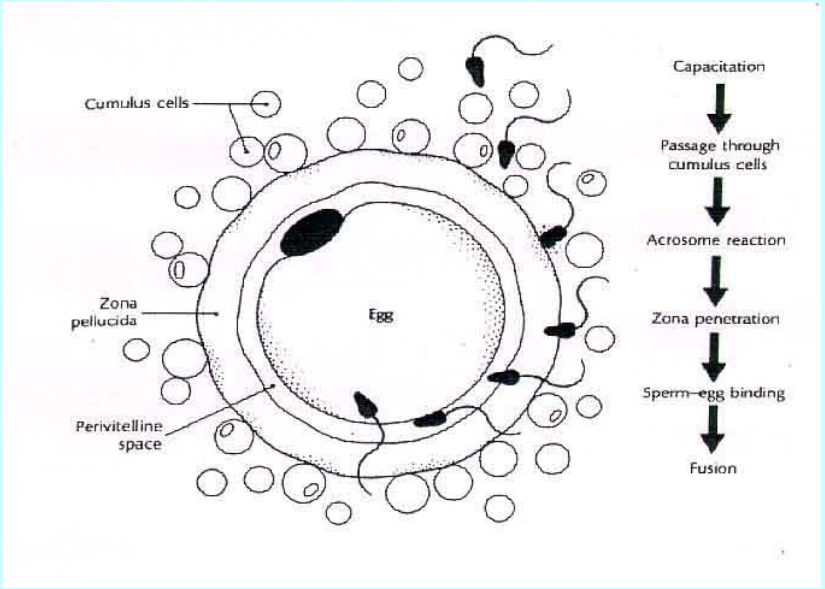
•If no zona binding, no 'power' or acrosome reaction significant chances of failure.

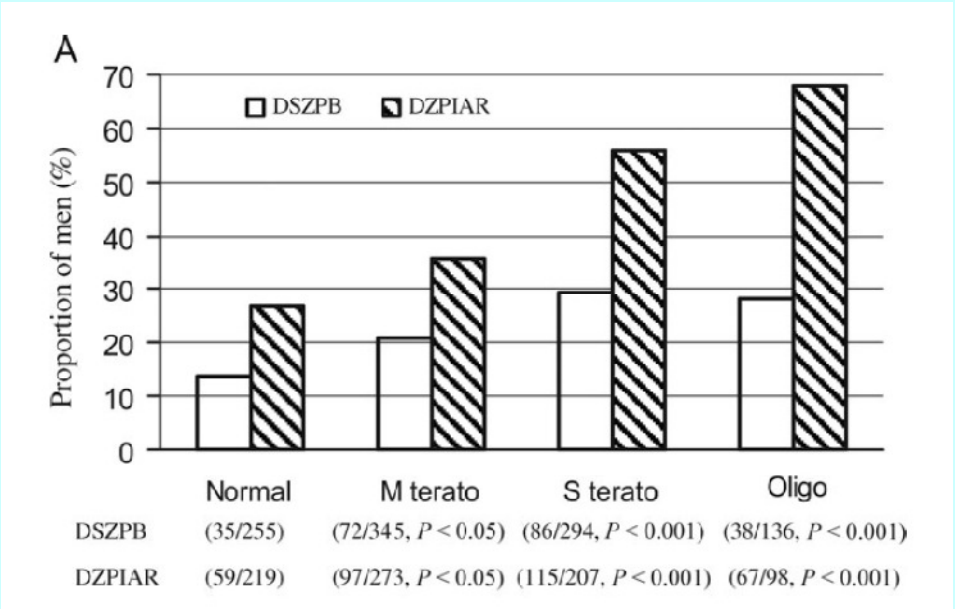
•If we could perform these with good R&R and at minimal cost would they be used/useful?

