Updated from pdf contact c.barratt@Dundee.ac.uk


First for Biological Science in UK


# Screening of man prior to IVF/ICSI

# Declaration of COI

- Fully employed by University of Dundee (UoD).
- WHO paid honorarium to be Chair of group and paid travel/accommodation/expenses.
- Editor in Chief of MHR
- Grant funding from MRC.
  - UoD Patent – sperm stimulation.
- Give occasional lectures that are company/society sponsored e.g. Vertex : pay travel/accommodation/ expenses.
- Cambridge University Press – 2 edited books.
- I'm not on any company board, advisory board or have a single share in anything or anybody.

# New – what's history ?

- Global infertility guidelines:
  Strategy for development and dissemination; Generating practice guidelines

- The revision and updating of the “WHO global Guidelines for infertility diagnosis, management and interventions for treatment” (1992) and the WHO manual for the investigation and diagnosis of the infertile couple” (1993)

- Initiated in January 2012 committee meeting


# Essentials and discussion points

- Physical examination and history
- **High quality Semen Analyses**
- **If abnormal spermatogenesis : potential screens.**

# As referral depends on Semen Analysis – make sure its high quality

- Cornerstone - Simple

# Potential groups [not a new discovery]

Fertile, Indeterminate and sub fertile ranges and corresponding odds ratio for infertility
All 3 abnormal. = 15.8 (8.7-29)

| **Variable** | **Concentration** x10$^6$/ml | **Motility** % | **Morphology** % |
|---|---|---|---|
| Fertile | >48 | >63 | >12 |
| Indeterminate | 13.5 - 48.0 | 32 - 63 | 9 -12 |
| Sub fertile | <13.5<br>2.2 | <32<br>2.5 | <9<br>2.9 |

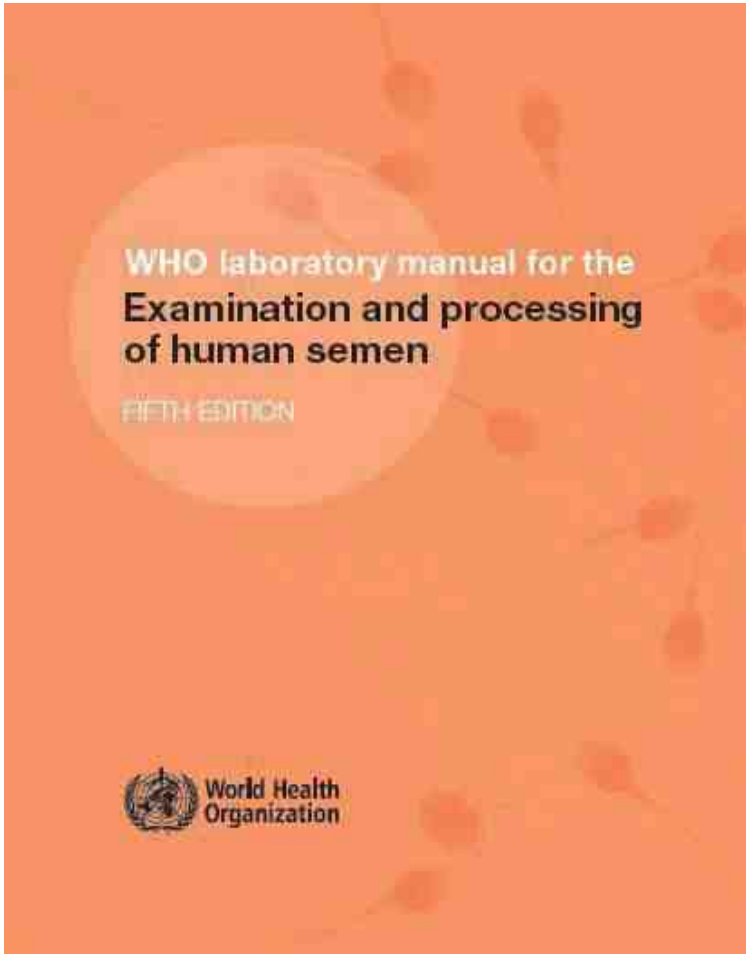
- 696 fertile couples, 765 infertile couples
- Considerable overlap between the groups
- 'none of the measures are diagnostic of infertility'

- *Minimal values similar to MacLeod and Gold in* **1951** "The greatest difference between the two groups [infertile and fertile] is seen at the count levels under 20million/cc. Only 5 per cent of fertile men compared with 16 per cent of the 'infertile' group fall into this category" (MacLeod and Gold, 1951). *Almost 60 years ago……'*

- *Remarkably : Data similar to new WHO 2009*
  Guzick *et al.,* (2001) NEJM 345, 1388-1399

# WHO Semen analysis

- Volume
- Concentration
- Motility
- Morphology



# Can we count sperm?

## The quality of the assessment is almost always poor….

**Semen analysis has a high degree of error**

**[or more correctly the person doing it does].**

UK NEQAS

UK NEQAS for Semen Analysis

Distribution : 40 Date : 17-Feb-2004

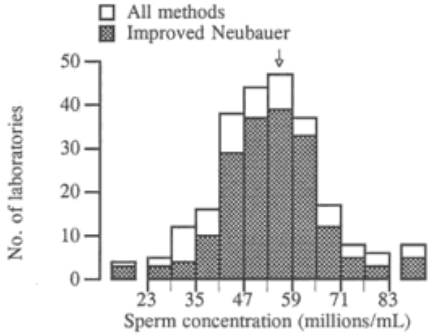
Analyte : Sperm concentration (millions/mL)

Laboratory :



## Specimen S157

| | n | Mean | SD | CV(%) |
|---|---|---|---|---|
| All methods | 242 | 53.47 | 9.74 | 18.2 |
| Horwell | 11 | 47.60 | 20.34 | 42.7 |
| Makler | 34 | 49.13 | 12.30 | 25.0 |
| Microcell | 6 | 53.34 | 7.12 | 13.3 |
| Improved Neubauer | 183 | 54.12 | 8.82 | 16.3 |
| Fast Read | 1 | | | |
| Fuchs Rosenthal | 2 | | | |
| Kova | 2 | | | |
| Mika | 1 | | | |
| No counting chamber | 1 | | | |
| Thoma | 1 | | | |




