Application of Male Oriented Technology to Reproduction

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Male Traits for Technological Improvement

Increase Bull Fertility

Improve Genetics

Increase Bull Fertility

- Breeding Soundness Exams (BSE)
  - Semen Analyses

Improve Genetics

- AI (frozen semen)
  - Sexed Semen

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- AI
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- Increase Bull Fertility
  - Breeding Soundness Exams (BSE)
    - Physical Exam
    - Health Evaluation
    - Semen Analysis

Semen Analysis

- Sperm concentration
- Percentage of Motile Sperm
- Morphological Evaluation

What does the semen analysis tell us?

Laboratory assays are not highly correlated to bull fertility

- Percentage of Motile Cells (.015-.84)
  - Percentage of all motile cells
  - Percentage of progressively motile cells
- Morphology (.06-.86)
- Percentage of live cells (.33-.66)

Graham et al. (1980)

Why do laboratory results correlate poorly with fertility?

Why do laboratory results correlate poorly with fertility?

- Definition of fertility
  - NRR
  - conception
  - pregnancy (day)
  - calves born
  - Sire fertility
    - binomial data

Why do laboratory results correlate poorly with fertility?

Definition of fertility

Factors not associated with semen

- herd management
  - herd health
    - estrus detection/ synchronization system
- proficiency of inseminator(s)
- semen care

Why do laboratory results correlate poorly with fertility?

Definition of fertility

Factors not associated with semen

Factors associated with the assay(s)

- bias
- inter- and intra-assay variability
- number of cells analyzed

Why do laboratory results correlate poorly with fertility?

Definition of fertility

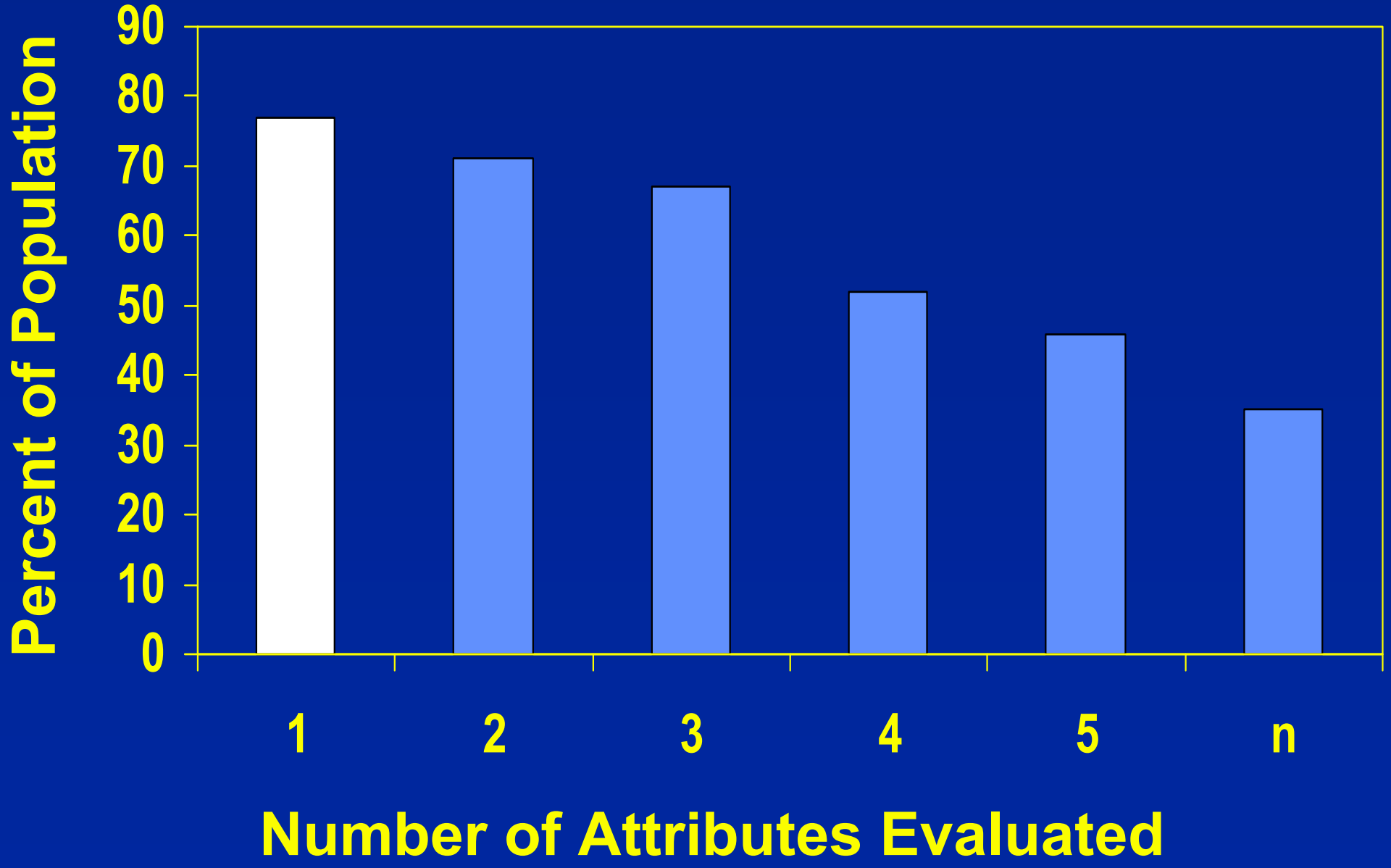
Factors not associated with semen

Factors associated with the assay(s)

Factors associated with the sperm
multifaceted cell

Attributes & Status	P. Fertility
A,B,C normal; D abnormal	Infertile
A,B D normal; C abnormal	Infertile
A,C,D normal; B abnormal	Infertile
B,C,D normal; A abnormal	Infertile
A,B,C D normal	FERTILE

For Population Mean	
A normal	Unknown
A abnormal	Infertile
B normal	Unknown
B abnormal	Infertile


Percent of Population
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
n
Number of Attributes Evaluated

Analysis of Sperm

Goal: Determine the fertility of a semen sample (fresh, cooled or frozen-thawed) using a rapid and inexpensive procedure.

We can identify poor quality samples; we can likely identify high quality samples, but not likely high fertility samples

Analysis of Sperm

Goal: Determine the fertility of a semen sample (fresh, cooled or frozen-thawed) using a rapid and inexpensive procedure.

