

21世紀的超選性凍精技術 及選性胚應用

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SexedULTRA™超選性工藝結合了最新生物技術、尖端科技設備以及先進的電腦軟體，使分離加工更具效率，並最大程度降低分離過程對精細胞的損害。使用特製的試劑可以讓精細胞保持健康與活力，同時運用我們最新研發的創世紀第三代(**Genesis III**)數字化分離設備進行分離。創世紀第三代(**Genesis III**)可以更精準、更迅速地分離精液，與其他分離設備相比可減少分離過程中的精子損失。

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- **SexedULTRA™**超選性凍精出現以前，選性凍精的受胎率通常比傳統凍精低15%-25%。經過多年研究探索和在美國、愛爾蘭、法國和新西蘭等國超過4萬牛頭次的實驗資料證明，
- **SexedULTRA™**超選性凍精產品的受胎率可以達到傳統凍精產品受胎率的95%（實際效果取決於動物個體的品種獨特性以及牧場管理）。

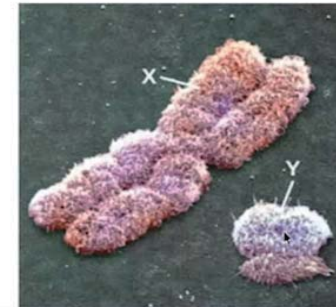
由於高成本與低受胎率的限制，選性產品以往僅用在於配種新女牛。鑒於**SexedULTRA™**超選性凍精近乎傳統凍精產品的受胎率水準，經產牛配種性控凍精如今也具有商業可行性。

SexedULTRA™超選性凍精的高受胎率表現在一項由哈佛大學研究人員參與的調查中更具有重要意義——調查顯示，母牛生子女牛時的產奶量要比生產公犢時高，且連續產下母犢的母牛在兩個泌乳期中的產奶量可增加445公斤。產奶量增長所帶來的經濟效益遠遠高於使用**SexedULTRA™**超選性凍精的支出。

SexedULTRA™超選性凍精產品僅由 **Sexing Technologies** 公司提供
感謝美國ST公司提供Dr. Vishwahath的資料

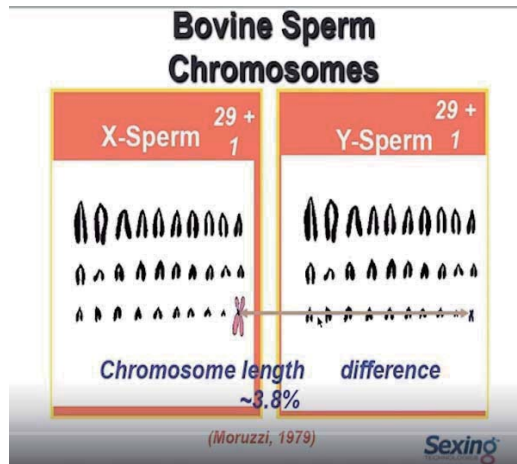
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SPERM DNA CONTENT
The principle on which sperm sorting through Flow Cytometry is based on