Your result 57

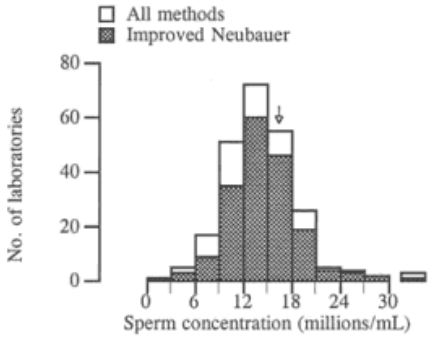
Designated value 53.47

Your bias (%) 6.60
Your BIS 33

Pool 140

## Specimen S158

| | n | Mean | SD | CV(%) |
|---|---|---|---|---|
| All methods | 241 | 14.24 | 3.57 | 25.1 |
| Horwell | 11 | 13.56 | 5.03 | 37.1 |
| Makler | 34 | 13.57 | 3.14 | 23.2 |
| Microcell | 6 | 11.90 | 4.70 | 39.5 |
| Improved Neubauer | 183 | 14.60 | 3.24 | 22.2 |
| Fast Read | 1 | | | |
| Fuchs Rosenthal | 2 | | | |
| Kova | 2 | | | |
| No counting chamber | 1 | | | |
| Thoma | 1 | | | |




Your result 17

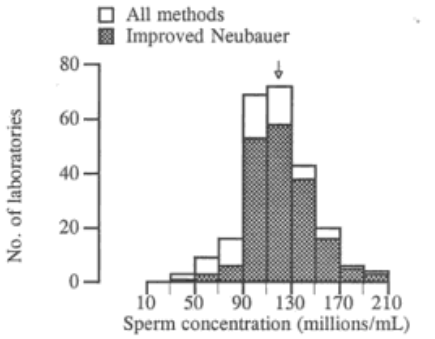
Designated value 14.24

Your bias (%) 19.37
Your BIS 39

Pool 141

## Specimen S159

| | n | Mean | SD | CV(%) |
|---|---|---|---|---|
| All methods | 242 | 118.65 | 19.17 | 16.2 |
| Horwell | 11 | 95.20 | 38.22 | 40.1 |
| Makler | 34 | 106.04 | 20.31 | 19.2 |
| Microcell | 6 | 82.50 | 17.90 | 21.7 |
| Improved Neubauer | 183 | 121.52 | 18.02 | 14.8 |
| Fast Read | 1 | | | |
| Fuchs Rosenthal | 2 | | | |
| Kova | 2 | | | |
| Mika | 1 | | | |
| No counting chamber | 1 | | | |
| Thoma | 1 | | | |




Your result 117

Designated value 118.65

Your bias (%) -1.39
Your BIS -7

Pool 142

# Adherence to WHO methods is very poor - throughout the world.

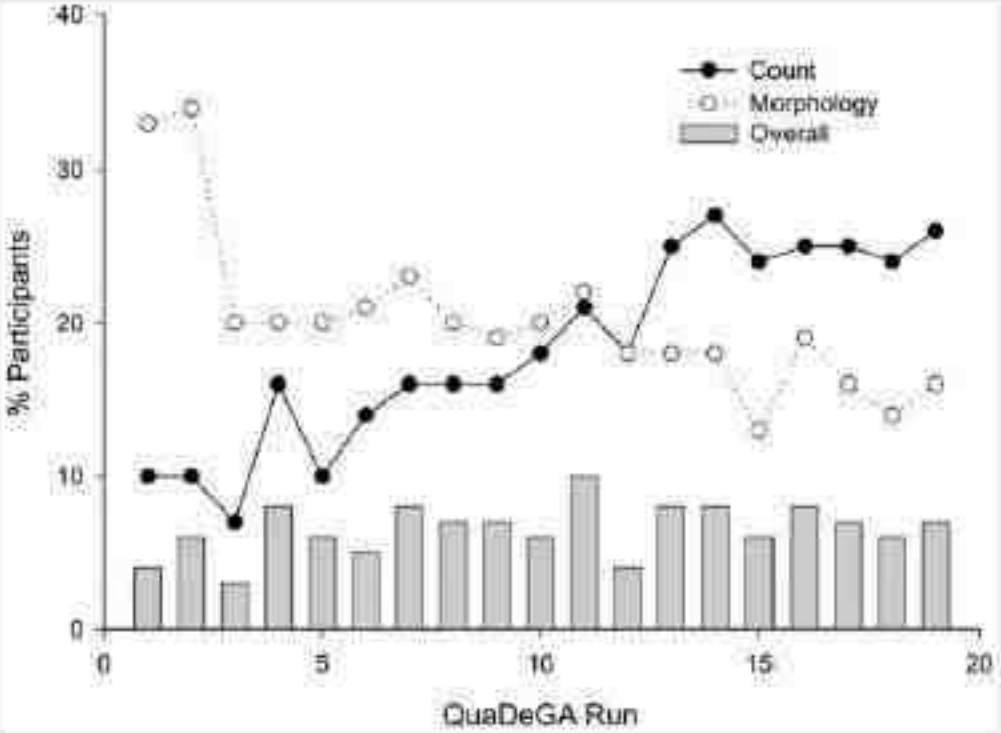
# Less than 10% UK laboratories are WHO compliant (From Riddell Hum Reprod [1995] 12, 3441-3445)

**Table III.** Summary of the number of 'specialist laboratories' (SL group) and 'DGH laboratories' (DGH group) that comply with the guidelines suggested in the WHO (1999) manual with regard to morphology assessment

| Criteria | SL group ($n = 19$) | DGH group ($n = 18$) | Total ($n = 37$) | Cumulative compliance[a] |
|---|---|---|---|---|
| Staining methods | 7 | 18 | 25 | 25 |
| Papanicolaou or Diff Quick | 5 | 12 | 17 | 17 |
| Sperm dimensions | 16 | 16 | 32 | 14 |
| ×100 oil immersion | 3 | 2 | 5 | 4 |
| Count ≥200 sperm | 6 | 5 | 11 | 3 |
| External quality control | 12 | 16 | 28 | 3 |
| Internal quality control | 8 | 8 | 16 | 2 |
| No. of compliant laboratories | 1 | 1 | 2 | 2 |