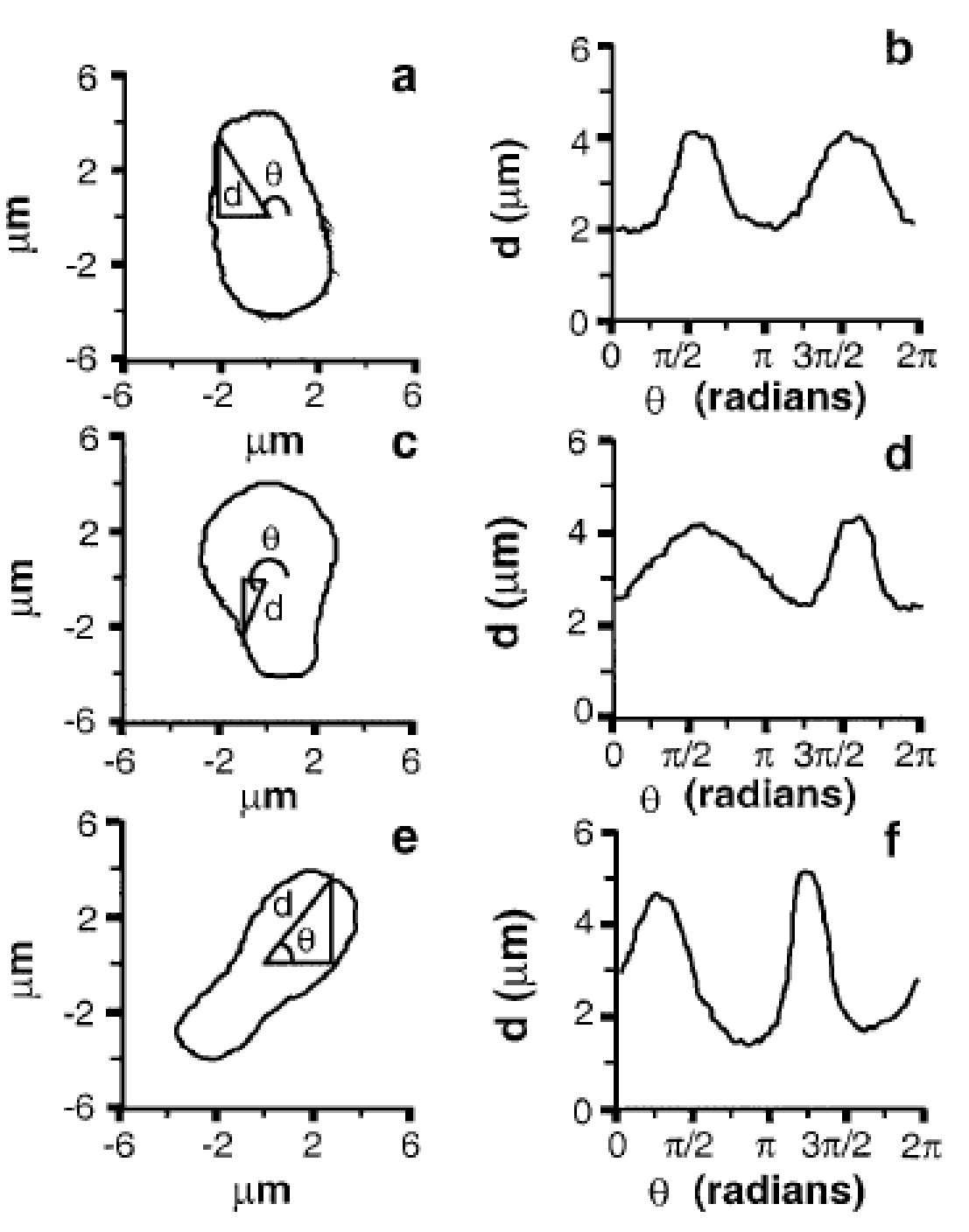
Unlikely that a single assay will enable differentiation of fertility.

New Assays

Accessory Sperm

Sperm Chromatin Structure Assay (SCSA)

Morphology: Fourier Harmonic Amplitudes (J.J. Parrish)


a
b
c
d
e
f
μm
d (μm)
θ (radians)
θ
d
6
2
-2
-6
0
4
π/2
π
3π/2
2π

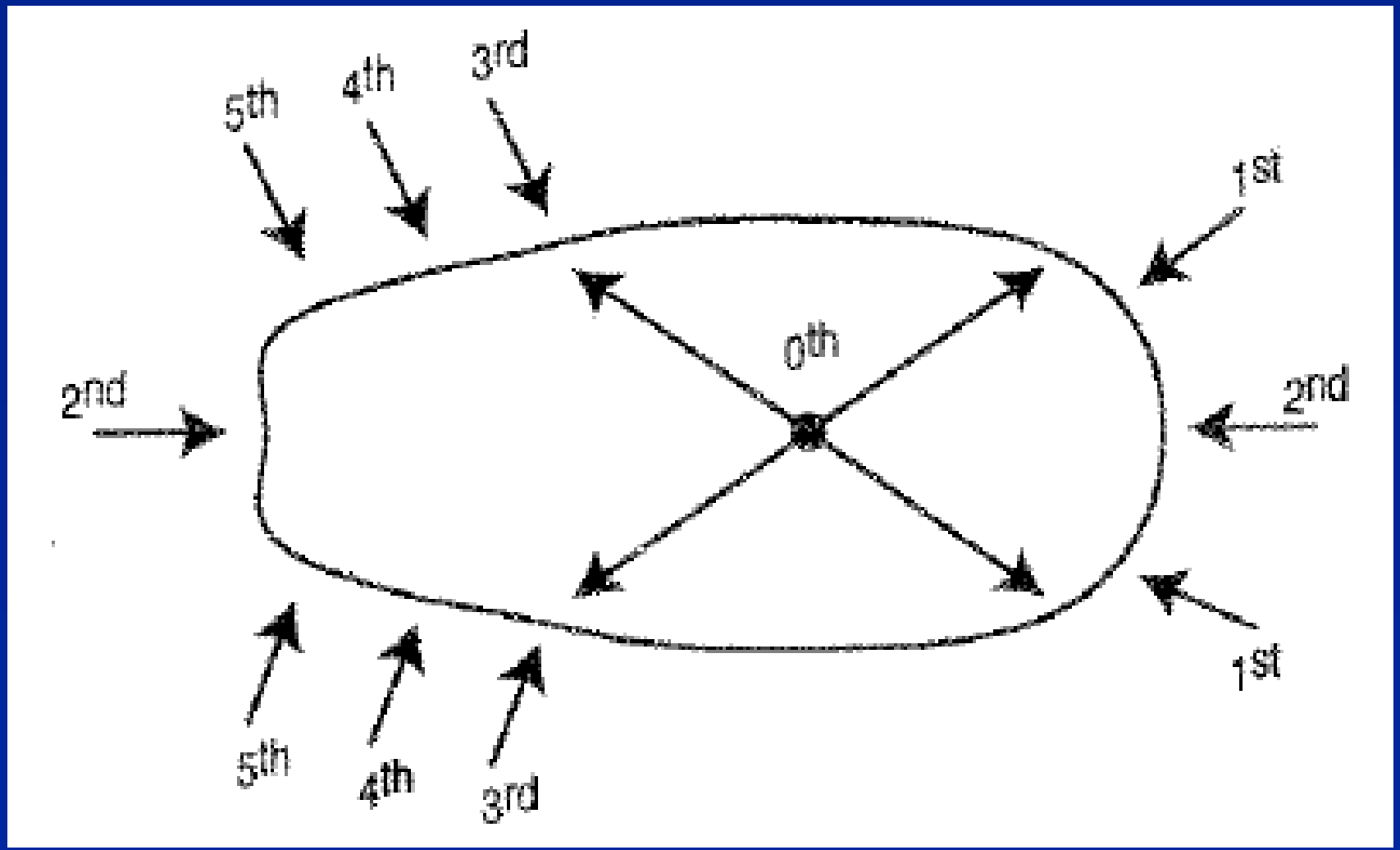

5th
4th
3rd
1st
2nd
0th
2nd
1st
5th
4th
3rd

Table 2. *Differences among bulls in harmonic amplitudes (mean ± SD) at Fourier frequencies 0–5**

Bull	Sperm Evaluated	Harmonic Amplitude Frequency 0	1	2	3	4	5
1	174	3.20 ± 0.09	0.15 ± 0.04	1.12 ± 0.09	0.17 ± 0.04	0.22 ± 0.05	0.10 ± 0.03
2	170	3.17 ± 0.11	0.15 ± 0.07	1.20 ± 0.10	0.14 ± 0.06	0.26 ± 0.07	0.14 ± 0.05
3	251	3.14 ± 0.12	0.13 ± 0.04	1.13 ± 0.10	0.16 ± 0.05	0.21 ± 0.06	0.09 ± 0.03
4	245	3.10 ± 0.09	0.13 ± 0.04	1.21 ± 0.09	0.13 ± 0.04	0.27 ± 0.06	0.10 ± 0.03
5	228	3.15 ± 0.09	0.13 ± 0.03	1.13 ± 0.07	0.15 ± 0.03	0.18 ± 0.05	0.10 ± 0.03
6	181	3.14 ± 0.10	0.13 ± 0.05	1.19 ± 0.08	0.12 ± 0.04	0.28 ± 0.07	0.12 ± 0.04
7	95	3.05 ± 0.10	0.12 ± 0.04	1.04 ± 0.10	0.16 ± 0.05	0.17 ± 0.05	0.09 ± 0.03
8	57	3.02 ± 0.14	0.15 ± 0.07	1.30 ± 0.12	0.13 ± 0.05	0.34 ± 0.11	0.13 ± 0.06
9	72	2.93 ± 0.11	0.10 ± 0.03	1.14 ± 0.07	0.10 ± 0.04	0.26 ± 0.05	0.07 ± 0.03
10	81	3.19 ± 0.09	0.19 ± 0.05	1.15 ± 0.10	0.21 ± 0.06	0.26 ± 0.07	0.13 ± 0.04
11	134	3.24 ± 0.12	0.14 ± 0.03	1.22 ± 0.09	0.14 ± 0.04	0.26 ± 0.05	0.11 ± 0.03
12	128	3.22 ± 0.09	0.16 ± 0.04	1.16 ± 0.08	0.17 ± 0.04	0.25 ± 0.06	0.11 ± 0.04

* Differences among these 12 randomly selected bulls were found in sperm nuclear shape as judged by mean harmonic amplitude at frequencies 0–5 using MANOVA; $p < .05$). The MANOVA approach was used because the harmonic amplitudes at each frequency were separate response variables and correlations would likely be present.