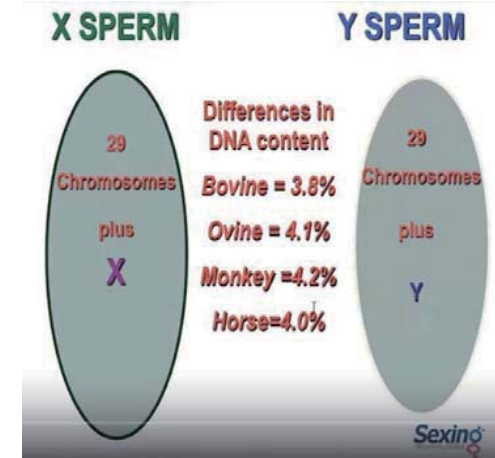
此圖可以看出放大後X及Y染色體大小差異

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乳牛有29對染色體，性染色體的質量差異約3.8%

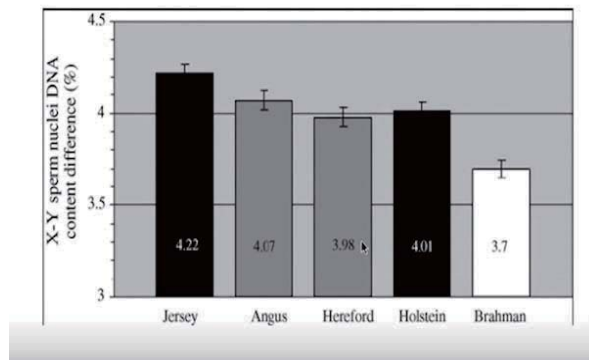
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牛、羊、猴、馬的性染色體的質量差異約3.8%~4.2%不等

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Breed differences in XY DNA differences in the Bovines



各乳/肉牛品種X及Y染色體差值自3.7%~4.22%不等

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HISTORICAL PERSPECTIVE

1976 Sperm DNA content (Gledhill et al.)

1983 X and Y sperm livestock (Garner et al.)

1986 Modification of sorter (Johnson et al.)

1987 Sorting of sperm heads (Johnson et al.)

1989 Progeny of X & Y sperm (Johnson et al.)

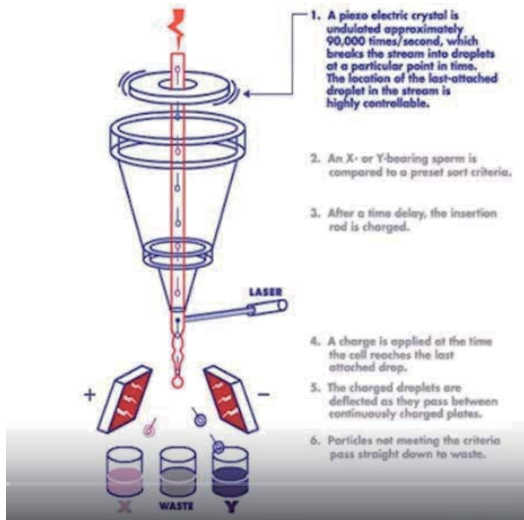
強森博士突破性的發現

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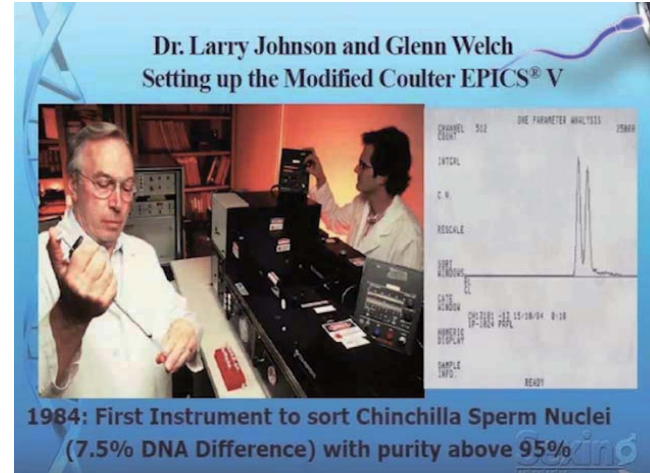
HISTORICAL PERSPECTIVE

- 1993 First use of sex sorted semen in IVF (Cran et al.)
- 1996 XY, inc created
- 1997 Low dose insemination- sex sorted semen (Seidel et al)
- 1999 Successful freezing of sex sorted semen (Schenk et al)
- 2002 Sexing Technologies
- 2012 Full automation by CytonomeST
- 2014 Sexed Ultra™