Conclusion : Some impressive data and there is a need for targeted sperm function testing but to who and which one(s) is unclear. None are universal and come with 'challenges

Objective : Keep trying→ IUI→IVF→ICSI

Use of zona binding/zona induced AR





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

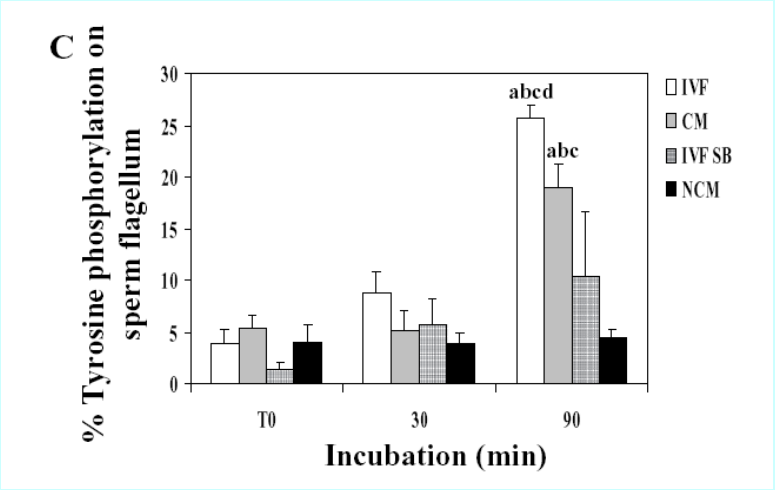
Human Reproduction Vol.22, No.7 pp. 1878–1884, 2007 doi:10.1093/humrep/dem087
Advance Access publication on April 23, 2007

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

De Yi Liu[1,4], Ming Li Liu[1], Claire Garrett[1] and H.W. Gordon Baker[1,2,3]

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]



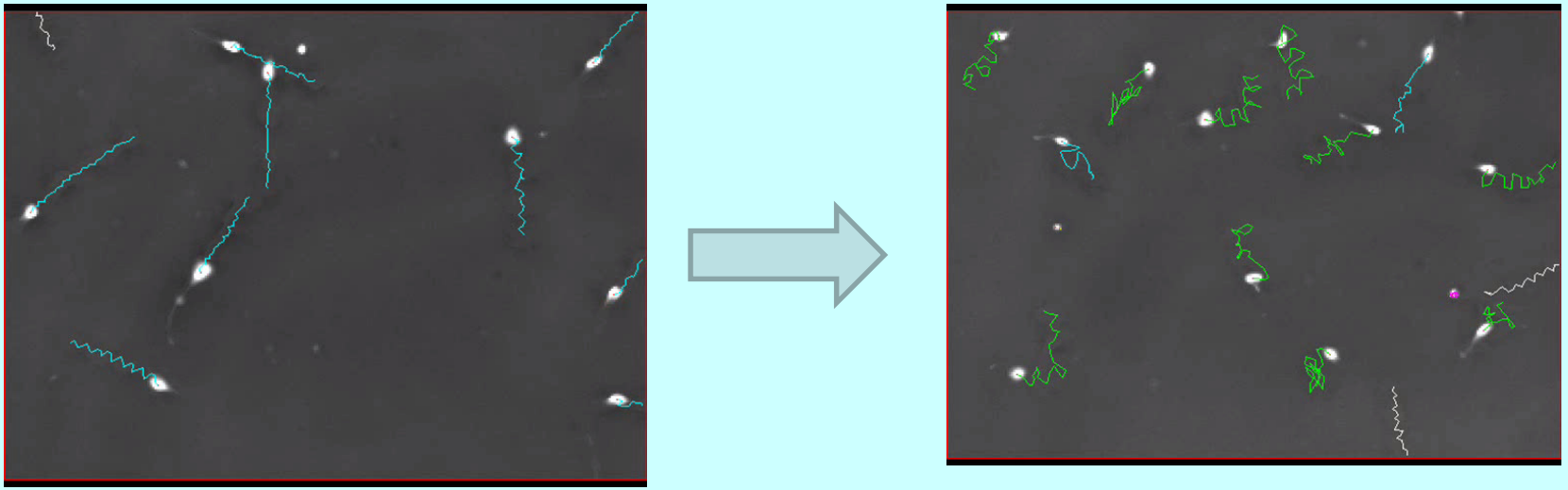
Moseley *et al.,* (2005) Mol. Hum. Reprod. 11, 523-9.

Is this the end of sperm function testing?

