Sexed sorted sperm – raising the fertility bar with SexedULTRA™

Background and recent developments

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BIOLOGY OF REPRODUCTION 41, 199–203 (1989)

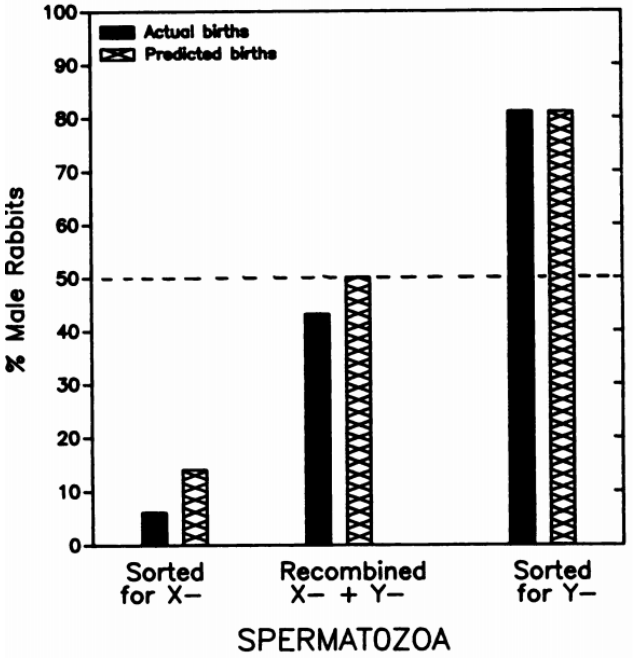
EXCEPTIONAL PAPER–RAPID PUBLICATION

Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting

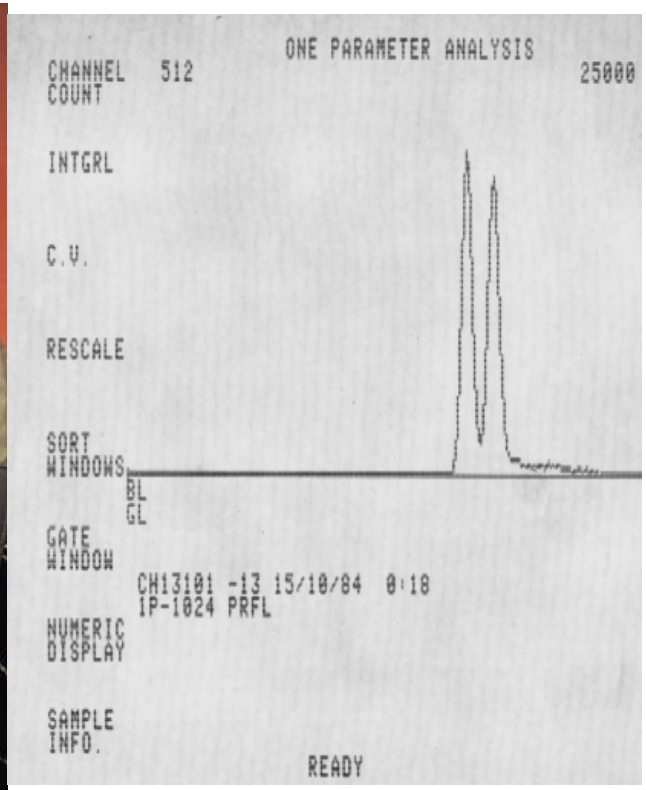
LAWRENCE A. JOHNSON,[1] JAMES P. FLOOK, and HAROLD W. HAWK

Reproduction Laboratory
Beltsville Agricultural Research Center
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland 20705

Surgical, oviductal AI with fresh sperm



Dr. Larry Johnson and Glenn Welch
Setting up the Modified Coulter EPICS® V



1984: First Instrument to sort Chinchilla Sperm Nuclei (7.5% DNA Difference) with purity above 95%

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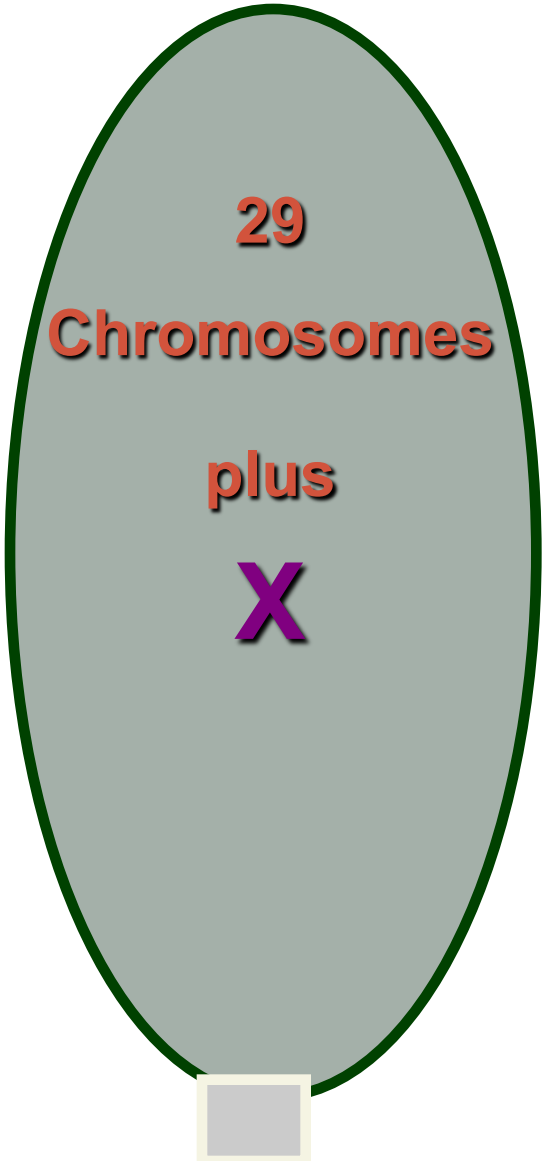
X SPERM