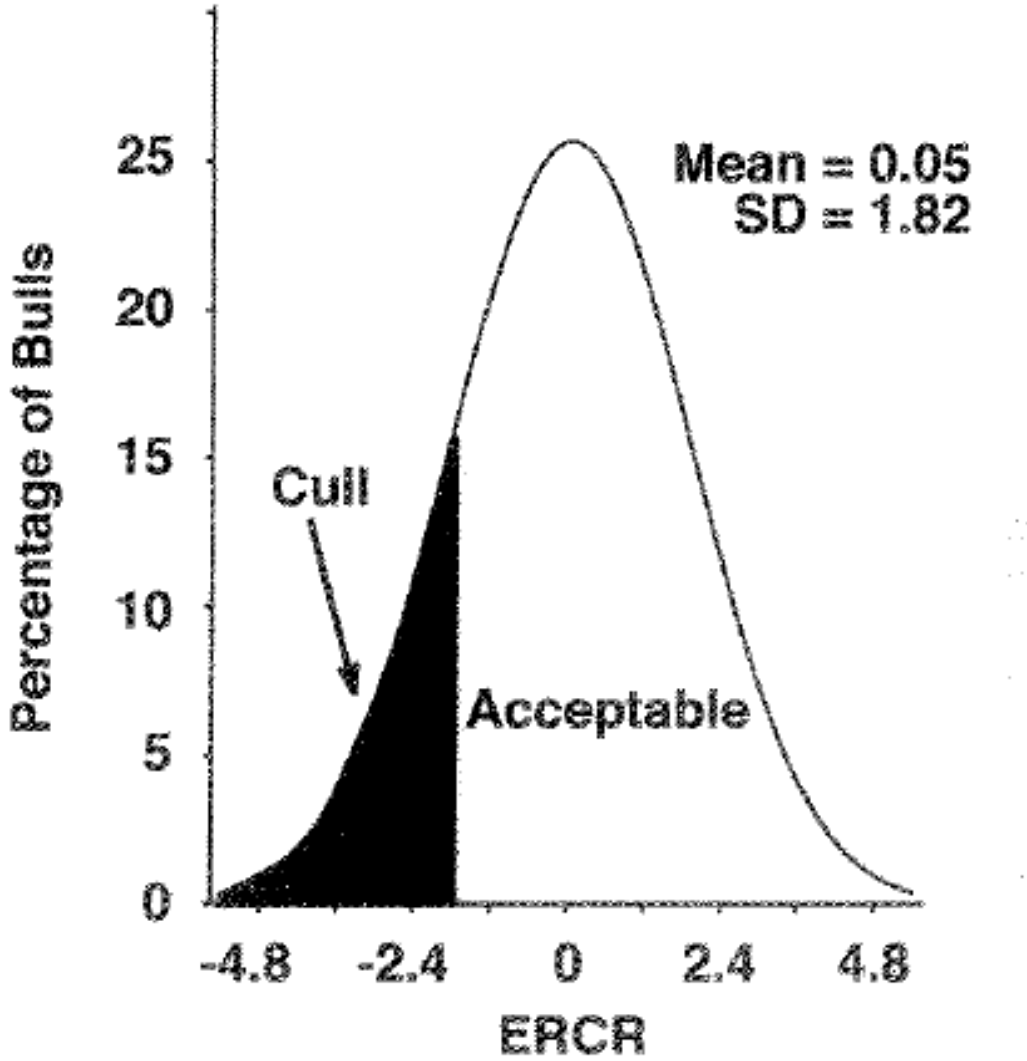
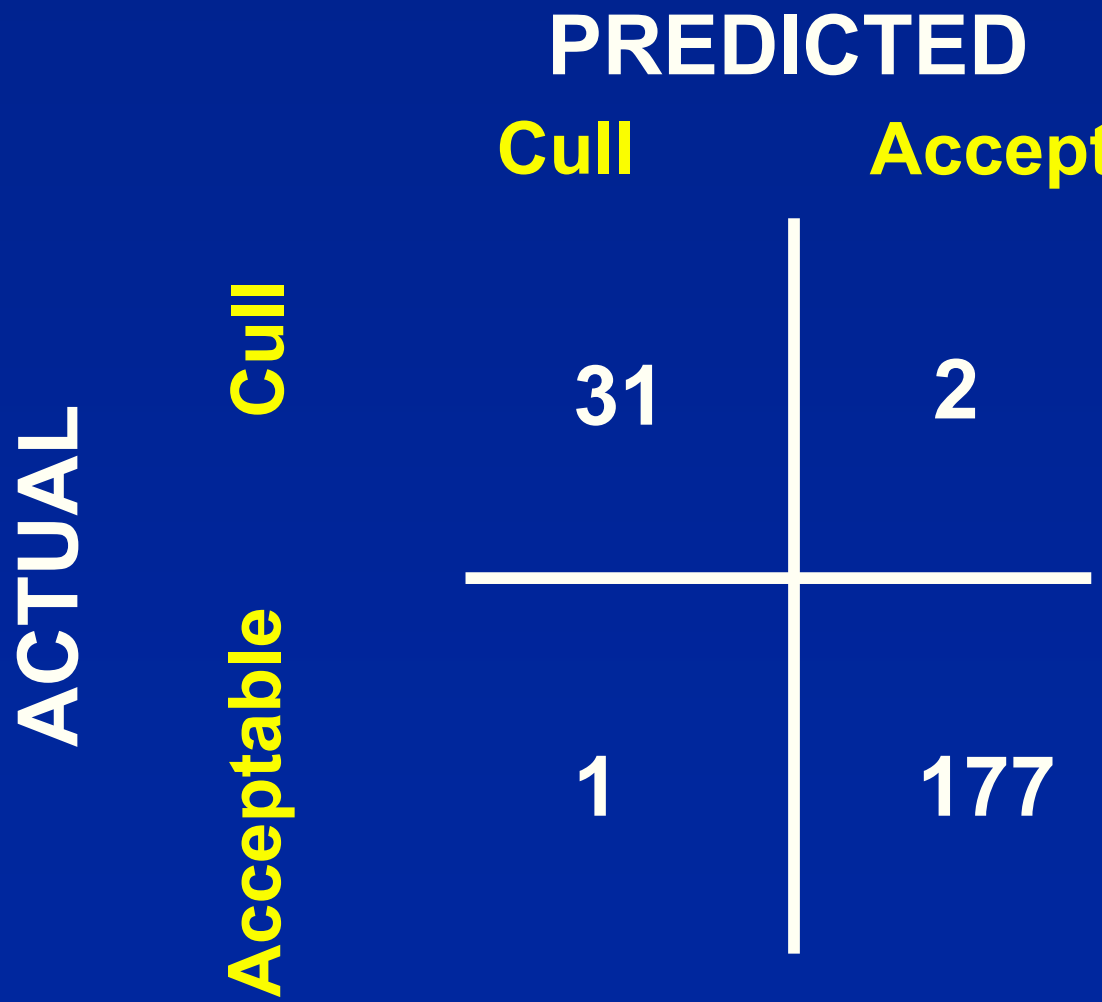


Figure 2. Fertility distribation of sampled bulls (n=210). Fertility was the estimated relative conception rate (ERCR). The cull fertility group was defined as <1 SD below the mean ERCR (.05 -1.82 = -1.77) with the remaining bulls with better fertility defined as acceptable fertility. There were 32 bulls (15%) in the Cull group and 178 (85%) in the Acceptable group.

