# Male Factors Affecting Pig Reproduction


- PJ Chenoweth
- F McPherson
- J Lievaart

*Charles Sturt University*

# The “Problem”

**Early pregnancy loss (EPL) is a major source of wastage/inefficiency in livestock.**

**Traditional view = this is a normal culling process for genetic “debris”**

**“Male” contribution estimated at 40% plus**

# Sire Differences

- Delayed return to estrus (*Salisbury et al 1951*)
- In-vivo access to ovum (*den Daas 1998*)
- Accessory sperm numbers (*Nadir et al 1993*)
- IVF rates
- Initiation/length of zygotic “S” phase
- Embryo cleavage and development (*Schneider et al 1993*)
- Sperm numbers for optimal litter size *(Flowers et al 2002)*

# Seminal Traits

Affect **both** fertilization and embryogenesis (*Saacke et al 2000*)

– Both sperm viability and morphology have been implicated in EPL

– Abnormal sperm morphology associated with recurrent abortion (*Hill et al 1994*)

# Pregnancy Wastage/Loss

- Fertilization rate ~ 90%
- 40% embryos/fetii lost early[1]
- 5% litters lost during gestation
- Litter size max. 4-5th parity[2]
- ≥ 8th parity, increased stillbirths

- *[1] Factors include – stress, high temp., overfeeding, disease, teratologic agents, genetic, disease,* ***MALE FACTORS??***
- *[2]Note uterine capacity effects*

# Factors Affecting Pregnancy and Litter Size

- O.R .
  - *Genetics*
  - *"Flushing"*
  - *Inc from pubertal to 3rd oestrus*
- Infection
- Biostimulation
- Uterine Capacity
  - *Genetics*
- Hormonal Regimes
  - *ImmunIsation against inhibin*
  - *GnRH*
  - *P4 inhibitor (epostane)*

- **Boar/A.I problems**
- **Infection**
- **Congenital problems**
- **Cystic ovaries**
- **Environment**
  - *heat,*
  - *hygiene,*
  - *ventilation*

# Pig Breeding Analyses

12,336 single-sire A.I. breedings (utilising 7 sire-lines and19 dam-lines) for 3 yrs

**Dependent variables**

•days to outcome (D),
•total pigs born (T),
•pigs born alive (A),
• still-born (S) and
•mummified (M) pigs.

**Study factors**

• month of mating (1-12),
•year of mating (2006-8),
•dam line (170),
•sire line (16),
•parity and season as well as their respective interactions.

# Results

**Sire line** influenced

Days to outcome (P< 0.000),

Pigs born alive (P < 0.001),

Still-born pigs (P < 0.000)

Mummified pigs (P < 0.033)

Sire line interacted with parity for pigs born alive (P < 0.000) but not with season.

# Plus

Dam line influenced
Pigs born alive (P < 0.000),
Stillborn pigs (P < 0.000)
Mummified pigs (P < 0.000).

Season influenced
Pigs born alive (P < 0.006),
Mummified pigs (P < 0.023)
Total pigs born (P < 0.008);

Month of mating influenced
Pigs born alive (P < 0.000)
Mummified pigs (P < 0.001)

Year of mating influenced stillborn pigs (P < 0.002).

# Concepts

- **Spermatogenic epithelium has limited responses to stress**
- **The major cause of sperm DNA damage is oxidative stress**
- **Sperm DNA problems cause male-factor infertility**
- **Routine sperm assessment methods are only partially successful in identifying such damage.**

# Sperm DNA Damage

- Sperm count
- Sperm morphology
- Sperm motility
- Fertilization rate
- Impaired pre-implantation development
- Increased abortion
- Elevated disease levels in offspring

