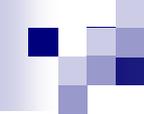




# Application of sexed sperm

2011 11/ 21 Feng Hsiang chu

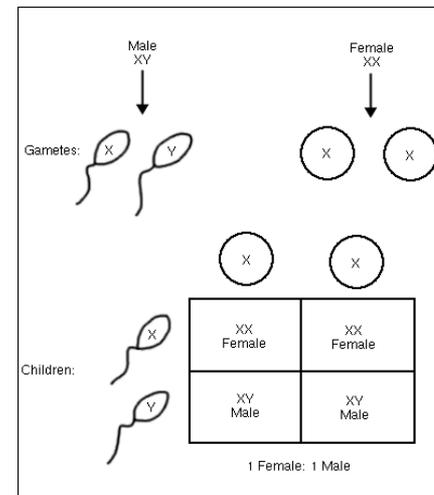


# Outline

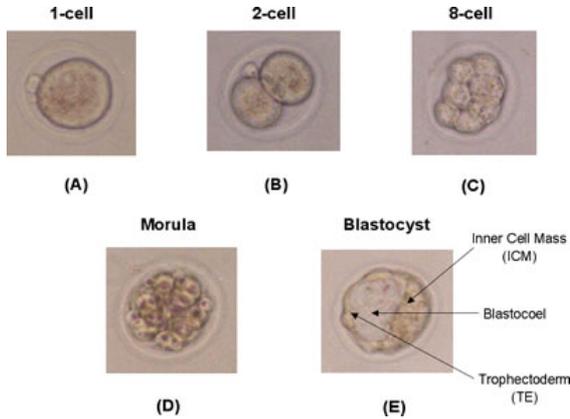
1. Introduction
2. Sexing preimplantation embryos by PCR
  - 2.1. Application of the HMG box of bovine SRY gene for sex determination
  - 2.2. Using loop-mediated isothermal amplification
3. Sexing of embryos by developmental arrest induced by H -Y antisera
4. Overview of sexing sperm
  - 4.1. AI with cryopreserved sexed sperm
  - 4.2. IVF of bovine embryos using sex-sorted sperm
5. Conclusions

# ■ Introduction

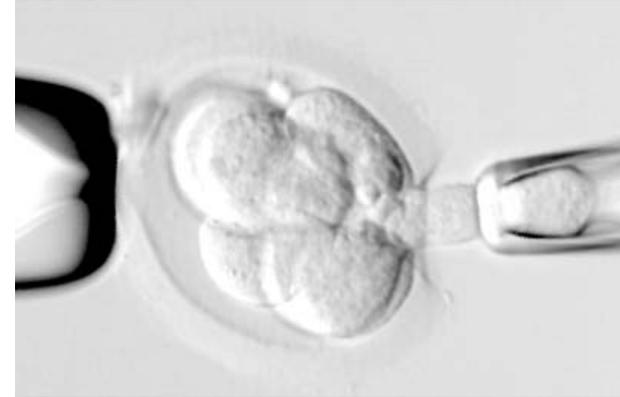
- For thousands of years, livestock owners have desired a methodology to predetermine the sex of offspring for their herds
- 1 Holstein heifer calves selling for \$ 510–590, 1 Holstein bull calves sold for \$ 170–208
- different techniques, such as the Quinacrine mustard **staining for Y-chromosome**, the Quantitative **Southern Blotting**, the semi-quantitative **PCR**, the multicolour fluorescence in situ hybridisation (**FISH**) have been developed



# Sexing preimplantation embryos by PCR



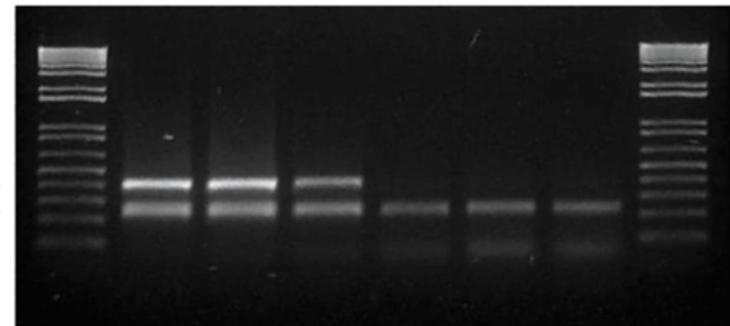
Embryos in vitro



micromanipulation



PCR mechanism



Gel electrophoresis

# Application of the HMG box of bovine SRY gene for sex determination

- Amplification of the bovine high motility group (HMG) box of the sex-determining region of the Y chromosome gene (SRY).
- The open reading frame (ORF) of human SRY gene is contained within a single exon and encodes a 204-amino-acid protein.
- The central 79 amino acids encode the HMG box, which functions as a DNA-binding and DNA-bending domain and also contains 2 nuclear localization signals.
- Comparison of the amino acid sequence of the HMG box of the SRY gene among human, mouse, rabbit, wallaby, marsupial mouse, and sheep revealed 70% identity.
- There is no sequence conservation outside the HMG box.

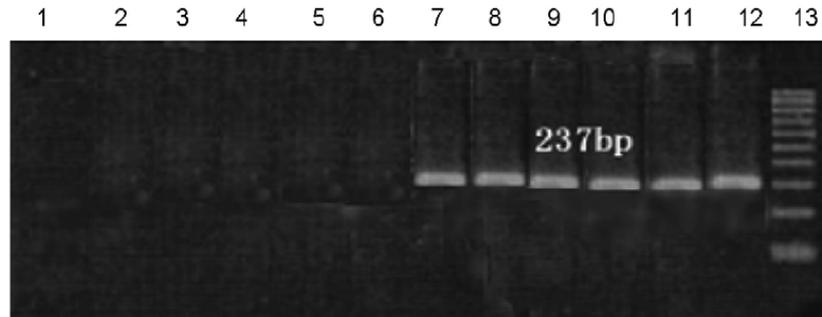


Fig. 1. Agarose gel electrophoresis of PCR products of genomic DNA extracted from male and female bovine, using HMG primers. Lanes 1–6 are PCR products from reactions using genomic DNA prepared from the testes of six female bovines. Lanes 7–12 are PCR products from reactions using genomic DNA prepared from six male bovines. Lane 13 is 1000 bp PCR low ladder (Sigma). The position of the 237 bp is male-specific PCR product.

Table 1  
Accuracy of sexing bovine embryos

Embryos	
Sexed	14
Transferred	14
Calves	
Born (%)	9/14 (64.3)
Accuracy (%)	9/9 (100)

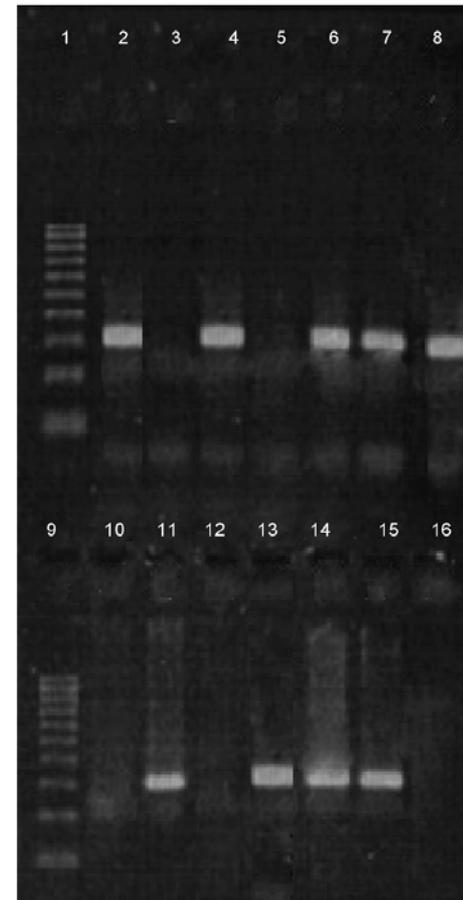
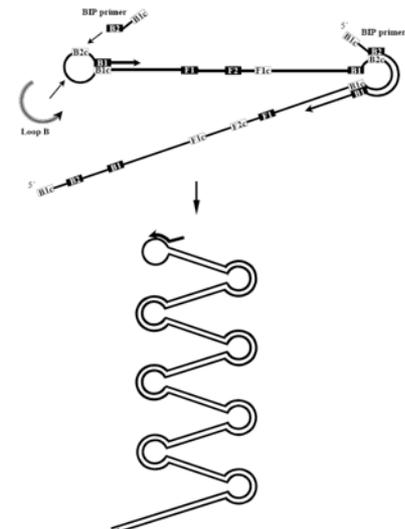
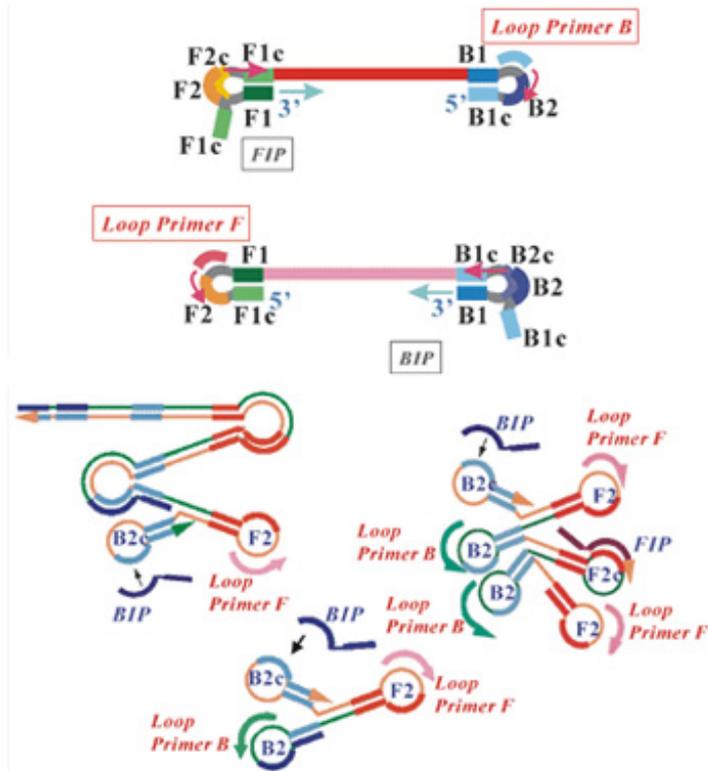
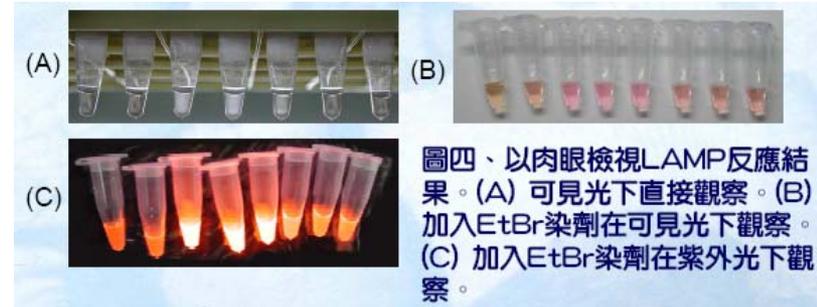


Fig. 2. Sexed embryonic cells using designed male-bovine specific primers by PCR. Lanes 1 and 9 are 1000 bp PCR low ladders (Sigma); Lanes 3, 5, 10, 12 and 13 are female embryos; Lanes 2, 4, 6, 7, 8, 11, 13, 14 and 15 are male embryos.

# Using loop-mediated isothermal amplification



The Loop Primers (either Loop Primer B or Loop Primer F), containing sequences complementary to the single stranded loop region (either between the B1 and B2 regions, or between the F1 and F2 regions) on the 5' end of the dumbbell-like structure, provide an increased number of starting points for DNA synthesis for the LAMP method. An example is shown in the figure where there is an amplified product containing six loops. In the original LAMP method, four of these loops would not be used, but through the use of Loop Primers, all the single stranded loops can be used as starting points for DNA synthesis.



圖四、以肉眼檢視LAMP反應結果。(A) 可見光下直接觀察。(B) 加入EtBr染劑在可見光下觀察。(C) 加入EtBr染劑在紫外光下觀察。

# sexing of bovine preimplantation embryos using loop-mediated isothermal amplification

Primer sequences for LAMP-based embryo sexing

Male-specific primers

Inner primer F 5'-AGCTATGTGGCATGTGGATCCTCCCTGGAAATGTTAAGTG-3'  
 Inner primer B 5'-TAAAGCCAGACACAGAGGTCACCTTTGCTTCTCTTCCTGCTTC-3'  
 Outer primer F 5'-AGCCAAGAAGTGGATGAATC-3'  
 Outer primer B 5'-GCAGTGCATTCCCTCCTC-3'  
 Loop primer F 5'-GGGATGAAACTGTGCAT-3'  
 Loop primer B 5'-ATTGCATGTGGAAGAACTGTAG-3'

Male-female common primers

Inner primer F 5'-GAGGAACATTGGCTTCTGGACAAGCTGGGGATTGCTCT-3'  
 Inner primer B 5'-AGTGGAAAGCAAAGAACCCACCCAGTGAGCTCCAA-3'  
 Outer primer F 5'-AGGCTGCCTCTTGTGTT-3'  
 Outer primer B 5'-CATGGCCTAGAGACCAATC-3'  
 Loop primer F 5'-CCTAGATGAGGTCTATTGGC-3'  
 Loop primer B 5'-CTGCTCTCGAATTGTGACG-3'

cycles of shuttle PCR at 98 °C for 8 s and at 66 °C for 20 s. The final extension step was followed by 5-min incubation at 72 °C.