Acceptable vs Not Acceptable



Analysis of Sperm

Goal: Identify poor quality samples versus high quality samples.

Semen Analysis

Cull bulls in the lowest 15-20%

Semen Analysis

Cull bulls in the lowest 15-20%

- Percentage of Motile or abnormal
- Concentration

Semen Analysis

Cull bulls in the lowest 15-20%

This is a cost effective approach

Increase Bull Fertility

Breeding Soundness Exams (BSE)

Semen Analyses

Improve Genetics

AI (frozen semen)

Sexed Semen

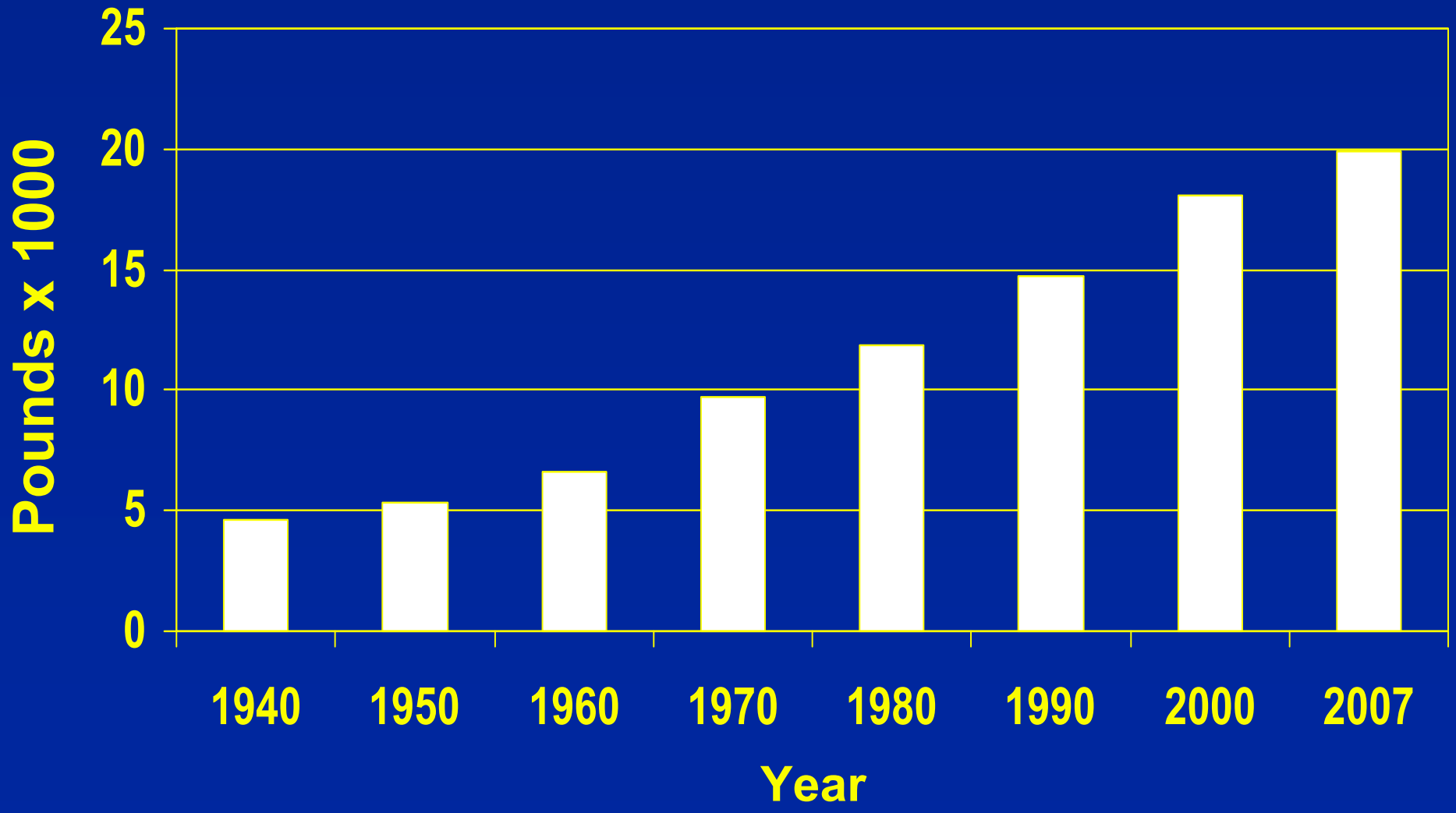
Improve Genetics of Male

Artificial Insemination

Dairy: Milk Production

Turkey and swine: growth rate, carcass quality

Milk Production per Cow in the U.S.