Differences in DNA content

Bovine = 3.8%

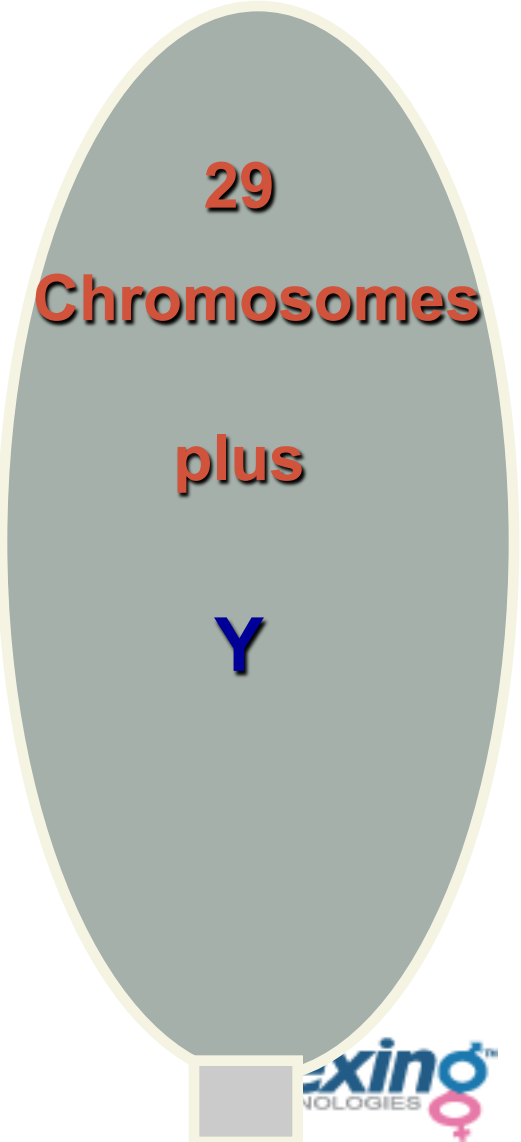
Ovine = 4.1%

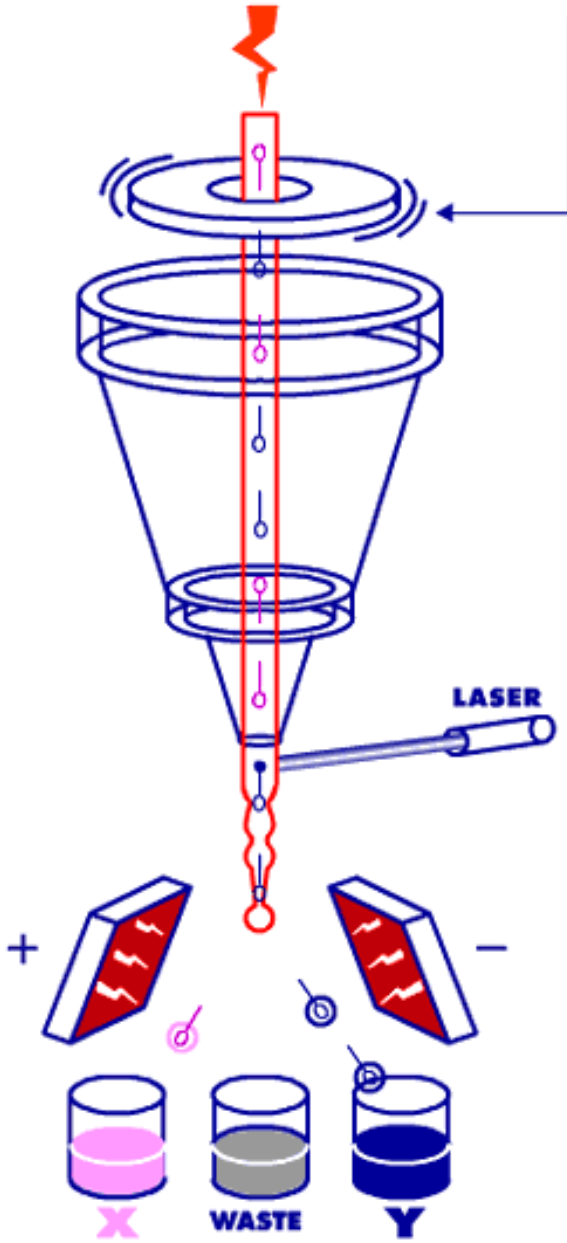
Monkey =4.2%

Horse=4.0%

Human=2.8%

Y 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.

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HISTORICAL PERSPECTIVE

1976 Sperm DNA content (Gledhill et al.)

1979 Orientation of sperm (Dean et al.)

1982 Bimodal DNA peaks (Pinkel 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.)

1988 Sperm viability (Johnson & Clarke)

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

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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)

