Can the Standard Semen Analysis be used to Decide between Insemination Methods for In Vitro Fertilization?

David H. McCulloh, Ph.D., H.C.L.D. (A.B.B.), C.C. (A.B.B.)
NYU Fertility Center
Research Associate Professor
Department of Obstetrics and Gynecology
New York University School of Medicine
New York, NY

- Disclosures
- I am an Embryologist

Disclosures for DHM:

Assistant Laboratory Director - NYU Langone MEDICAL CENTER | NYU Fertility Center

Andrology Laboratory Director - BIOGENETICS corporation

Director of Clinical Science - რეპროარტი
ქართულ-ამერიკული
რეპროდუქციული კლინიკა
REPROART
GEORGIAN-AMERICAN CENTER
FOR REPRODUCTIVE MEDICINE

President & Principal Scientist - MA McCulloh Associates
Quality Management: Quality Control, Quality Assurance, Quality Improvement
Data Management: Database Creation, Statistical Analysis

The Problem:

- Does the Semen Analysis Assess the sperm's ability to perform those critical steps that are necessary to fertilize an egg?
- What are the steps?

McCulloh's View of the Steps

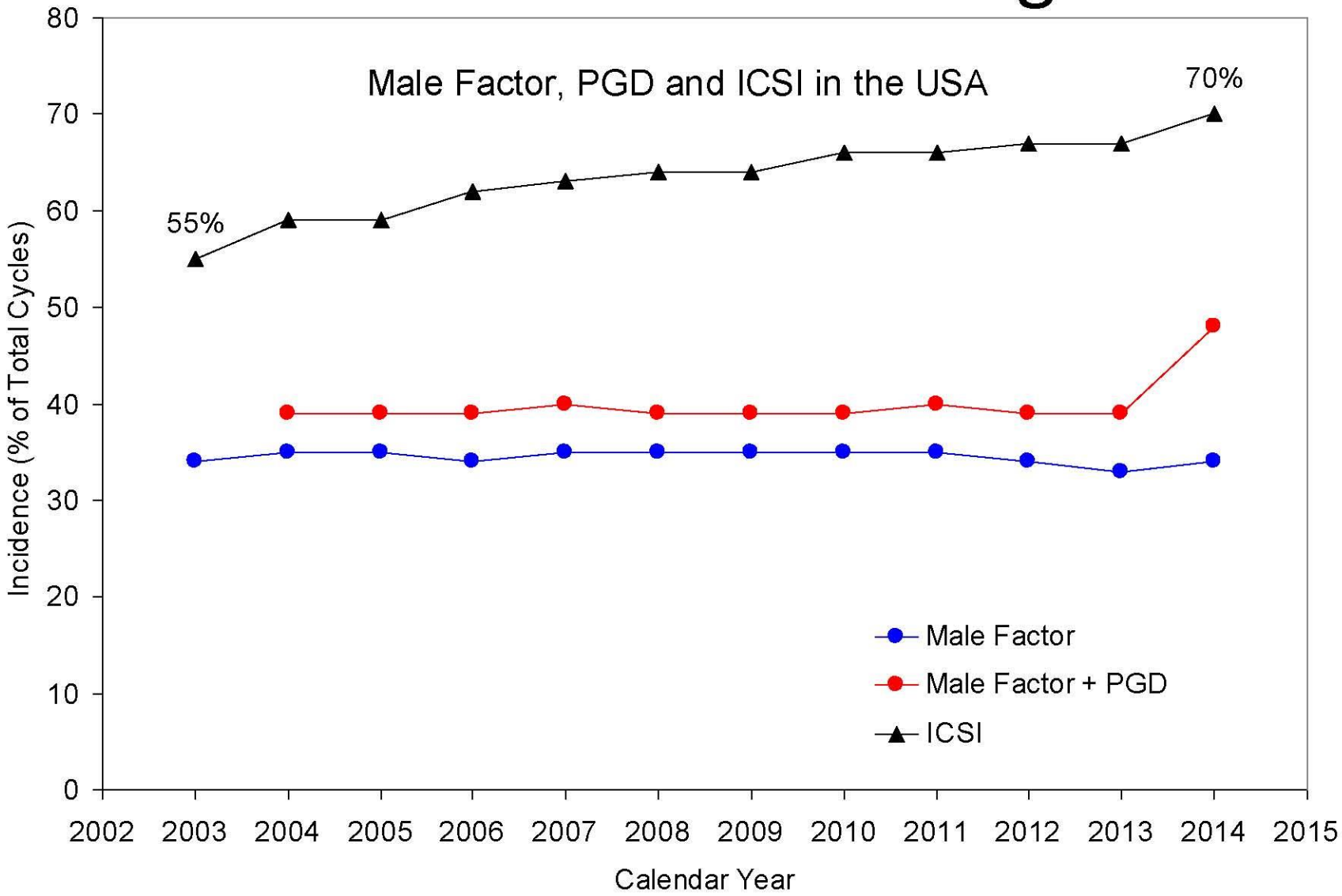
- Sperm must be present (sperm count > 0)
- Must gain access to the vicinity of the egg (motility > 0)
- Must penetrate the oocyte's investments (cumulus, zona)
  - Capacitation?, hyperactivation?
  - Acrosome reaction (release of hyaluronidase, acrosin, trypsin-like protease)
    - (must be adequate acrosome – morphology > 0)
- Must fuse with the oolemma
- Must "inject" the sperm contents into the egg
  - Nucleus (DNA, chromatin)
  - Centriole (microtubular organizing center)
- Must activate the oocyte
  - Phospholipase C zeta
- Genome must integrate with the egg's genome
  - Full complement of 23 chromosomes sufficiently undamaged.