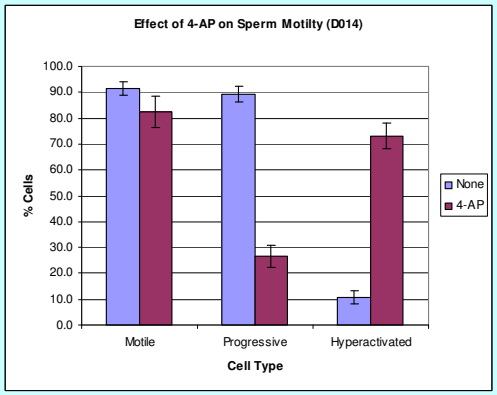
- If we could perform these [or new ones] with good R&R and at minimal cost would they be used/useful?
- So…[worse case scenario –usual question] [ignoring IUI]:
  - Assume at IVF FF rate 1.5% (<10% FR in 3%) and test cost €10 to perform.
    - Identify 3% [not all males] patients = €1000 for 100 patients.
    - If test pick out 2 in 100 (at €1000).
    - Average IVF clinic in UK approx 450 cycles thus < €4500 pa (50 : 50 IVF/ICSI).

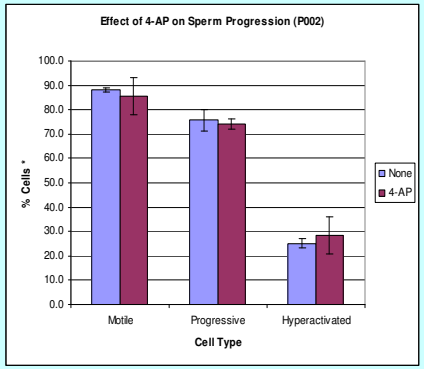
Is it worth doing?

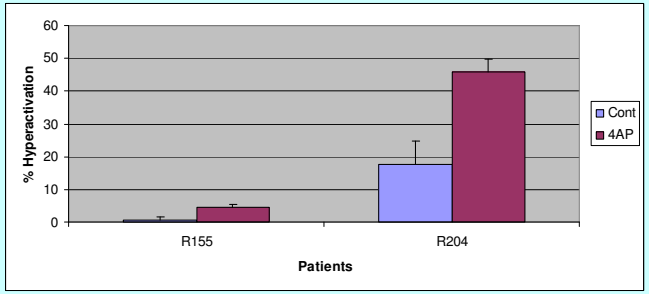
Simpler [robust] methods – to detect failure before [ART] IUI/IVF?



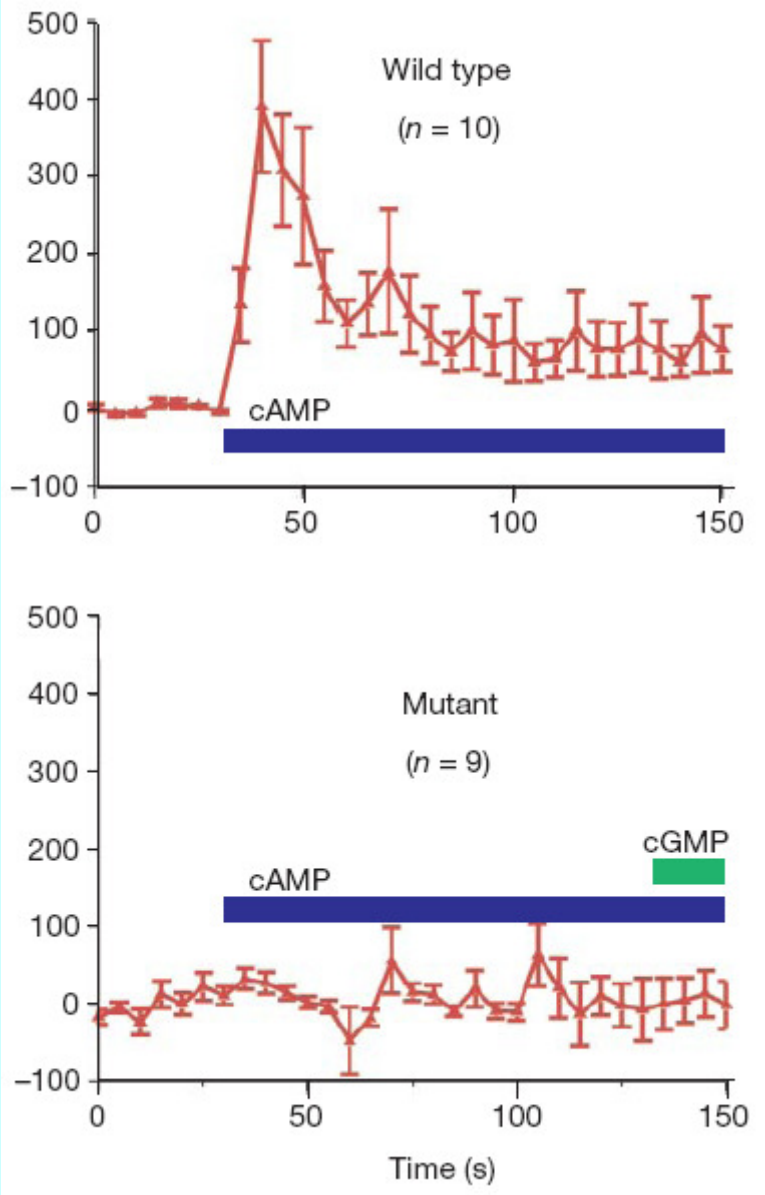
Hyperactivated motility necessary to fertilise the egg