1998 High speed flow-cytometers and sex semen (Rens et al)

1999 Successful freezing of sex sorted semen (Schank et al)

2002 Sexing Technologies

2005 Decisive program by Monsanto (Digital Electronics)

2010 Micro fluidics program by CytonomeST

2012 Full automation by CytonomeST
2014 Sexed Ultra ™

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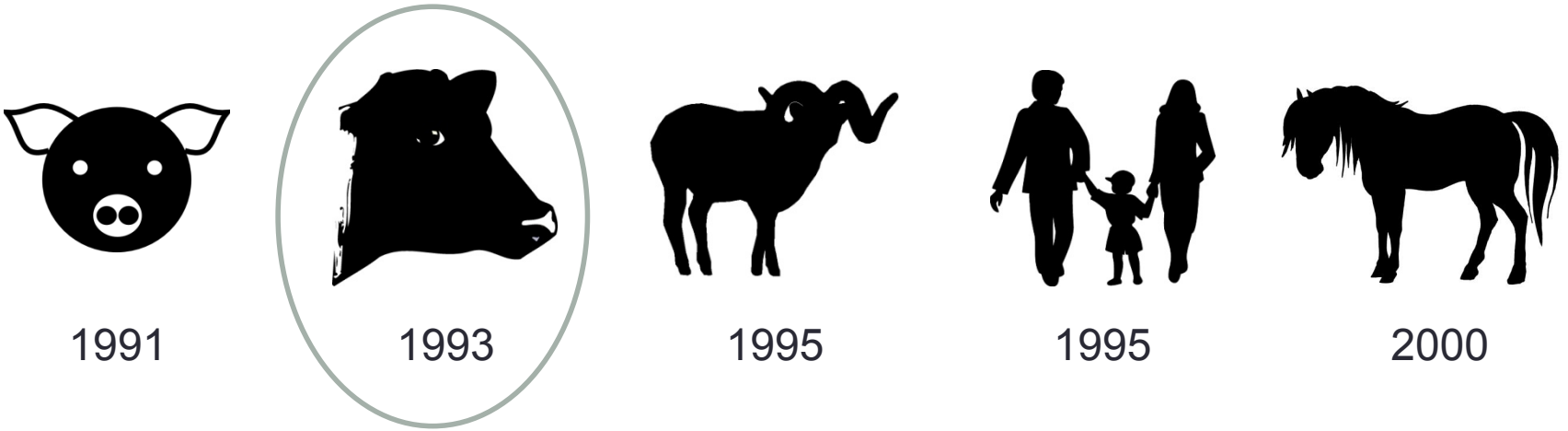
Development of commercial sperm sexing

- Continued in other species



1991 1993 1995 1995 2000

Main commercial focus

Dr George Seidel and team at Colorado State University and XY Inc

Sexing™ TECHNOLOGIES

Primary issue with sexed sperm

Fertility

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The common theme

sex sorted semen is lower in fertility compared with unsorted semen.

On average has been estimated to be around 75 – 80% of that of unsorted semen.

Schenk et 2009; Seidel et al 2009, DeJarnette et al 2010, Seidel, 2012, 2013.

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Sexed semen CR is 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

Sexing™ TECHNOLOGIES

Increasing sperm numbers does not compensate for this sub fertility

Sex sorted		Conventional		
Sperm concentration	Conception rate	Sperm concentration	Conception rate	Relative fertility
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

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The educated conclusion:

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

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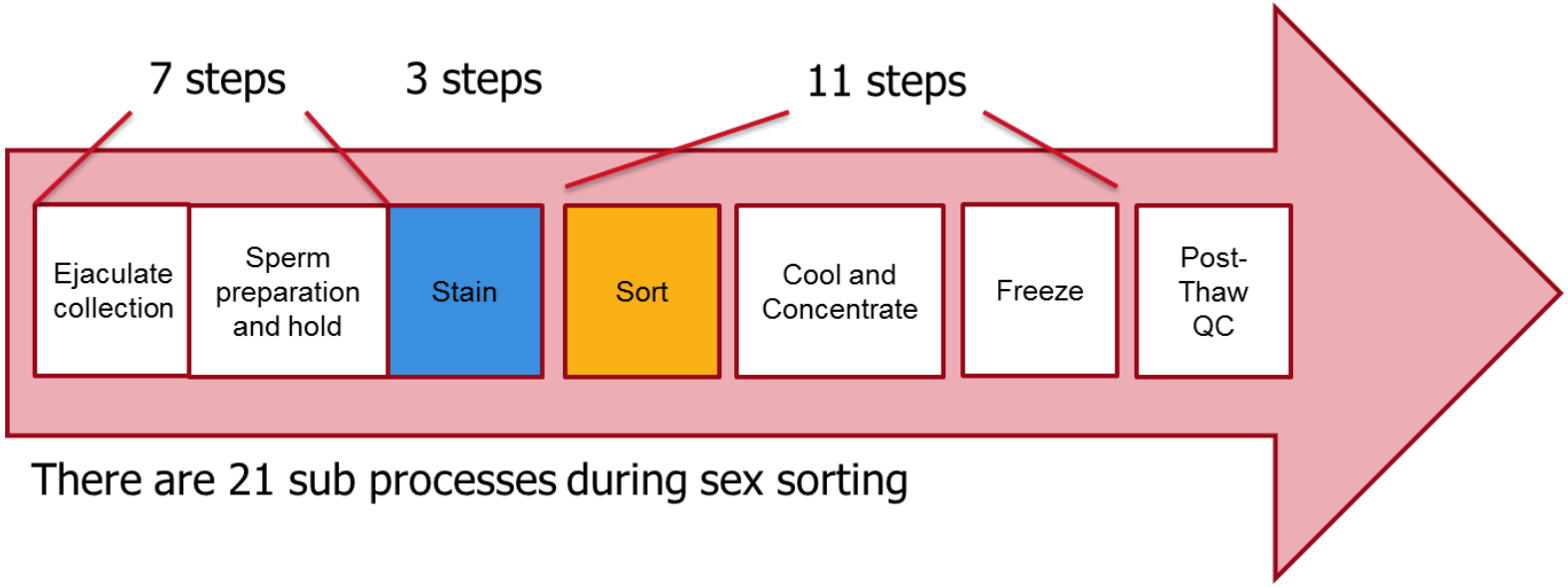
The cause of diminished functional capacity of sex-sorted sperm is multifactorial



- High dilution (up to 5000×)
- Nuclear staining and incubation
- Mechanical forces (pressure)
- Exposure to UV laser & electric charge
- Projection into collection medium (80-90km/h)
- Post-sorting centrifugation
- Post-sorting freezing/thawing