**Lewis & Aitken 2005** **Erenpreiss et al 2006**

# Bio-Markers for Spermatogenic Damage

## Abnormal chromatin

- Morphology *(Diadem/crater)*
- SCSA *(acridine orange)*
- Other fluorochromes

## "Abnormal" Sperm

- Ubiquitin ***("tag" or "chaperone" protein)***

## Abnormal membranes

- Morphology
- L/D stain
- HOS

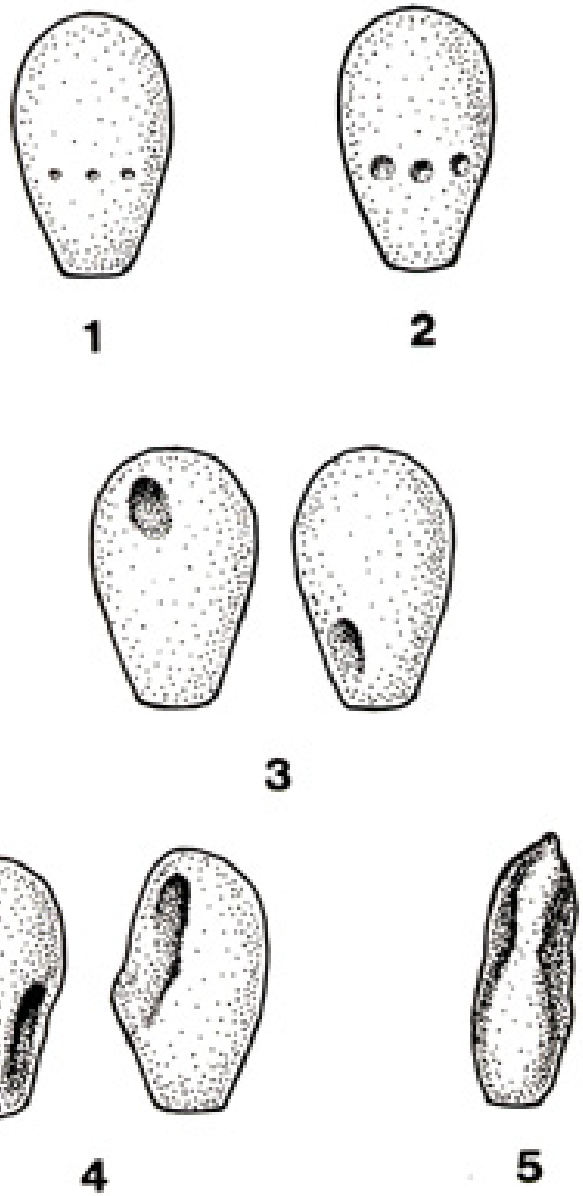
## Acrosome Loss/Dysfunction

- Morphology *(PIA)*
- Targeted fluorochromes

## Mitochondrial Dysfunction

- Morphology
- Targeted fluorochromes

## The Crater/Diadem Defect & its Sequelae.



# Embryo Effects of Cratered Sperm Insemination

| Cow # | Embryo # | Semen | Exc. | Good | Fair/Poor | Deg/UFO |
|---|---|---|---|---|---|---|
| 7 | 99 | Control | 47.5 | 20.0 | 10.0 | 22.0 |
| 7 | 109 | Cratered | 9.2 | 11.9 | 7.4 | 62.3 |

Saacke 1991

# An Effective Sperm Dose?

- Is a function of both “quality” & numbers
- Varies with individuals, species, insemination site
- Should be “consistent”

- <u>Assumptions:</u>
  - *female “eligibility”*
  - *good bio-security (animals and semen)*
  - *Appropriate, “normal” delivery*

# An Effective Sperm Dose for Pigs?

## Objective: ≥ 2.5 billion effective sperm/dose

| *At Collection/ Packaging* | *Minimal Acceptable* | *Optimal* |
|---|---|---|
| **% Progressive Motility** | 70% | 90% |
| **% Normal Sperm** | 70% | 90% |
| **Sperm Concentration/Dose** | 25 x $10^6$/ml | 40 x $10^6$/ml |
| **PIA** | 70% | 90% |
| **Total Effective Sperm/dose** | **2 x $10^9$** | **3 x $10^9$** |

| BOAR NO. | SAMPLE NO. | VOL. mL | A. MOT. % IVOS | B. CONC. x $10^6$ HEM. 1:10 | D. % NORMAL | E. % PIA | D1 A x B | D2 B x D2 | Dose 1 B x Vol | Dose 2 A x B x Vol | Dose 3 B x D x Vol |
|---|---|---|---|---|---|---|---|---|---|---|---|
| UG12 | 1 | 90 | 79 | 32.25 | 60 | 90 | 25.4775 | 19.35 | 2.9025 | 2.292975 | 1.7415 |
| UG12 | 1a | 90 | 69 | 34.75 | 64 | 94 | 23.9775 | 22.24 | 3.1275 | 2.157975 | 2.0016 |
| UM12 | 2 | 90 | 80 | 35 | 80 | 96 | 28 | 28 | 3.15 | 2.52 | 2.52 |
| UM12 | 2a | 91 | 86 | 30.25 | 80 | 96 | 26.015 | 24.2 | 2.75275 | 2.367365 | 2.2022 |
| TX55 | 3 | 89 | 91 | 37.75 | 75 | 98 | 34.3525 | 28.3125 | 3.35975 | 3.057373 | 2.519813 |
| TX55 | 3a | 91 | 94 | 34.5 | 72 | 98 | 32.43 | 24.84 | 3.1395 | 2.95113 | 2.26044 |
| UG15 | 4 | 91 | 53 | 30.5 | 56 | 99 | 16.165 | 17.08 | 2.7755 | 1.471015 | 1.55428 |
| UG15 | 4a | 87 | 62 | 30.75 | 52 | 94 | 19.065 | 15.99 | 2.67525 | 1.658655 | 1.39113 |
| TX49 | 5 | 88 | 84 | 37.5 | 58 | 96 | 31.5 | 21.75 | 3.3 | 2.772 | 1.914 |
| TX49 | 5a | 87 | 84 | 42 | 64 | 98 | 35.28 | 26.88 | 3.654 | 3.06936 | 2.33856 |
| **MEAN** | | **89.4** | **78.2** | **34.525** | **66.1** | **95.9** | **27.22625** | **22.86425** | **3.083675** | **2.431785** | **2.078003** |
| **±SD** | | **1.57762** | **13.028175** | **3.79043** | **10.07141** | **2.685351** | **6.373782** | **4.378831** | **0.309451** | **0.558799** | **0.400932** |

# Pork CRC Objectives

Improved pig production/economics via

- *increasing pigs/sow/yr (PPSY) via improved AI boar semen quality and consistency.*
- *improving diagnostics of male-factor reproductive problems.*
- *potentially reducing both sperm dose and average numbers of inseminations/sow* .