## Effect of cell number on sensitivity and accuracy of LAMP-based embryo sexing

Number of blastomeres used for assay	Number of embryos examined	Number (%) with satellite sequence detected	Number (%) correctly determined
1	15	12 (80.0)	9 (75.0)
2	28	26 (92.9)	23 (88.5)
3	16	13 (81.3)	13 (100)
4	16	16 (100)	16 (100)
5	17	17 (100)	17 (100)

DNA of blastomeres was extracted with treatment at 95 °C for 5 min (heat method).



# Sexing of embryos by developmental arrest induced by H -Y antisera

- embryos at the late morula stage were cultured in medium containing high-titer rat H-Y antisera
- After 12 h of incubation, embryos blocked at the late morula stage were classified as males and those at the blastocyst stage were classified as females.
- that 83% of the embryos classified as males and 82% of those classified as females had their sex correctly predicted
- was an efficient strategy for non-invasive embryo sexing

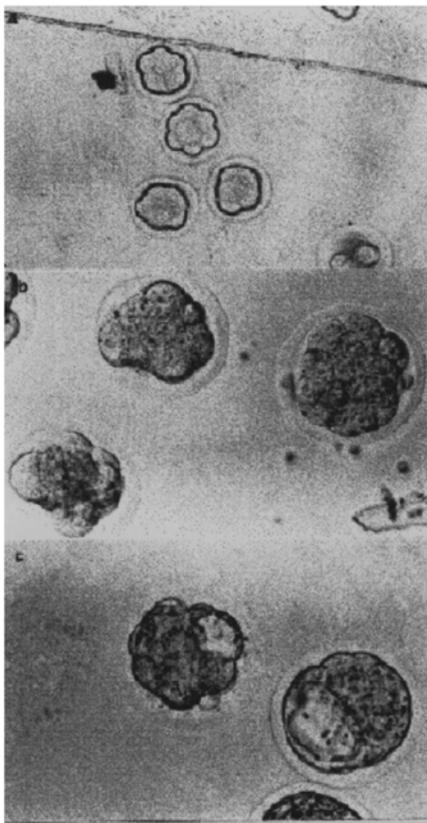


Fig. 1. (a) Morulae and compact morulae before treatment with high-titer rat H-Y antisera; (b) embryos at the compact morula stage after being cultured for 12–24 h in the presence of high-titer rat H-Y antisera (classified as males); and (c) embryos at the blastocyst stage after being cultured for 12–24 h in the presence of rat H-Y antisera (classified as females).

#### Sexing murine embryos by inducing developmental arrest with high-titer rat H-Y antisera

Blastocoele formation (presumptive sex)	No. (%) embryos	No. (%) embryos sexed by chromosomal analysis	
		Male	Female
No (male)	60 (51.3)	50 (83.3)	10 (16.7)
Yes (female)	57 (48.7)	9 (15.8)	48 (84.2)
Total	117	59	58

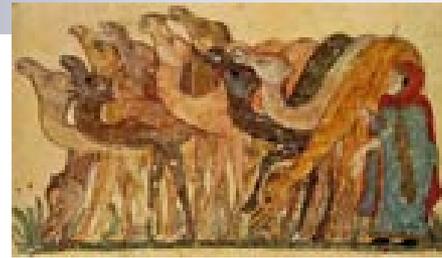
The genetic sex was confirmed by chromosomal analysis.



# Overview of sexing sperm

8,000-6,000 B.C.

**Humans Begin Herding Animals**  
Mesopotamia – sheep  
Egypt – cattle & goats  
China – ponies  
Mid-East – camels  
South America - llamas



1780

**First well-documented artificial insemination using dogs.**



1910

**Artificial Insemination**

Humans place bull semen into a cow's reproductive tract.  
Today, more than 65% of U.S. dairy herds, 85% of U.K. dairy herds and 90% of Scandinavian dairy herds are bred by A.I.



1950s

**Frozen Sperm**

Sperm that is deep-frozen then thawed is shown to produce healthy offspring.

Today, 99% of all A.I.'s in the U.S. and U.K. dairy industries use frozen/thawed semen.



1970s

**Flow Cytometer**

Equipment first developed to sort living cells at high speed.

1992

**First Sex-Selected Calf**

Mastercalf, Ltd., of Cambridge, U.K., produces world's first sex-selected calf by in-vitro fertilization.



## Mid-1990s

### Separation of X- and Y-Bearing Sperm

Further advances in flow cytometry and low-dose insemination permit Colorado State University researchers to produce the world's first sex-selected calf by artificial insemination.



1997

**XY Inc. Acquires Mastercalf of U.K. and achieves world control of the sexing technology of animals. XY Inc. also produces its first sex-selected calf by artificial insemination.**



2005

**World's First Sex-Selected Dolphin.** In October 2005 the world's first sex-selected marine mammal, an Atlantic bottlenose dolphin, is born at SeaWorld San Diego via XY® Inc. sex-selection technology.



2006

### World's First Sex-Selected Kittens

In October 2006, the world's first sex-selected domestic cats were born with their sex predetermined. The litter, produced from embryos fertilized with sexed sperm, was born at Audubon Center for Research of Endangered Species in New Orleans.



2007

### World's First Sex-Selected Dogs

In January 2007, the world's first sex-selected dogs were born, demonstrating XY® Sex Selection Technology works in the canine world.

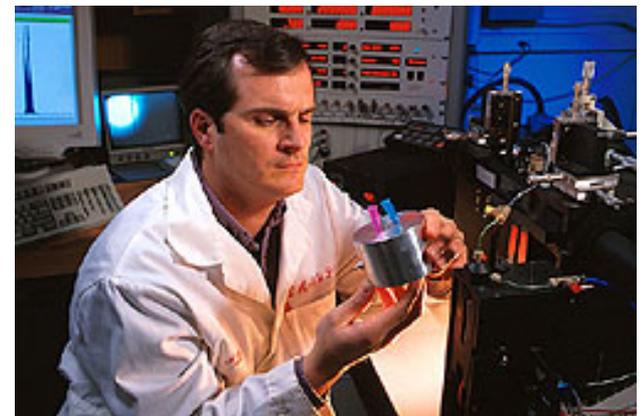
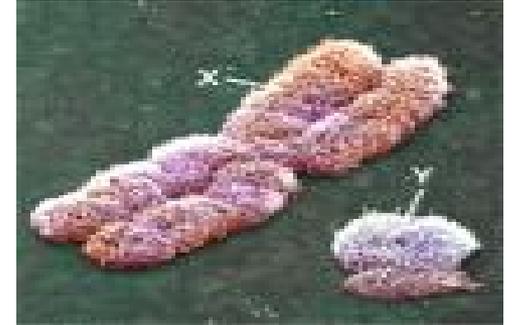


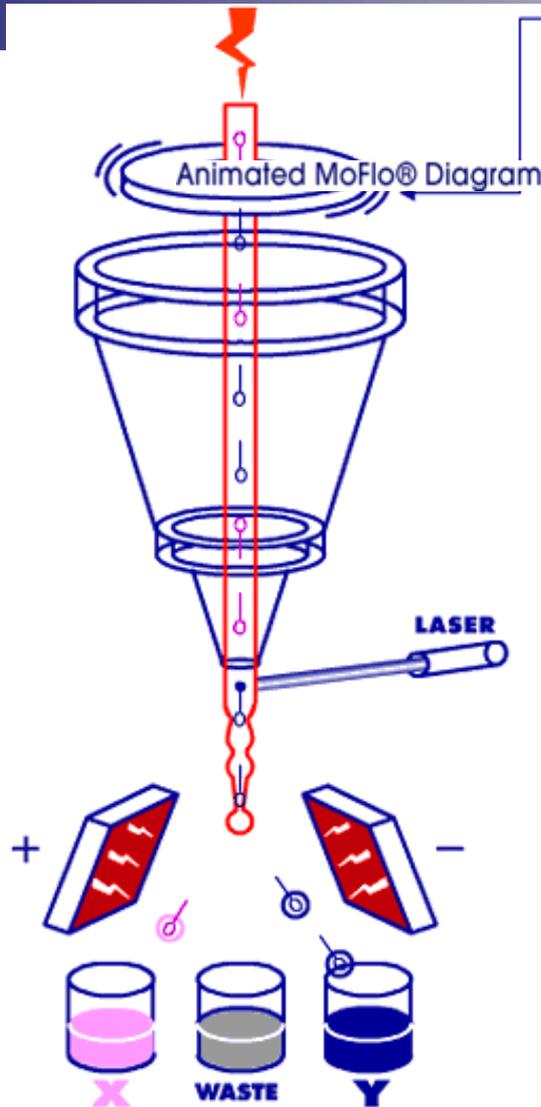
# separated X- and Y-sperm by sperm sorter

- The ability to sort individual sperm cells into viable X- and Y-chromosome-bearing fractions made producers' sex selection dream a reality in the 1990s
- Semen can be sexed with greater than 90% accuracy with use of a flow cytometric cell sorter
- There are, however, slight differences in the sexing accuracy between X-sorted sperm (87.8%) and Y-sorted sperm (92.1%) in calves born
- Semen sexing, involving the separation of X- from Y-chromosome bearing sperms, implies its application in artificial insemination (AI) or in *in vitro* fertilisation (IVF) with the subsequent embryo transfer (ET).

# How sperm are sexed

- the X-sperm contains more DNA than the Y-sperm (approximately 4% more in the case of cattle)
- X-sperm bind more dye than Y-sperm, they give off 4% more fluorescence, which the computer can recognize flow cytometric cell sorter
- this technology is characterized by high costs, complexity of implementation and lower pregnancy rates than with control sperm.

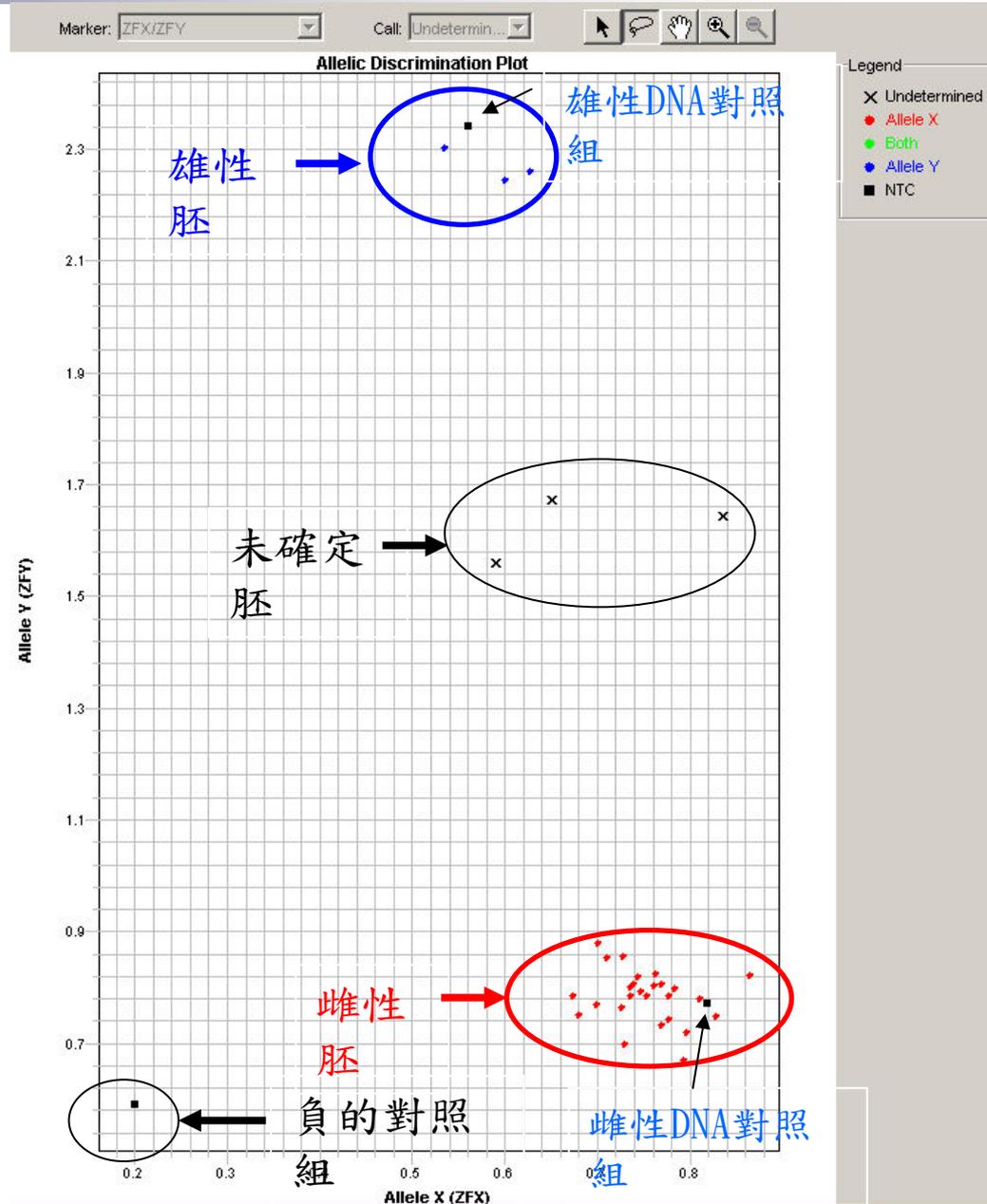




1. A piezo electric crystal is undulated approximately 90,000 times/second, which breaks the stream into droplets at a particular point in time. The location of the last-attached droplet in the stream is highly controllable.
2. An X- or Y-bearing sperm is compared to a preset sort criteria.
3. After a time delay, the insertion rod is charged.
4. A charge is applied at the time the cell reaches the last attached drop.
5. The charged droplets are deflected as they pass between continuously charged plates.
6. Particles not meeting the criteria pass straight down to waste.