Sexing™ TECHNOLOGIES

Diminished functional capacity of sex-sorted sperm is multifactorial

Biggest impact is sorting process itself

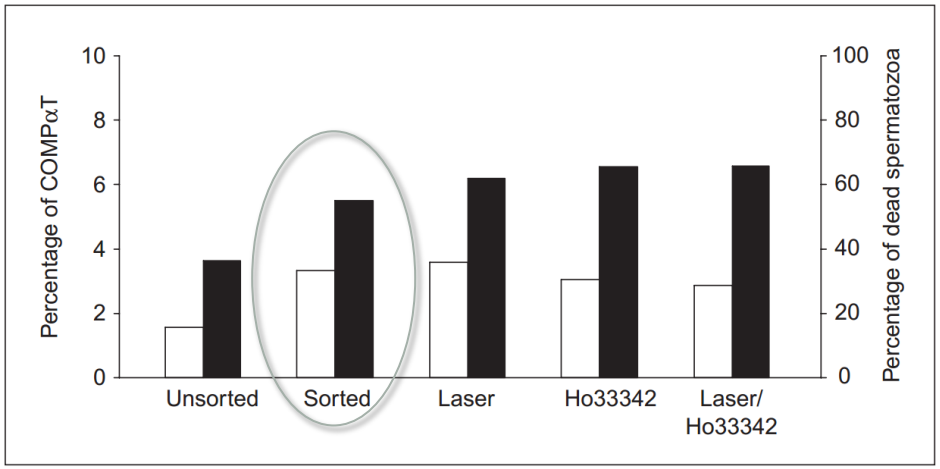
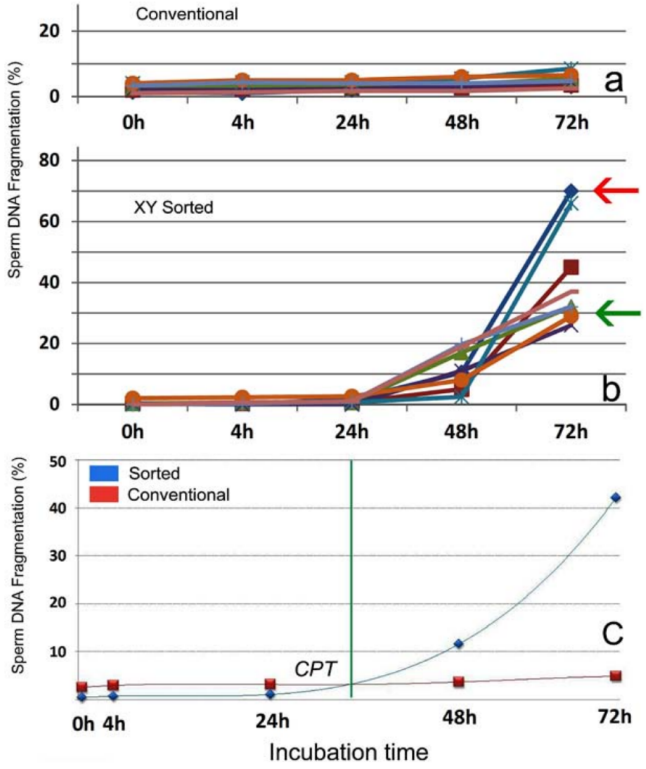


Fig. 4. Percentages of (■) dead spermatozoa and (□) spermatozoa with damaged DNA after thawing as determined by the spermatozoa chromatin stability assay (percentage of COMPαT, cells outside of the main population) after (1) unsorted control, (2) passing spermatozoa through the sorter without laser or staining, (3) with laser but no staining, (4) with staining, but no laser, and (5) with both staining and laser (modified from Garner *et al.*, 2001).

Seidel and Garner (2002) *Reproduction* **124**, 733-743.

DNA fragmentation accelerated in sorted-bull sperm



Gosalvez et al. (2011) *Therio* **75**, 206-211.

Sexing Technologies

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.**

Sexing™ TECHNOLOGIES

SPERM HETEROGENEITY

Sperm population in the ejaculate is made up of distinct sub-populations

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- **Structural heterogeneity**
  - Variations in morphology and structural elements (Ballachey, Evenson and Saacke, 1988)
- **Functional heterogeneity**
  - Sexual selection, sperm competiton, (Heterospermic inseminations Beatty et al, 1969, Parrish and Foote 1985, Holt and Van Look 2004)
- **Physiological heterogeneity**
  - Discrete packets of sperm are physiologically ready for fertilisation at different times post insemination – Rodriguez-Martinez, 2006, 2007)

Sexing™ TECHNOLOGIES

Exploiting heterogeneity

Through encapsulating sperm, discrete packets of sperm available for fertilisation for extended periods.

- (Bovine) Nebel et al 1993, Vishwanath et al, 1996, 1997,
- (Pig) Faustini 2011,
- (Sheep) Maxwell et al 1996.