# Quote:

"*The 2 major factors increasing US pigs born/sow/year are improved:*

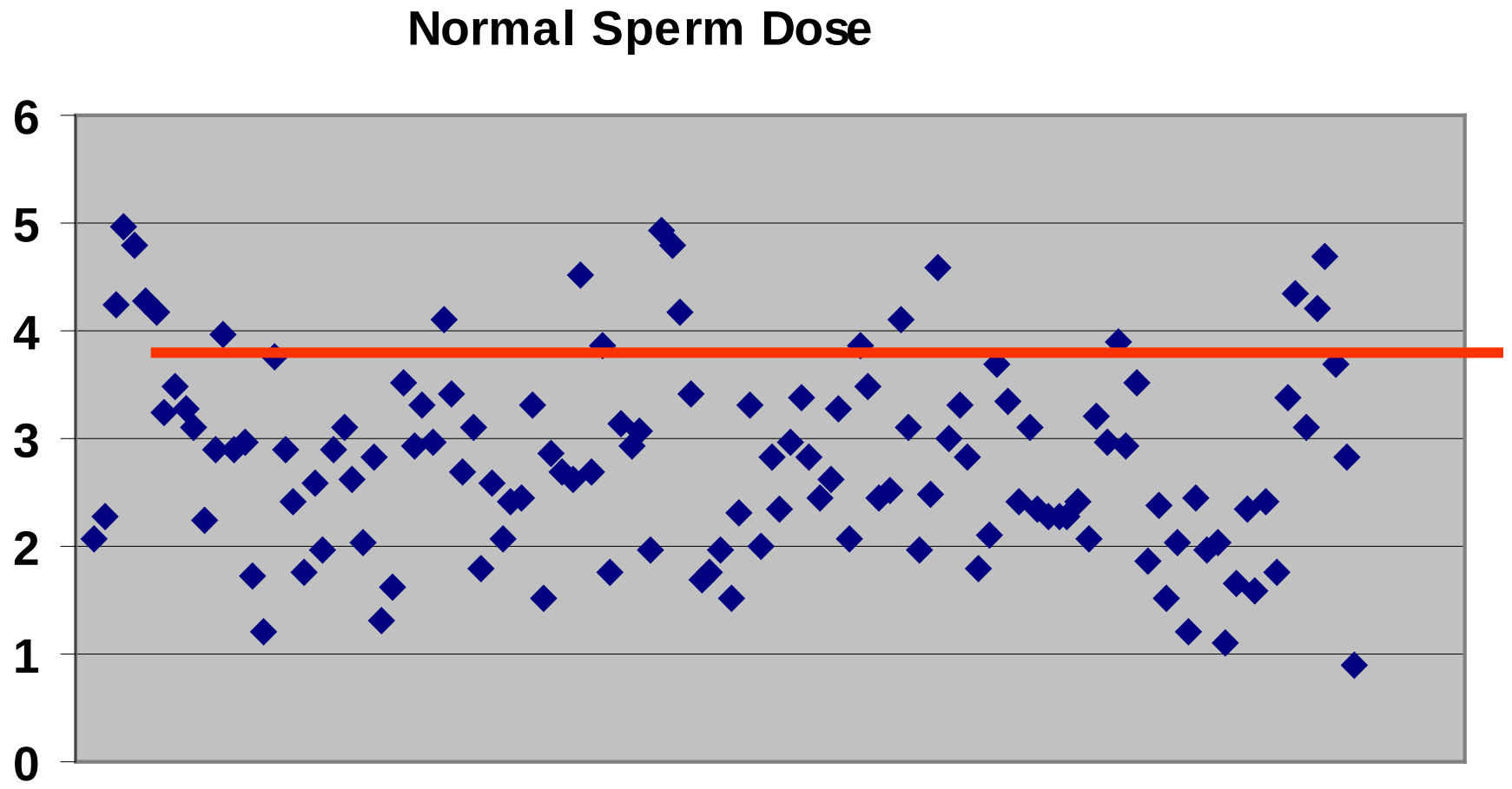
- *Genetics*

*&*

- *AI boar semen quality and consistency".*

Mark Wilson,
2006 Pan Pacific Pig Congress

# Survey Data – Routine Boar Stud submissions 2005-7



# Results

**Table 1. Statistical Analyses of Survey Data**

| Semen Trait | Mean | SD | Min | Max |
|---|---|---|---|---|
| **Arrival Temp. ( $^0$C)** | 19.77 | 2.23 | 15 | 23.1 |
| **Sperm Motility (%)** | 69.98 | 20.21 | 10 | 99 |
| **Sperm Concentration ($10^6$/ml)** | 28.4 | 14.54 | 11.5 | 92 |
| **Normal sperm (%)** | 75.88 | 14.29 | 26 | 96 |
| **PIA (%)** | 93.51 | 4.15 | 70 | 100 |

# Validation of Tests

| Precision | Accuracy |
| --- | --- |
| = Consistency or lack of variability, | = Ability to measure desired trait. |
| Tested via multiple repetitions. | Compared with a “gold standard” |
| Coefficient of Variation | Multiple statistical approaches. |

# “Gold Standards” for Livestock Semen Assessment

## Sperm

- Motility – C.A.S.A
- Concentration – Haemocytometer
- Morphology – D.I.C. (1000X)