Semen Analysis

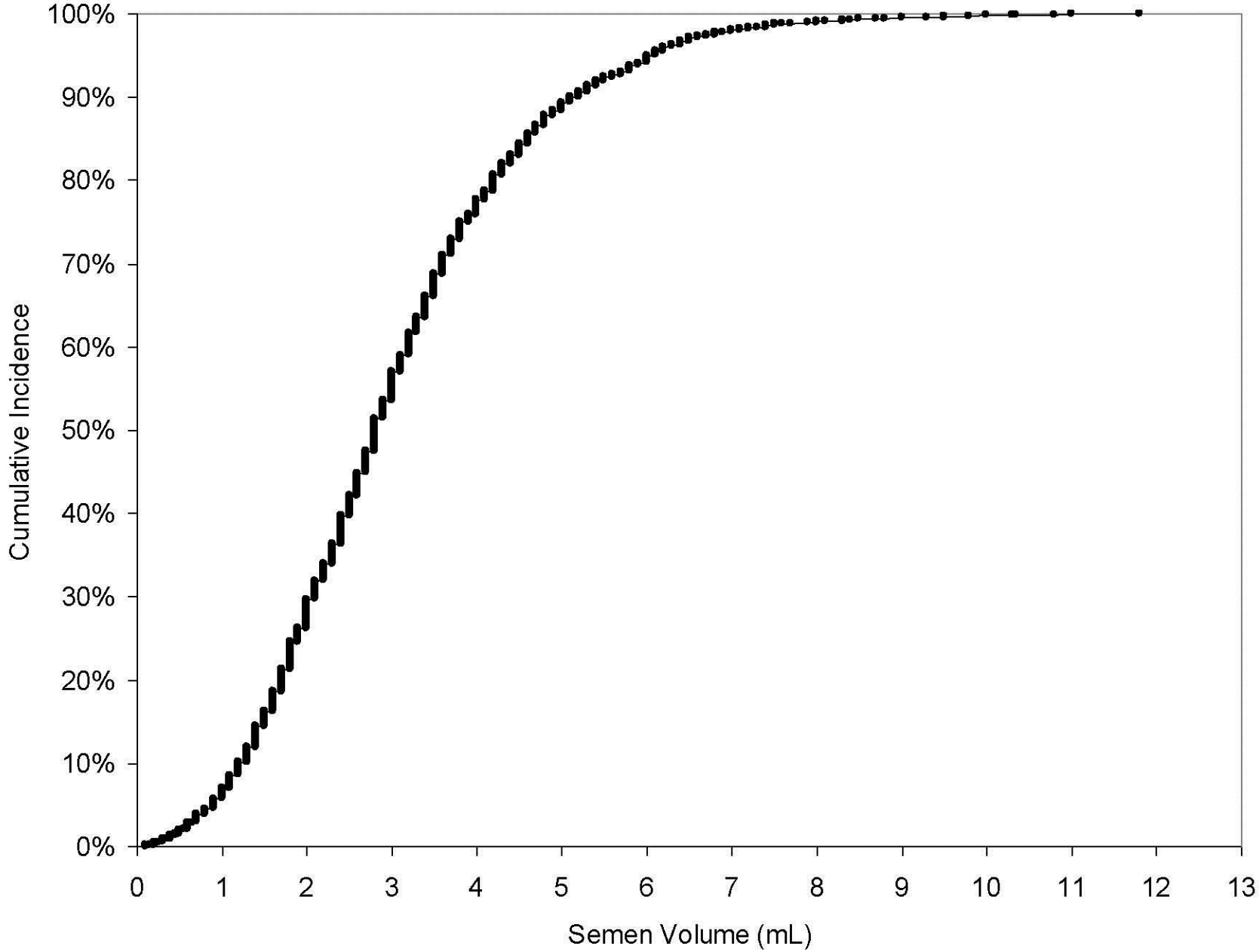
- Semen Volume (ml)
- Viscosity
- pH
- Sperm Count (M/ml)
- Motility (%)
- Progression (1 – 4) (not A, B, C, D)
- Kruger (% Normal Morphology - Strict Criteria)

- *Some consideration of sperm following wash using semen overlaid on 80% Pureception (~Isolate ~Puresperm ~ Percoll) followed by wash in 3 ml modified HTF.*
  - *Motility*
  - *Progression*
  - *Recovery (Motile sperm recovered/Motile sperm washed)*

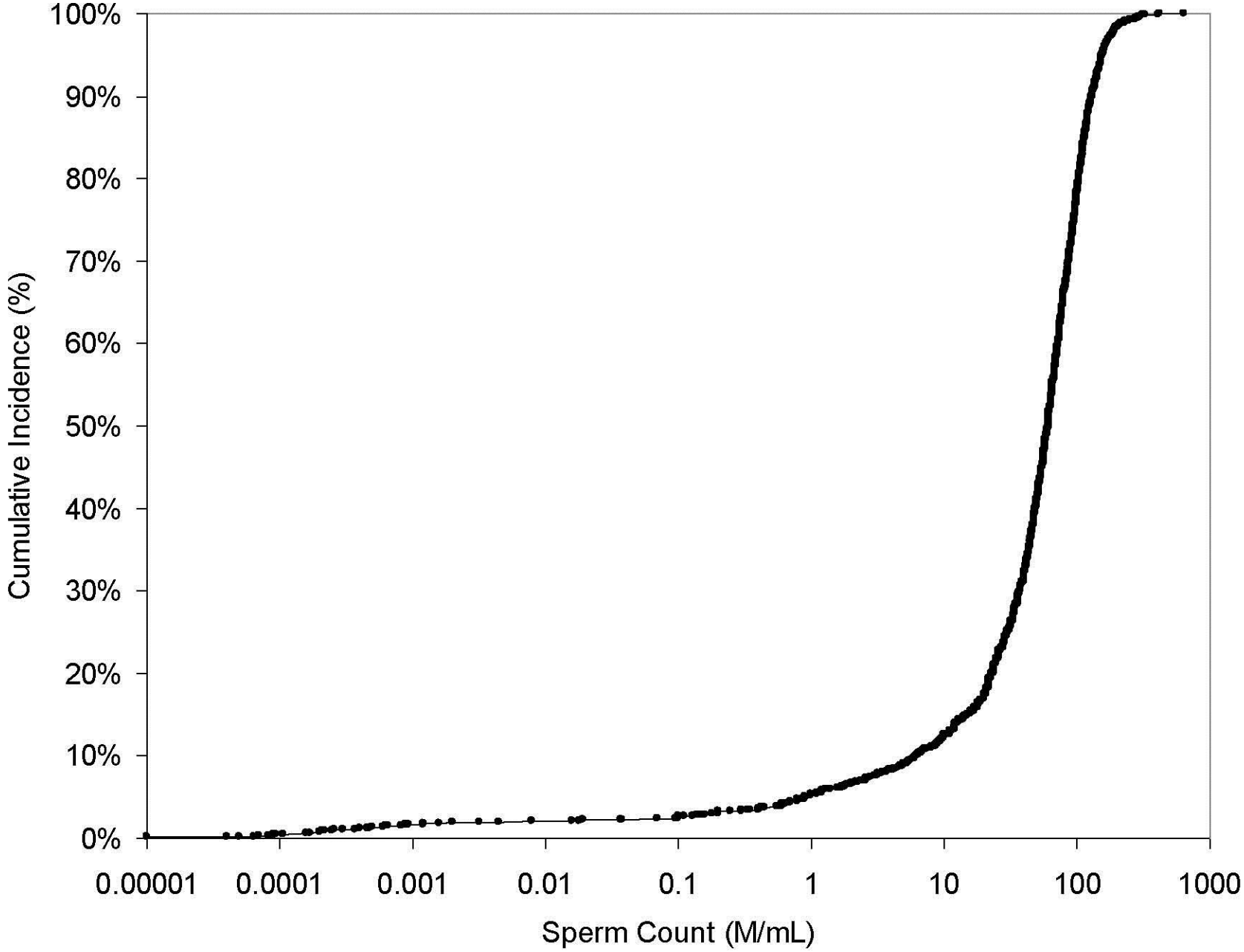
No Change in Male Factor, but Use of ICSI is Increasing!