Sexing™ TECHNOLOGIES

Theory disproved

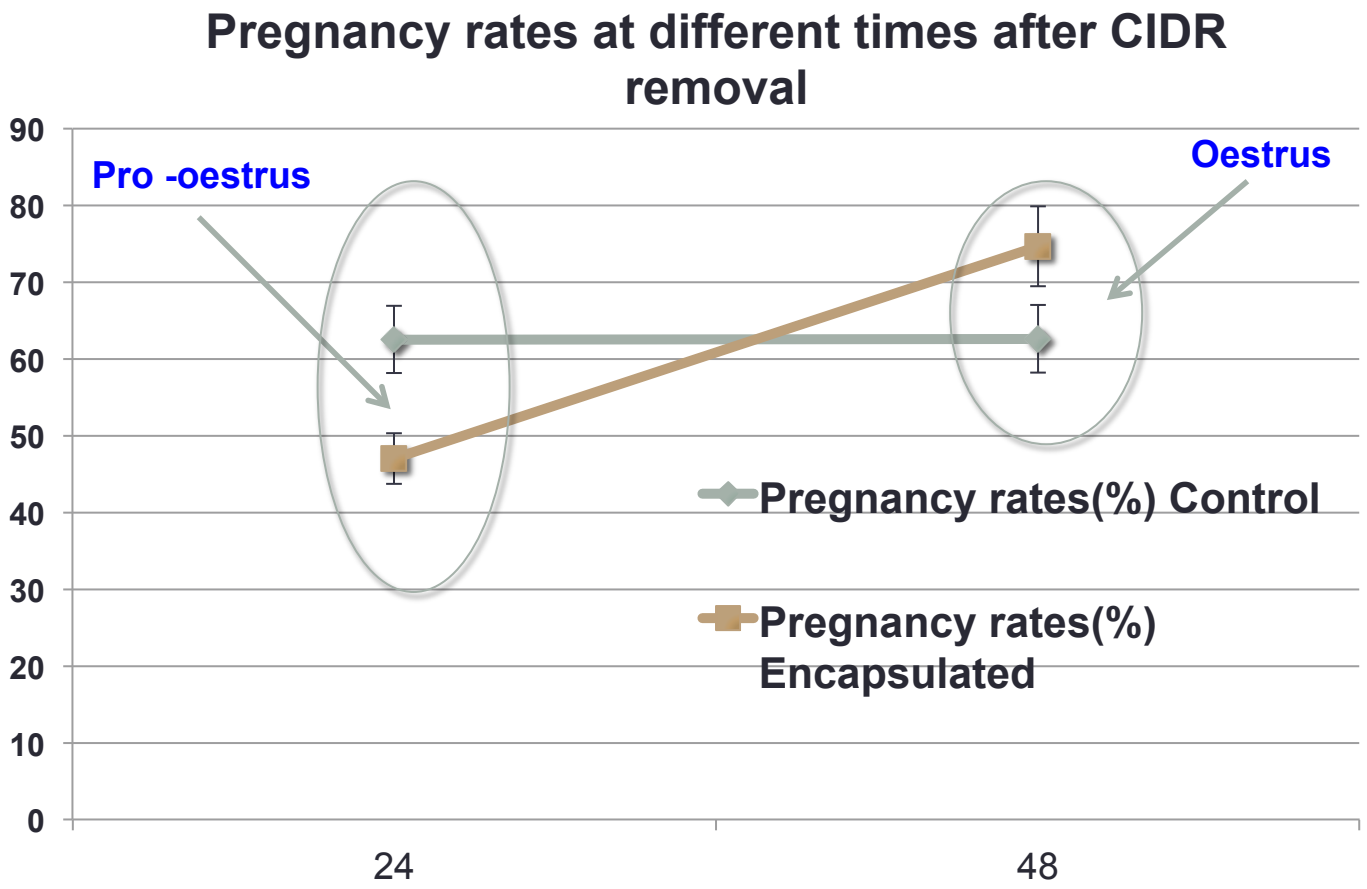
Table 2B. Pregnancy rates (%) to first inseminations for only those animals visually detected in estrus.

Semen type	Interval to insemination					
	24 h			48 h		
	number of insems	number pregnant	%	number of insems	number pregnant	%
Control	96	60	62.5[b]	91	57	62.6[b]
Trial	94	43	45.7[a]	91	68	74.7[c]*

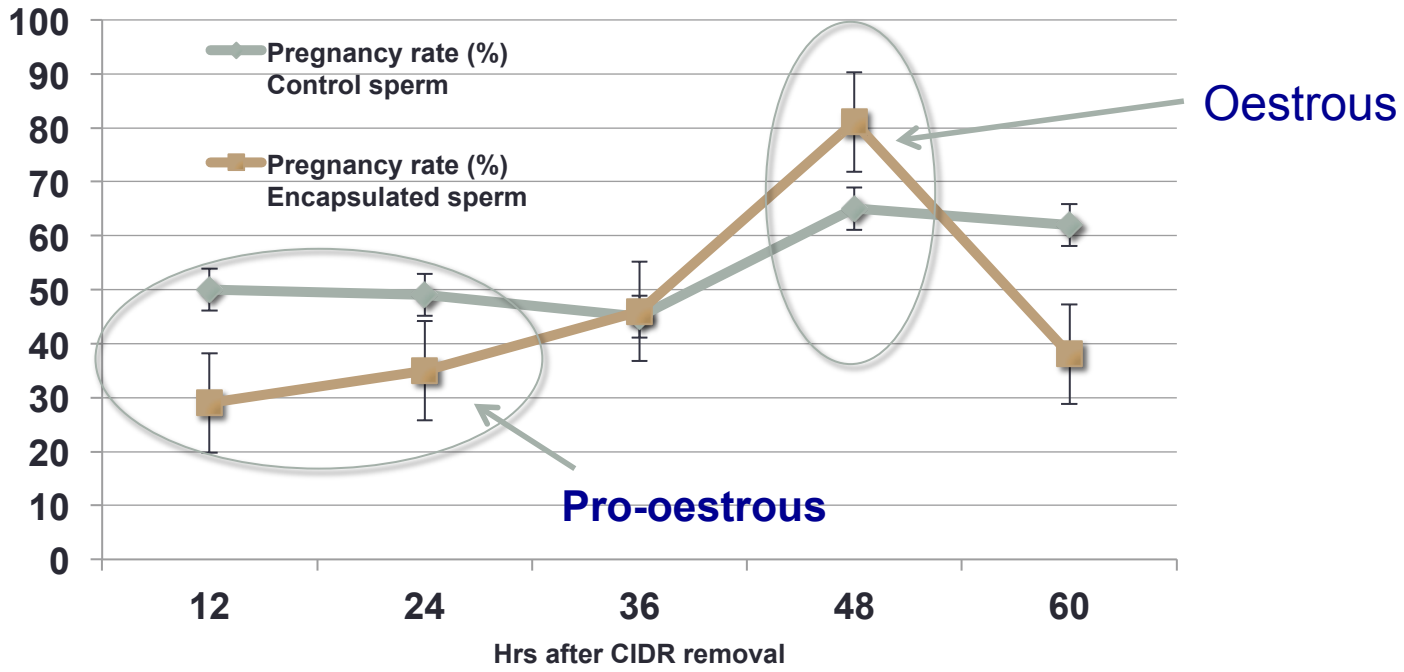
Means with different superscripts differ significantly ($P<0.05$).
*Signifies different from Control at both 24 hours and 48 hours ($P<0.08$).