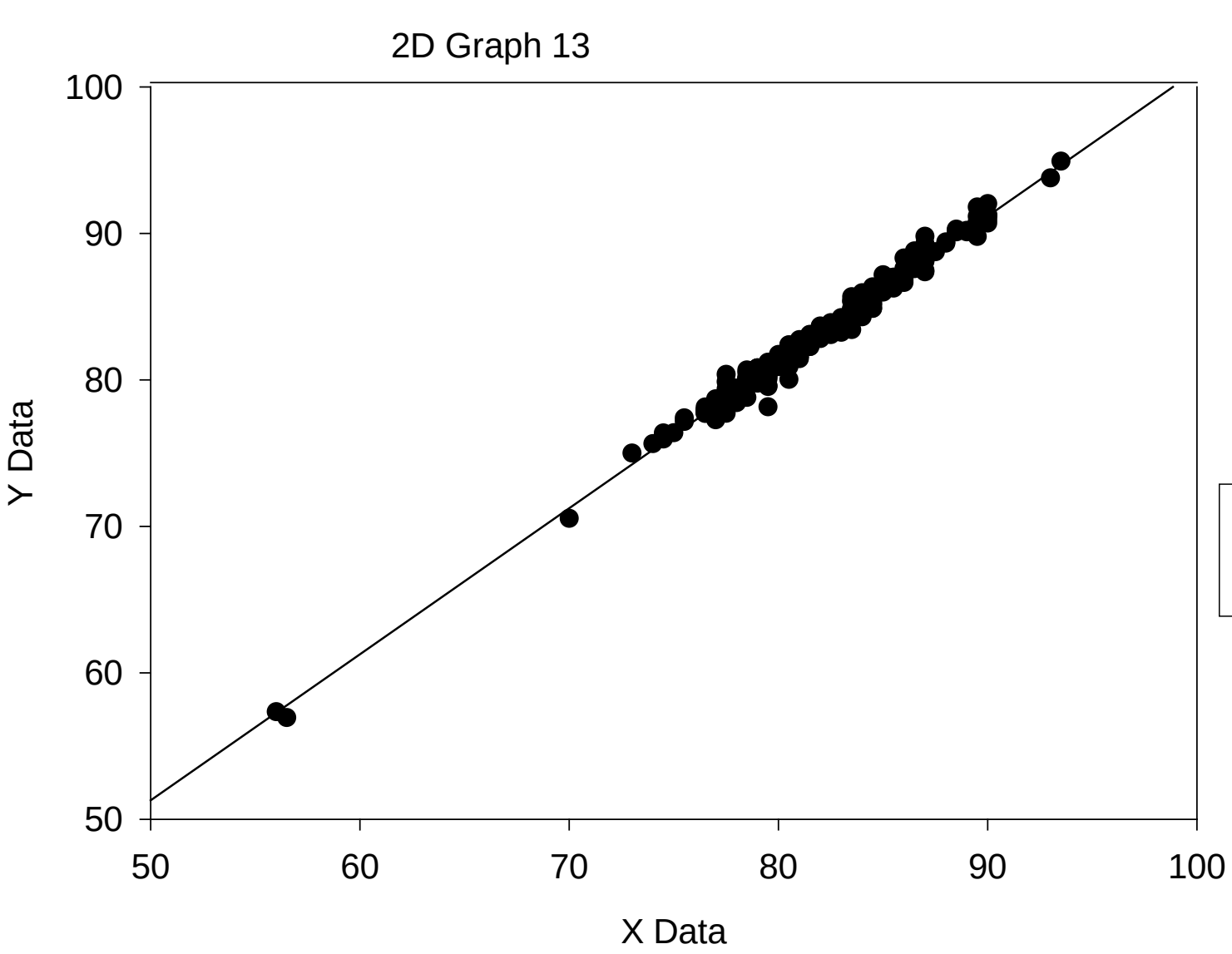
# Conclusions..... so far

| Instrument | FACS | Improved Neubauer | Corning | Sperm Vision | Ultimate | SP 100 |
|---|---|---|---|---|---|---|
| **CV** | *2.72 %* | *7.14 %* | *10.4 %* | *8.13 %* | *5.40 %* | *3.11 %* |
| **95 % confidence interval** | *2.45 – 3.05* | *6.42 – 8.03* | *9.40 – 11.7* | *7.32 – 9.14* | *4.87 – 6.06* | *2.81 – 3.49* |

*Comparison of FACSCount AF System, Improved Neubauer hemacytometer, Corning 254 photometer, Sperm Vision CASA System, Hamilton Thorne Ultimate CASA System and NucleoCounter SP-100 for determination of sperm concentration of boar semen*

*C. Hansen [a], *, T. Vermeiden[b], J. Vermeiden , C. Simmet, B.C. Day, H. Feitsma[b]*

*a The National Committee for Pig Production, Danish Bacon and Meat Council, Axeltorv 3, DK-1609 Copenhagen V, Denmark*