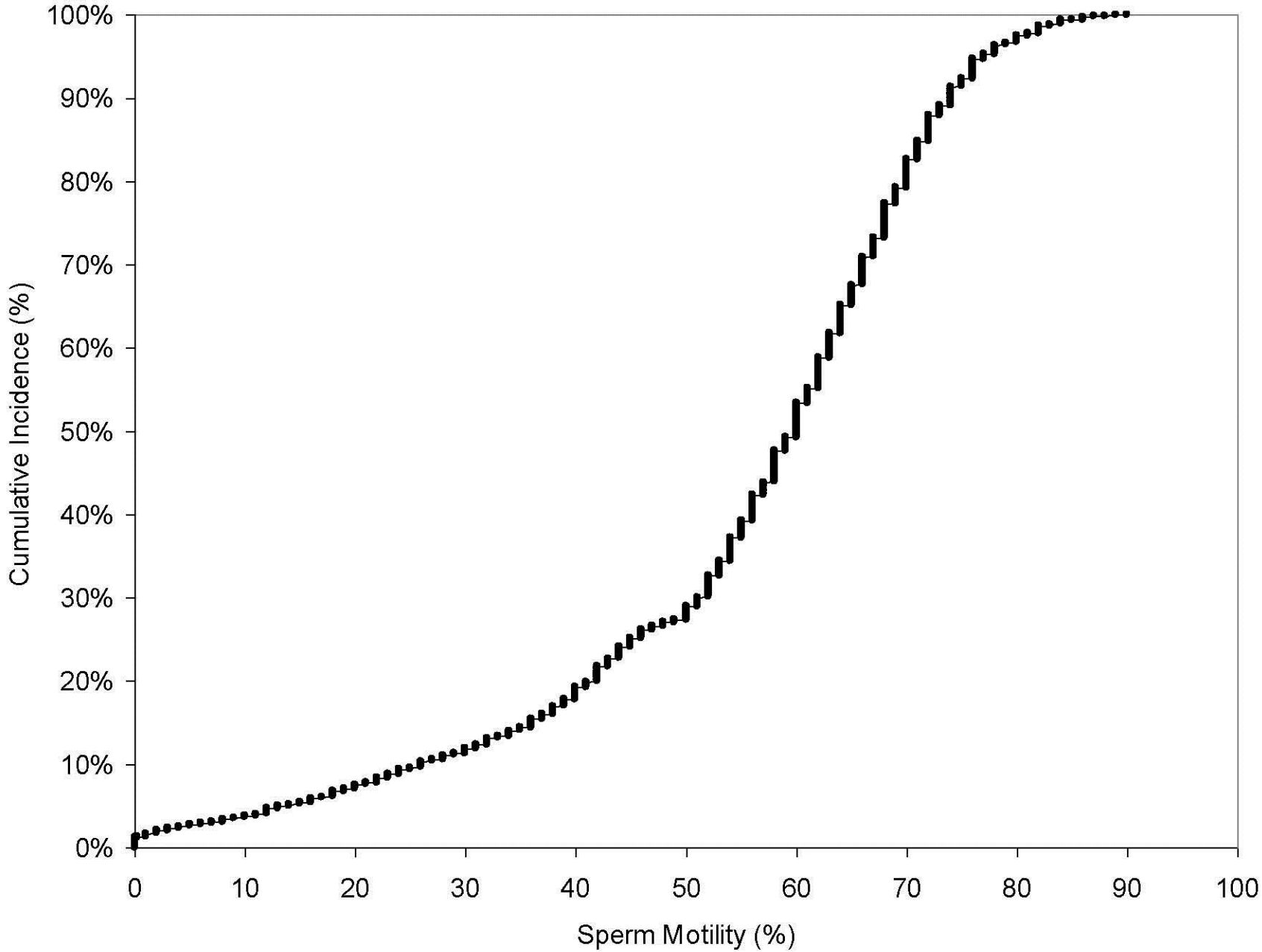
Semen Volume



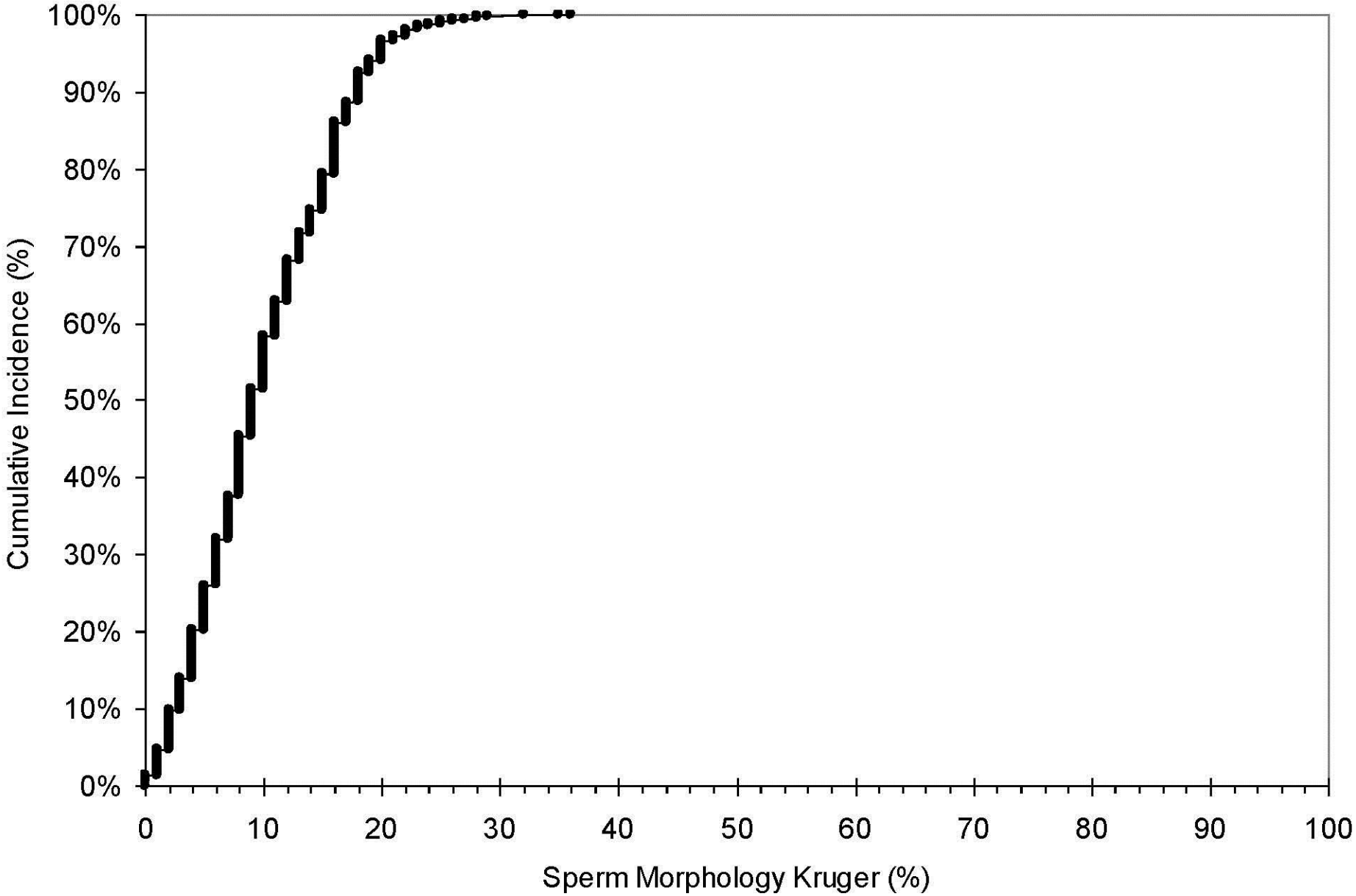
Sperm Count


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Incidence (%)
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Sperm Count (M/mL)

Motility


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Incidence (%)
0
10
20
30
40
50
60
70
80
90
100
Sperm Motility (%)