# *Similar in Germany…and not improving …*

**SUPPLEMENTAL FIGURE 1**

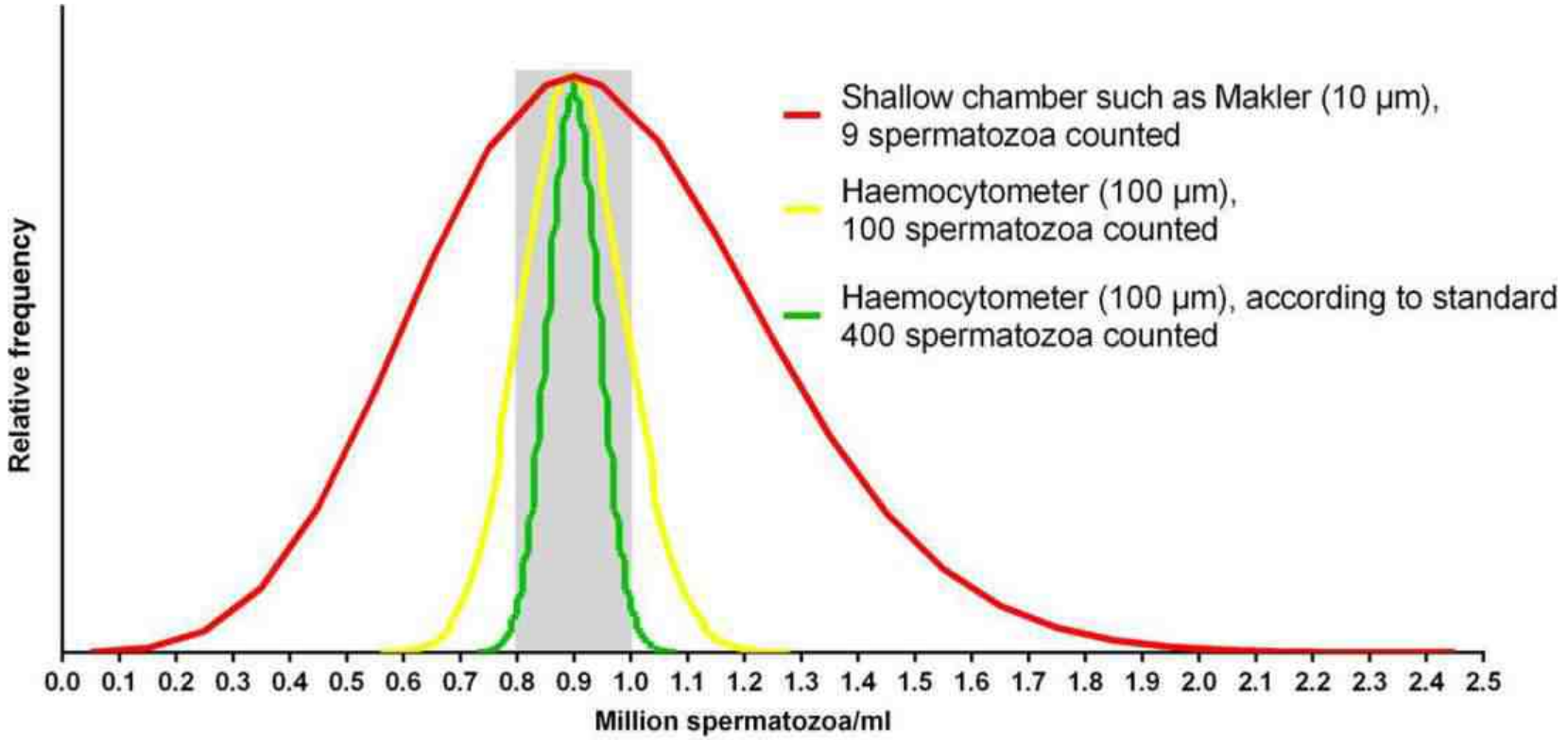



Adherence to WHO guidelines by the QuaDeGA participants in sperm counting (filled circles) and morphology (open circles). The bar graph indicates the percentage of laboratories that follow all the recommended procedures for the evaluation of sperm concentration/number, morphology and motility.

*Mallidis. Ten years of semen analysis EQC. Fertil Steril 2012.*

# Its important in context of IVF ICSI

# A schematic representation of the uncertainties associated with different numbers of spermatozoa counted with results of sperm counting in the borderline zone (grey box)—50:50 IVF/ICSI.




**Lars Björndahl et al. Hum. Reprod. 2016;31:227-232**

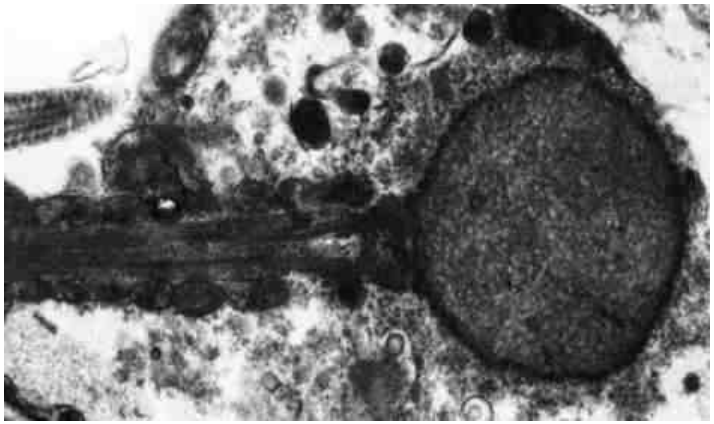


human reproduction

# Unfashionable but ….How can we improve it?

Perform it with more rigour……

# Not just an issue of numbers - Globozoospermia



- Incidence of globozoospermia 1:???????? sub-fertile men.
- Generally limited data as few cases reports

# Screening - When spermatogenesis goes wrong

- OA vs NOA

# NOA not as successful as OA

From Osmanagaoglu *et al.,* Hum Reprod 18, 1836-1840.