Data from Vishwanath et al 1996

Sexing TECHNOLOGIES


Pregnancy rates at different times after CIDR removal
90
80
70
60
50
40
30
20
10
0
Pro -oestrus
Oestrus
Pregnancy rates(%) Control
Pregnancy rates(%) Encapsulated
24
48

Pregnancy rate of Control or Encapsulated sperm inseminated into cross bred heifers at varying times (12h to 60h) after CIDR removal



McMillan and Vishwanath 1994

Sexing TECHNOLOGIES

Data from Jordan et al J. Anim Sci 2014

Table 3. Pregnancy rate to fixed-time artificial insemination (FTAI) based on estrous response and treatment[1]

Estrous response[3]	Pregnancy rate to FTAI[2]					
	Treatment 1		Treatment 2		Treatment 3	
	Proportion	%	Proportion	%	Proportion	%
Estrous	81/105	77%[a]	53/104	51%[b]	47/111	42%[bc]
Nonestrous	42/113	37%[d]	3/113	3%[e]	40/110	36%[cd]
Combined	123/218	56%	56/217	26%	87/221	39%

[a–e]Pregnancy rates with different superscripts within rows or columns are different, $P < 0.0001$.

[1]Cows received a controlled internal drug release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 µg, i.m.) on d 0. On d 7, the CIDR insert was removed and $PGF_{2\alpha}$ (25 mg, i.m.) was administered. At 66 h after CIDR insert removal and $PGF_{2\alpha}$, the cows received GnRH (100 µg, i.m.). Cows were assigned to 1 of 3 treatments: 1) FTAI (concurrent with GnRH, 66 h after CIDR removal) with conventional semen regardless of estrous expression, 2) FTAI with sex-sorted semen regardless of estrous expression, or 3) FTAI with sex-sorted semen for cows having expressed estrus and delayed AI 20 h after final GnRH for cows failing to express estrus.

[2]Pregnancy rate to FTAI determined by ultrasound 60 d after AI.

[3]Estrous response by 66 h after $PGF_{2\alpha}$ administration, as determined by activation of an estrus detection aid (Estrotect; Spring Valley, WI).

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Observation:

Similar to encapsulation, heterogeneity of the sperm cell population altered during sex sorting process

Fertile in a narrow window and requires optimisation of time of insemination.

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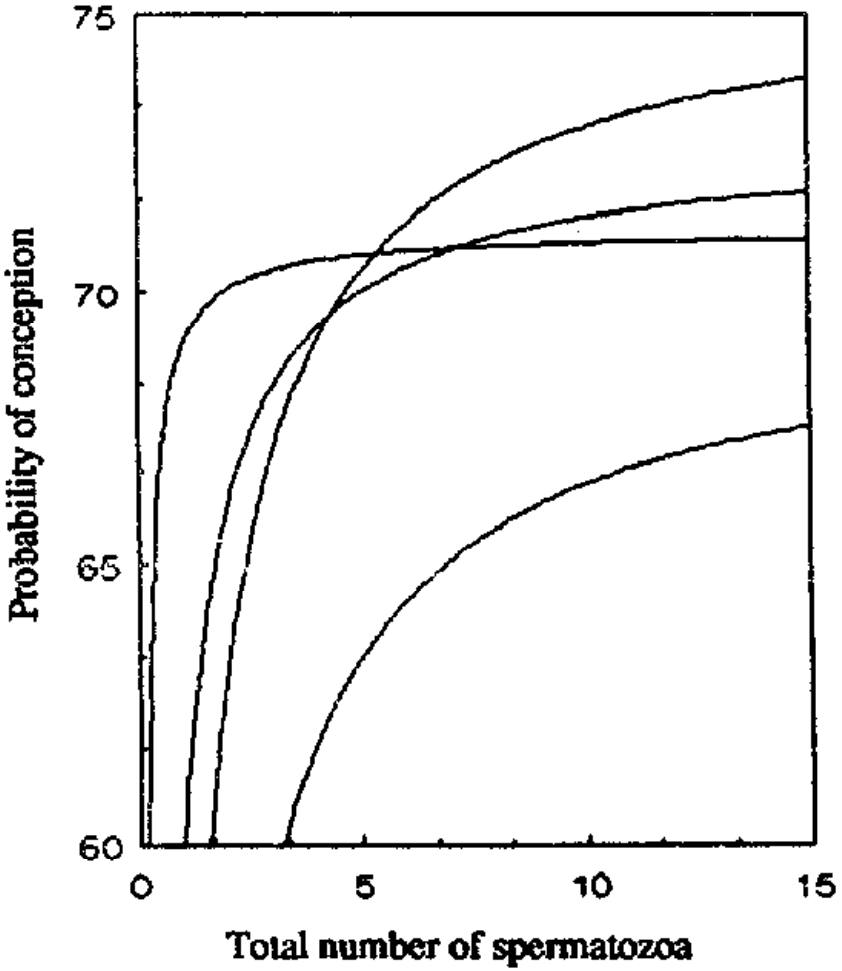
Sexed sperm – effect of sperm numbers and cryopreservation