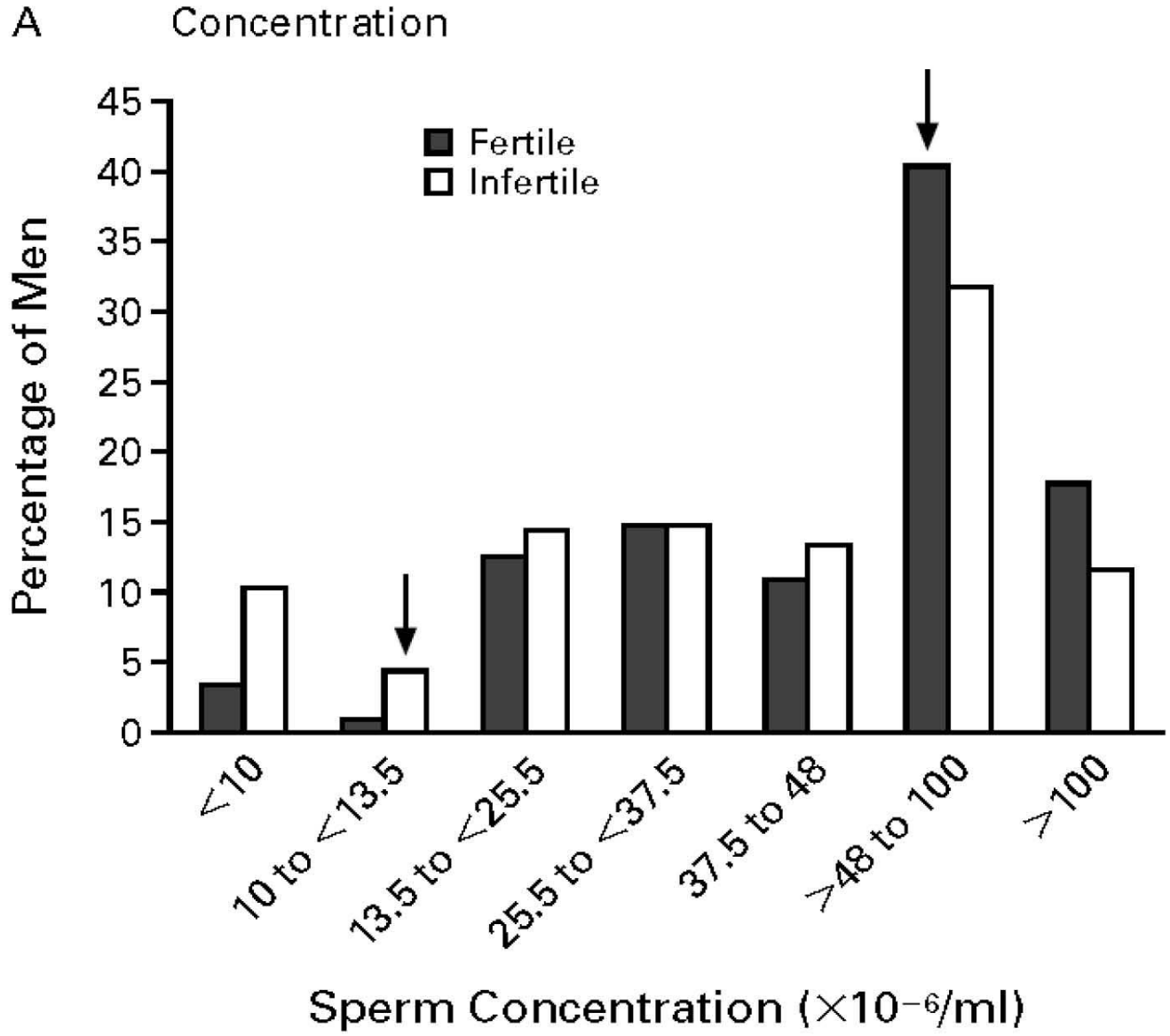
Morphology (Strict, Kruger)



Changing Criteria for Male Factor

(applied to NYU Semen Analyses 2009-2012)

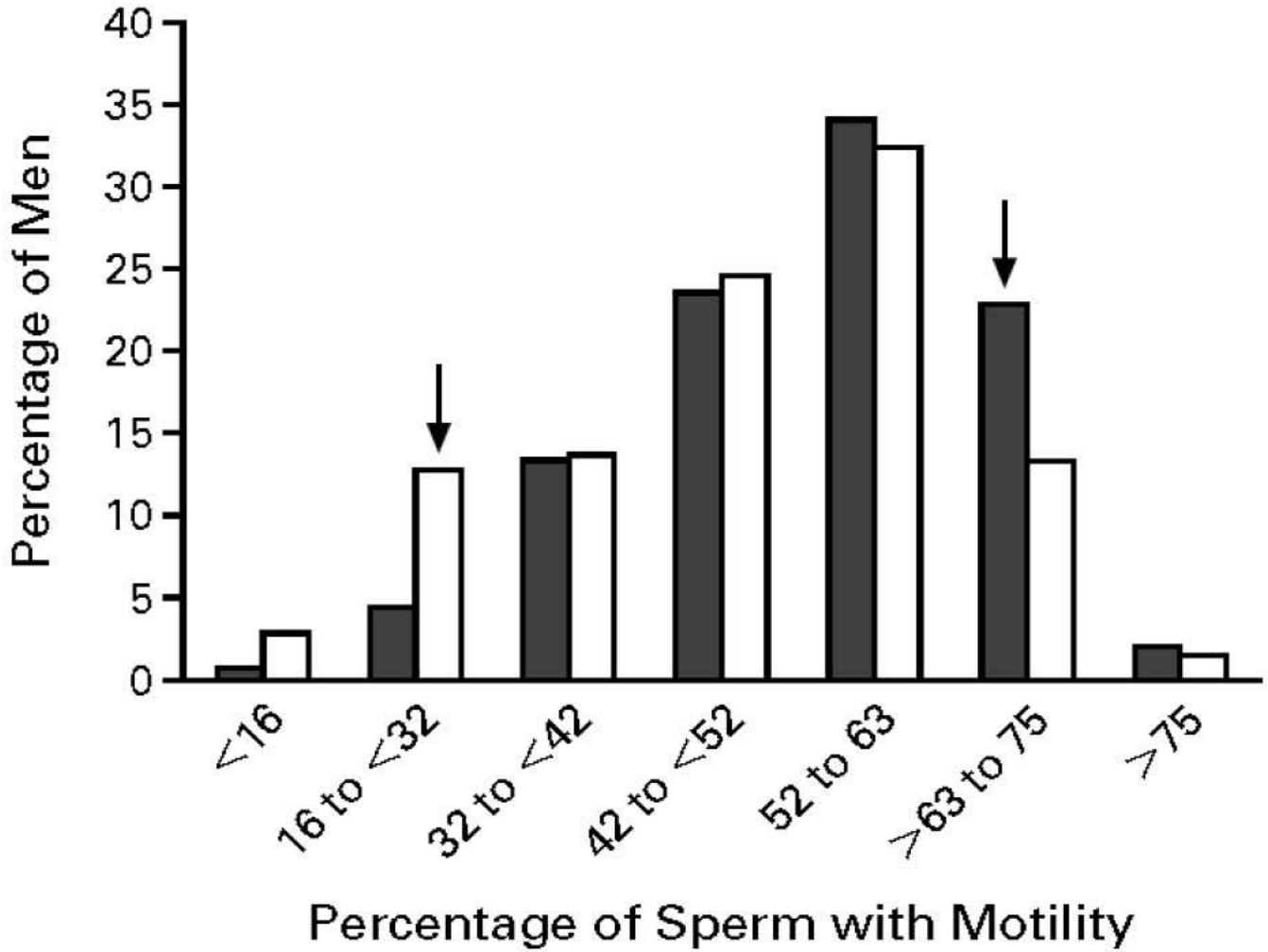
Source		Count	Motility	Morph	>/= 1	>/=2	3
WHO 1999	criterion (</=)	20	50	14			
	%	**17.4%**	**28.9%**	**74.6%**	**77.6%**	**31.2%**	**12.2%**
Guzick 2001	criterion (<)	13.5	32	9			
	%	**14.4%**	**12.4%**	**45.4%**	**48.6%**	**16.0%**	**7.5%**
WHO 2010	criterion (<)	15	40	4			
	%	**14.8%**	**17.8%**	**14.0%**	**28.2%**	**13.2%**	**5.1%**