K.Osmanagaoglu *et al.*

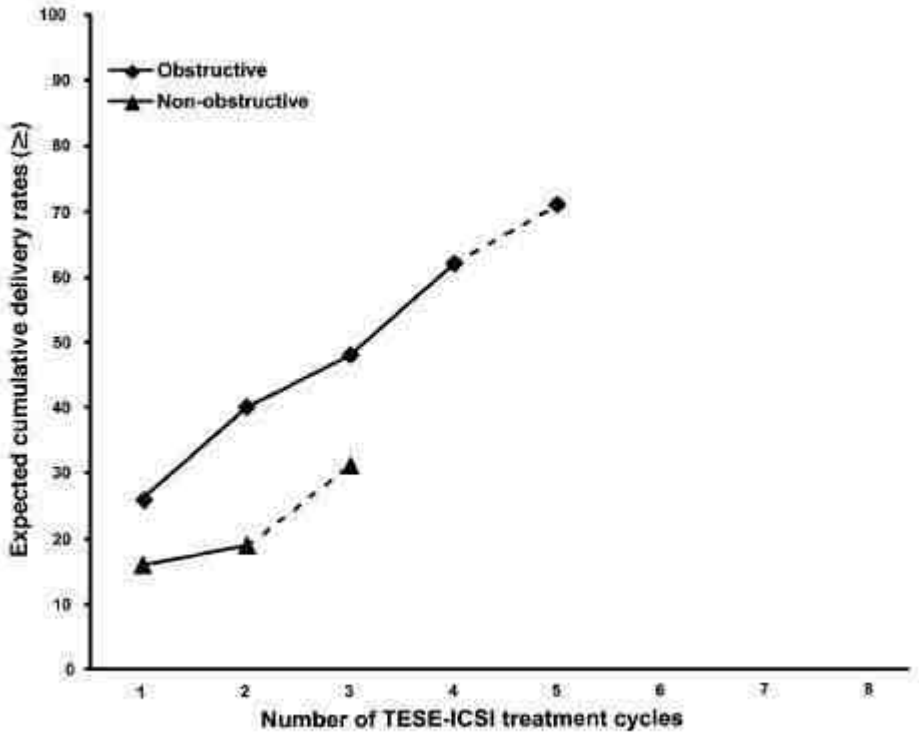



Figure 1. Comparing expected cumulative delivery rates in patients

**Table I.** Causes of obstruction and spermatogenetic defects

| | Obstructive azoospermia [*n* (%)] | Nonobstructive azoospermia [*n* (%)] | Hyposperm atogenesis [*n* (%)] |
|---|---|---|---|
| Anejaculation or retrograde ejaculation | 1 (0.3) | 1 (0.2) | 1 (1.8) |
| Testicular tumour (benign or malign) | 1 (0.3) | 3 (0.5) | 0 (0) |
| Inguinal hernia | 3 (0.8) | 3 (0.5) | 0 (0) |
| Mumps orchitis | 0 (0) | 9 (1.4) | 0 (0) |
| Congenital bilateral absence of the vas deferens | 80 (20.9) | 1 (0.2) | 4 (7.0) |
| Post-chemotherapy or radiotherapy | 4 (1.0) | 24 (3.8) | 1 (1.8) |
| Cryptorchidism | 20 (5.2) | 99 (15.8) | 10 (17.5) |
| Hydrocele | 3 (0.8) | 1 (0.2) | 0 (0) |
| Hypogonadotropic hypogonadism | 0 (0) | 4 (0.6) | 0 (0) |
| Infection | 32 (8.4) | 19 (3.0) | 5 (8.8) |
| Karyotype anomaly | 1 (0.3) | 65 (10.3) | 0 (0) |
| Failed reversal | 88 (23.1) | 10 (1.6) | 4 (7.0) |
| Trauma or testicular torsion | 3 (0.8) | 1 (0.2) | 0 (0) |
| Varicocele | 9 (2.4) | 45 (7.2) | 4 (7.0) |
| Yq deletion | 0 (0) | 26 (4.1) | 0 (0) |
| Steinert disease | 0 (0) | 2 (0.3) | 2 (3.5) |
| Congenital anomaly genital tract | 3 (0.8) | 0 (0) | 0 (0) |
| Diabetic neuropathy | 1 (0.3) | 0 (0) | 0 (0) |
| Post-vasectomy | 12 (3.1) | 0 (0) | 0 (0) |
| Toxic | 0 (0) | 0 (0) | 1 (1.8) |
| Young syndrome | 1 (0.3) | 0 (0) | 0 (0) |
| Unexplained | 119 (31.2) | 315 (50.1) | 25 (43.8) |

From Vernaeve et al., 2006
Hum Reprod 21, 1551-1554

# Specific conditions

# Screening and ART success in Non Mosaic KS


1. Causes still remain to be finally resolved e.g. why reduction in germ cell development?

2. Consistent pregnancies in couples where man has non mosaic KS using testicular cells. At least 101 children born (Fullerton)

3. Prediction of recovery and success remains difficult. Recovery ~44% of the time. Most predictive factor - age of man at recovery (younger being better).

4. Suggestion 3 groups of patients :

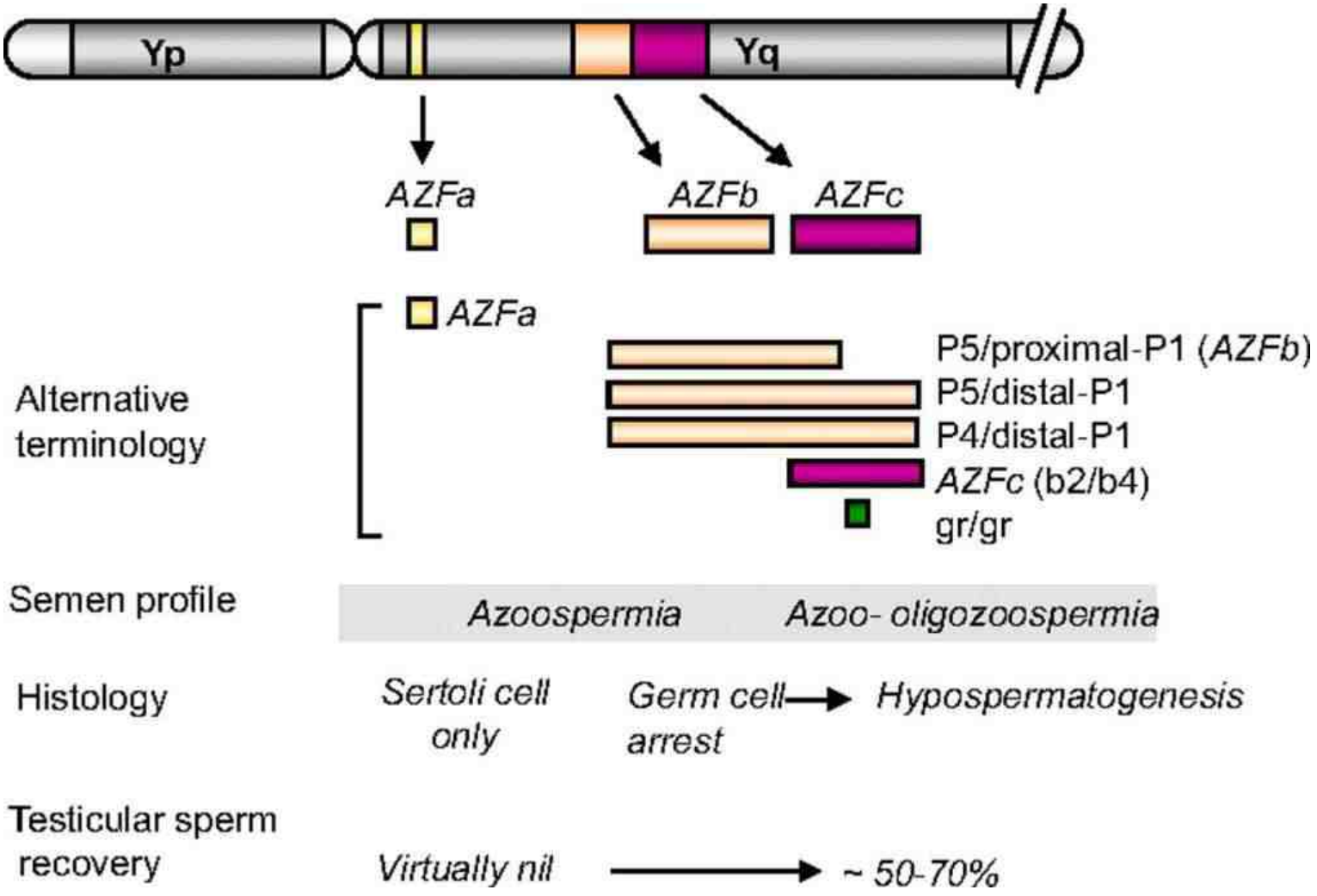
    1. With focal spermatogenesis
    2. No sperm but spermatogonia
    3. No germ cells at all