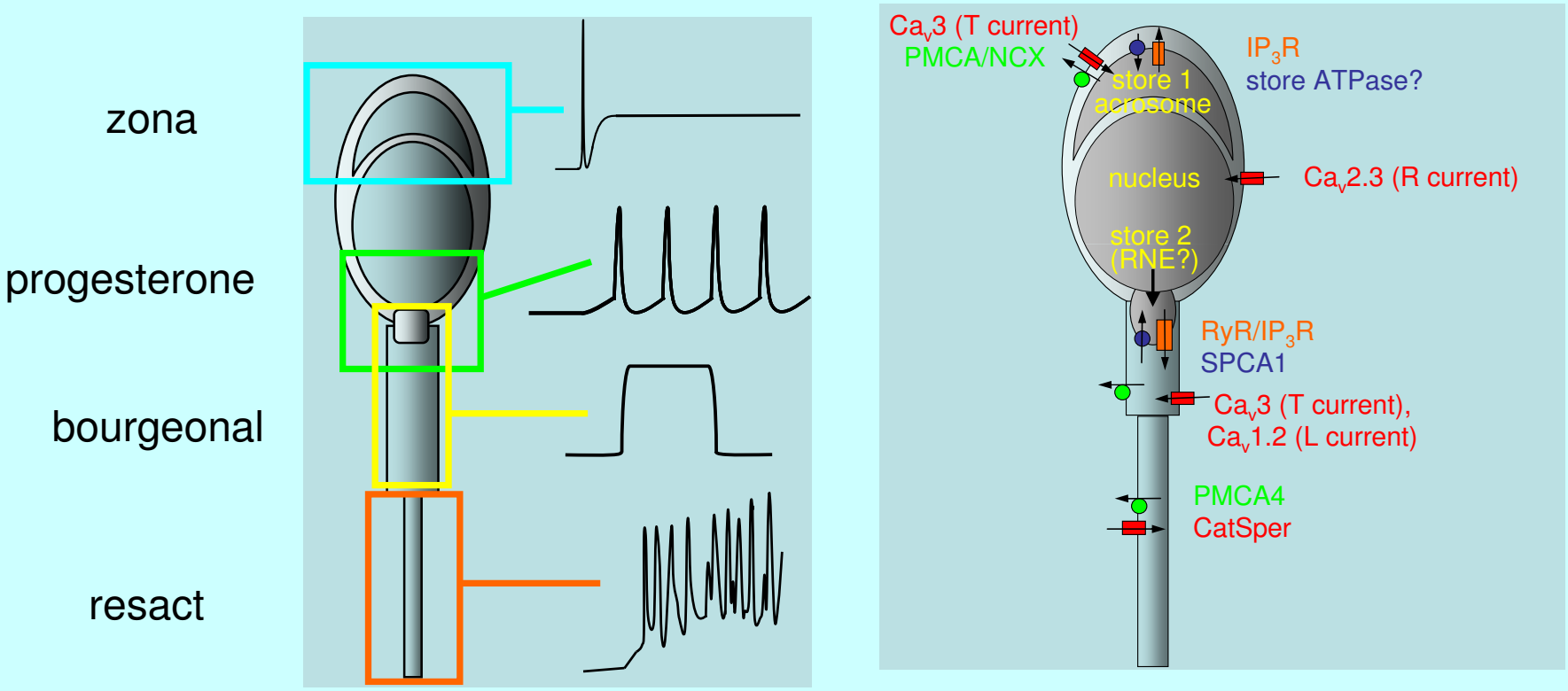


CatSper KO mice have impaired Ca^{2+} signalling



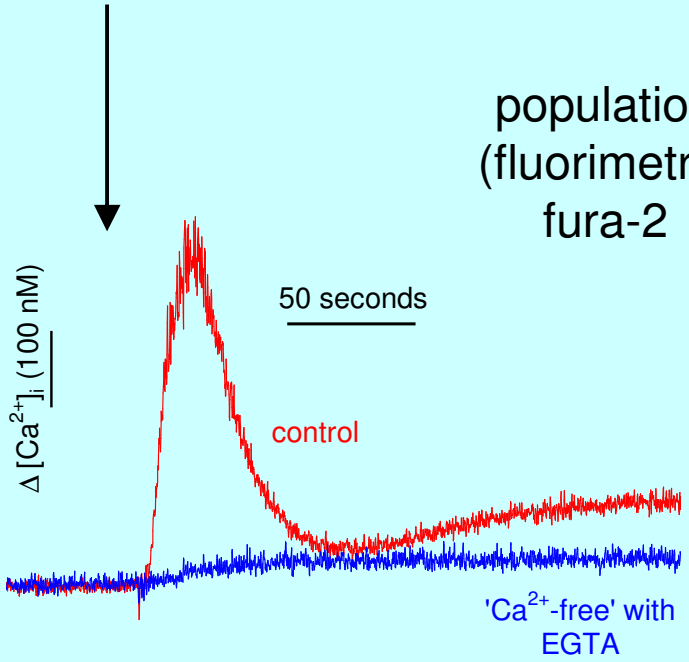
Current Thoughts - Calcium Regulation in Sperm

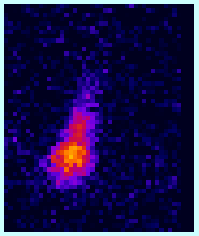
Publicover, Harper and Barratt - Nature Cell Biology (2007) 9 235-42