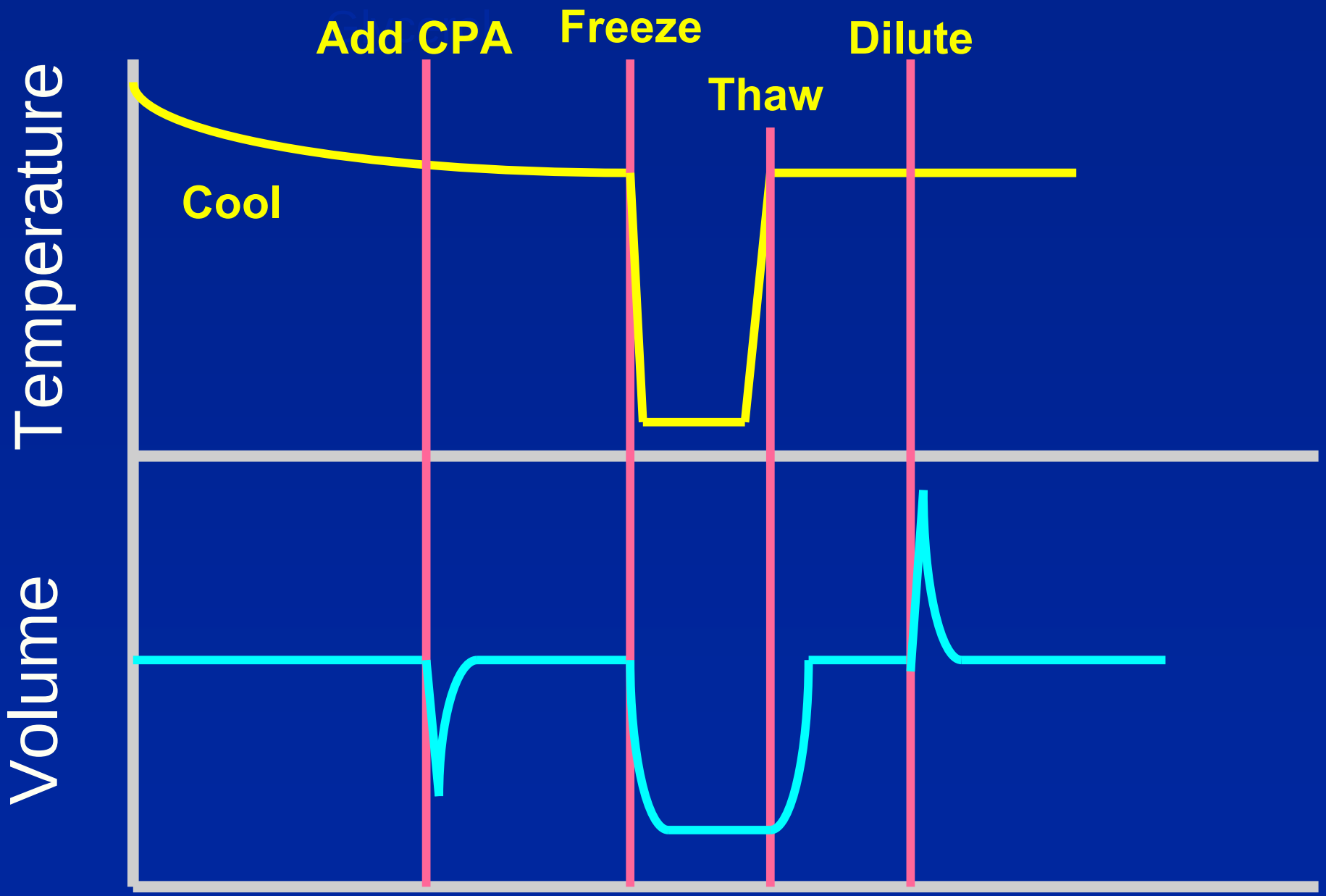


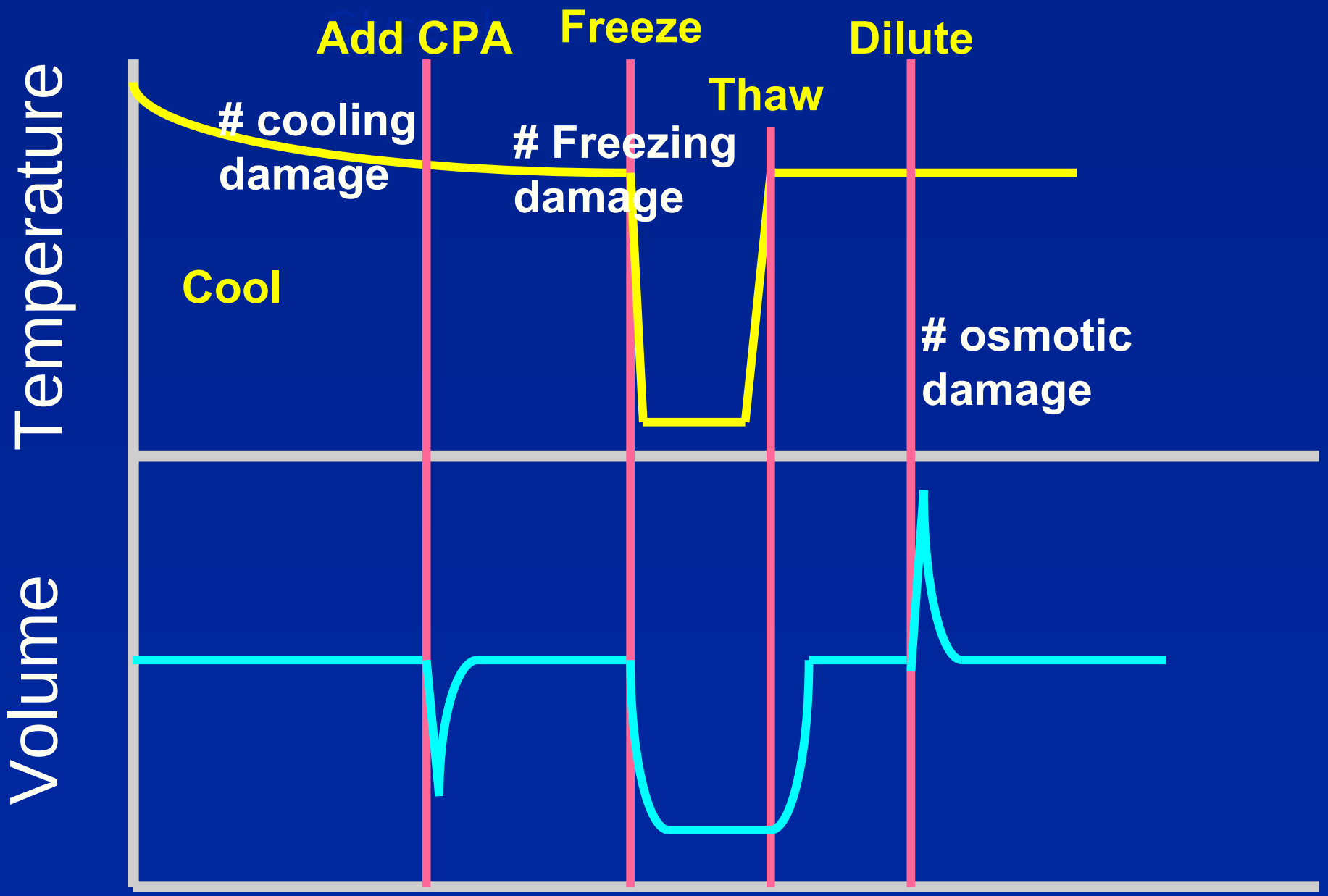
Advantages to Using AI

Decrease spread of sexually transmitted diseases

Improve genetics – Higher selection pressure on males

Frozen Semen


Add CPA
Freeze
Thaw
Dilute
Cool
Temperature
Volume


Add CPA
Freeze
Thaw
Dilute
Temperature
Volume
cooling damage
Freezing damage
Cool
osmotic damage

Frozen Semen

Holstein Bulls

- 95% produce semen that freezes well
- Fertility is similar to cooled semen

Stallions

- 25% produce sperm that freeze well
- 50% produce sperm that freeze okay
- 25% produce sperm that do not freeze
- Fertility is lower than cooled semen

Why do Holstein and Stallion Sperm cryopreserve so differently?

Shape

Chemical composition

- cooling damage
- cryoprotectant membrane permeability
  - swelling and shrinking (osmotic)

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Chemical composition

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- cryoprotectant membrane permeability
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Sixty years of selection

Improving Stallion Sperm Cryosurvival

Different diluent composition

Improving Stallion Sperm Cryosurvival

Different diluent composition

Alternative Cryoprotectants

Glycerol < DMF

Effect of Cryoprotectants on Cryosurvival of Stallion Sperm

Treatment	%Motile	%Viable
Glycerol	52	45
M Formamide	54	36
DM Formamide	60*	51
Ethelyne Glycol	43	45

Improving Stallion Sperm Cryosurvival

Different diluent composition

Alternative Cryoprotectants

- Glycerol < MF = DMF

Adding Cholesterol to sperm membranes

- Human sperm cholesterol/P-lipid = .99
- Stallion sperm chol/P-lipid = .36

Cyclodextrin Conc. Optimizing Cryosurvival

	Post-Thaw Viability (%)	
CLC (mg)	Stallion	Ram
Control (0)	43	24
0.75	52*	35
1.0	--	45*
1.5	53*	--
2.0	--	47*
3.0	52*	--
4.5	55*	36
6.0	54*	--
SEM	3	5

Improving Bull Sperm Cryosurvival

Different diluent composition

Alternative Cryoprotectants

Adding Cholesterol to sperm membranes

Improving Bull Sperm Cryosurvival

Different diluent composition

Alternative Cryoprotectants

Adding Cholesterol to sperm membranes

Cyclodextrin Conc. Optimizing Cryosurvival

	Post-Thaw Viability (%)		
CLC (mg)	Bull	Stallion	Ram
Control (0)	42	43	24
0.75	58	52*	35
1.0	--	--	45*
1.5	60*	53*	--
2.0	--	--	47*
3.0	57	52*	--
4.5	--	55*	36
6.0	53	54*	--
SEM	4	3	5