AND INFERTILE MEN

DAVID S. GUZICK, M.D., PH.D., JAMES W. OVERSTREET, M.D., PH.D., PAM FACTOR-LITVAK, PH.D., CHARLENE K. BRAZIL, B.S., STEVEN T. NAKAJIMA, M.D., CHRISTOS COUTIFARIS, M.D., PH.D., SANDRA ANN CARSON, M.D., PAULINE CISNEROS, PH.D., MICHAEL P. STEINKAMPF, M.D., JOSEPH A. HILL, M.D., DONG XU, M.PHIL., AND DONNA L. VOGEL, M.D., PH.D., FOR THE NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK*

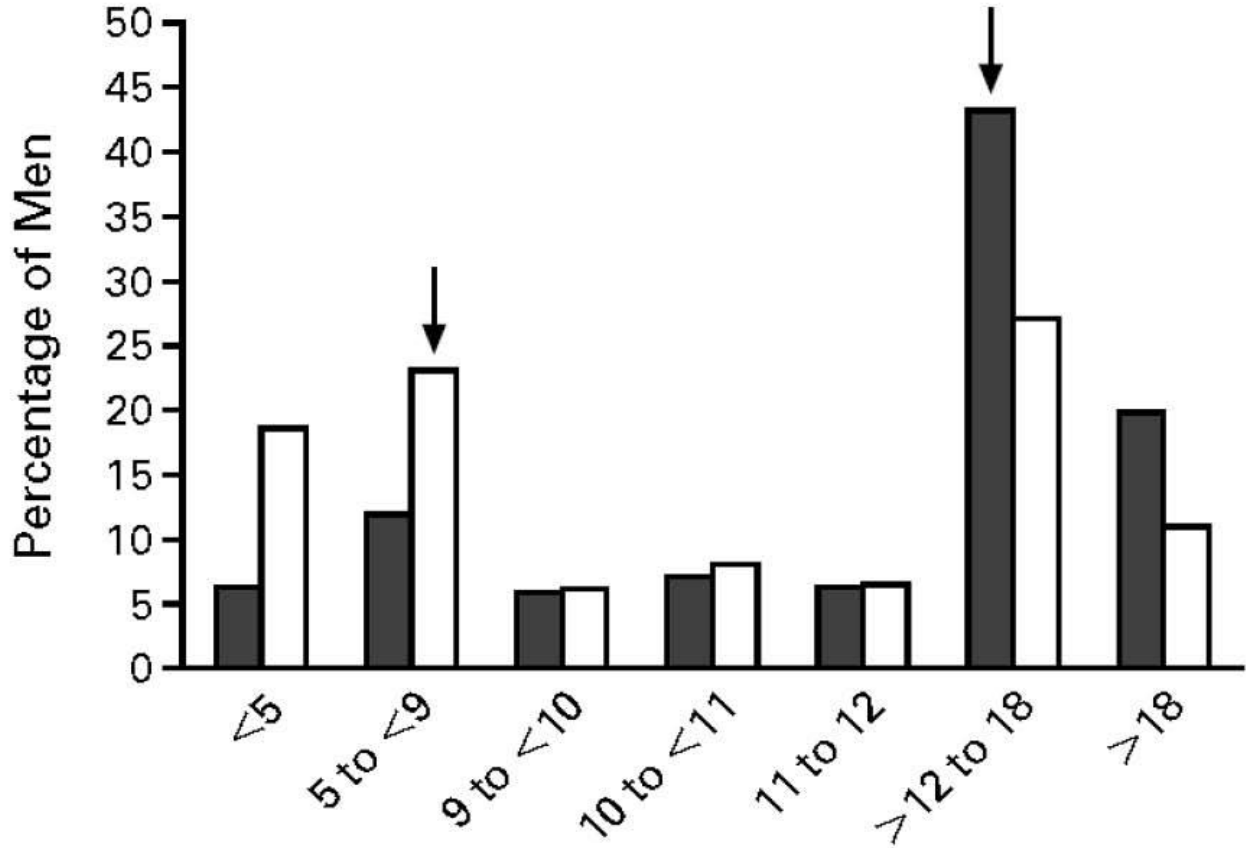
B Motility



SPERM MORPHOLOGY, MOTILITY, AND CONCENTRATION IN FERTILE AND INFERTILE MEN

DAVID S. GUZICK, M.D., PH.D., JAMES W. OVERSTREET, M.D., PH.D., PAM FACTOR-LITVAK, PH.D., CHARLENE K. BRAZIL, B.S., STEVEN T. NAKAJIMA, M.D., CHRISTOS COUTIFARIS, M.D., PH.D., SANDRA ANN CARSON, M.D., PAULINE CREGHEAD, PH.D., MICHAEL P. STEINKAMPF, M.D., JOSEPH A. TIEF, M.D., DONG XU, M.PHIL., AND DONNA L. VOGEL, M.D., PH.D., FOR THE NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK*

C Morphology



Percentage of Sperm with Normal Morphologic Features

SPERM MORPHOLOGY, MOTILITY, AND CONCENTRATION IN FERTILE AND INFERTILE MEN

DAVID S. GUZICK, M.D., PH.D., JAMES W. OVERSTREET, M.D., PH.D., FAN FACTOR-LITVAK, PH.D., CHARLENE K. BROWN, D.D.S., SHEVA L. NAKAJIMA, M.D., CHRISTOS COUTIFARIS, M.D., PH.D., SANDRA ANN CARSON, M.D., PAULINE CASTROS, PH.D., MICHAEL P. STEINKAMPF, M.D., JOSEF T.A. HILL, M.D., DONG XU, M.P.H., AND DONNA L. VOGEL, M.D., PH.D., FOR THE NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK*

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
	$\times 10^{-6}$/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.

DAVID S. GUZICK, M.D., PH.D., JAMES W. OVERSTREET, M.D., PH.D., PAM FACTOR-LITVAK, PH.D., CHARLENE K. BRAZIL, B.S., STEVEN T. NAKAJIMA, M.D., CHRISTOS COUTIFARIS, M.D., PH.D., SANDRA ANN CARSON, M.D., PAULINE CISNEROS, PH.D., MICHAEL P. STEINKAMPF, M.D., JOSEPH A. HILL, M.D., DONG XU, M.PHIL., AND DONNA L. VOGEL, M.D., PH.D., FOR THE NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK*

Analysis of Variance

- Compared Variance AMONG Patients
  - With Variance WITHIN Patients' Repeated Analyses
- For 502 patients who had ≥ 2 semen analyses (1198 total) at URA using visual scoring of
  - sperm count,
  - motility,
  - progression, and
  - morphology
- For each parameter, AMONG Patient variability was significantly greater than WITHIN Patient variability ($P < 0.05$ with 501/695 d.f.)
  - Parameters: Volume, viscosity, pH, count, %motile, progression, %normal (Kruger), %normal acrosome

What does Significant ANOVA mean?