- 第一張商業執照發給了XY公司，最初的業務在複製動物上，因為當時各大乳牛AI公司對Sexed精液的受孕率不具信心。



選性分離儀器的簡易藍圖



1984年強森博士首先分離出栗鼠的X/Y精蟲（DNA差值7.5%）

Sex sorting technology-progress through the years

| | 1990~1995 | 1995~2002 | 2002~2012 | 2012~2014 |
|--------------------|-----------|-----------|-----------|--------------|
| 篩選速度:精蟲數目/秒 | 200~400 | 1,000 | 5,000 | 7,000~10,000 |
| 純度 | 83% | 85% | 85% | > 93% |
| 相對於傳統精液的受孕率 | 70% | 80% | 80% | 92%~98% |
| 每1000劑傳統精液可得選性精液劑量 | 10劑 | 50劑 | 400劑 | 1,100劑 |

在1995年~2012年，只有產能上的提昇，受孕率僅維持在80%，在2012~2014年，產能及相對受孕率達90%以上了。



創世紀第二代及第三代（右圖），第三代創世紀的每位操作員可以同時管理前、左、右三台機組，每一機組有3個分離漏斗/電腦監控

GENESIS II 2nd Generation and Genesis III



產能全開可以年產6萬劑/每台

In 2010 Sexed semen CR was 75 to 80% of that of conventional semen

| Treatment | Conception rate % | Proportion compared to conventional |
|----------------------|-------------------|-------------------------------------|
| 2.1 mill Sex Sorted | 45% | 74% |
| 3.5 mill Sex sorted | 47% | 78% |
| 15 mill conventional | 62% | |

DeJarnette et al 2010

之前一般認知，選性精液只能達到相對於傳統精液75%~80%的受孕率，即便提昇每劑的精蟲數也效果不大。

Increasing sperm numbers did not compensate for this sub fertility in the old process

| Sex sorted | | Conventional | | Relative fertility |
|-----------------------|-----------------|-----------------------|-----------------|--------------------|
| Sperm concentration | Conception rate | Sperm concentration | Conception rate | |
| 2.1 x 10 ⁶ | 38% | 2.1 x 10 ⁶ | 55% | 70% |
| 10 x 10 ⁶ | 44% | 10 x 10 ⁶ | 60% | 73% |

DeJarnette et al 2011

Similar observations in other studies as well Seidel and Schenk, 2008; DeJarnette et al, 2010, Lucena et al 2014

2008, 2011, 2010, 2014 的專家報告也是這樣的結論

The educated conclusion:

- Cytometry alters functional capacity
- Possible fertilisation failure??
- Early embryonic death??
- **Increasing sperm numbers did not alter this probability of fertilisation**

所以，當時的看法既定的結論及推論，認為選性精液有了天花板，不易突破，因此，只建議用在女牛配種計劃上。

The challenge

- Improve sorting techniques – new hardware and software.
- Improve the biochemical processes involved in the sex sorting process

Identify the primary lesion for reduced fertility.

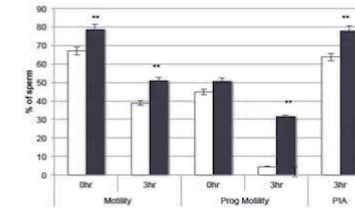
選性凍精的主要的挑戰在於: 找出導致受孕率不及傳統精液的主要因素, 改進硬/軟體的條件, 改進分離試劑的配方。

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Recent progress

- Semen Biochemistry
- Machine improvements

SexedULTRA™ method improves in vitro sperm characteristics compared with the XY method



Open bars XY method, Close bars, SexedULTRA™ method
** significantly different to XY method n = 12, P < 0.01

最新第三代機型所生產的超選性凍精(黑柱圖型), 在活力及前進活力都優於XY機型所生產的選性凍精。

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Trials with SexedUltra™ with frozen sex sorted semen – Select Sires

| Process method | Number of inseminations | Scanned pregnancy rate |
|-----------------|-------------------------|------------------------|
| XY (old method) | 3384 | 41.6% |
| SexedULTRA™ | 3546 | 46.1%* |