In practice, about 20% of sperm end up in the X-fraction, 20% in the Y-fraction and 60% are damaged or not sexable

- 乳牛選性冷凍精液受精後，發育至8-16細胞期33個牛胚利用 Real time PCR分析結果。雌性胚比率為90%。



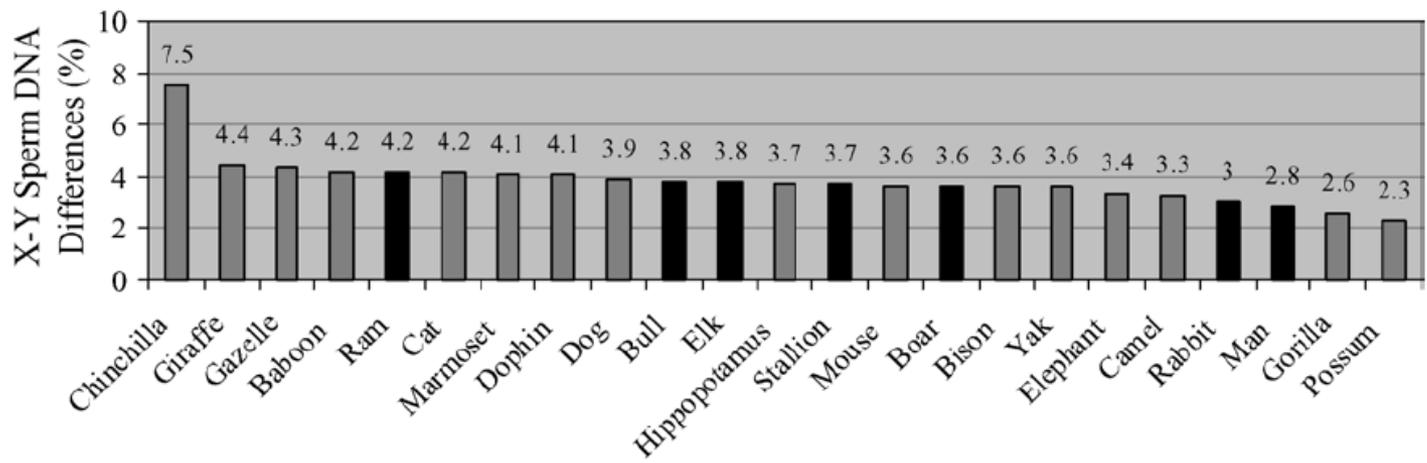
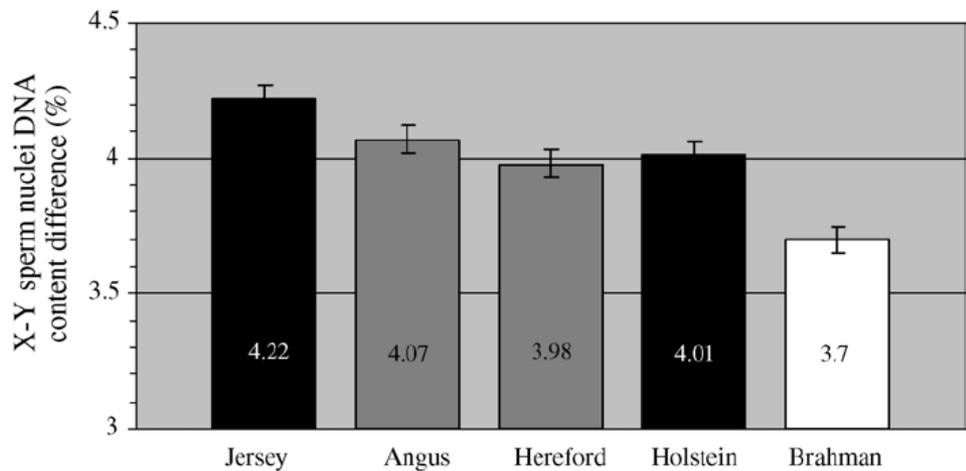


Table 1  
Dimensions and profiles of sperm heads and flow cytometric sorting indices for some domestic mammals and man<sup>a</sup>

Dimension	Bull	Boar	Ram	Rabbit	Cat	Dog	Horse	Man
Length ( $\mu\text{m}$ )	9.1	9.0	8.1	7.7	7.7	7.0	6.5	4.6
Head sagittal section								
Width ( $\mu\text{m}$ )	4.7	5.0	4.0	4.5	3.2	3.5	3.4	3.2
Head profile								
Area ( $\mu\text{m}^2$ )	34.5	37.5	26.6	28.0	19.0	20.9	15.2	10.8
X-Y difference (%)	3.8	3.6	4.2	3.0	4.2	3.9	3.9	2.8
Sorting index <sup>b</sup>	131	115	112	84	80	82	59	31

<sup>a</sup> Compiled from Mann [67] Mann and Lutwak-Mann [68], Johnson [7], Welch and Johnson [6], Garner [11], Garner and Seidel [13] and Seidel and Garner [14].

<sup>b</sup> An approximation of the ability to flow cytometric sort sperm consisting of the head profile area ( $\mu\text{m}^2$ )  $\times$  X-Y Sperm DNA difference (%).

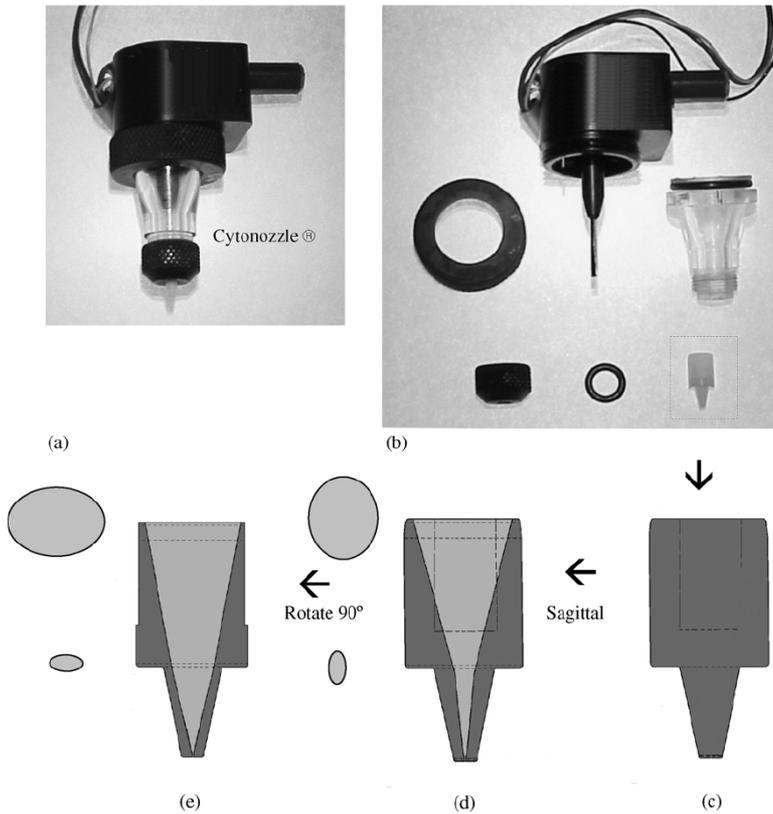
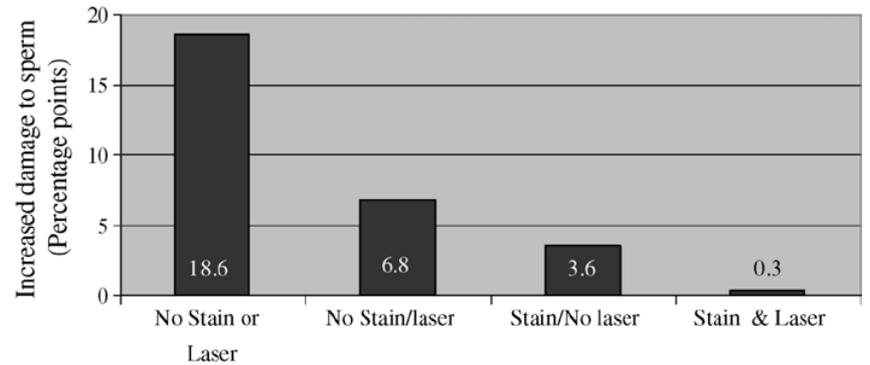


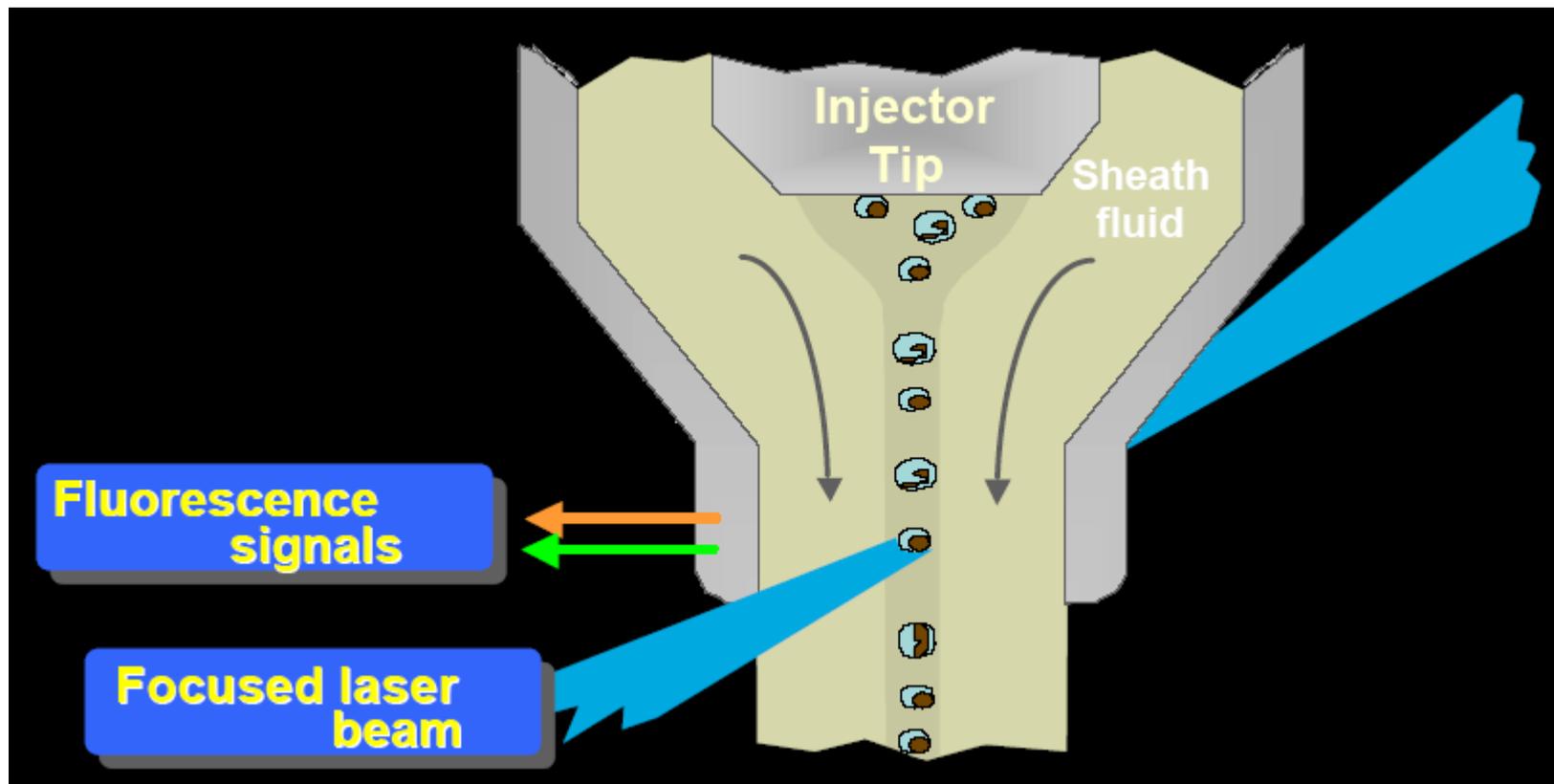
Fig. 3. Illustration of an assembled Cytonozzle<sup>®</sup> (a); a disassembled nozzle showing the flow chamber, tapered injection needle and the ceramic tip [surrounded by dotted lines] (b); a profile of the ceramic tip (c); sagittal section of the tip showing the narrowest elliptical orienting configuration [shaded cross sections of the tip interior are shown on the immediate left of the tip] (d); and a sagittal section after rotation of the tip 90° the illustrate the widest portion of the elliptical internal bore of the tip [shaded cross sections of the tip interior are shown on the immediate left of the tip] (e).