- Patient-to-patient Variability (AMONG)
  - Bob versus Ted

    was significantly greater than
- Variability of Each Individual when compared with himself (WITHIN)
  - Bob versus Bob and/or
  - Ted versus Ted
- Mean Square WITHIN =(Variance) is the average variability of values for a patient
  - Used to calculate Standard Deviation (within)

“Within Patient” Variability is Large

(patients with 2 or more semen analyses at U.R.A. – visual count microcells)

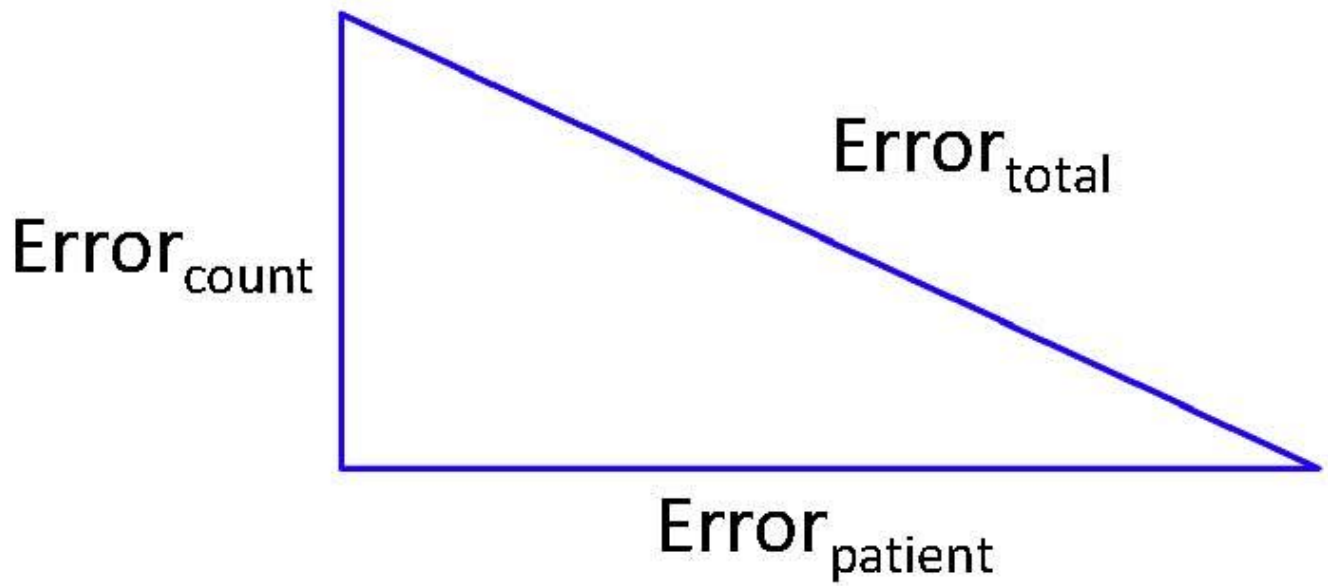
Parameter (units)	mean	Within pt. Std. Dev.	Coeff. of Variation (Std.Dev./mean)
Volume (ml)	2.86	0.86	30.2%
Viscosity	1.08	0.32	29.6%
pH	8.04	0.40	4.9%
Sperm Count (M/ml)	34	18	54.0% **
Motility (%)	52	13	25.4%
Progression (1 – 4)	2.02	0.99	49.1% **
Kruger (%Normal)	5.6	4.7	84.3% **
Acrosome (%Normal)	64	16	24.7%

Counting Error Affects Semen Analysis!

- Errors are related to $\sqrt{(1/N)} = (1/N)^{1/2} = 1/\sqrt{N}$
  - **Where N = the number of sperm counted**
  - Numbers counted: Sperm Count
    - Std. Dev. = √(mean) [Poisson assumptions]
      - » Mean = N / #squares
      - » Std. Dev. = √(N / #squares)
      - » S.E.M. = (√N) / #squares
    - **S.E.M./Mean = 1/√N**
  - Percentages: Motility, Morphology
    - **S.E.M. = √(pq/N)** [Binomial assumptions]
      - » Morphology (p = normal : q = NOT normal)
      - » Motility (p = motile : q = NOT motile)

Counting Error and Patient Error Add

$$Error_{total} = \sqrt{(Error_{patient}^{2} + Error_{count}^{2})}$$



Relative Contributions of Counting Error and Patient Error

Parameter	Std. Dev. Within	Counting Error	Patient Error
Sperm Count (M/ml)	18.4	4.0[1]	18.0
Motility (%)	13.2	5.9[2] **	11.8
Progression (1 – 4)	0.99	0.14[3]	0.98
Kruger (% Normal)	4.7	2.3[4] **	4.1
Acrosome (%Normal)	15.8	4.8[4]	15.1

[1] assuming 10 squares in triplicate (~71 sperm counted)
[2] assuming ~71 sperm counted
[3] average counts with ~100 sperm counted
[4] assuming 100 sperm counted

What do all these numbers mean?

- Values reported in a Semen Analysis are ESTIMATEs
- No value in the semen analysis is absolute or without error!
- Errors of these estimates are quite large, especially for
  - Kruger Morphology
  - Sperm Count
- Errors come from at least two sources:
  - Counting Error (especially for Motility, Kruger)
    - we have control over this (via # sperm counted)
  - Within Patient variability (esp. Count, Progression, Kruger)
    - we have NO control over this
  - It makes little sense to decrease counting error too much since within-patient variability is large

Does Semen Analysis assess Function?

- What functions are required of sperm?
  - Must be present (Count > 0)
  - Must Pass through investments
    - Motility – to pass through cumulus/corona
    - Acrosome – to pass through zona pellucida (morphology)
  - Must Fuse (sperm plasma membrane – oocyte plasma membrane) probably involves "docking macromolecules on sperm surface (no SA param)
  - Must Activate Oocyte (sperm cytoplasmic activators like Phospholipase C zeta)(no SA param)
  - Must contribute a normal, functional Genetic Complement (no SA param)

Multiple Regression:

Are sperm parameters associated with outcome?
or restated: Do any semen parameters matter?

Outcomes considered:

- Fertilization
  - Standard Insemination
  - Following Intracytoplasmic Sperm Injection (ICSI)
- Implantation (Clinical Pregnancy)
- Implantation Time
- Live Birth
- Pregnancy Loss = 1/(Live Birth per Clinical Pregnancy)

Parameters Investigated

(All were examined… red were associated)

- Female patient age (years)
- Male patient age (years)
- Semen volume (ml)
- Sperm Count (Semen)
- Motility (Semen)
- Progression (Semen)
- Kruger (Semen)
- Acrosome (Semen)
- Motility (Harvest)
- Recovery (% of motile sperm recovered post processing)
- Progression (Post Processing)

Let’s Start at the End and work back

- Are Semen Parameters associated with:
  - Clinical Pregnancy?
  - Live Birth?
  - Pregnancy Loss?

Clinical Pregnancy (N = 605)

$\ln(\text{Odds Ratio}_{\text{Clin Preg}}) =$

- $0.117 \text{ X Age}_{\text{female}}$ (-49.08%)
- $0.289 \text{ X Progression}_{\text{Semen}}$ (-14.87%)
- + 4.54

Live Birth (N = 606)

$$\ln(\text{Odds Ratio}_{\text{Live Birth}}) =$$
$$- 0.150 \text{ X } \text{Age}_{\text{female}} \text{ (-52.59\%)}$$
$$- 0.314 \text{ X } \text{Progression}_{\text{Semen}} \text{ (-13.33\%)}$$
$$+ 5.19$$

Live Birth per Clinical Pregnancy (N = 250) (= 1/[Clinical Pregnancy Loss])

$$\ln(\text{Odds Ratio}_{\text{Live Birth/Clinical Pregnancy}}) = -0.155 \text{ X } \text{Age}_{\text{female}}{}^{a} \text{ (-48.87\%)} + 6.349$$

- NO semen analysis parameter was associated with Live Birth per Clinical Pregnancy (or with Clinical Pregnancy Loss)

[a] $\text{Age}_{\text{female}}$ correlated with Age_{male}

Implantation Time (N = 237)

(note: multiple linear regression)

$$Time_{Implantation} =$$
$$- 0.564 \times Progression_{Semen}{}^{a} \text{ (-1.24 days)}$$
$$+ 0.0565 \times Kruger^{b} \text{ (1.07 days)}$$
$$+ 10.052$$

[a] $Progression_{semen}$ correlated with $Progression_{harvest}$, $Motility_{semen}$ and $Motility_{harvest}$

[b] Kruger correlated with Acrosome

So, Is Semen Analysis Predictive?