Sexing™ TECHNOLOGIES

Increasing sperm numbers increased fertility – den Daas 1992



Increasing sperm numbers with sex sorted semen does not compensate for this sub fertility

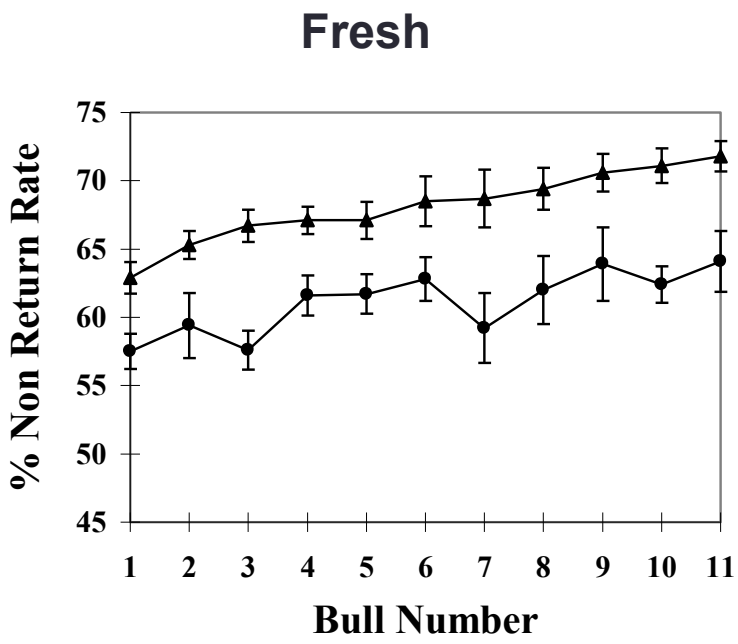
Sex sorted		Conventional		
Sperm concentration	Conception rate	Sperm concentration	Conception rate	Relative fertility
2.1×10^6	38%	2.1×10^6	55%	70%
10×10^6	44%	10×10^6	60%	73%

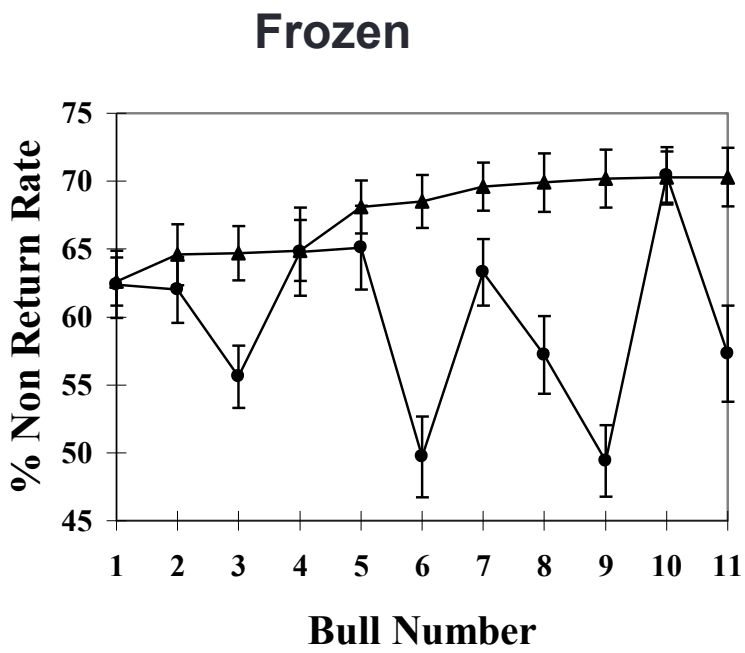
DeJarnette et al 2011

Sire effect, P < 0.01
Concentration effect P < 0.01
Semen type effect P < 0.01
Sire x Concentration - NS

Sexing TECHNOLOGIES

Comparison of fresh and frozen NRR's at optimal and sub-optimal sperm concentrations





Fresh – optimal dose rate 2.5 million / straw and sub-optimal 0.5 million / straw

Frozen – optimal dose rate 20 million / straw and sub-optimal 5 million / straw

Sexing™ TECHNOLOGIES

Observation:

Lower fertility with sex sorted semen is partially due to dose rate

Increasing dose rates does not fully restore fertility of sex sorted sperm.

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Lessons from fresh sex sorted sperm – New Zealand

Sexing TECHNOLOGIES

NRR of fresh sex sorted (1 million) or conventional semen (2 million)

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

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Calving statistics with fresh sex sorted or conventional semen

	2011			2012		
	SS	Conv	SS-Conv	SS	Conv	SS-Conv
No of AI	14,239	17,372		31,051	31,294	
Calving / AI %	51.2	54.3	-3.1	49.7	52.6	-3.0

Data from Z Xu, JDS in press
Calving / AI %, is adjusted calving taking into account AI in culled cows and AI in non pregnant cows.