* Process method differs P < 0.01

兩年前, 第一次超選性凍精與XY選性凍精的美國田間試驗 (46.1% VS 41.6%)。

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Field trials with a new and improved SexedULTRA™

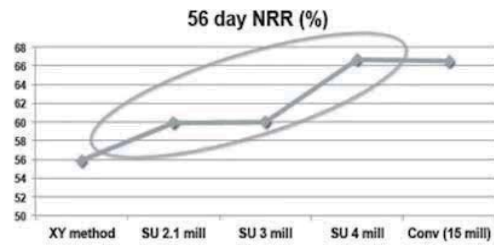
| Treatment | # inseminations | Pregnancy rate (%) |
|-----------------|-----------------|--------------------|
| New SexedULTRA™ | 3189 | 52.9* |
| SexedULTRA™ | 2833 | 50.4 |

*Significant treatment effect P < 0.05
Significant bull effect P < 0.01
Significant farm effect P < 0.01

更新一代配方的超選性凍精 New SexedULTRA™ 的比較實驗

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Dose rate trials with new SexedULTRA™



| Treatment | Number of Inseminations | 56 day NRR (%) |
|----------------|-------------------------|-------------------|
| XY old method | 1953 | 55.9 ^A |
| SU 2.1 mill | 1999 | 59.9 ^B |
| SU 3 mill | 2013 | 60.0 ^B |
| SU 4 mill | 1890 | 66.7 ^C |
| Conv (15 mill) | 62,398 | 66.5 ^C |

- Trial with heifers and cows
- NRR with different superscripts are significantly different P < 0.01

Sexing

新超選性凍精，每劑4佰萬精蟲數，幾乎可達到相較一般傳統精液 66.7% VS 66.5%的受孕率。

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Lessons from fresh sex sorted sperm – New Zealand NRR of fresh sex sorted or conventional semen

| Season | SS | | Conv | | SS – Conv | SS / Conv |
|--------|--------|-------|--------|-------|-----------|-----------|
| | Insems | NRR % | Insems | NRR % | NRR % | % |
| 2011 | 8,848 | 69.4 | 10,981 | 73.6 | -4.2 | 94.3 |
| 2012 | 18,760 | 68.1 | 19,915 | 72.3 | -4.2 | 94.2 |
| 2013 | 26,104 | 69.9 | 26,189 | 73.4 | -3.6 | 95.1 |
| Total | 51,712 | 69.1 | 57,085 | 73.1 | -3.9 | 94.6 |

Data from Z Xu 2014, Livestock Improvement, In press JDS
Results are 18-24 day NRR
All inseminations in lactating dairy cows

2014年，在紐西蘭季節配種中，新鮮超選性凍精只比一般傳統精液略差3.6~4.2%，幾乎可達到相對94%的受孕率。

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Australia FTAI Sexed Semen Trial

- Day 0 – 2mg ODB and insert CIDR
- Day 7 – inj PG
- Day 10 – remove CIDR
- Day 12 – FTAI.
- 2013
 - weighed calves on Day 0
 - recorded time of CIDR removal and applied scratchy
 - Recorded heat and time of AI

Jon Kelly, Warnambool Vet Clinic, Victoria Australia

在澳洲進行的同期化AI計劃中，女牛可以達到55%受孕率。

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Fresh Sexed Heifers - 2014

- 852 heifers
- 6 farms
- 9 different joinings
- 5 sires
- **55% CR(44-69)**
 - Old sexed semen Frozen - 40% CR(26-51)
 - Sexed Ultra – 57% CR(48-63)
 - 2 farms, 2 sires and 77 matings so far.

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Fresh Sexed 2014 - Cows

- 293 cows
- 4 farms
- 2 sires

• **37% CR(30-45)**

- Old sexed semen Frozen - 30% CR(25- 42)
- Sexed Ultra – 46%CR(25-55)
 - 2 farms, 2 sires and 110 cows so far.