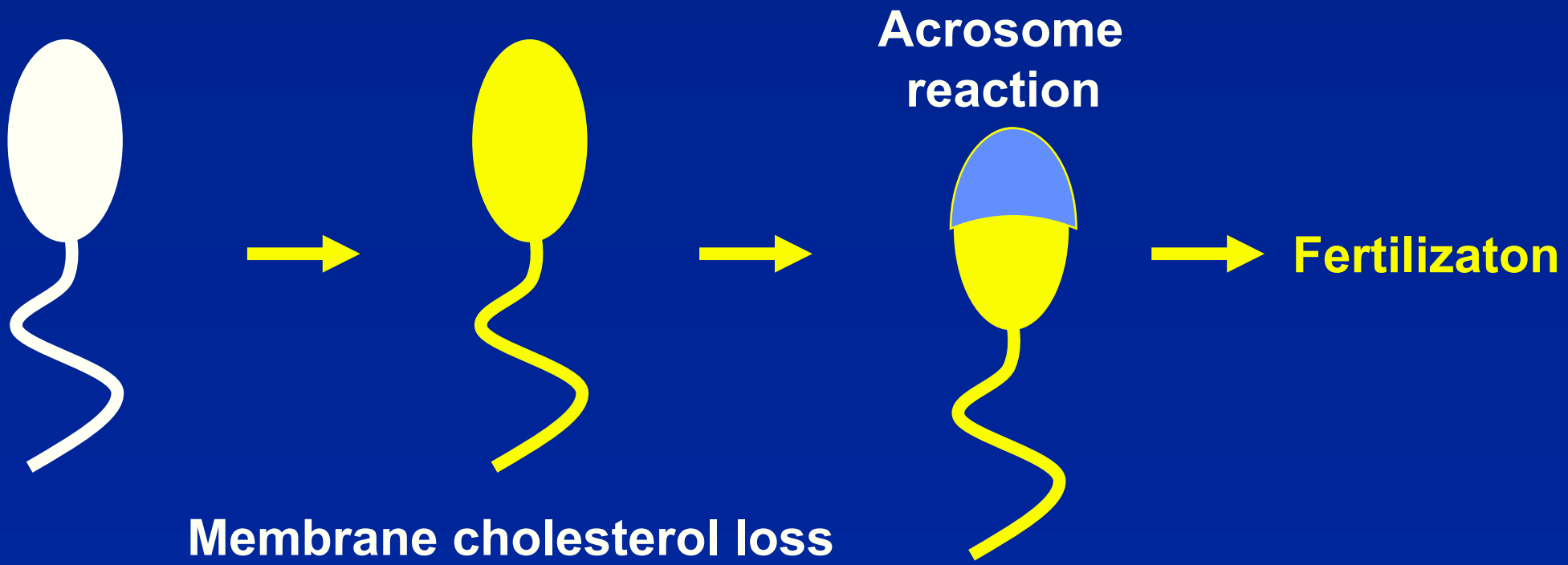
Cholesterol Concentration in Sperm

	Cholesterol:P-Lipid Ratio	
	Control	CLC-treated
Stallion	0.36	0.86
Ram	0.38	0.89
Bull	0.45	0.90
Human	0.99	

Does Cholesterol Effect Fertility?

- Studies to date using bull, ram & stallion sperm
  - Equal or less than control sperm

Why does cholesterol treatment reduce fertility?



Membrane cholesterol loss
Increase intracellular Ca^{++}
Increase cAMP
Increase intracellular pH
Increase Tyr-phosphorylation

Why does cholesterol treatment reduce fertility?

- Sperm capacitation
  - Removal of cholesterol required
    - Added cholesterol increases time
  - Perhaps should inseminate earlier
    - Cosynch vs Ovsynch?

Why does cholesterol treatment reduce fertility?

- Sperm capacitation
  - Removal of cholesterol required
    - Added cholesterol increases time
  - Perhaps should inseminate earlier

  - Mixture of untreated and cholesterol?
    - Widen window of viable sperm?

Increase Bull Fertility

- Breeding Soundness Exams (BSE)
- Semen Analyses

Improve Genetics

- AI
- Sexed Semen

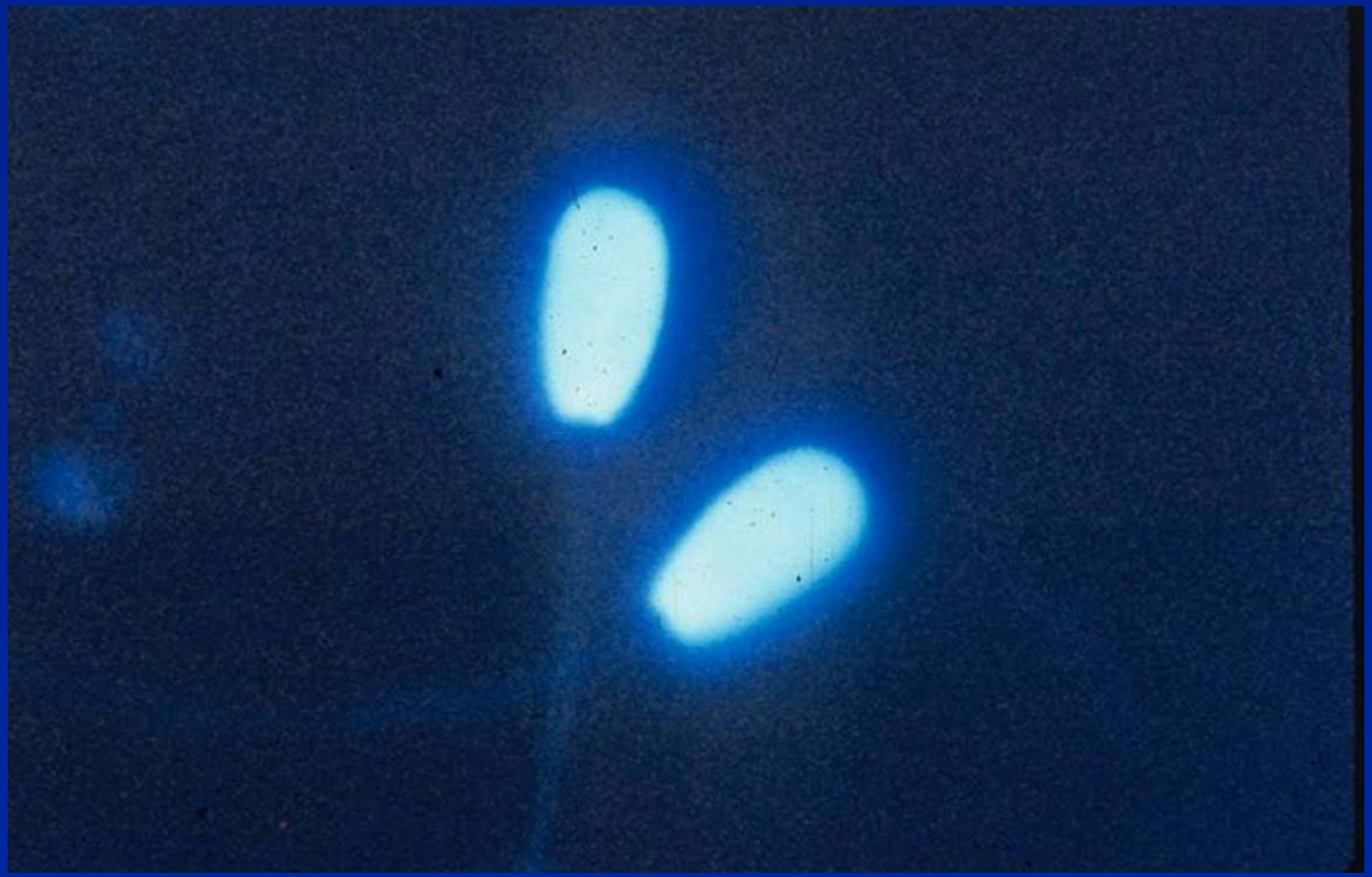
Methods for Sexing Semen

Differences in DNA content

- X chromosome is larger than Y chromosome
- X-sperm have 3.8% more DNA than Y-sperm