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

3 μM progesterone





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

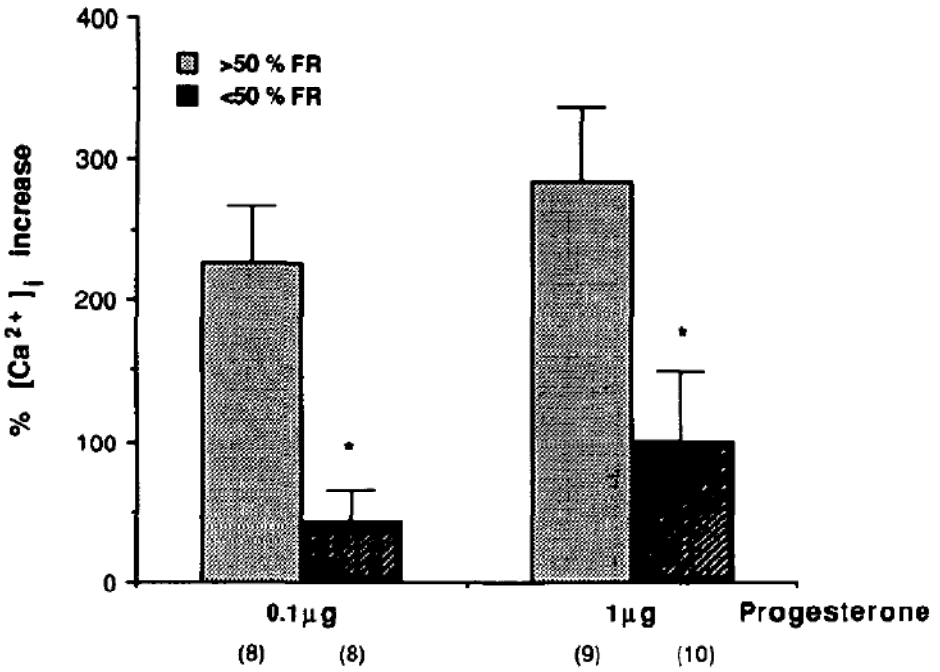


Fig. 1. Percentage increase of sperm $[Ca^{2+}]_i$ following 0.1 and 1.0 µg/ml progesterone challenge in patients with a fertilization rate (FR) ≥50 and <50%. Numbers of patients shown in parentheses. $P < 0.005$ versus ≥50% FR.

What about DNA ?
Assessment of DNA integrity of the cell.

Landmark study : Evenson *et al*., 1980 Science 210, 1131-1133.

'a new and independent determinant of male fertility'

HOWEVER

'The small but statistically significant association between sperm DNA integrity test results and pregnancy in IVF and ICSI cycles is not strong enough to provide a clinical indication for routine use of these tests in infertility evaluation of men'

Collins et al (2008) Fertil Steril 89, 823-31.

New potential biomarkers?

Proteomics : the sperm proteome, it's modification and differences between men.

Sperm are ideal for proteomic analysis - basis of sperm dysfunction

No transcription and translation [currently]

Three strategies :

1. The sperm proteome [or compartments 2300+].
2. Dynamic studies i.e. capacitation related changes. Use ‘biological tools’ Nitric oxide.
3. Unbiased comprehensive [global] comparison of normal with pathology e.g. failure to fertilize at IVF

The sperm proteome ….

- We've identified ~1900 proteins[$] too much data
- So……What's interesting?
  - Significant number of histones [?epigenetic modification]
  - Full proteosome [implying turnover?]
  - Significant complement of heat shock proteins (25 +) [chaperone, stress]

$ in Triton x100 fraction

.

Tulloch & Lefievre Unpublished

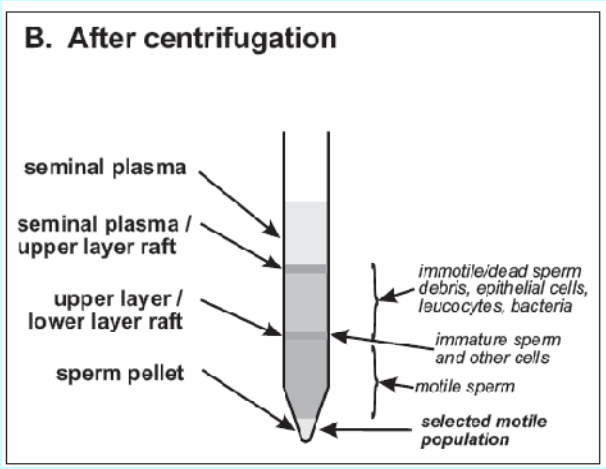
Comparison Good and Bad sperm - preliminary data [40/80 fraction]

Over represented in 40% fraction:

- *Valyl-tRNA synthetase [translational control]*
- *Tripeptidyl-peptidase 2*
- *Hypoxia up regulated protein [stress related]*
- *Alanyl-tRNA synthetase*
- *Endoplasmin precursor [stress]*
- *Elongation factor 2 [translational control]*
- *Histone1 H2AA Histone H2A type 1*