5. The majority of births healthy.

Reference: Van Saen et al., (2012) Fertil Steril , 97, 319 - 323

# Possible use of Y deletions….

# Yq microdeletions.




McLachlan R I , O'Bryan M K JCEM 2010;95:1013-1024

The Journal of Clinical Endocrinology & Metabolism

# Y deletions provide valuable information recovery of sperm

**TABLE 2**

**Outcomes of microdissection TESE in azoospermic men stratified by Y microdeletion status.**

| Etiology of azoospermia | Sperm retrieved | Sperm not retrieved | Total | Retrieval rate |
|---|---|---|---|---|
| AZFa | 0 | 2 | 2 | 0% |
| AZFb | 0 | 7 | 7 | 0% |
| AZFb+c | 0 | 7 | 7 | 0% |
| AZFa+b+c | 0 | 4 | 4 | 0% |
| AZFc | 15 | 6 | 21 | 71.4%[a] |
| Nondeleted, idiopathic | 188 | 197 | 385 | 48.8%[a] |

*Note:* TESE = testicular sperm extraction; AZF = azoospermic factor.

[a] Comparison of retrieval rates in AZFc deleted men and idiopathically azoospermic nondeleted men, $P < .05$ (Fisher's exact test).

*Stahl. Y microdeletion screening is essential. Fertil Steril 2010.*

# Y deletions provide valuable information – success

**TABLE 3**

**Clinical pregnancy outcomes in idiopathically azoospermic men and in azoospermic AZFc deleted men in whom sperm were surgically retrieved.**

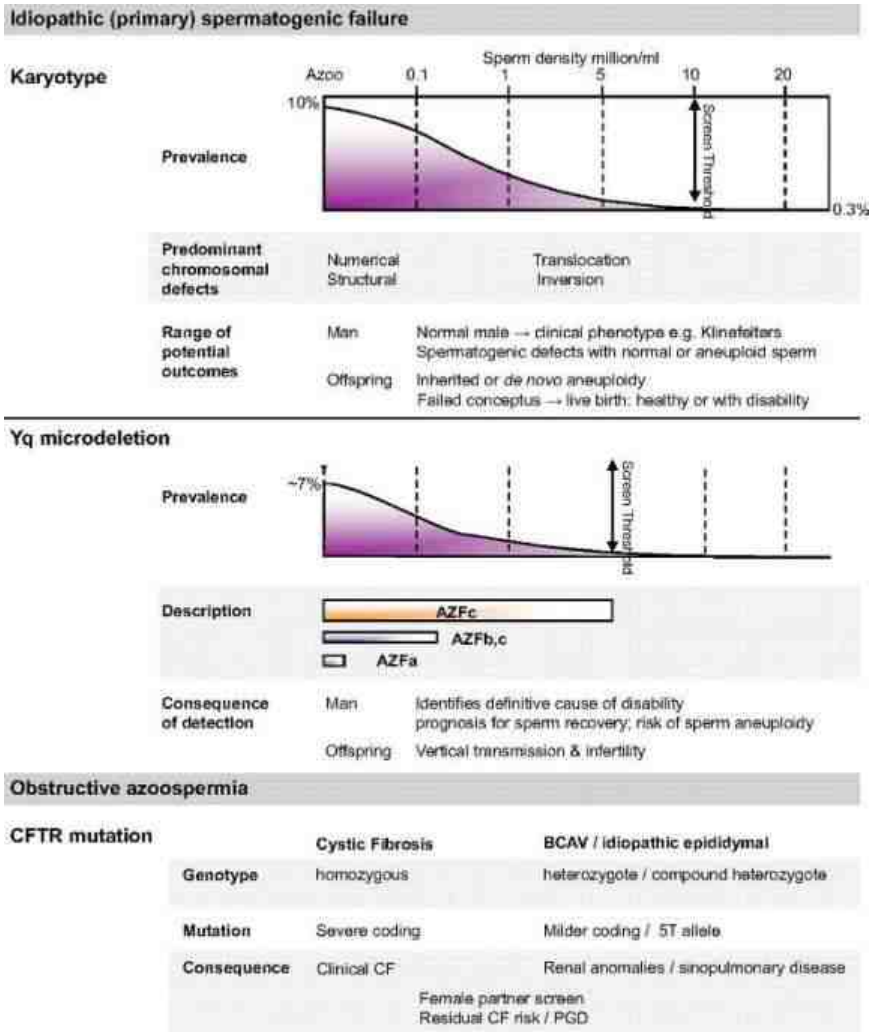
| Etiology of azoospermia | Sperm retrieved | Clinical pregnancies | Clinical pregnancy rate |
|---|---|---|---|
| Idiopathic | 188 | 91 | 48.4%[a] |
| AZFc deletion | 15 | 10 | 66.7%[a] |

[a] Not significantly different ($P = .19$, Fisher's exact test).