# Fluid System: Hydrodynamic Focusing

讓細胞在管路中排路隊

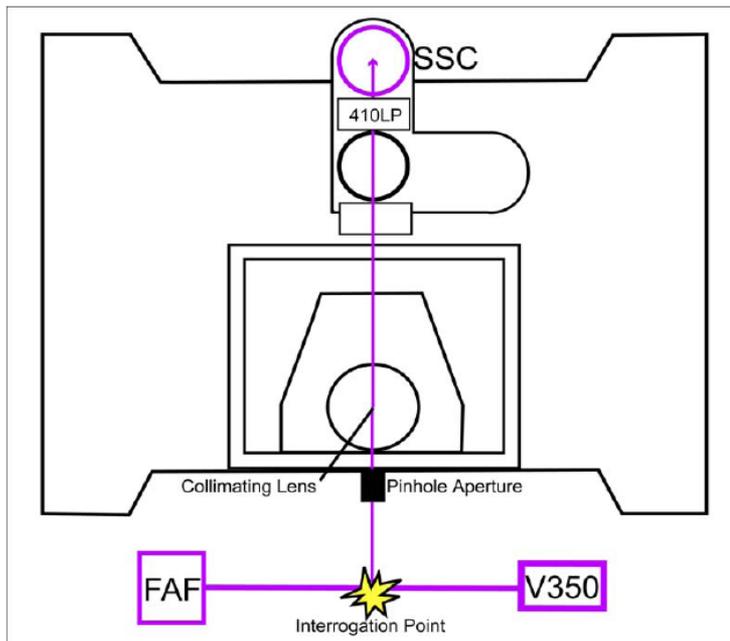
**Hydrodynamic Focusing** (流體動力聚焦)



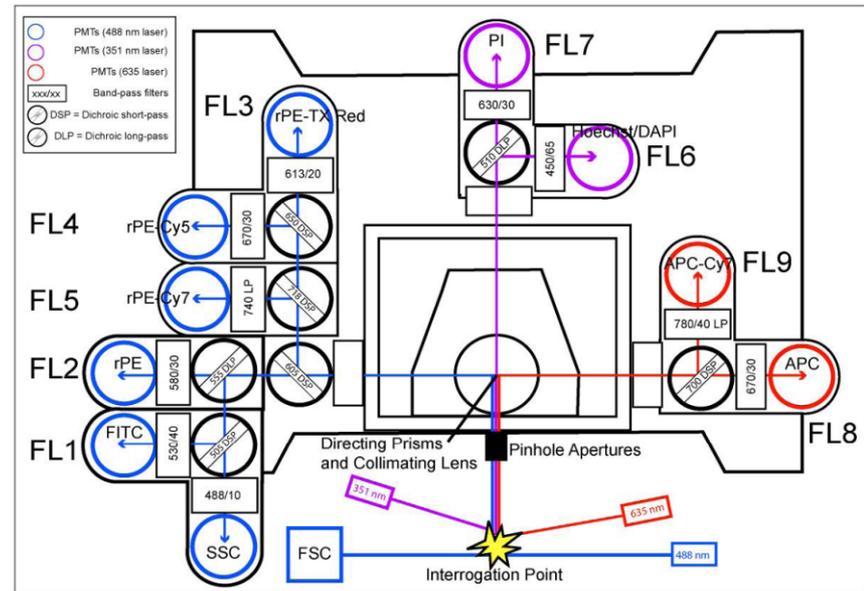
# Sperm sorting V.S. Sorter 差異

## 1. 光學配置

Sperm sorting

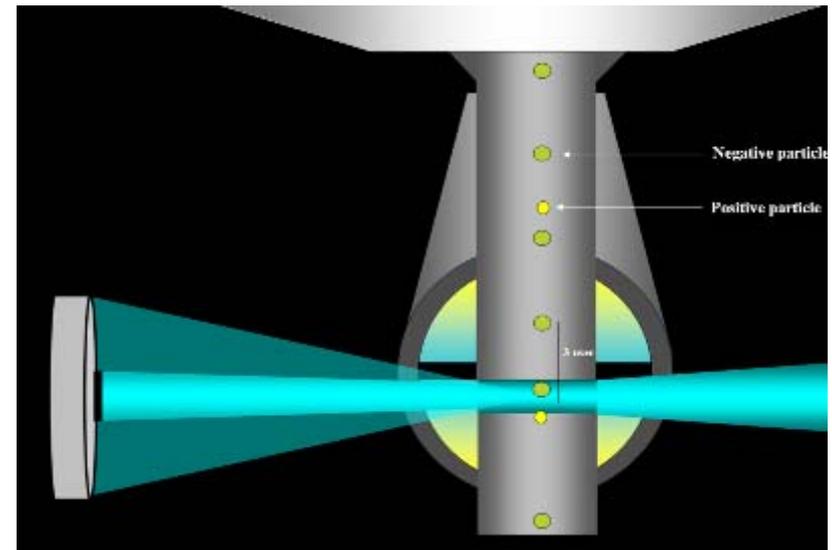
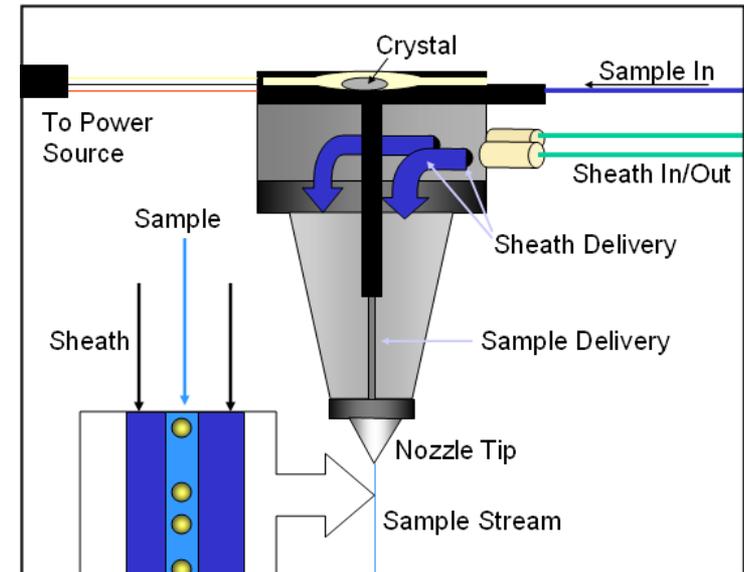


Sorter



# Fluidic Design

- **Jet-in-Air stream design**
- **Faster velocity** of stream to achieve high speed analysis and sorting (Sheath pressure: 4~100 psi)
- The Faster velocity generated **very short signal pulse** to improve the cell distinguished



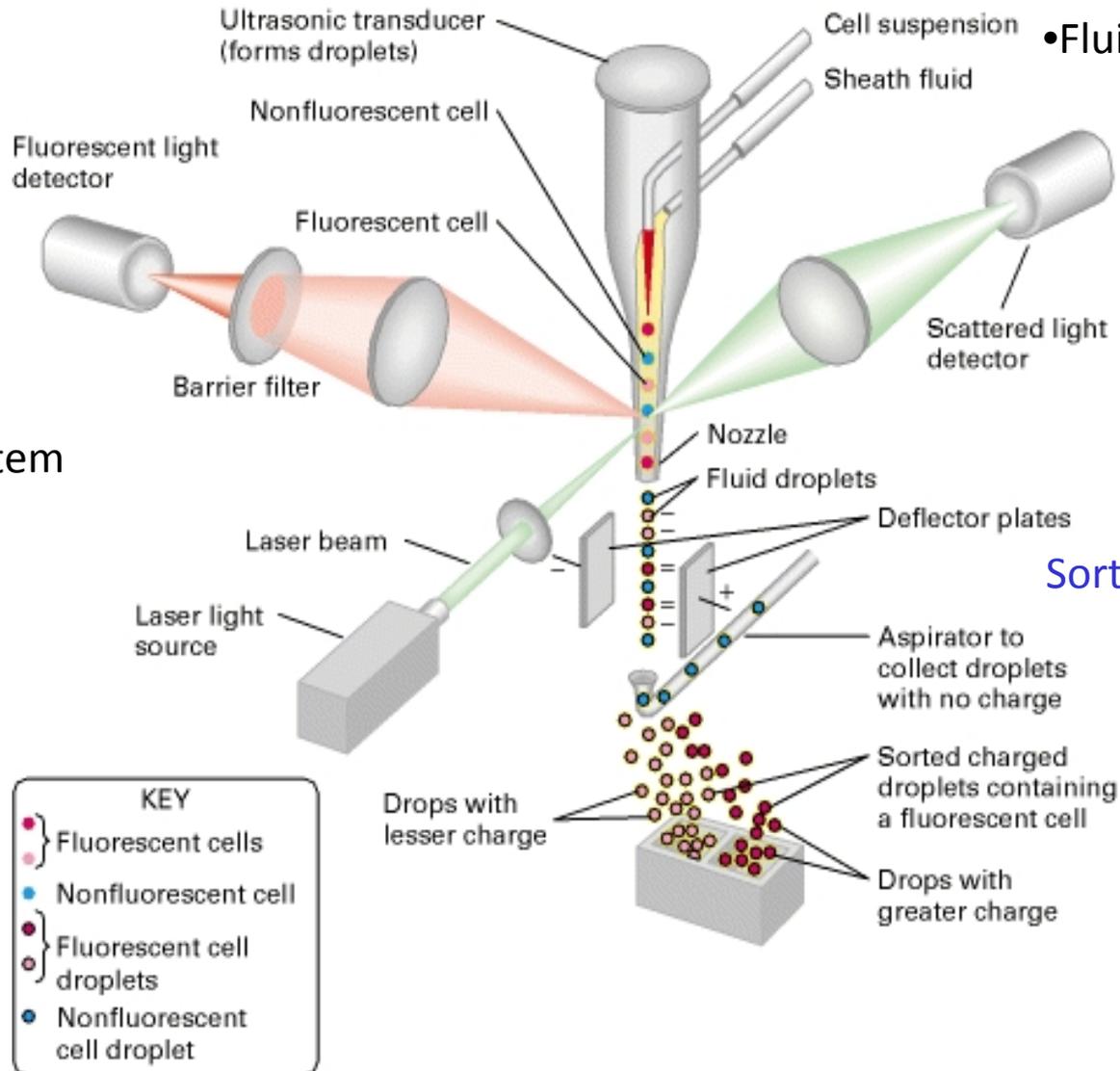
# Flow Cytometry Is Made Of

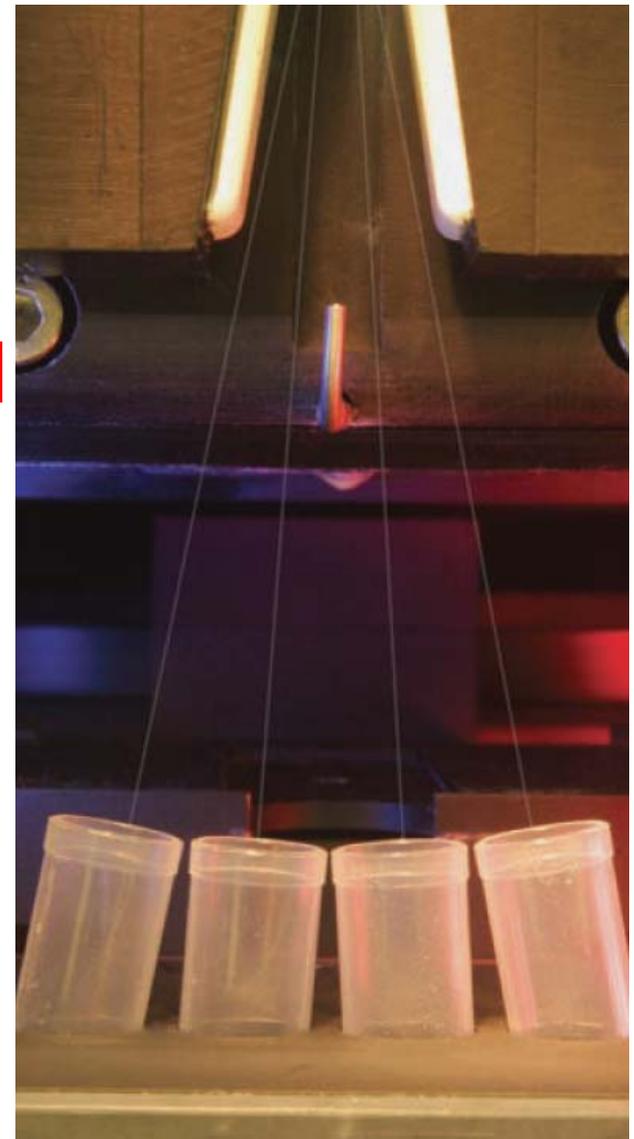
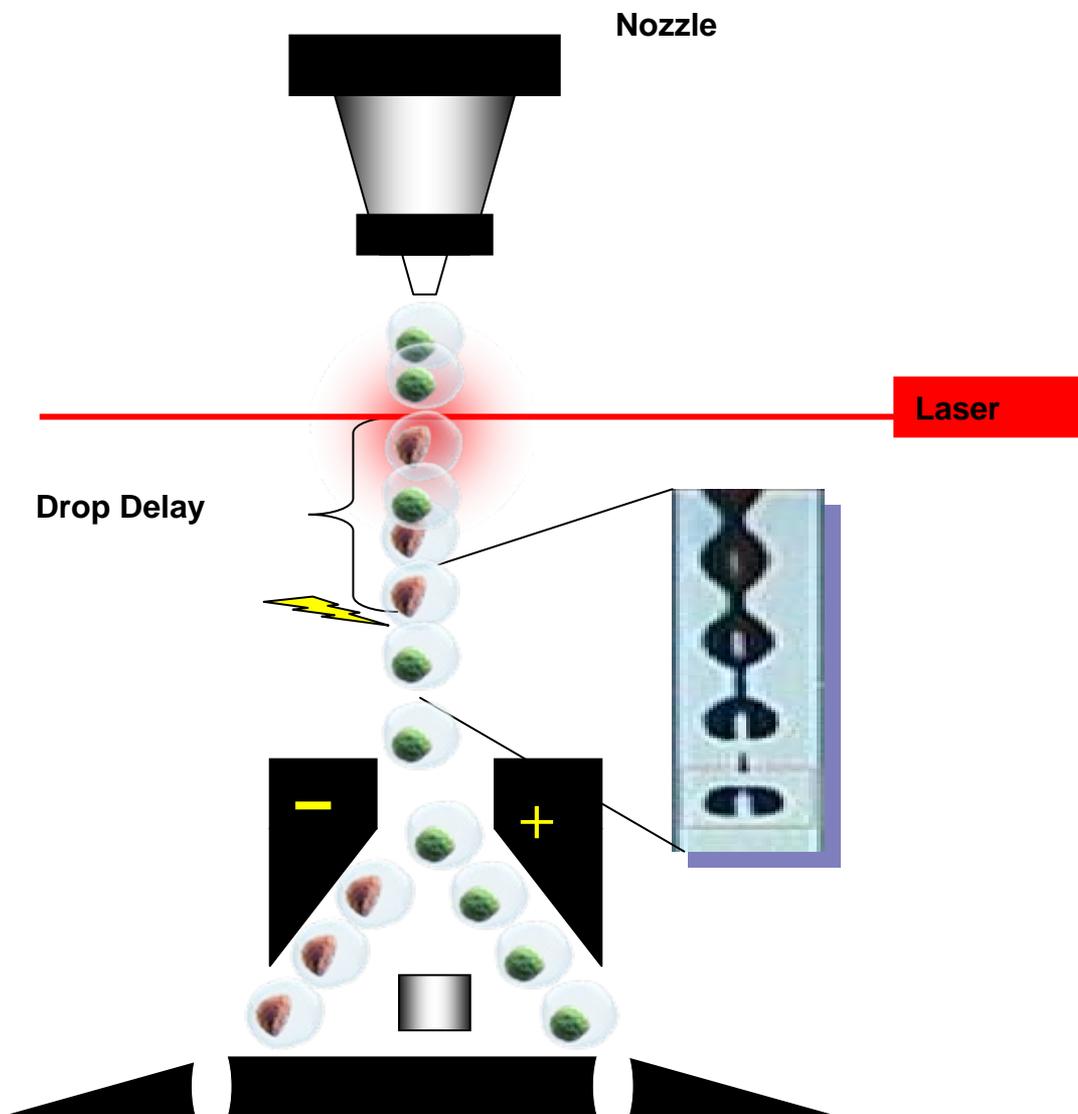
Electronic System