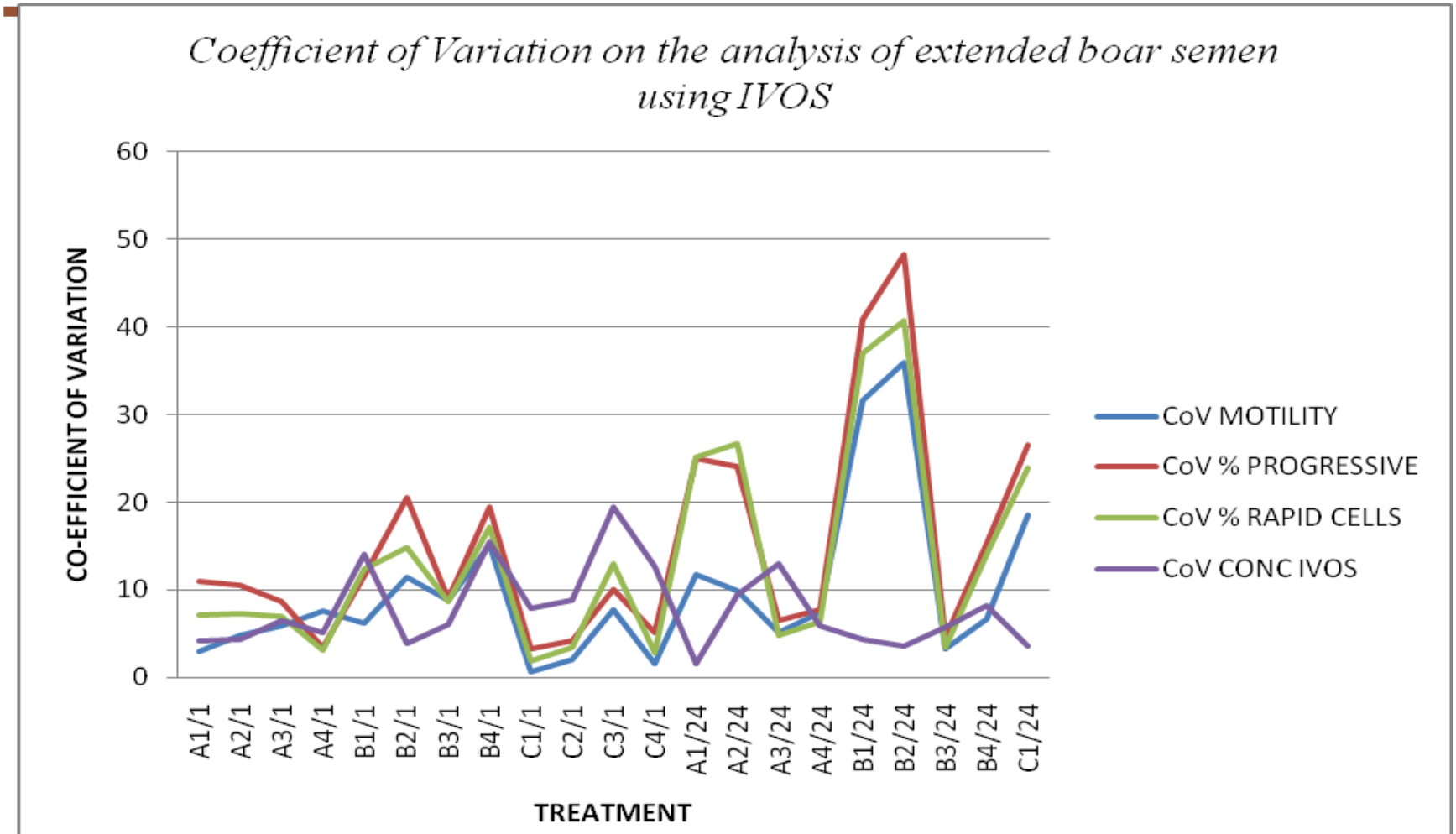


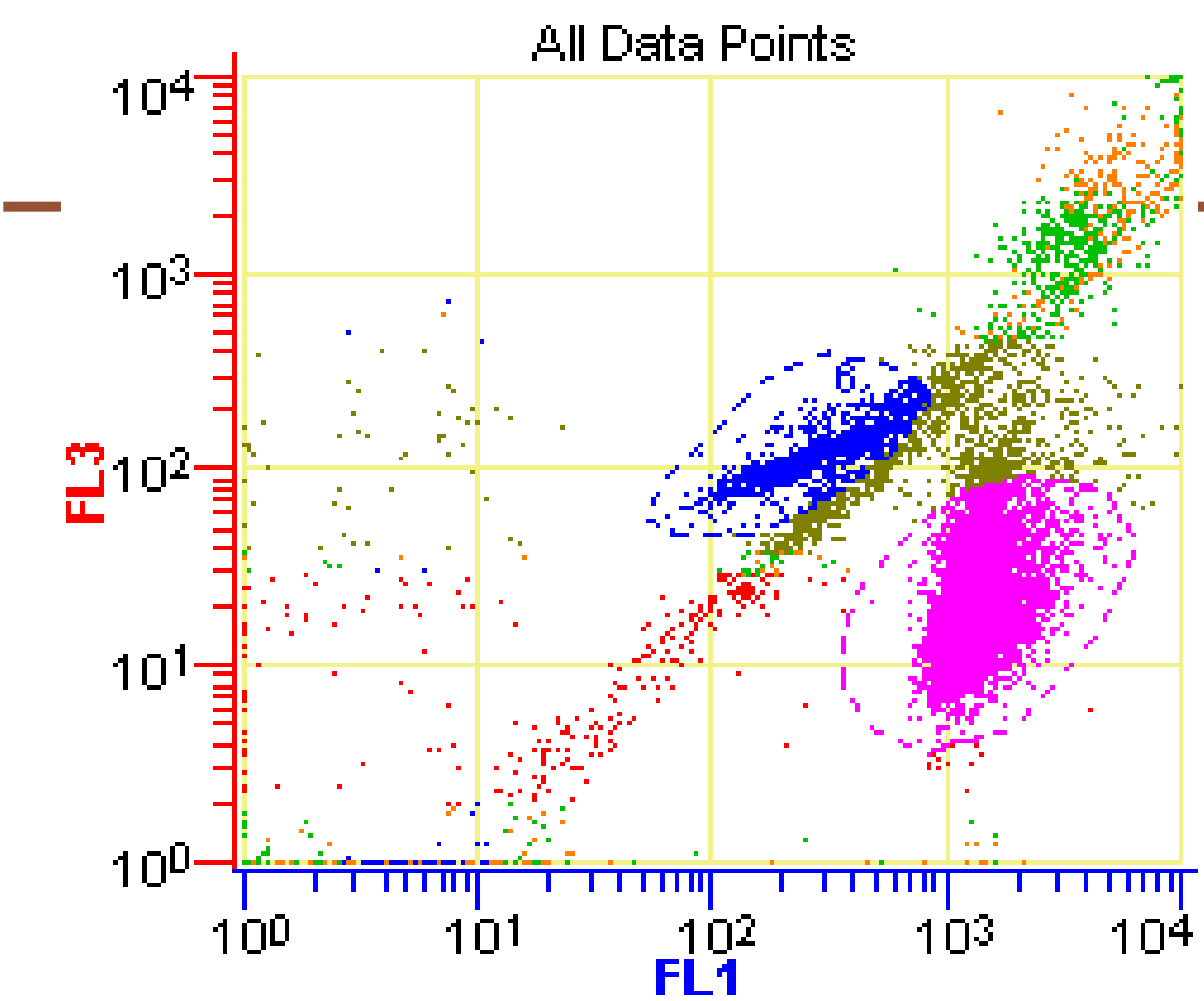

**Volume (ml) Vs Weight (g)**

| BOAR ID | CoV % MOTILITY | CoV % PROGRESSIVE | CoV % RAPID CELLS | CoV CONC IVOS |
|---|---|---|---|---|
| A1/I | 3.01 | 10.98 | 7.21 | 4.22 |
| A2/I | 31.83 | 31.06 | 31.41 | 31.94 |
| A3/I | 5.94 | 8.58 | 7.04 | 6.42 |
| A4/I | 7.71 | 3.43 | 3.23 | 5.05 |
| B1/I | 6.20 | 11.63 | 12.44 | 14.05 |
| B2/I | 11.43 | 20.57 | 14.92 | 3.87 |
| B3/I | 8.85 | 8.88 | 8.65 | 6.07 |
| B4/I | 15.17 | 19.49 | 17.18 | 15.40 |
| C1/I | 0.73 | 3.23 | 1.94 | 7.87 |
| C2/I | 2.15 | 4.12 | 3.48 | 8.78 |
| C3/I | 7.82 | 10.06 | 13.09 | 19.45 |
| C4/I | 1.68 | 5.08 | 2.91 | 12.63 |
| A1/24 | 11.73 | 24.98 | 25.16 | 1.54 |
| A2/24 | 9.90 | 23.99 | 26.76 | 9.34 |
| A3/24 | 5.20 | 6.42 | 4.94 | 12.96 |
| A4/24 | 7.42 | 7.74 | 6.41 | 5.79 |
| B1/24 | 31.65 | 40.85 | 37.04 | 4.26 |
| B2/24 | 35.90 | 48.30 | 40.72 | 3.58 |
| B3/24 | 3.37 | 4.45 | 3.54 | 5.71 |
| B4/24 | 6.79 | 15.34 | 14.11 | 8.12 |
| C1/24 | 18.59 | 26.57 | 23.99 | 3.60 |
| C2/24 | 16.58 | 13.86 | 21.03 | 23.21 |
| C3/24 | 13.48 | 13.40 | 15.10 | 12.26 |