*Stahl. Y microdeletion screening is essential. Fertil Steril 2010.*

# Potential summary

# Essential genetic investigations in male infertility – a Model – 2010.




McLachlan R I , O'Bryan M K JCEM 2010;95:1013-1024

THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

# Whilst data limited there appears no significant cause for concern over heath of the children

# Neonatal outcome

Belva *et al.*, (2011) Hum Reprod 26, 1752-1758

**Table III** Low birthweight, very low birthweight and prematurity rates according to sperm origin.

| | Testicular sperm[a] (*n* = 512) | Epididymal sperm[a] (*n* = 182) | Ejaculated sperm (*n* = 2477) |
|---|---|---|---|
| Low birthweight | | | |
| Singletons | 26/314 (8.2) | 5/96 (5.2) | 101/1299 (7.8) |
| Multiples | | | |
| Twin | 92/168 (55.4) | 32/55 (58.1) | 588/1098 (53.5) |
| Triplet | 16/21 (76.2) | 17/18 (94.4) | 70/80 (87.5) |
| Very low birthweight | | | |
| Singletons | 4/314 (1.3) | 0/96 (0) | 21/1299 (1.6) |
| Multiples | | | |
| Twin | 8/168 (4.8) | 4/55 (7.3) | 54/1098 (4.9) |
| Triplet | 5/21 (23.8) | 4/18 (22.2) | 30/80 (37.5) |
| Prematurity | | | |
| Singletons | 32/316 (10.1) | 12/93 (12.9) | 126/1299 (9.7) |
| Multiples | | | |
| Twin | 104/160 (65) | 33/58 (56.9) | 647/1098 (58.9) |
| Triplet | 21/21 (100) | 21/21 (100) | 80/80 (100) |

Data are given as numbers (%).

[a]No difference was found comparing low birthweight, very low birthweight and prematurity rate in the testicular and epididymal sperm group versus the ejaculated sperm group.

- Questionnaire of 530 children born after ICSI with testicular cells and 194 with epididymal sperm. Compared to 2516 with ejaculated cells
- Overall neonatal health in terms of birth parameters, major abnormalities and chromosomal aberrations was ‘reassuring’

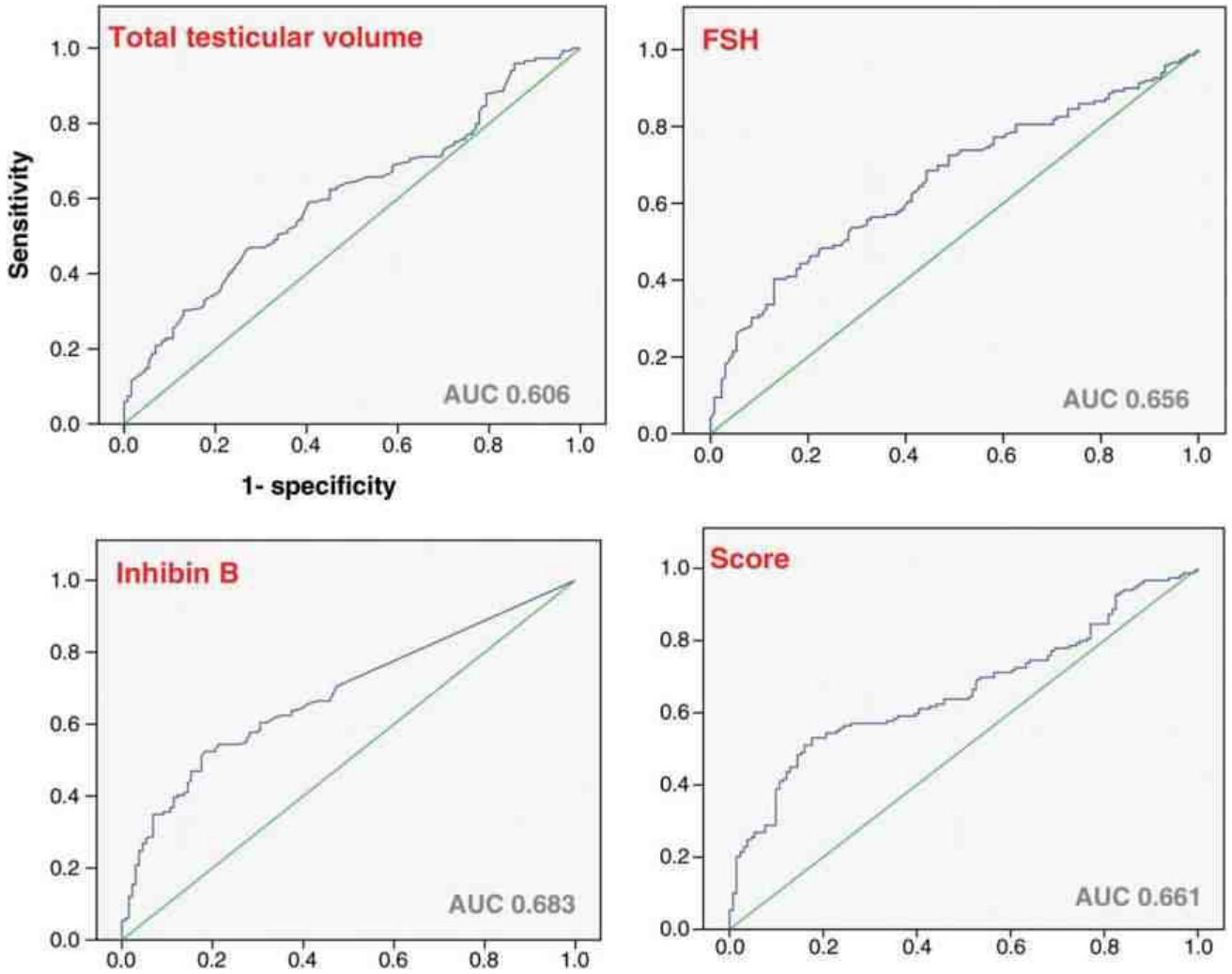
# Predicting successful outcomes particularly for NOA (aside KS and Y deletions)

## What screens?

- Traditionally techniques have limited usefulness (e.g. volume, FSH, inhibin B).

- Possibility of placing them together n= 280. Positive likelihood ratio~3.