Methodology for Sorting Sperm

Stain the sperm with Hoeschst 33342 dye



Hoeschst + Red Food coloring

Blue = live

Blue + Red = Dead

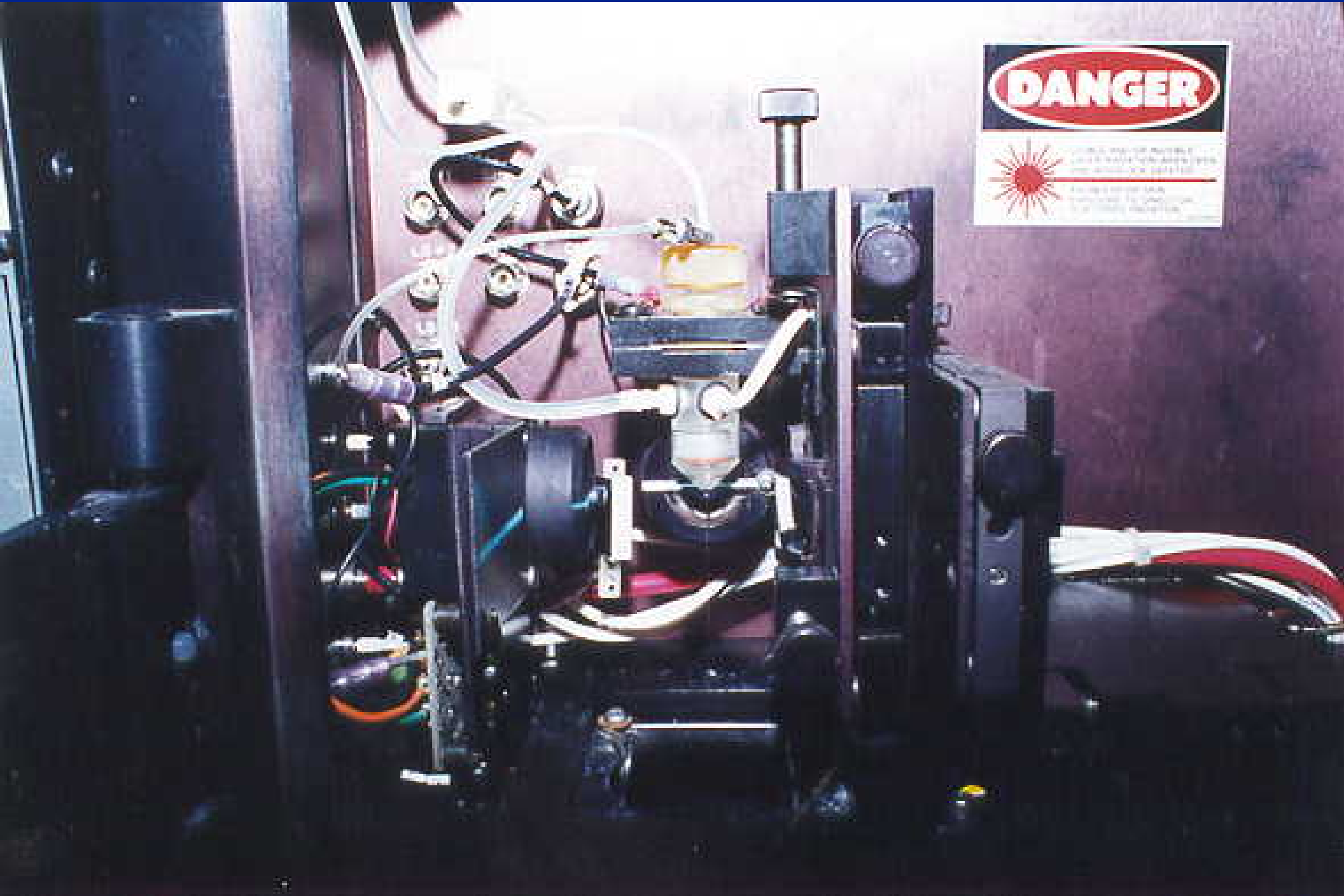
Methodology for Sorting Sperm

Stain the sperm with Hoeschst 33342 dye

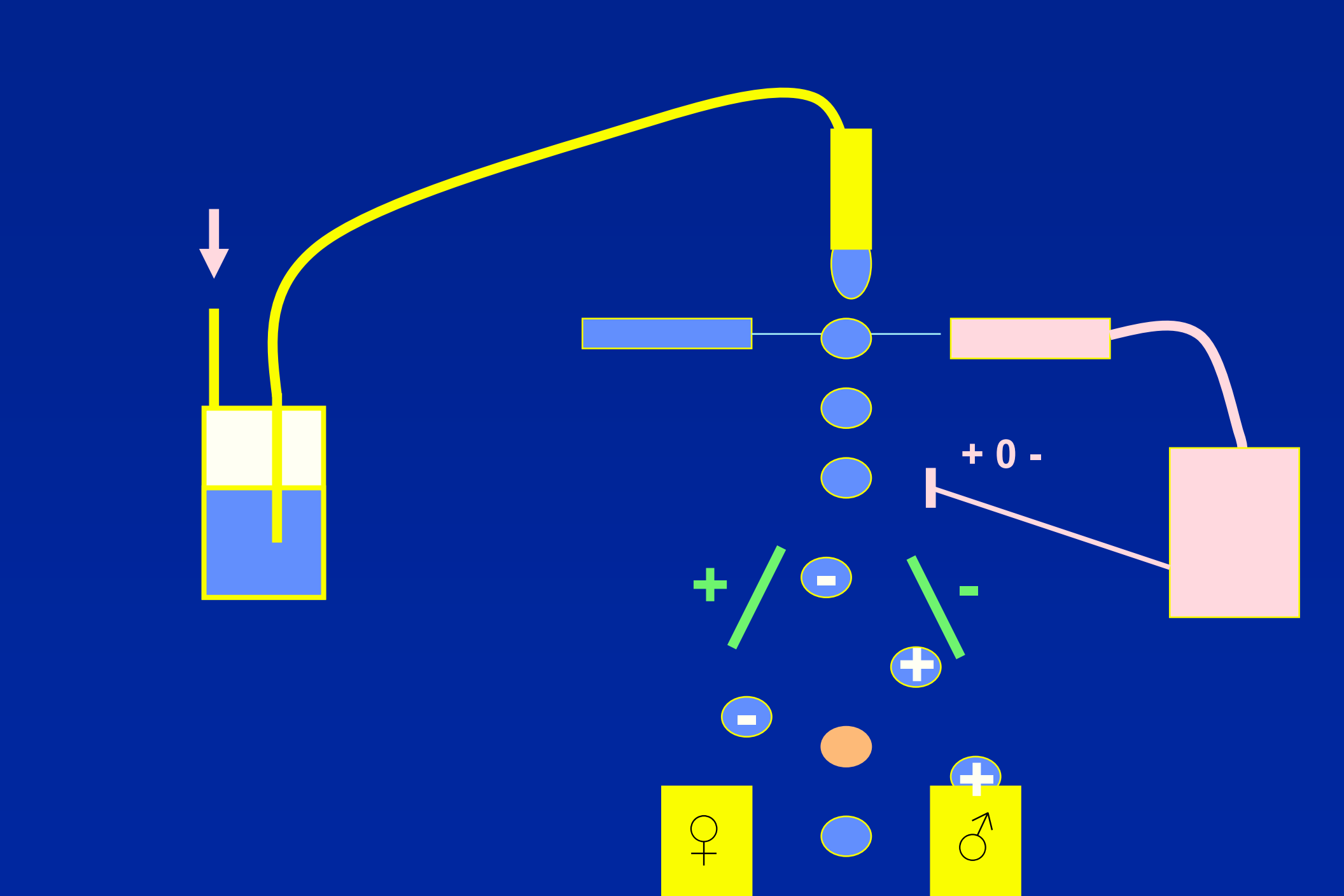
Place in flow cytometer

Sort (3000-4000 sperm/sec)