•Optical System

•Fluid System

Sorting System



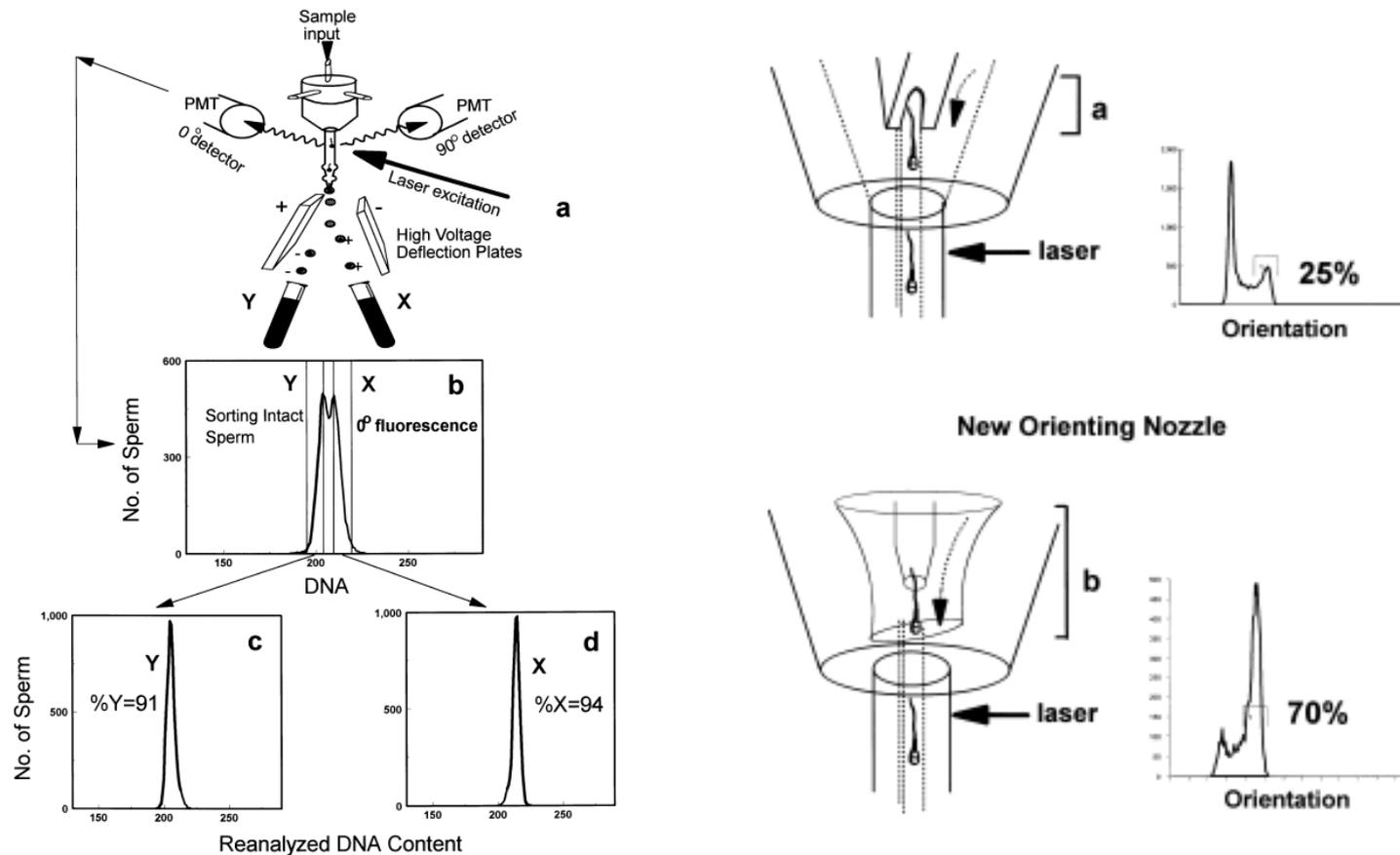




# Sperm sex selection

## The Beltsville Sperm Sexing Technology: High-Speed Sperm Sorting Gives Improved Sperm Output for In Vitro Fertilization and AI Lawrence A. Johnson, Glenn R. Welch and Wim Rens

*J Anim Sci* 1999. 77:213-220.

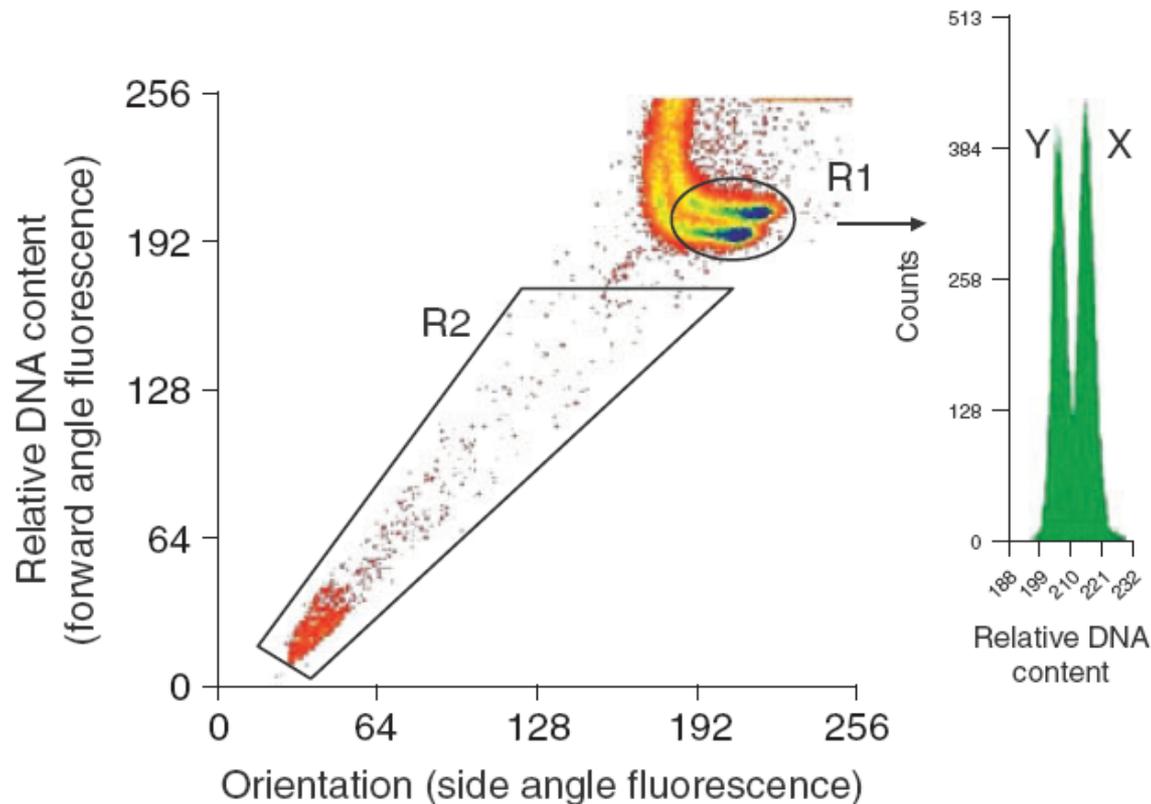


# Sperm sex selection

*Reproduction, Fertility and Development*, 2006, 18, 319–329

## Development of sperm sexing and associated assisted reproductive technology for sex preselection of captive bottlenose dolphins

J. K. O'Brien<sup>A,B,C</sup> and T. R. Robeck<sup>B</sup>



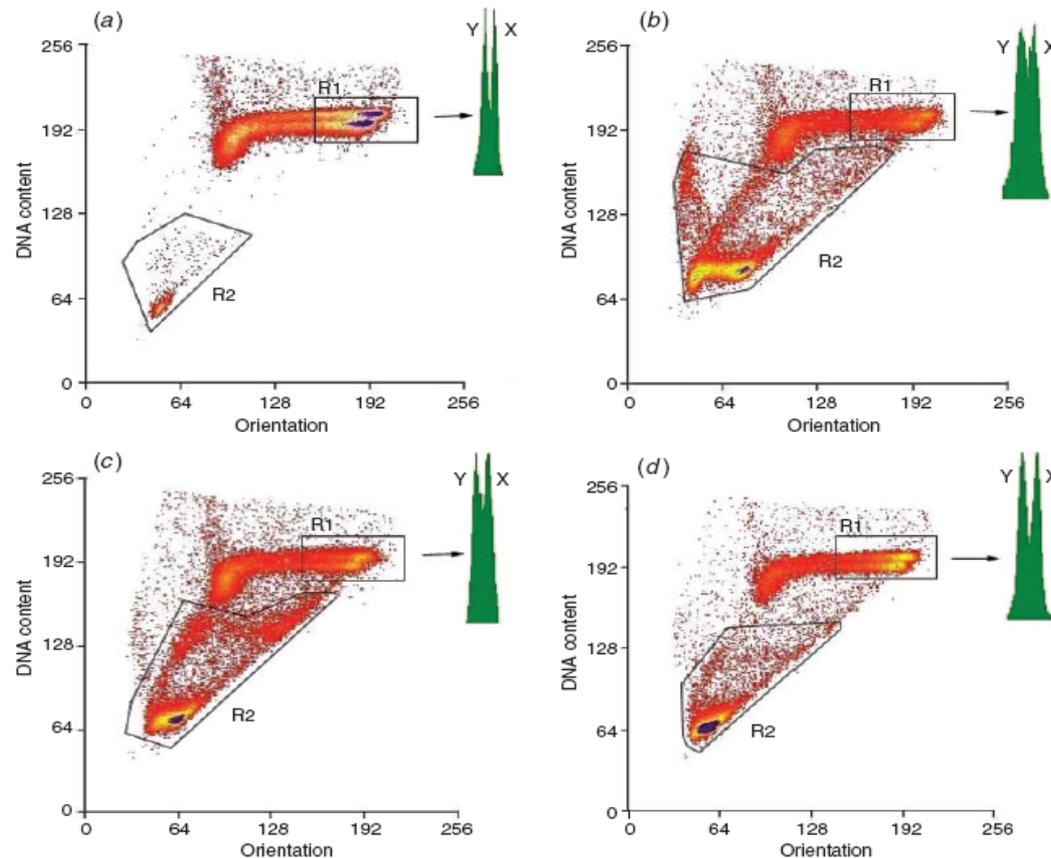
## Flow cytometric sorting of frozen–thawed spermatozoa in sheep and non-human primates