- Clinical Pregnancy & Live Birth – YES
  - $Progression_{semen}$
- Pregnancy Loss – NO
- Implantation Time – YES
  - Kruger and $Progression_{semen}$

How About for Fertilization?

- Standard Insemination?
- ICSI?

$$\text{Fertilization}_{\text{Standard Insem}}\ (N=485)$$

$$\ln(\text{Odds Ratio}_{\text{fertilization}}) =$$

$$.0295\ X\ \text{Motility}_{\text{harvest}}\ (29.28\%\%)$$

$$+.0753\ X\ \text{Kruger}\ (26.71\%)$$

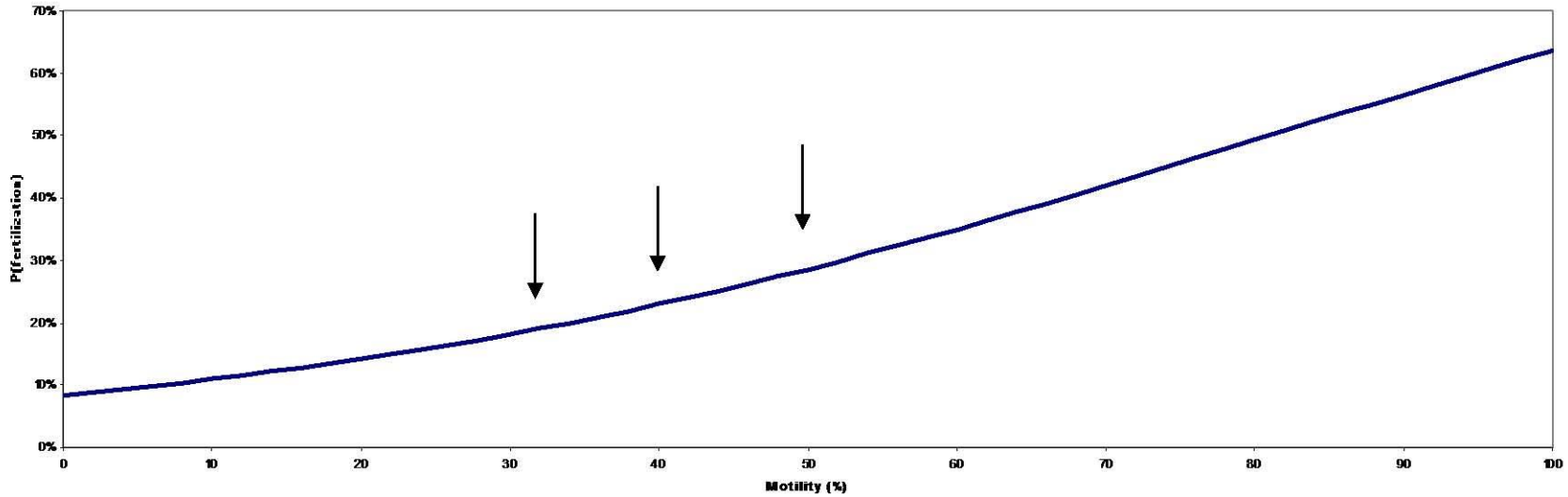
$$+1.49\ X\ \text{Recovery}\ (29.81\%)$$

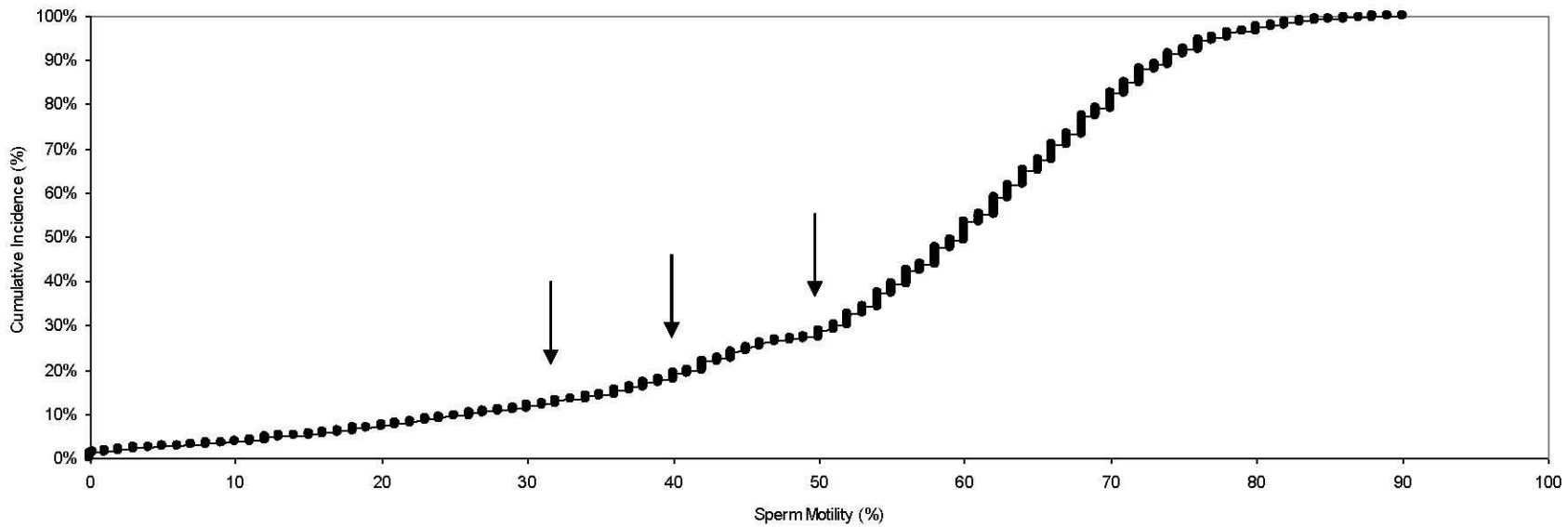
$$-.0608\ X\ \text{Age}_{\text{female}}\ (-22.18\%)$$

$$-.503\ X\ \text{Progression}_{\text{semen}}\ (-20.06\%)$$

$$-.456$$

Motility (Criteria and Prevalence)