# ROC curves for TTV, FSH, inhibin B and our score as a guide to the presence of spermatozoa in 280 men with NOA.




Boitrelle F et al. Hum. Reprod. 2011;26:3215-3221



human reproduction

# However…

- Still limited use but may help provide realistic chances for patients

**Human Reproduction, Vol.26, No.12 pp. 3213–3214, 2011**
Advanced Access publication on September 18, 2011 doi:10.1093/humrep/der316

human reproduction

EDITORIAL COMMENTARY *Andrology*

## How to predict fatherhood for men with non-obstructive azoospermia opting for TESE–ICSI?

**Herman Tournaye***

Vrije Universiteit Brussel and Centre for Reproductive Medicine, 101 Laarbeeklaan, B-1090 Brussels, Belgium

*Correspondence address. E-mail: tournaye@uzbrussel.be

# Potential new markers

- One example would be seminal plasma proteins

Research



## Verification of Male Infertility Biomarkers in Seminal Plasma by Multiplex Selected Reaction Monitoring Assay*

Andrei P. Drabovich‡, Keith Jarvi§¶‖, and Eleftherios P. Diamandis‡§**‡‡§§

Seminal plasma is a promising biological fluid to use for noninvasive clinical diagnostics of male reproductive system disorders. To verify a list of prospective male

ential diagnosis of azoospermia. *Molecular & Cellular Proteomics 10: 10.1074/mcp.M110.004127, 1–13, 2011.*

# What types of germ cells from the testis can we inject? Any change?

Stages of mature spermatozoa?

Elongated spermatids?

Round spermatids?

How do we judge success?

# Round Spermatids

## ASRM (2008) summary

(ASRM Practice Committee Fertil Steril 90, Supplement 3 S199-200

1. Generally unsuccessful – clinically used in rare circumstances
2. Experimental technique
3. Identification an issue
4. Oocyte activation
5. Genetic abnormalities 1 specific paper of concern
6. Poor embryonic development

Vloeberghs V, Verheyen G, Tournaye H. Clinics (Sao Paulo). 2013;68 Suppl 1:151-6. Review

# Yana -Round spermatids may work…..

**Table 1. Clinical outcome of ROSI partners of 76 women in this table had round spermatids as their most advanced spermatogenic cells**

| Assisted fertilization | Cryopreservation of embryos | Total no. of women | Total no. of treatment cycles | Percentage of oocytes fertilized (fert/total) (%) | Percentage Oocytes cleaved (cleaved/total) (%) | Total no. of transferred embryos | No. and percentage of recipients (%) | | | | Total no. of live offspring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total transferred cycles | Pregnancy* | Miscarriage | Live offspring delivered | |
| ROSI | No | 58 | 162 | 55.6 (330/594) | 44.9 (267/594) | 152 | 121 | 20 (16.5) | 13 (65.0) | 7 (5.8)§§ | 9 |
| (present study) | Yes | 28† | 42 | 76.4 (107/140) | 71.4 (100/140) | 56 | 42 | 10 (23.8) | 5 (50.0) | 5 (11.9)¶ | 5 |
| TESE and ICSI | No | 260 | 540 | 44.9 (833/1855) | 43.5 (807/1,855) | 562 | 468 | 134 (28.6) | 27 (20.1) | 107 (22.9)§ | 121 |
| | Yes | 140 | 295 | 68.2 (825/1210) | 65.3 (790/1,210) | 377 | 290 | 174 (60.0) | 14 (8.0) | 160 (55.2)§§,¶¶ | 176 |

Partners of 76 women in this table had round spermatids as their most advanced spermatogenic cells. To compare the efficiencies of ROSI and ICSI, part of the data collected in our clinic after TESE and ICSI are included in this table. § vs. §§ and ¶ vs. ¶¶, $P < 0.05$; Fisher's protected least significant difference test.
*No and percentage of recipients with positive germinal sacs detected by ultrasound scanning between 4 and 5 wk of gestation.
†Ten of these 28 patients failed to get pregnancy previously when they received nonfrozen ROSI embryos.

**Fourteen babies born after round spermatid injection into human oocytes**

Atsushi Tanaka[a,1], Motoi Nagayoshi[a], Yosuke Takemoto[a], Izumi Tanaka[a], Hiroshi Kusunoki[a], Seiji Watanabe[a], Keiji Kuroda[a], Satoru Takeda[a], Masahiko Ito[a], and Ryuzo Yanagimachi[b,1]

[a]Saint Mother Obstetrics and Gynecology Clinic, Institute for ART, Fukuoka 807-0825, Japan; [b]Animal Diversity Sciences, Graduate School of Agriculture, Kobe University, Kobe 657-8501, Japan; [c]Department of Anatomical Science, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan; [d]Department of Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo 113-8033, Japan; [e]Department of Infectious Diseases, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan; and [f]Department of Anatomy, Physiology, and Biochemistry, University of Hawai Medical School, Honolulu, HI 96822

Contributed by Ryuzo Yanagimachi, September 29, 2015 (sent for review July 30, 2015; reviewed by J. Michael Bedford and Martin M. Matzuk)

# Summary

## Essentials and discussion points

- Physical examination and history
- **High Quality Semen Analyses**
- **If abnormal spermatogenesis : potential screens.**
  - **Genetics e.g. Y deletions**
  - **Routine ? FSH**
  - **Possible new seminal  plasma**
  - **? Spermatids**