Over represented in 80%

- *TBC*

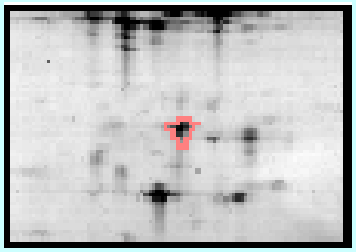


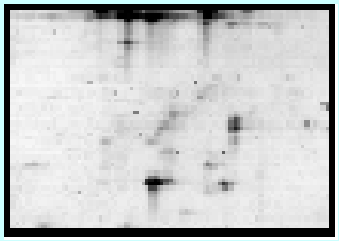
2 donors both consistent 1 sample each

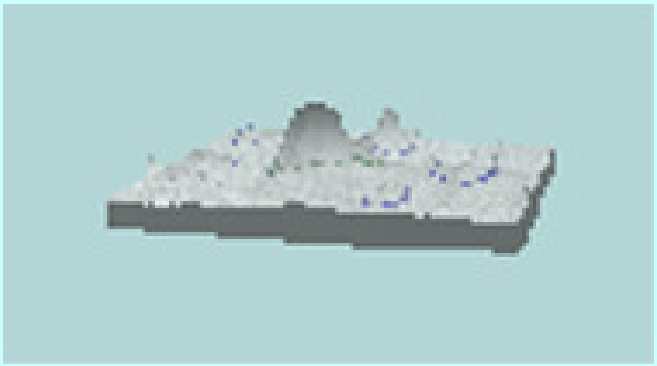
Excluding 'most' WBC with dynabeads and CD 45.

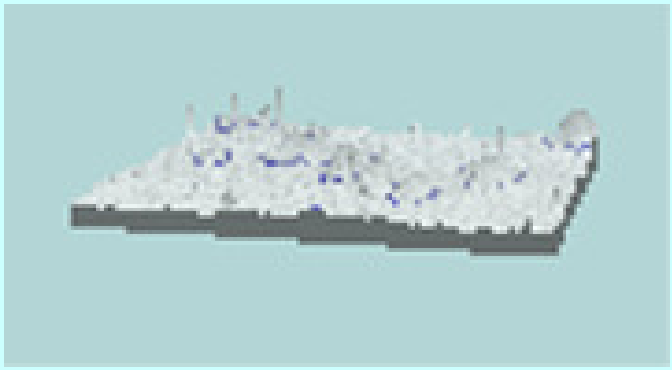
Tulloch & Lefievre Unpublished

Differences between men can be easy to identify.