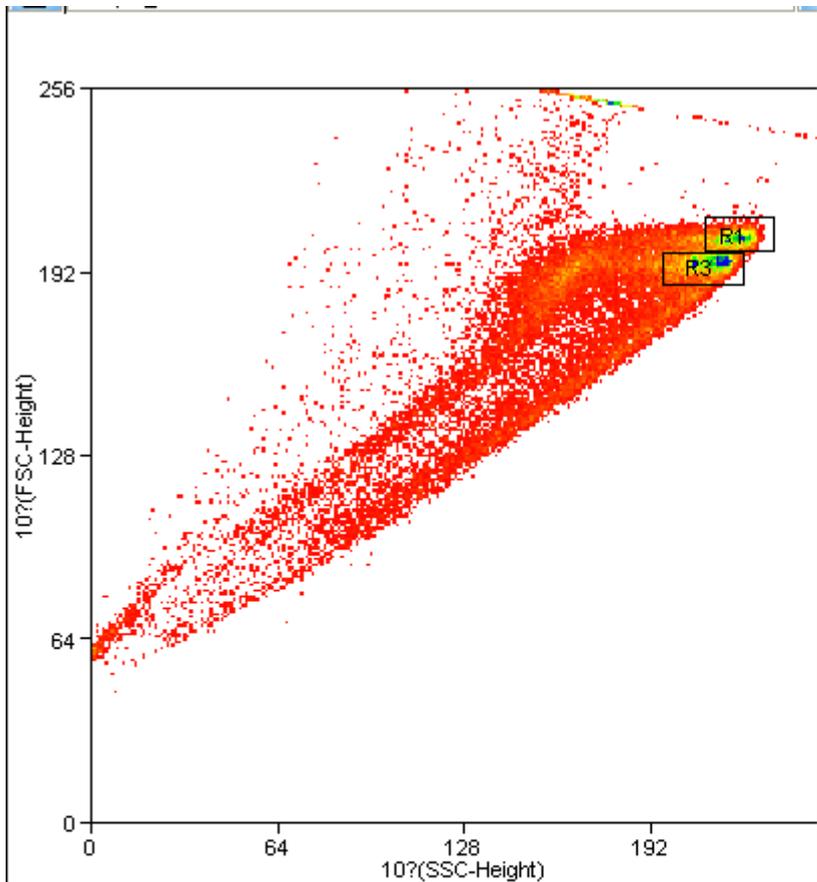
*J. K. O'Brien<sup>A,C</sup>, F. K. Hollinshead<sup>A</sup>, K. M. Evans<sup>B</sup>, G. Evans<sup>A</sup> and W. M. C. Maxwell<sup>A</sup>*

<sup>A</sup>Centre for Advanced Technologies in Animal Genetics and Reproduction, Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia.

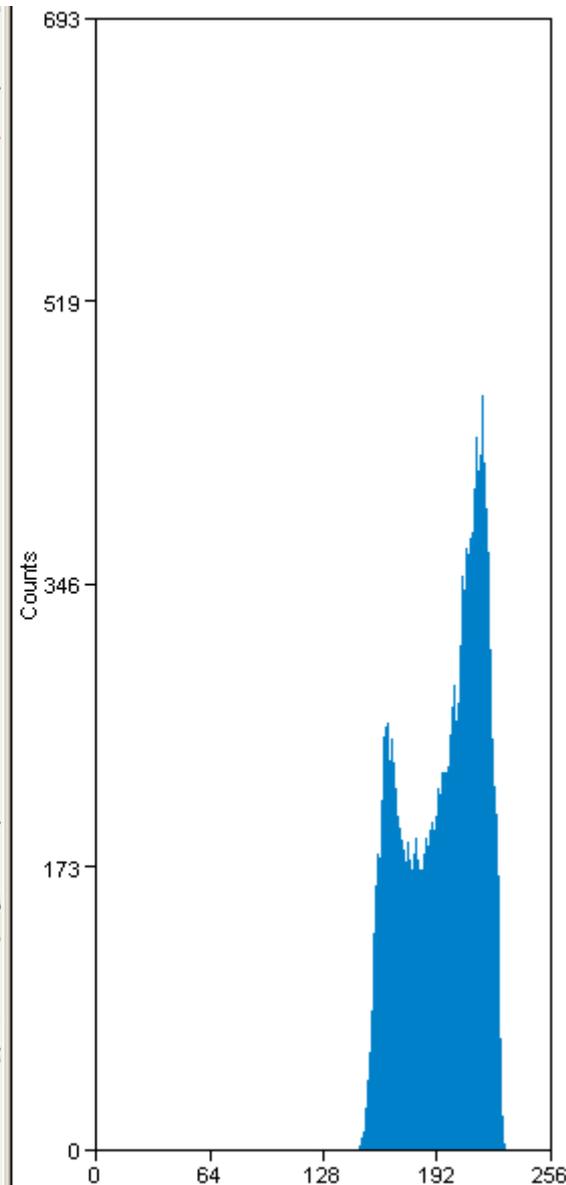
<sup>B</sup>XY Inc., Fort Collins, CO, USA 80523.

<sup>C</sup>To whom correspondence should be addressed. email: justineo@vetsci.usyd.edu.au





Region	Count	% Hist	% All	Bounds	Mode
Total	25369	100.00	100.00	(0.00,255.00)	(255.00...
R1	2963	11.68	11.68	(210.00,210.00)	(234....
R3	4930	19.43	19.43	(196.00,198.00)	(224....

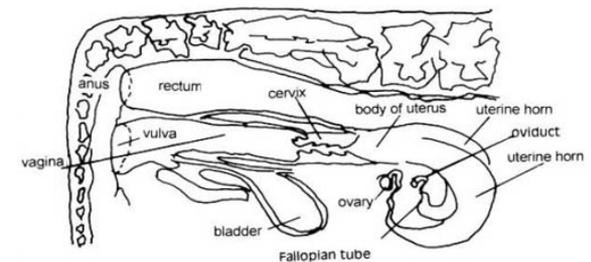


# AI with cryopreserved sexed sperm

Results of Trial 5—2001. Pregnancy rates in Holstein heifers following insemination of unsexed or sexed sperm

Bull	Treatment	No. heifers	Pregnant (%)	Sexed as % of control
HO007	20 × 10 <sup>6</sup> unsexed	119	(67%)	78
	6.0 × 10 <sup>6</sup> sexed	75	(57%)	
	1.5 × 10 <sup>6</sup> sexed	101	(48%)	
HO014	20 × 10 <sup>6</sup> unsexed	19	(32%)	72
	6.0 × 10 <sup>6</sup> sexed	59	(24%)	
	1.5 × 10 <sup>6</sup> sexed	24	(21%)	
HO015	20 × 10 <sup>6</sup> unsexed	48	(69%)	57
	6.0 × 10 <sup>6</sup> sexed	58	(40%)	
	1.5 × 10 <sup>6</sup> sexed	92	(39%)	
HO016	20 × 10 <sup>6</sup> unsexed	72	(49%)	77
	6.0 × 10 <sup>6</sup> sexed	61	(34%)	
	1.5 × 10 <sup>6</sup> sexed	81	(40%)	
Average	20 × 10 <sup>6</sup> unsexed	263	(62%) <sup>a</sup>	70
	6.0 × 10 <sup>6</sup> sexed	246	(41%) <sup>b</sup>	
	1.5 × 10 <sup>6</sup> sexed	288	(43%) <sup>b</sup>	

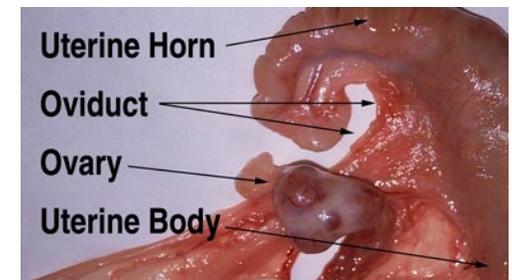
<sup>a,b</sup>Means without common superscript letters differ ( $P < 0.05$ ).



Results of Trial 6—2000. Pregnancy rates in lactating Angus cows following insemination of unsexed sperm deposited into the uterine body or sexed sperm deposited into either the uterine horns or uterine body

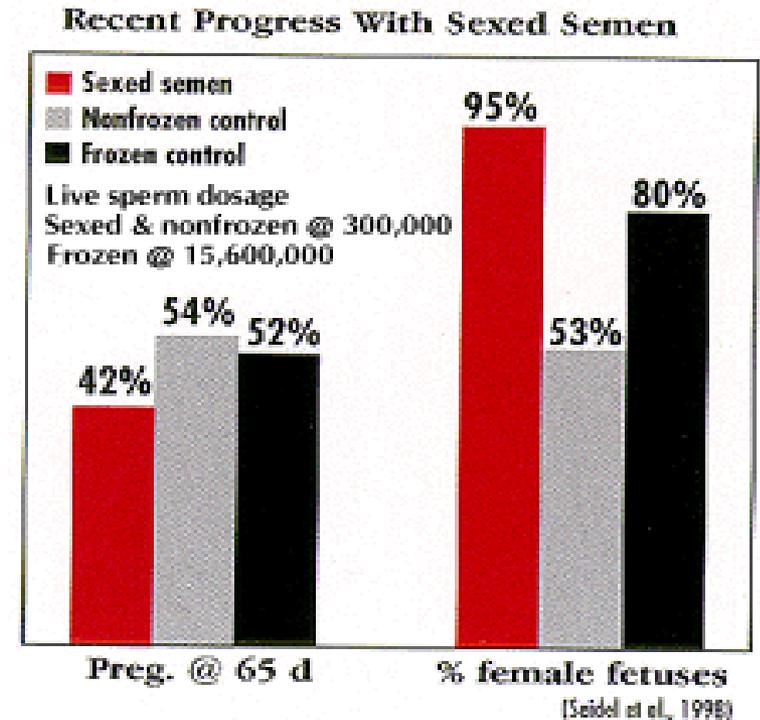
Treatment/site	No. cows	No. (%) pregnant day 60	No. (%) calved	% Male
20 × 10 <sup>6</sup> unsexed/body	21	16 (76%) <sup>a</sup>	15 (71%)	53
3.0 × 10 <sup>6</sup> sexed/body	42	24 (57%) <sup>a,b</sup>	23 (55%)	91
3.0 × 10 <sup>6</sup> sexed/horn	42	21 (50%) <sup>b</sup>	21 (50%)	90

<sup>a,b</sup>Means without common superscript letters differ ( $P < 0.05$ ). There were no significant treatment differences in calving rates. However, calf sex for sexed treatments differed from the unsexed control ( $P < 0.01$ ).



G.E. Seidel et al 2008

- lower fertility of sorted sperm
- lower survival of sorted sperm after cryopreservation
- reduced number of sperm that could be separated in a specified time period



# IVF of bovine embryos using sex-sorted sperm

Means ( $\pm$  S.E.M.) for rates of embryo cleavage and blastocyst development from unsorted and sex-sorted sperm using ovaries obtained from anonymous donor cows at a commercial abattoir

	Unsorted spermatozoa	Sex-sorted spermatozoa
Total no. of oocytes	3312	1577
No. of replicates	24	19
No. of oocytes per replicate	138	83
Cleavage rate (%)	67.3 $\pm$ 3.5	65.0 $\pm$ 3.6
Blastocyst development rate (%)	20.1 $\pm$ 2.9 a	12.2 $\pm$ 2.3 b

Within a row, means with different letters differ ( $P < 0.05$ ).

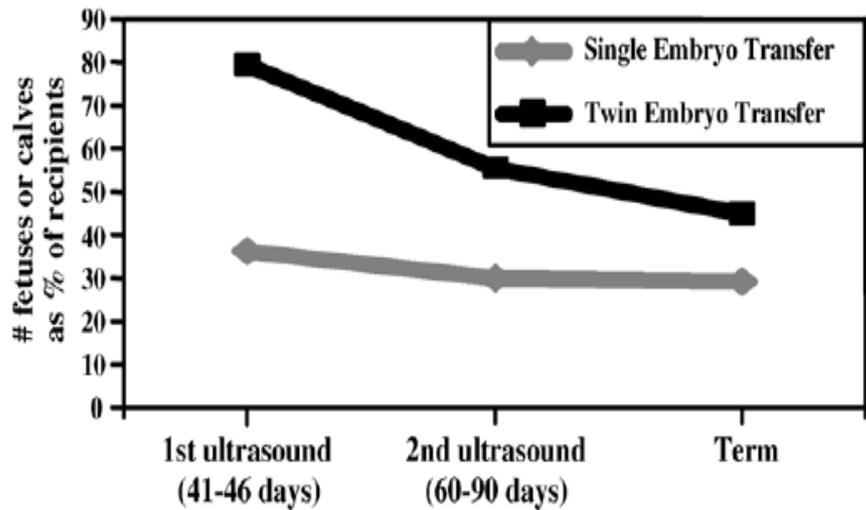


Fig. 1. Offspring production per recipient [29].

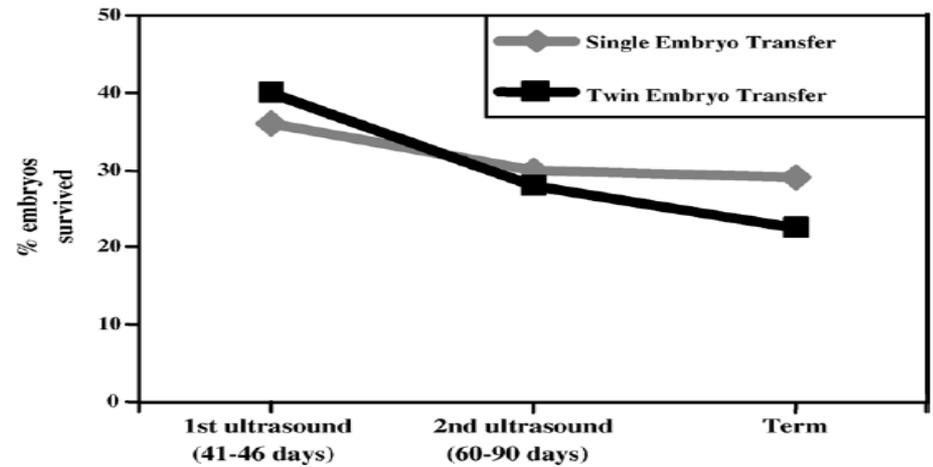


Fig. 2. Individual embryo survival [29].