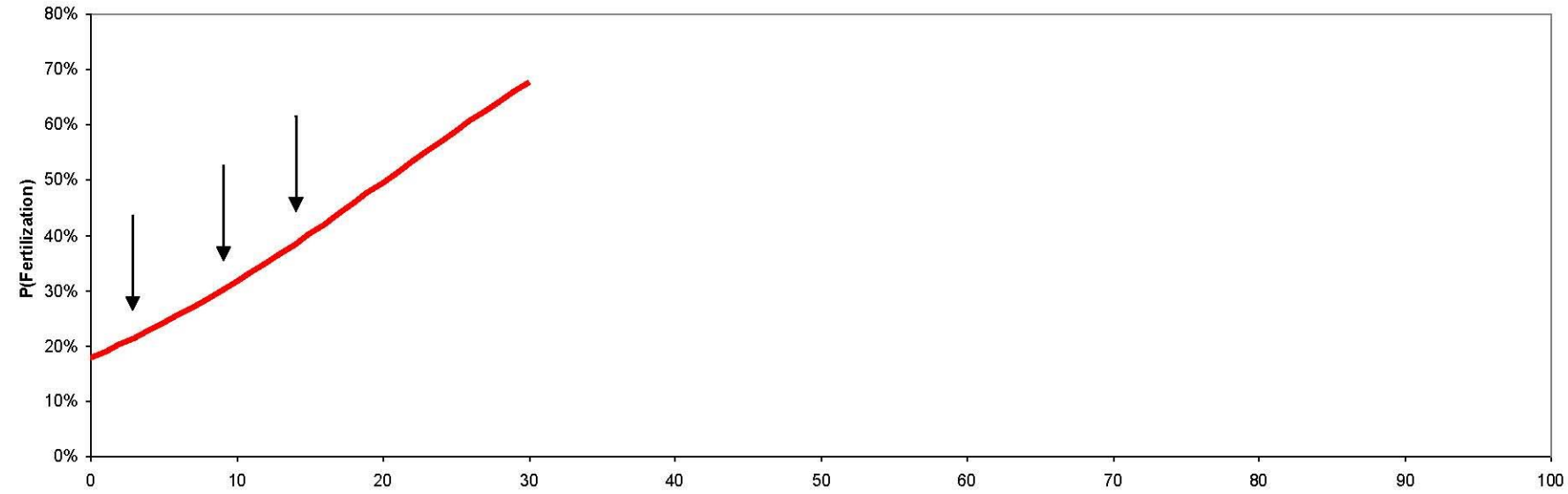


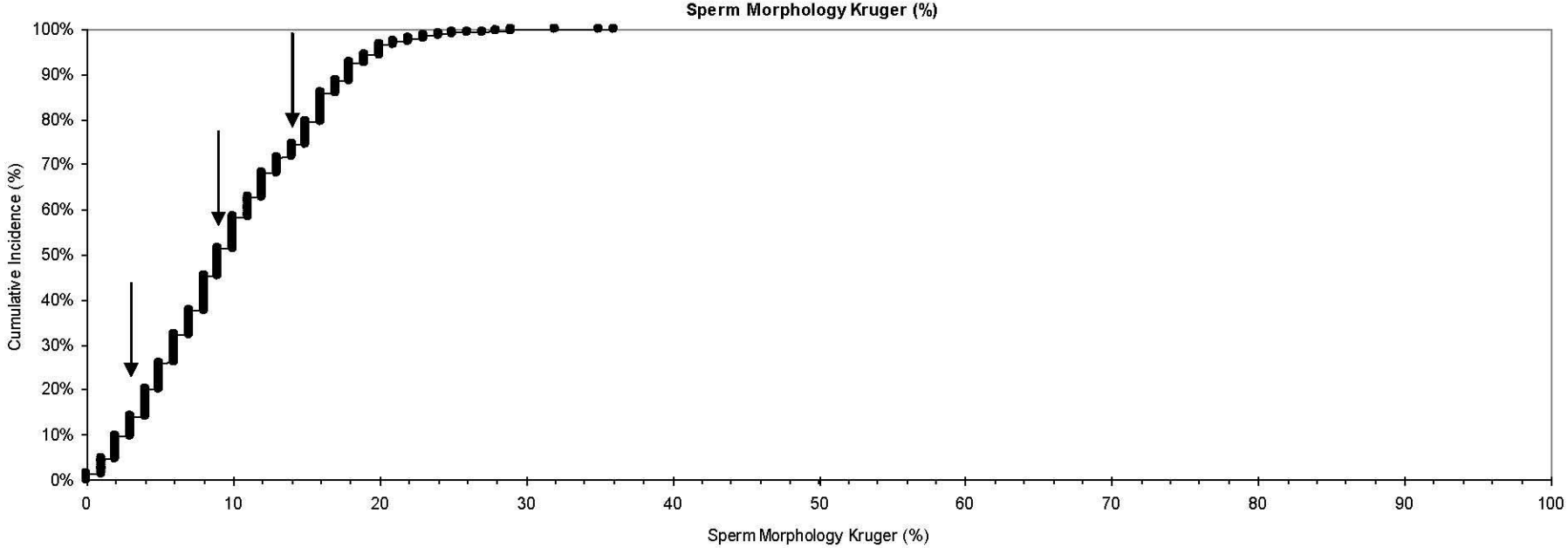


Conclusions about the criterion?

- Not a lot of difference between incidences of fertilization at the different criterion levels
- Not a lot of difference between the percentage of patients included at these different criterion levels
- Probably because motility while significantly associated is not terribly causal (at least directly) in the relationship with fertilization

Kruger Morphology (Criteria and Prevalance)





Conclusions about the criterion?

- Not a lot of difference between incidences of fertilization at the different criterion levels
- Large difference between the percentage of patients included at these different criterion levels
- Probably because morphology while significantly associated is not terribly causal (at least directly) in the relationship with fertilization

$Fertilization_{ICSI}$ (N = 5012)

$\ln(Odds\ Ratio_{fertilization}) =$

.00562 X Sperm Count (10.92%)

-.00554 X $Motility_{harvest}$ (-6.62%)

+.0259 X Age_{female} (9.11%)

-.0173 X Age_{male} (-7.81%)

-.0127 X Kruger (-4.20%)

+.189 X Recovery (3.40%)

+1.145

Magnitude of Effect: Standard Insemination > ICSI

	Motility	Kruger	Recovery	Age
Standard Insem.	.0295 (Δ = 29.3%)	.0753 (Δ =26.7%)	1.49 (Δ =29.8%)	-.0608 (Δ =-22.1%)
ICSI	-.0055 (Δ =-6.6%)	-.0127 (Δ =-4.2%)	.189 (Δ =3.4%)	.0259 fem. (Δ =9.1%) -.0173 male (Δ =-7.8%)
Std/ICSI	-5.32X	-5.93X	7.88 X	2.34X 4.51X

Parameters Associated with Outcome

Positive Association Negative Association

Outcome	1st	2nd	3rd	4th	5th	6th
Fert (Std Insem)	$Motility_{harvest}$	Kruger	Recovery	Age_{female}	$Prog_{semen}$	
Fert (ICSI)	Sperm Count	$Motility_{harvest}$	Age_{female}	Age_{male}	Kruger	Recovery
Clinical Pregnancy	Age_{female}	$Prog_{semen}$				
Live Birth	Age_{female}	$Prog_{semen}$				
Pregnancy Loss	Age_{female}					
Implant'n Time	$Prog_{semen}$	Kruger				

So, Is Semen Analysis Predictive?

- Fertilization (Standard Insem) – YES
  - Kruger and $Progression_{semen}$
  - Many associated params are not in the usual S/A
- Fertilization (ICSI) – Not So Much
- Clinical Pregnancy & Live Birth – YES
  - $Progression_{semen}$
- Pregnancy Loss – NO
- Implantation Time – YES
  - Kruger and $Progression_{semen}$

Statistical Significance:
Yes, but is it clinically useful?

Parameter	$S.D._{within}$	$S.D._{among}$	Ratio (within/among)
Progression	**0.99**	**1.12**	**0.89**
Kruger	**4.7**	**6.7**	**0.70**

With repeated semen analyses, $S.D._{.within}$ will decrease by a factor $1/\sqrt{\#S.A.}$

With 2 S.A.'s - Ratios: Progression 0.63 ; Kruger 0.50

With 3 S.A.'s - Ratios: Progression 0.51 ; Kruger 0.40

With 4 S.A.'s - Ratios: Progression 0.44 ; Kruger 0.35

Conclusions

- Two types of error for semen parameters
  - Counting error
  - Within-patient varibility (this is larger!)
- Semen analysis parameters are associated with outcome
  - Progression semen (Std Fert, Clin Preg, Live Birth, Implantation time)
  - Kruger (Std Fert, Implantation time)
- Other non-semen analysis characteristics also related
  - (Age, $\text{Motility}_{\text{harvest}}$, Recovery)
- Large variability in semen parameters make semen parameters from one analysis poor (nearly useless) predictors for outcome!

Conclusions

- If Semen Analysis Parameters aren't very predictive, then what is?
- Are we missing the real solutions by concentrating on Semen Parameters?
- Are there better function tests?