# Repeated attempts can be successful.

**Table II.** Consecutive sperm retrieval in patients with nonobstructive azoospermia

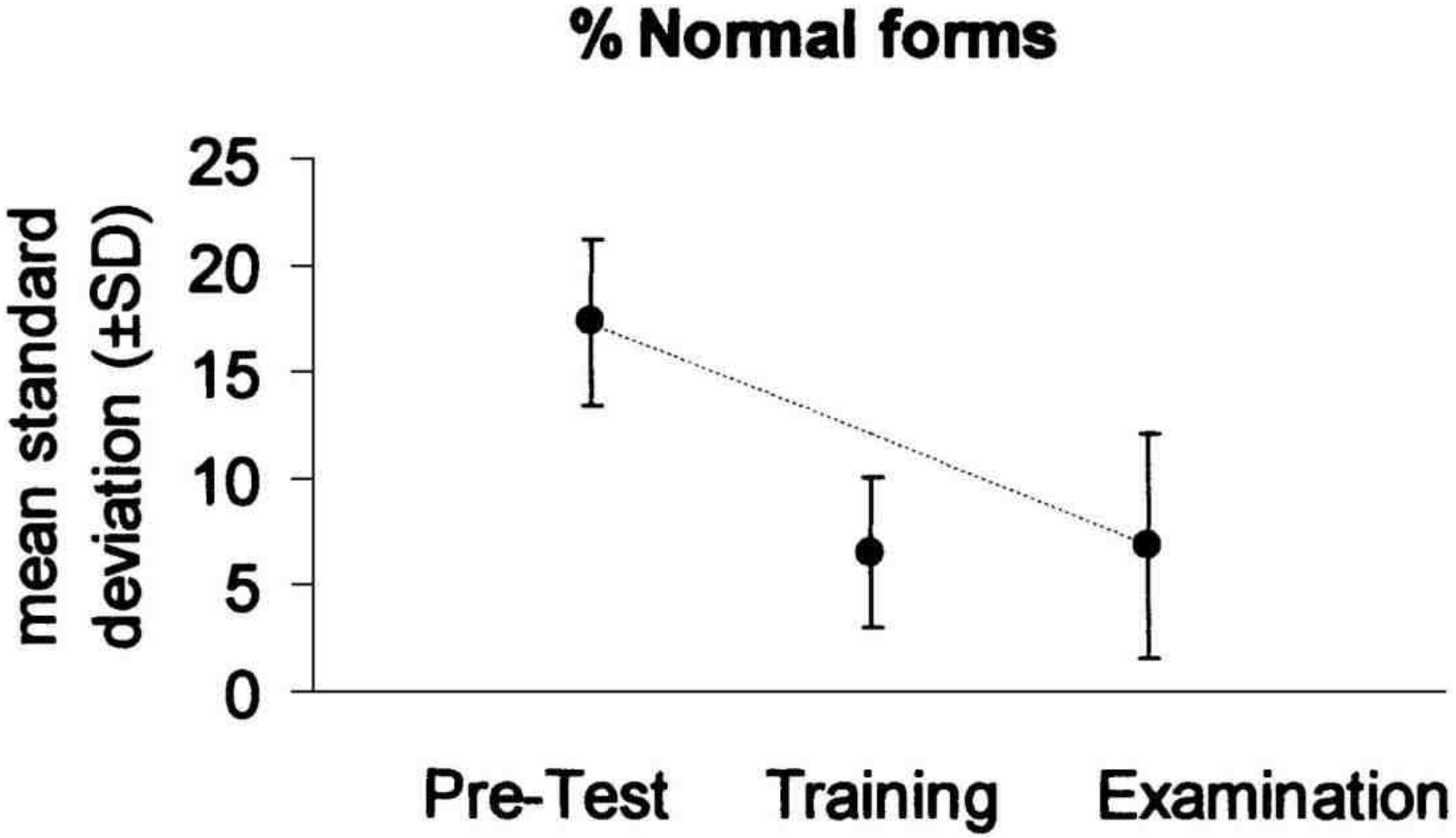
| Rank | *n* | Successful sperm recovery (*n*) | Successful sperm recovery (%) |
|---|---|---|---|
| Total | 784 | 384 | 49 |
| 1 | 628 | 261 | 41.6 |
| 2 | 103 | 77 | 74.7 |
| 3 | 34 | 28 | 82.3 |
| 4 | 11 | 11 | 100 |
| 5 | 6 | 5 | 83.3 |
| 6 | 2 | 2 | 100 |

From Vernaeve et al., 2006
Hum Reprod 21, 1551-1554

**Also issue of how to train and improved compliance**

***High quality consistent training does help***

**Decrease in course participants' variability, as revealed by average SD of results from assessments of proportion of morphologically normal sperm in test samples, from pre-test (n = 16), through training (n = 15) to examination (n = 15).**




**Björndahl L et al. Hum. Reprod. 2002;17:1299-1305**



human reproduction

# High quality consistent training does help

**TABLE 1**

Descriptive statistics of 290 participants' morphology scores during 18 pretraining and posttraining sessions.

| | Normal cells recorded during pretraining (%) | | Normal cells recorded during posttraining (%) | |
|---|---|---|---|---|
| | Slide 1 | Slide 2 | Slide 3 | Slide 4 |
| Reference slide value | 15 | 5 | 14 | 9 |
| Lowest value | 0.0 | 0.0 | 0.0 | –8.0 |
| Highest value | 98.0 | 75.0 | 60.0 | 33.0 |
| Mean | 29.7 | 12.6 | 13.2 | 4.4 |
| 95% confidence interval | 26.6–33.2 | 10.5–14.7 | 12–14 | 3.4–5.4 |
| Median | 17.0 | 6.5 | 13.0 | 2.0 |
| SD | 26.6 | 16.2 | 8.0 | 5.6 |
| Percent difference from mean | 104.0 ± 189.1[a,b] | 13.0 ± 15.8[c,d] | 13.0 ± 8.1 | 4.2 ± 5.7 |

[a] Compared with slide 3, $P = .0001$, Mann-Whitney test for independent samples.
[b] Compared with slide 4, $P = .001$, Mann-Whitney test for independent samples.
[c] Compared with slide 3, $P$ = not significant, Mann-Whitney test for independent samples.
[d] Compared with slide 4, $P$ = not significant, Mann-Whitney test for independent samples.

*Franken. Semenology training. Fertil Steril 2010.*

# Generally NOA and OA

- Cochrane meta analysis suggests no hard evidence to suggest one technique over another (van Peperstraten et al., (2008) Cochrane Database Syst Rev (16)CD002808)

- Men with spermatogenesis e.g. vasectomy reversal – possible from epididymis – motile sperm have similar success rates to testicular cells (Nicopoullos *et al.,* 2004 Fertil Steril 82, 691-701) and can be cryopreserved.

- NOA : TESE but maybe via microsurgery.
  - Recovery rates are very variable: 40-50% (Tournaye 2012 Asian J Andrology 14, 103-108)
  - Meta analysis: Deruyver Y, Vanderschueren D, Van der Aa F. Andrology. 2014 Jan;2(1):20-4)

# EAU Guidelines

(Jungwirth et al., 2012 Eur Urol. 62:324-32)

| Diagnosis | Unselected 12,945 | Azoospermic 1446 |
|---|---|---|
| Idiopathic | 30.3 % | 13.3% |
| KS (XXY) | 2.6% | 13.7% |
| Y deletions | 0.3% | 1.6% |

Significant component unknown.