*M.B. Wheeler et al. / Theriogenology 65 (2006) 219–227*

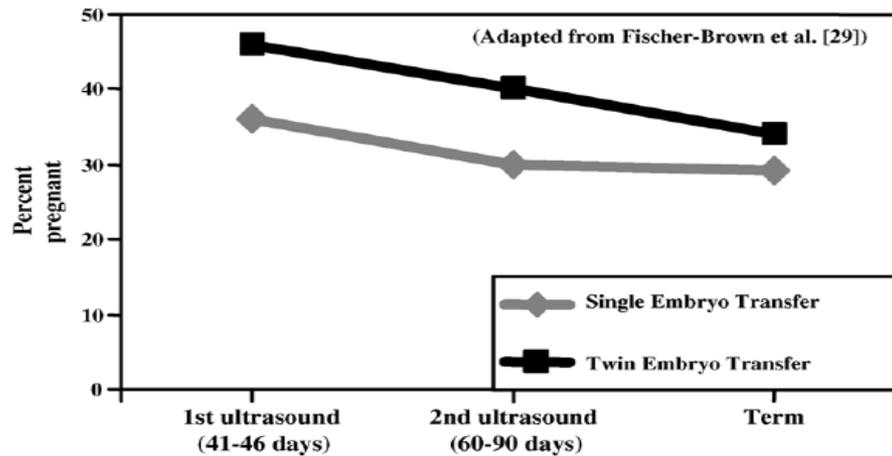
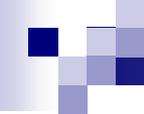


Fig. 3. Proportion of pregnant recipients irrespective of number of offspring [29].

*M.B. Wheeler et al 2006*



One very appealing attribute of using flow-sorted sperm for IVP is that considerably fewer sperm are needed for IVF

- lower fertilization rates
- lower cleavage rates
- lower blastocyst rates
- lower pregnancy rates
- partial capacitation of the sperm
- dilute sperm samples
- sire variation

# Conclusions

- Optimization the parameters (temperature, primers and cycles) for the PCR procedure made the present method rapid and reliable. Accuracy of sex prediction was 100%.
- Selective developmental arrest of male embryos induced by high-titer H-Y antisera. Under these conditions, selective embryonic developmental arrest may prove to be a commercially viable non-invasive method for sexing embryos.
- Sperm sorting by flow cytometer provides a powerful tool for artificial insemination and production of predefined sexed embryos but, an accurate verification of the yield of sperm separation remains essential for a field application of this technique or for improvement and validation of other related semen sexing technologies.

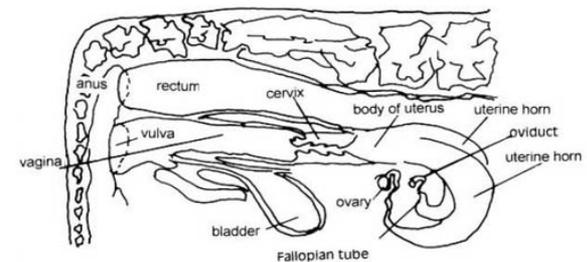
# AI with cryopreserved sexed sperm

Results of Trial 5—2001. Pregnancy rates in Holstein heifers following insemination of unsexed or sexed sperm

Bull	Treatment	No. heifers	Pregnant (%)	Sexed as % of control
HO007	20 × 10 <sup>6</sup> unsexed	119	(67%)	78
	6.0 × 10 <sup>6</sup> sexed	75	(57%)	
	1.5 × 10 <sup>6</sup> sexed	101	(48%)	
HO014	20 × 10 <sup>6</sup> unsexed	19	(32%)	72
	6.0 × 10 <sup>6</sup> sexed	59	(24%)	
	1.5 × 10 <sup>6</sup> sexed	24	(21%)	
HO015	20 × 10 <sup>6</sup> unsexed	48	(69%)	57
	6.0 × 10 <sup>6</sup> sexed	58	(40%)	
	1.5 × 10 <sup>6</sup> sexed	92	(39%)	
HO016	20 × 10 <sup>6</sup> unsexed	72	(49%)	77
	6.0 × 10 <sup>6</sup> sexed	61	(34%)	
	1.5 × 10 <sup>6</sup> sexed	81	(40%)	
Average	20 × 10 <sup>6</sup> unsexed	263	(62%) <sup>a</sup>	70
	6.0 × 10 <sup>6</sup> sexed	246	(41%) <sup>b</sup>	
	1.5 × 10 <sup>6</sup> sexed	288	(43%) <sup>b</sup>	

<sup>a,b</sup>Means without common superscript letters differ ( $P < 0.05$ ).

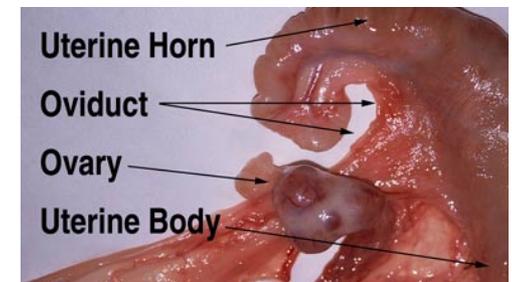
選性精液每劑AI精子數量  
對懷孕率的影響



Results of Trial 6—2000. Pregnancy rates in lactating Angus cows following insemination of unsexed sperm deposited into the uterine body or sexed sperm deposited into either the uterine horns or uterine body

Treatment/site	No. cows	No. (%) pregnant day 60	No. (%) calved	% Male
20 × 10 <sup>6</sup> unsexed/body	21	16 (76%) <sup>a</sup>	15 (71%)	53
3.0 × 10 <sup>6</sup> sexed/body	42	24 (57%) <sup>a,b</sup>	23 (55%)	91
3.0 × 10 <sup>6</sup> sexed/horn	42	21 (50%) <sup>b</sup>	21 (50%)	90

<sup>a,b</sup>Means without common superscript letters differ ( $P < 0.05$ ). There were no significant treatment differences in calving rates. However, calf sex for sexed treatments differed from the unsexed control ( $P < 0.01$ ).



選性精液AI時置放位置對懷孕率的影響

G.E. Seidel et al 2008

# IVF of bovine embryos using sex-sorted sperm 利用選性精液進行牛胚體外生產

Means ( $\pm$  S.E.M.) for rates of embryo cleavage and blastocyst development from unsorted and sex-sorted sperm using ovaries obtained from anonymous donor cows at a commercial abattoir

	Unsorted spermatozoa	Sex-sorted spermatozoa
Total no. of oocytes	3312	1577
No. of replicates	24	19
No. of oocytes per replicate	138	83
Cleavage rate (%)	67.3 $\pm$ 3.5	65.0 $\pm$ 3.6
Blastocyst development rate (%)	20.1 $\pm$ 2.9 a	12.2 $\pm$ 2.3 b

Within a row, means with different letters differ ( $P < 0.05$ ).

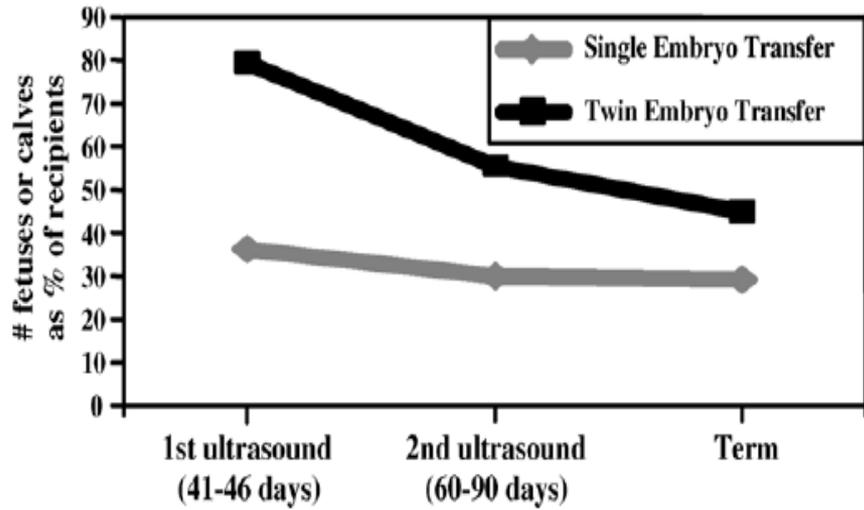


Fig. 1. Offspring production per recipient [29].

*M.B. Wheeler et al. /Theriogenology 65 (2006) 219–227*

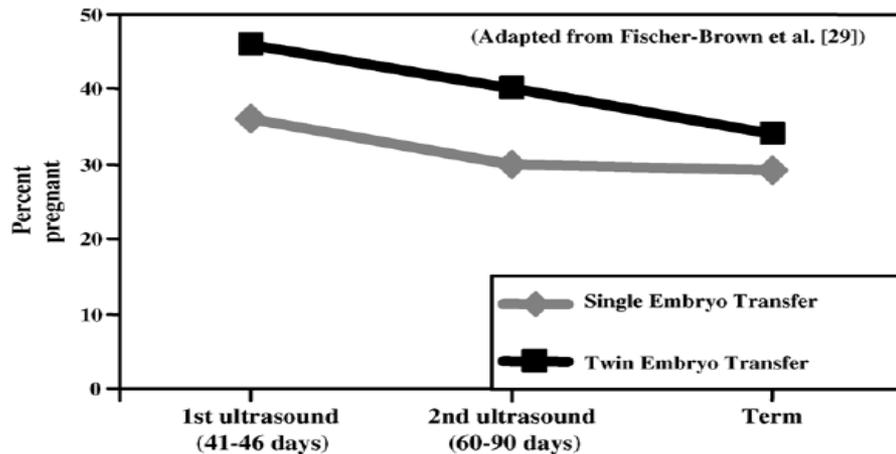


Fig. 3. Proportion of pregnant recipients irrespective of number of offspring [29].

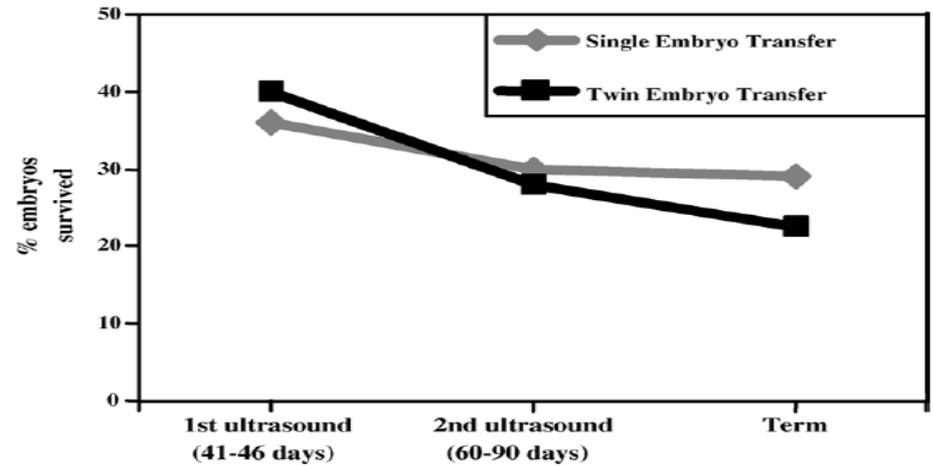


Fig. 2. Individual embryo survival [29].