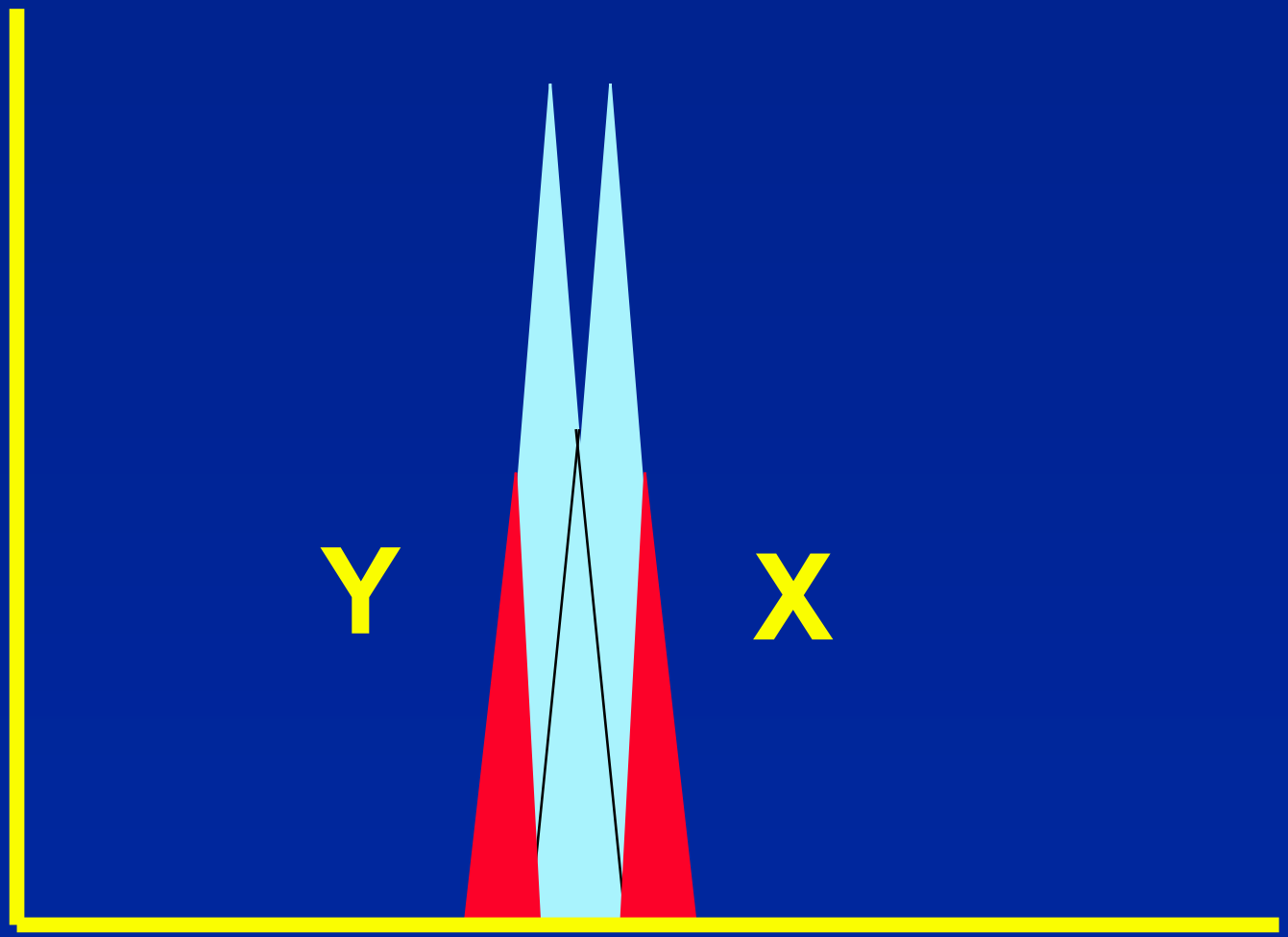

COULTER
VIABLE SAMPLE HANDLING SYSTEM


DANGER

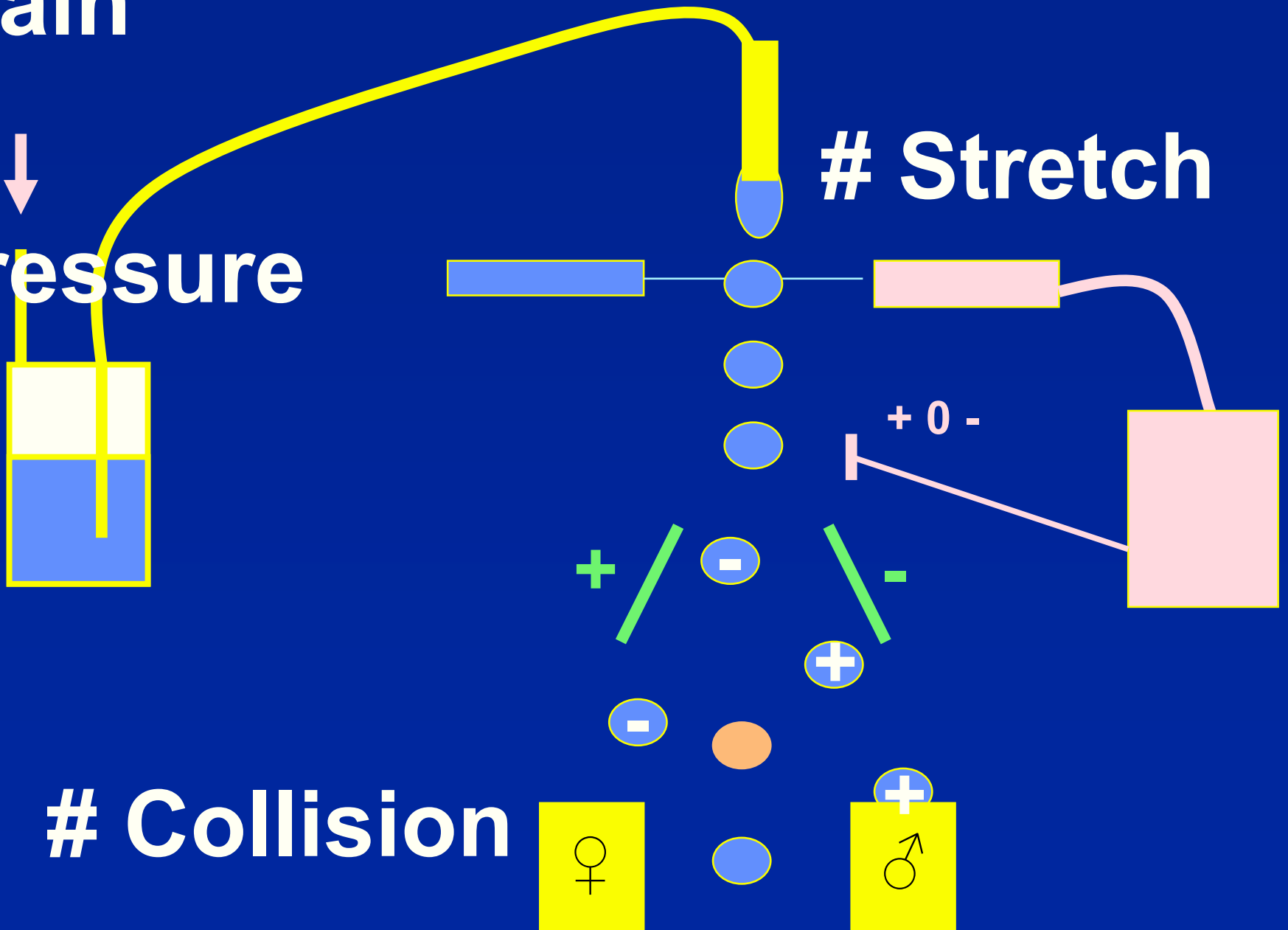

COULTER
VIABLE SAMPLE HANDLING SYSTEM


+ 0 -
+
-
-
♀
♂


COULTER
VIABLE SAMPLE HANDLING SYSTEM


Y
X

stain



Methodology for Sorting Sperm

Stain the sperm with Hoeschst 33342 dye*

Place in flow cytometer and sort

- Pressure*
- Orient properly
- Form droplets*
- Laser
- Determine ‘sex’ and charge
- Sort past electrical plates
- Catch*

Methodology for Sorting Sperm

Stain the sperm with Hoeschst 33342 dye*

Place in flow cytometer and sort

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- Orient properly
- Form droplets*
- Laser
- Determine ‘sex’ and charge
- Sort past electrical plates
- Catch*

Sorted sperm are “different”

Methodology for Sorting Sperm

- Stain the sperm with Hoeschst 33342 dye*
- Place in flow cytometer and sort
  - Pressure*
  - Orient properly
  - Form droplets*
  - Laser
  - Determine ‘sex’ and charge
  - Sort past electrical plates
  - Catch*
- Post Sorting Manipulations
  - Centrifuge**
  - Cryopreserve**

Bull Effects & Sorting Sperm

1. Sperm from some bulls will not sort
   - orient properly
   - stain properly

2. Sorted sperm from some bulls will not freeze
   - centrifugation
   - cryopreservation

3. There are few sorted cells to work with

Cost Effectiveness of Sex-Sorted Sperm

Increase Bull Fertility

- Breeding Soundness Exams (BSE)
  - Semen Analyses

Improve Genetics

- AI (frozen semen)
  - Sexed Semen