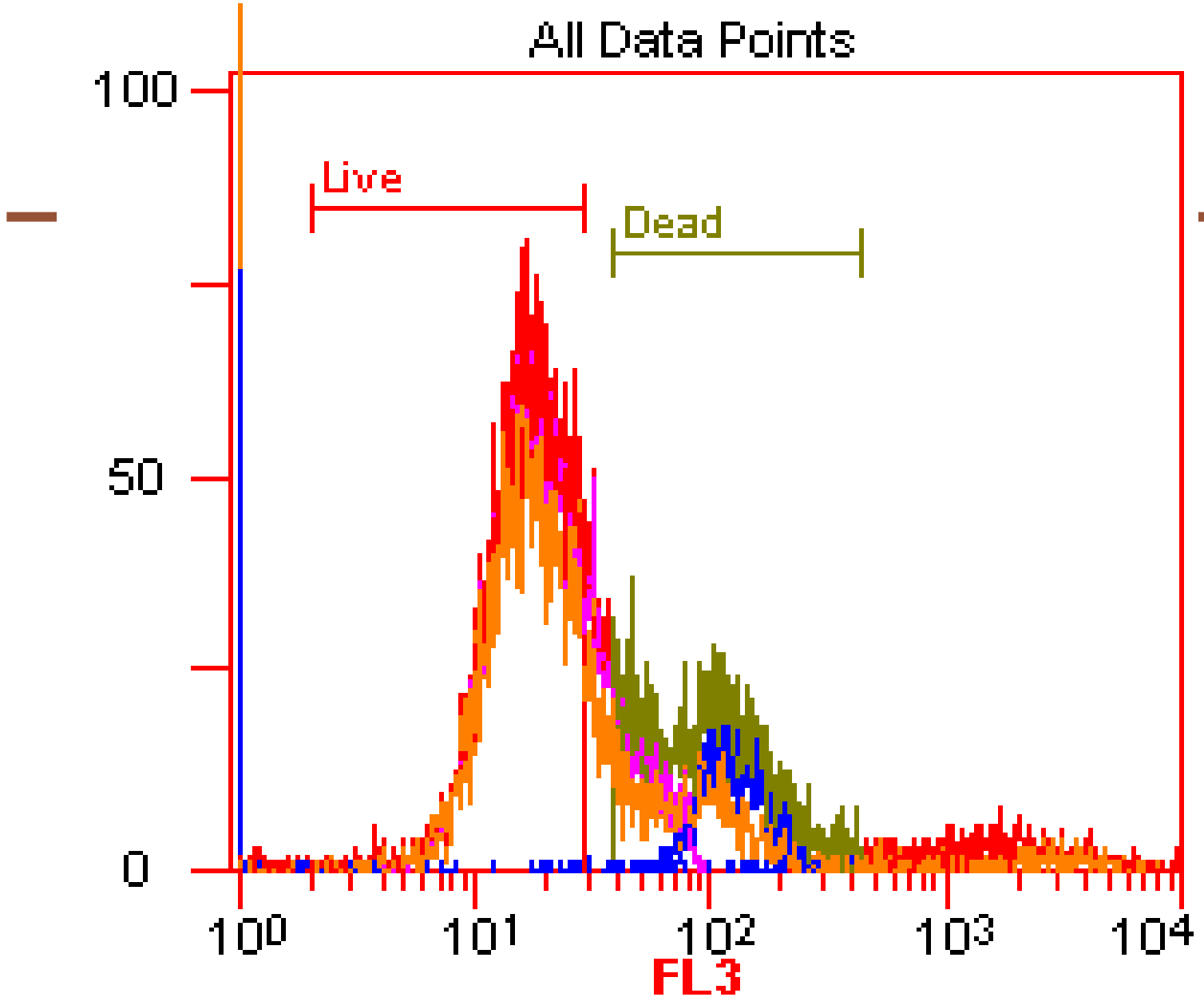
**CoVs For IVOS Traits**

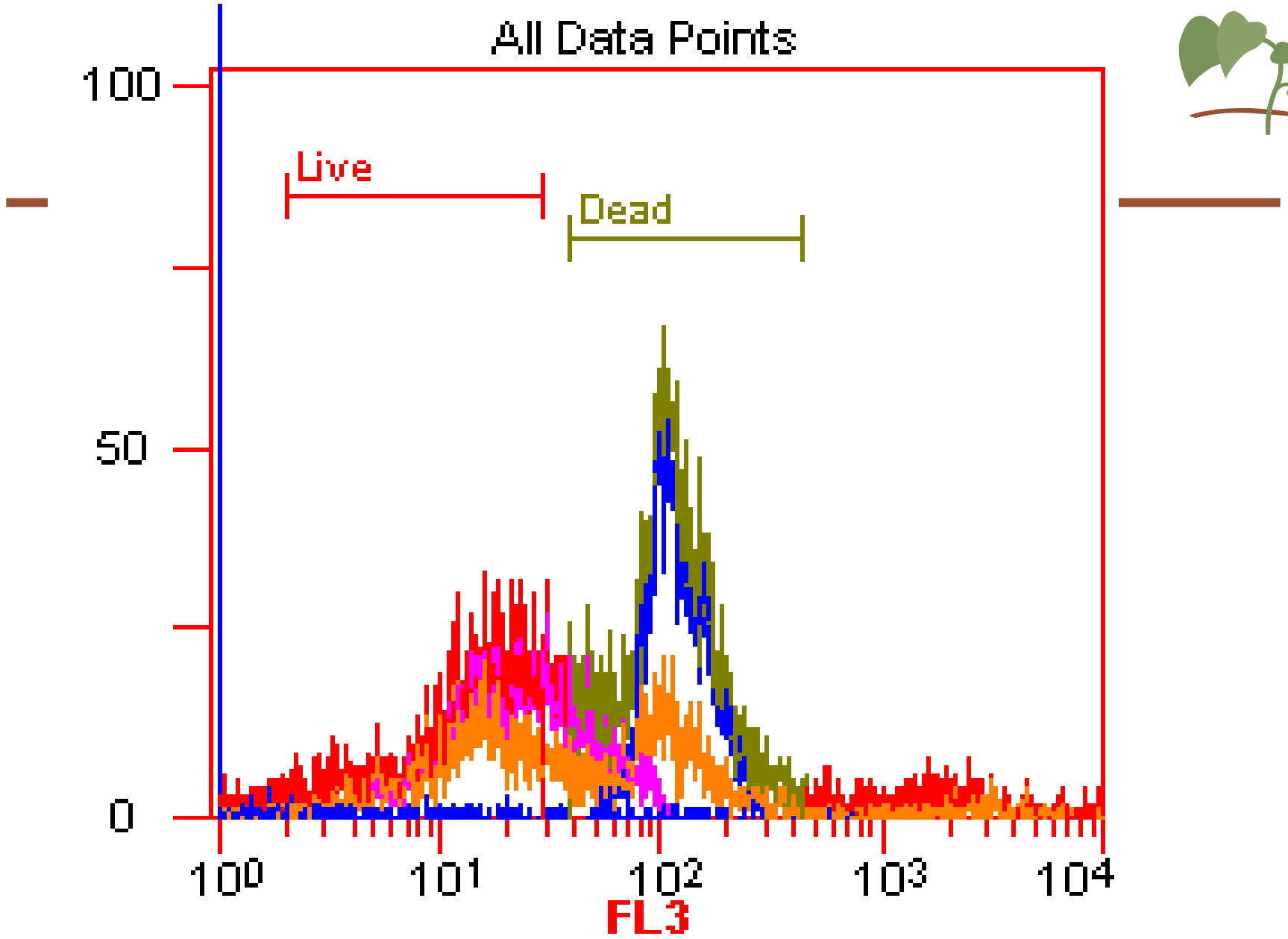
# Validation of Tests



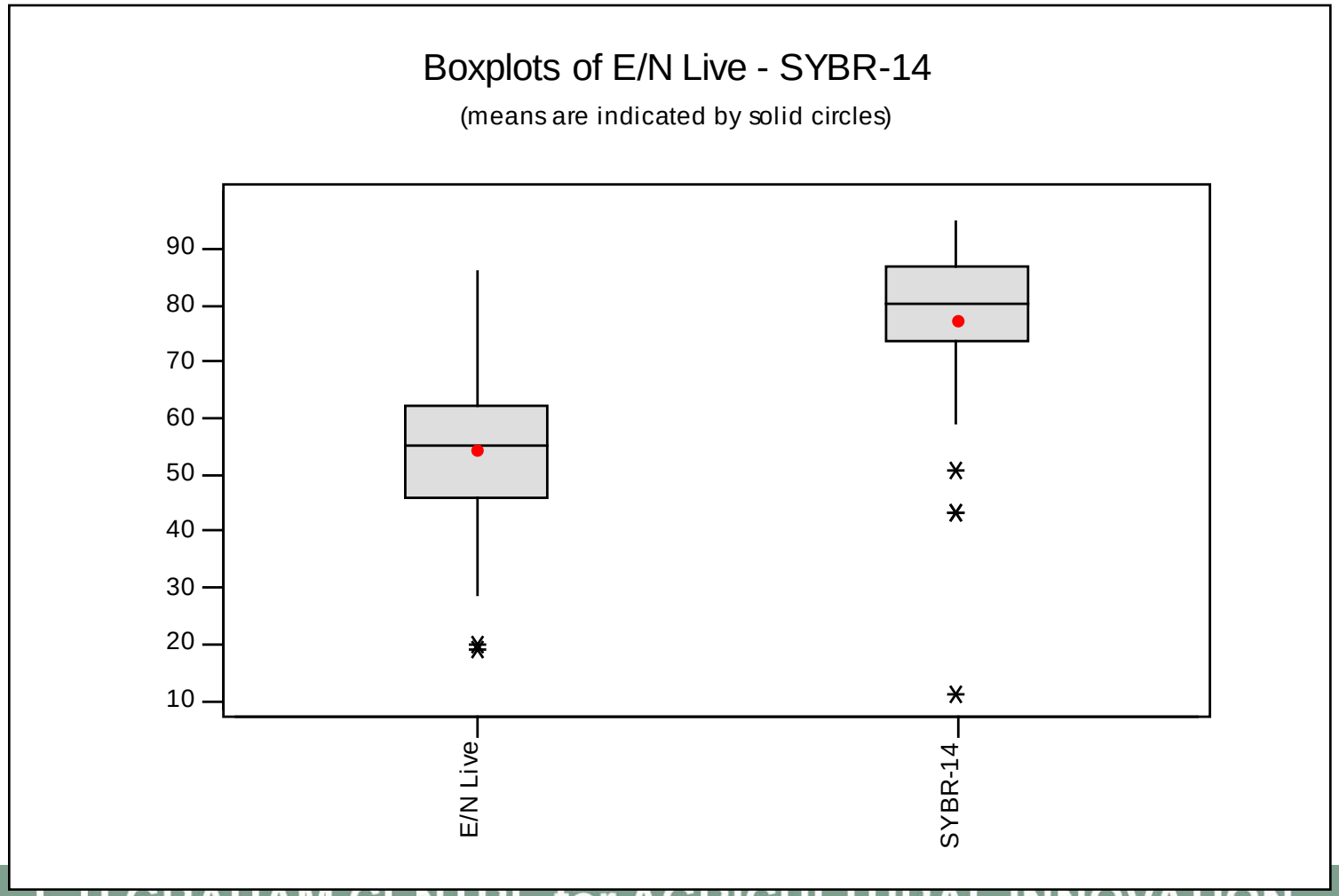

All Data Points
FL3
FL1
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
6

| Location | Total Count | Live | Dead |
|---|---|---|---|
| | | Percent Total Reg(5) | Percent Total Reg(6) |
| D2 | 12868 | 68.95% | 10.37% |
| D3 | 11279 | 68.03% | 13.10% |
| D4 | 11956 | 58.90% | 12.64% |
| D5 | 11521 | 65.21% | 11.73% |
| D6 | 7277 | 67.90% | 13.23% |
| D7 | 12268 | 33.93% | 31.06% |
| D8 | 11002 | 37.23% | 35.74% |


All Data Points
100
50
0
Live
Dead
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
FL3


All Data Points
100
50
0
Live
Dead
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
FL3

# RESULTS…Eosin/Nigrosin Viability



# Thank You!

Questions?