Patient

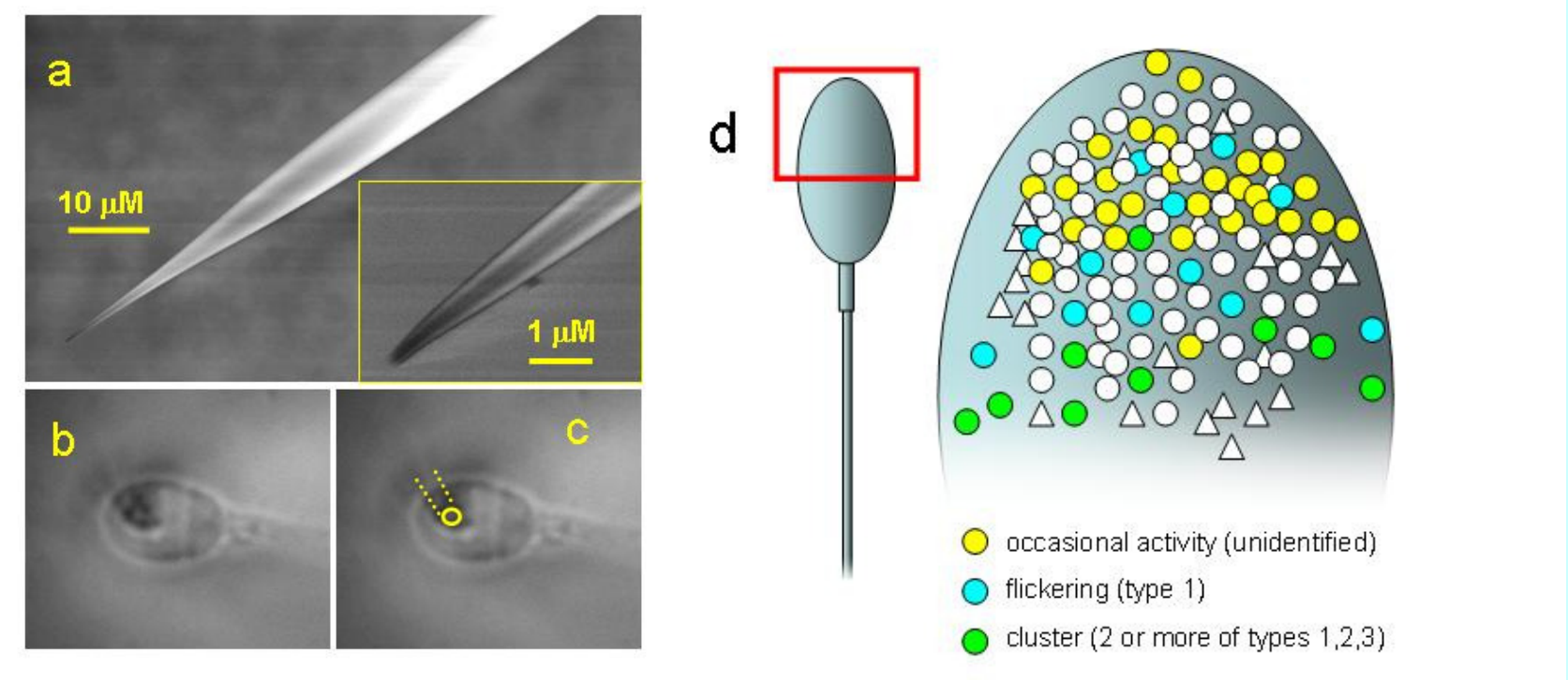
Normozoospermic donor

Challenges :

1. Quantification : iTRAQ
2. Clearer pathology.

Understanding a sperm – technological advances

Patch Clamping the Human Spermatozoon –the first steps



247 seals from 454 attempts. Active channels in 49 duration 2-40 mins. 3 types found.
In the main where inside/out achieved anion channel but not Cl^- selective.
?? regionalisation and clustering.
To date we can only record what is present
This can't be done in mice

Gu *et al* 2004 Dev Biol 274, 308-17 . Gu *et al.,* 2007, 'clustering' J Cell Physiol 213, 801-8**.**

Can we select higher quality cells for ART ?

- Density Gradient selection – well proven.
- Sperm selected by various binding techniques
  - Annexin V [preliminary data exciting] table below,
  - hyaluronate
- More detailed morphology [x6000]
- Electric charge

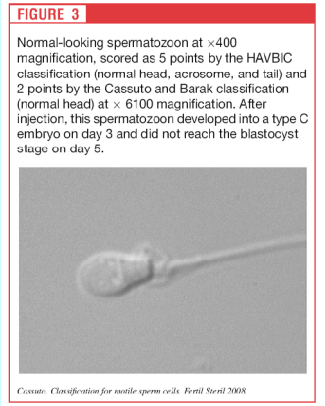


FIGURE 3

Normal-looking spermatozoon at ×400 magnification, scored as 5 points by the HAVBIC classification (normal head, acrosome, and tail) and 2 points by the Cassuto and Barak classification (normal head) at × 6100 magnification. After injection, this spermatozoon developed into a type C embryo on day 3 and did not reach the blastocyst stage on day 5.

Cassuto. Classification for motile sperm cells. Fertil Steril 2008

- Cassuto *et al.*, 2008 Fertil Steril In Press.
- *Said et al., 2008 J Androl 29, 134-42;*
- *Dirican et al., 2008 JARG 25, 375-381.*
- *Fleming et al., Hum Reprod 2008 23, 2646-2651*
- Nasr-Esfahani *et al.,* 2008 JARG 25, 197-203.

Table 4 Pregnancy and implantation rates among two groups

	Study group	Control group	P-Value
N of cases	122	74	
N of chemical pregnancies (%)	75 (61.47%)	34 (45.95%)	<0,05**
N of clinical pregnancies (%)	59 (48.36%)	27 (36.49%)	0,052*

•Dirican et al., 2008 JARG 25, 375-381.

Summary of where we are.

- Current tools - semen analysis - is a blunt instrument [lower end of the scale] and is of [almost] no value when done under ‘uncontrolled‘ conditions.

- Sperm function testing (including DNA assessments) remains limited. Generally blighted by poor technical control, robust methods and/or low quality clinical studies.

- New tools (*or more intelligent workings of old ones*) are necessary to complement the above. Proteomics is an exciting example but is in it’s infancy. ? Patching. FUTURE