性別控制胚可一次移置2個以增加代理孕母的懷孕率

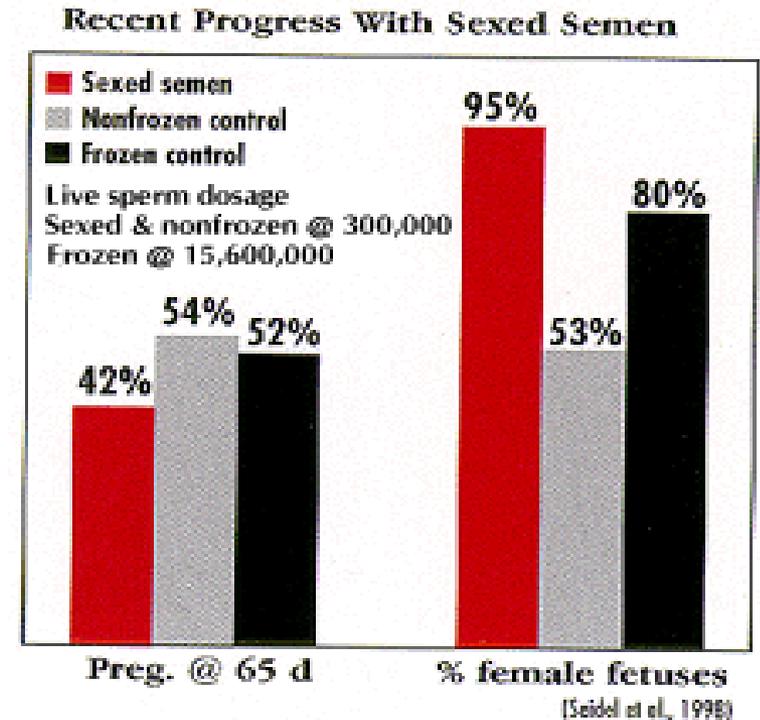
*M.B. Wheeler et al 2006*

One very appealing attribute of using flow-sorted sperm for IVP is that considerably fewer sperm are needed for IVF

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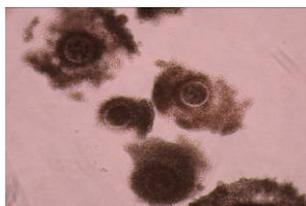
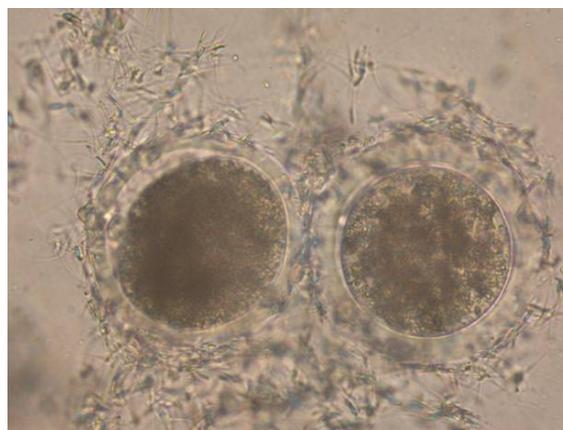
IVF相對使用較少量的精子就可受精 600隻/每個卵

- lower fertility of sorted sperm
- lower survival of sorted sperm after cryopreservation
- reduced number of sperm that could be separated in a specified time period



# IVF of bovine embryos using sex-sorted sperm

體外卵母細胞收集 ⇒ 少量選性精液體外授精 ⇒ 選性胚胎移置



## ■ 優點

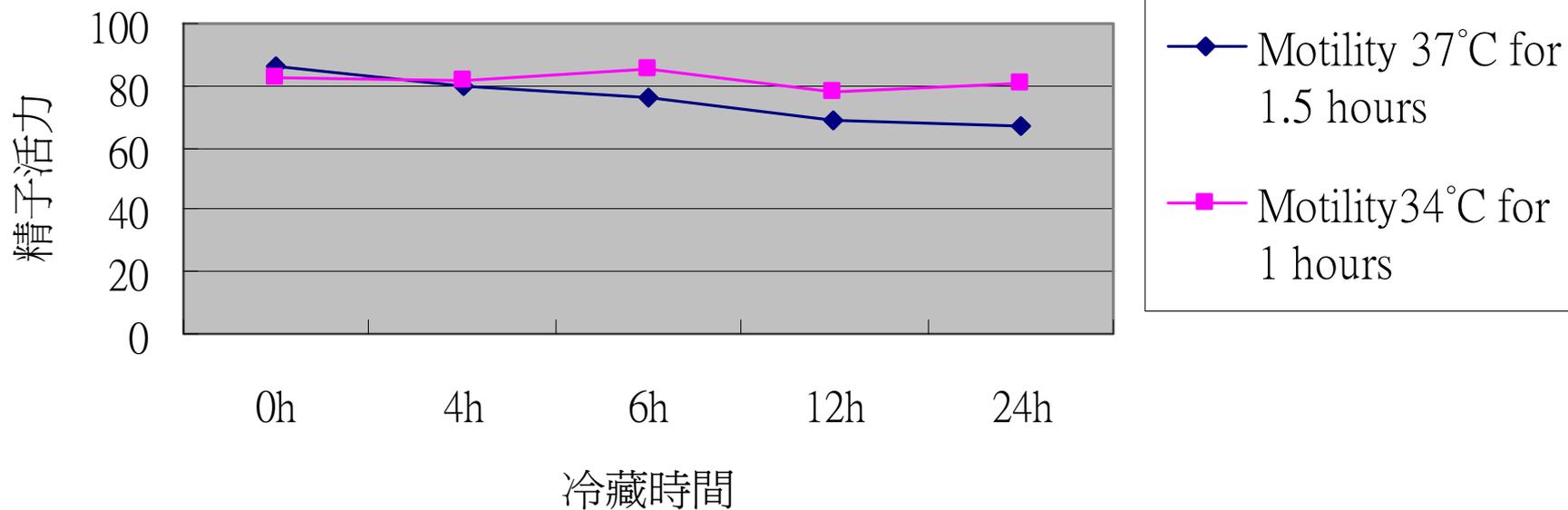
1. 成本低(平均5000個選性精子可讓1個卵授精)
2. 收集大量之卵母細胞，可經過嚴格篩選增加牛胚後續的發育能力

## ■ 缺點

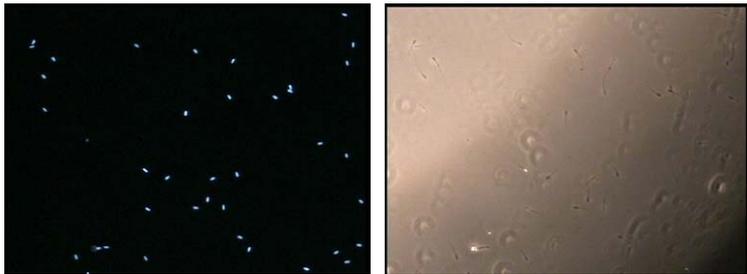
1. 卵母細胞來源不明(無母系遺傳背景資料)
2. 卵母細胞體外成熟效果不如體內成熟

# Sperm standing before sorting

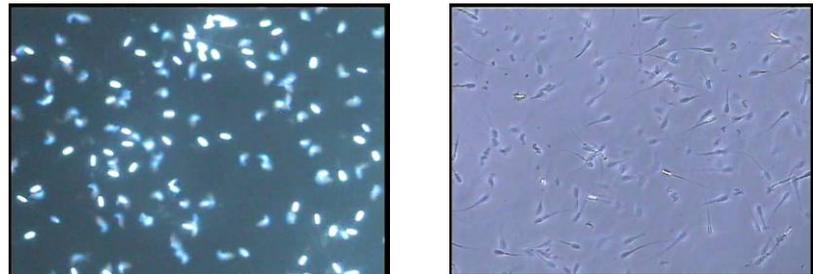
染色時間溫度對精子冷藏保存活力影響



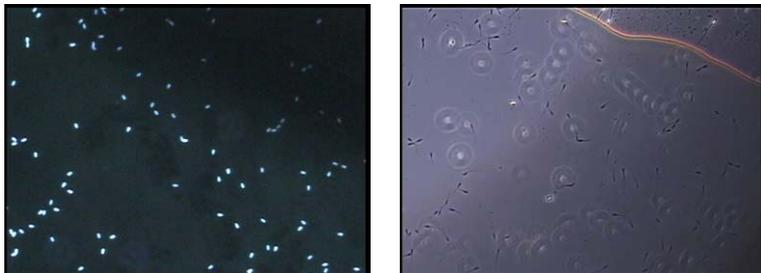
# Sperm standing before sorting



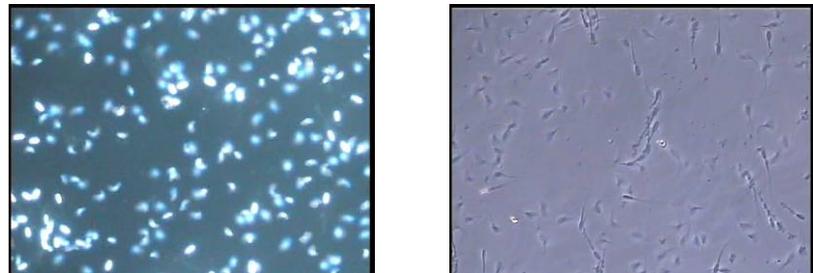
染色後 6 hours 小時



染色後 0 hours 小時



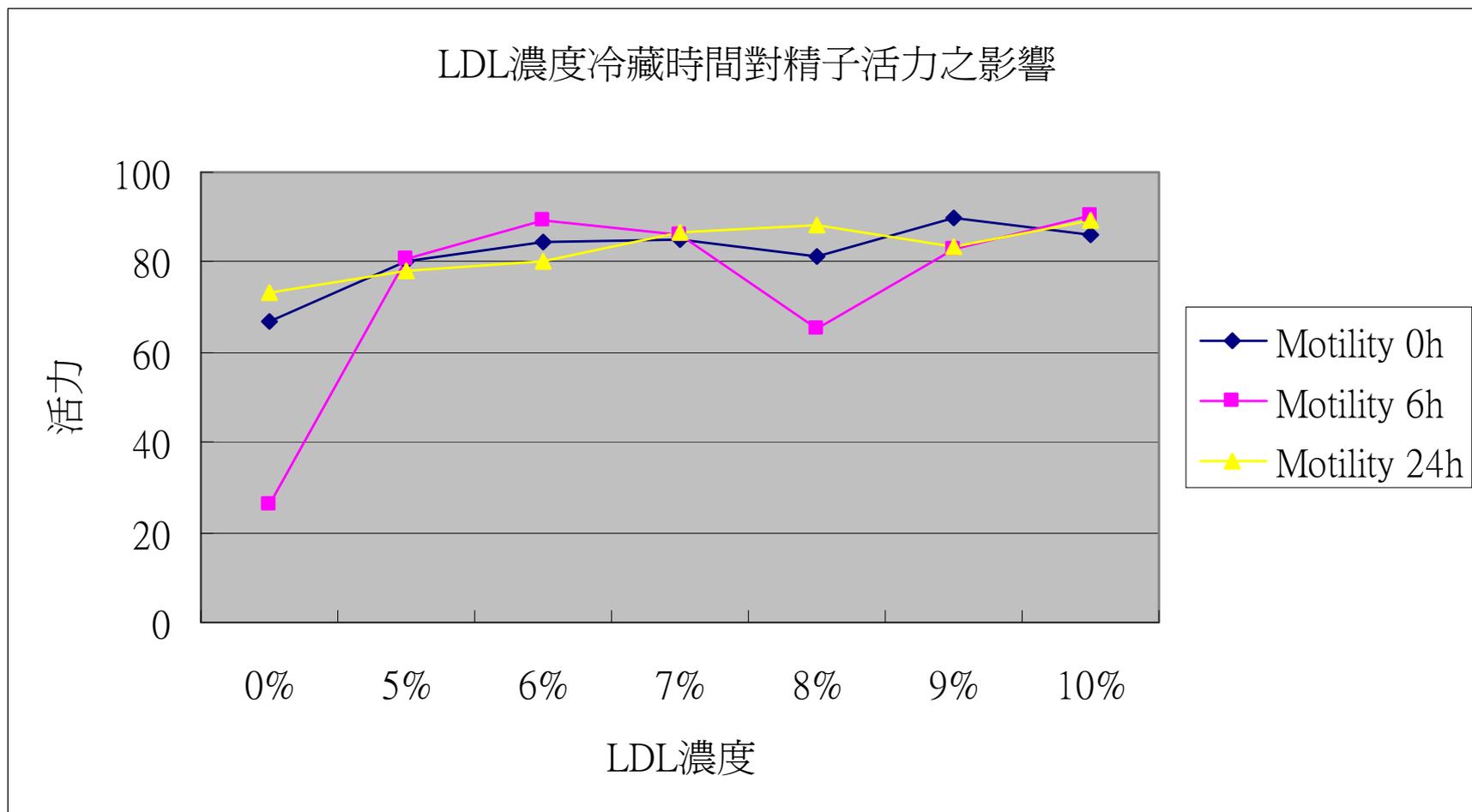
染色後 12 hours 小時



染色後 24 hours 小時

8.12 mM Hoechst 33342 solution and  
incubated at 34 °C for 1 hours

# 精子性別分離前稀釋液LDL的適當濃度



# 選性繁殖技術平台之相關人工生殖技術

1. 選性精液家畜人工授精



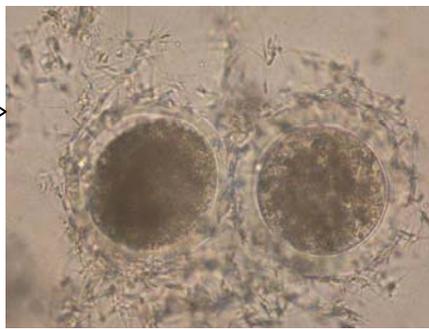
選性精子人工授精



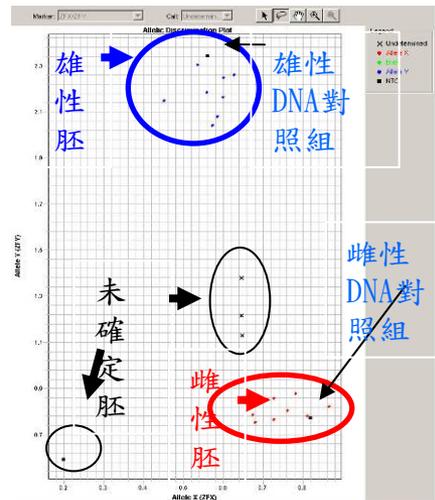
理想性別仔畜生產

精液性別分離

2. 選性胚體外生產



少量選性精液體外授精

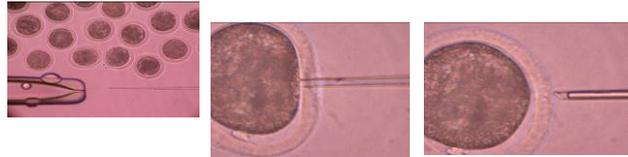


RT PCR 胚性別鑑定

3. 少量精子受精技術



山羊少量精液腹腔鏡人工授精技術



成熟卵母細胞單一選性精子注入



代理孕母胚移置



**Thanks for Your Attention**