經產牛的同期化AI計劃使用超選性凍精，可以達到平均37%的受孕率

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Other Results from field trials with frozen sex-sorted sperm SexedUltra™ in 2013 -14

- 13,500 AI's in Ireland (in cows sex frozen was 86% of conventional fresh semen).
- Trials in France and Germany show that SexedUltra™ was over 93% of that of conventional semen – dose rate trials compared with conventional.

在愛爾蘭的實驗中，13,500 次AI次數中，經產牛可達到傳統精液的相對86%受孕率。

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Why use sexed semen

Replacements – selection pressure

- Opportunity to cull
- Drive herd structure younger
- Extra income stream
 - Excess heifers
 - Sale of pregnant late cows?
- Calving ease
 - Heifers and cows
- Unwanted male calves

使用選性凍精的優點

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Available Sexed Embryos

| Dam Reg No ID | Dam Name | Sire Reg No ID | Sire | Breed | Type | Sex | Tank | Qty | DTP1 | STPI | PATPI | NM | PTAM |
|-------------------|------------------------------|-------------------|-----------|-------|------|-----|---------|-----------|-------------|-------------|-------------|------------|-------------|
| HOU5A000071441989 | ZAHBULLS JEROD ROSIE-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 3 | 2416 | 2784 | 2600 | 755 | 1497 |
| HOU5A000071632208 | VISION-GEN SHAN CP14995-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 6 | 2405 | 2784 | 2594 | 737 | 1550 |
| HOCAN000108535232 | SUNVIEW GOLD DIAMOND-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 5 | 2392 | 2784 | 2588 | 723 | 1535 |
| HO840003013923847 | MS PREDESTINE MACY-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 3 | 2345 | 2784 | 2584 | 758 | 1019 |
| HOU5A000072504216 | MARITA ENFORCER MAYA-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 1 | 2318 | 2784 | 2551 | 729 | 1557 |
| HOU5A000072504216 | MARITA ENFORCER MAYA-ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 8 | 2318 | 2784 | 2551 | 729 | 1557 |
| HO840003011538173 | S-S-I STRING TABBY 8774-ET | HO840003010363769 | CORSAIR | HO | Col | F | CHINA-1 | 5 | 2443 | 2650 | 2546 | 754 | 1102 |
| HO840003013473977 | EDG BOO BOO JACK 2540- ET | HO840003010353355 | MEGA-DUKE | HO | Col | F | CHINA-1 | 1 | 2237 | 2784 | 2510 | 688 | 1457 |
| HO840003012130335 | S-S-I OLYMPIC STEFFI 9066-ET | HO840003010356026 | DELCO | HO | Col | F | CHINA-1 | 8 | 2331 | 2674 | 2502 | 730 | 1650 |
| HO840003012130335 | S-S-I OLYMPIC STEFFI 9066-ET | HO840003010356026 | DELCO | HO | Col | F | CHINA-1 | 7 | 2331 | 2674 | 2502 | 730 | 1650 |
| HOU5A000072438085 | EDG 4858 OAK 2538-ET | HOU5A000071088720 | MEGASIRE | HO | Col | F | CHINA-1 | 3 | 2399 | 2605 | 2502 | 647 | 1011 |
| HO840003009276218 | ACON-HILL PETRONE MAISE-E | HO840003010356026 | DELCO | HO | Col | F | CHINA-1 | 4 | 2288 | 2674 | 2481 | 685 | 1807 |
| HOU5A000070609811 | PRUCE-HAVEN BHP BJ1937-E | HO840003010356026 | DELCO | HO | Col | F | CHINA-1 | 2 | 2209 | 2674 | 2441 | 631 | 1388 |
| HOCAN000011883944 | THURLER MOGUL TINA | HO840003010365126 | MEGA-WATT | HO | Col | F | CHINA-1 | 2 | 2174 | 2632 | 2403 | 569 | 1497 |
| | | | | | | | | 58 | 2329 | 2719 | 2524 | 705 | 1448 |

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