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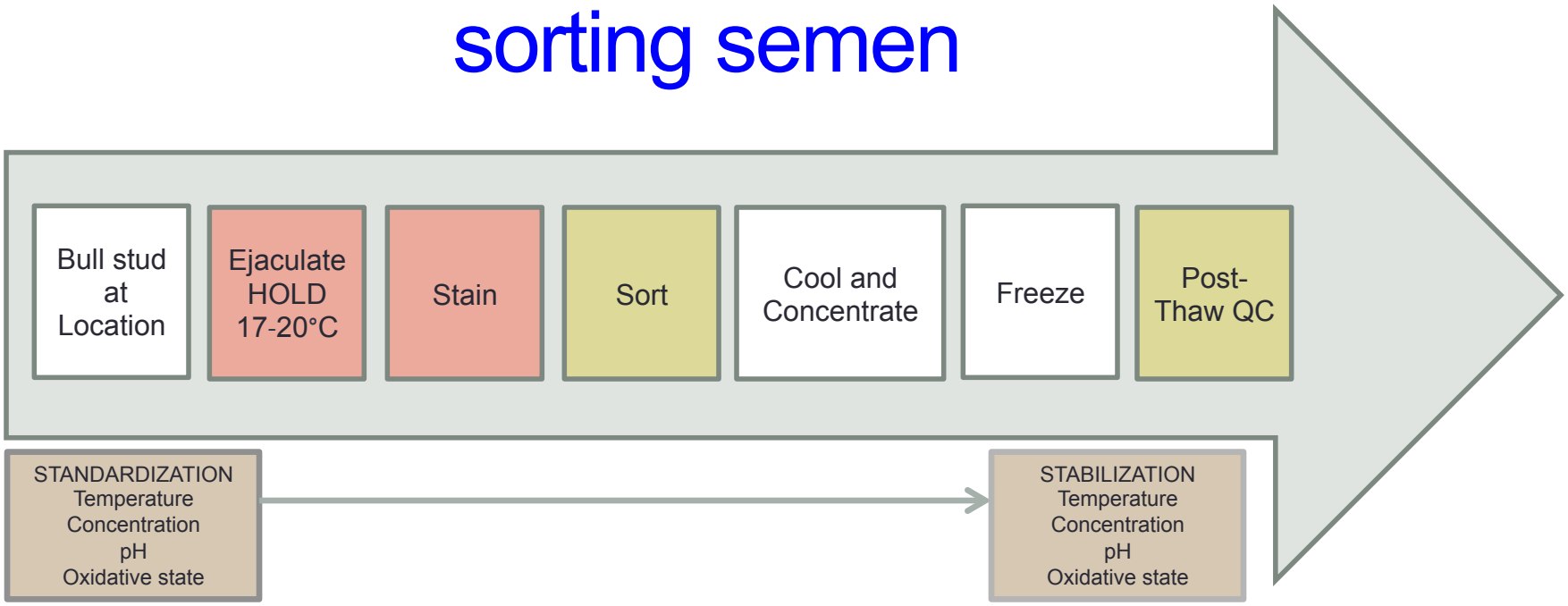
Observations

- Fresh sex sorted sperm has almost comparable fertility with that of conventional sperm at half the sperm concentration

- The sex sorting process per se is not detrimental to sperm fertilising ability

Sexing™ TECHNOLOGIES

Recent changes in processes with sex sorting semen

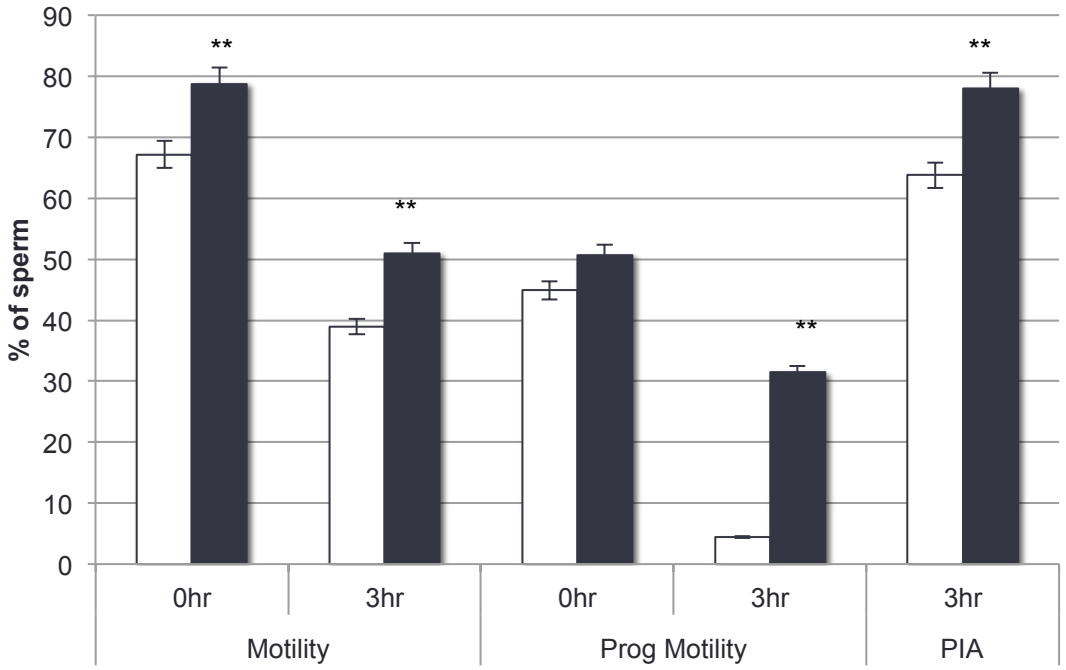


- A system that **maintains consistency in temperature** through the entire process.
- A system that **standardizes the pH and concentration** of all the ejaculates as soon as they are collected.
- A system that **reduces oxidative damage** at each step
- A buffer system that **stabilizes and maintains the pH** along the sorting process.

The collective process is termed SexedUltra™

Sexing Technologies

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



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

Preliminary trials with SexedULTRA™

	XY Method		SexedULTRA™ method	
	Inseminations	Pregnancy rate (%)	Inseminations	Preganacy rate (%)
Jersey	803	50.7	603	57.2
Holstein	363	39.7	354	50.6
Overall	1166	47.3	957	54.7**

**Significant differences in overall pregnancy rate XY compared with SexedUltra™ $P < 0.01$

Sexing Technologies

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

Process method	Number of inseminations	Scanned pregnancy rate
XY	3384	41.6%
SexedULTRA™	3546	46.1%*

*** Process method differs P < 0.01**

Sexing TECHNOLOGIES

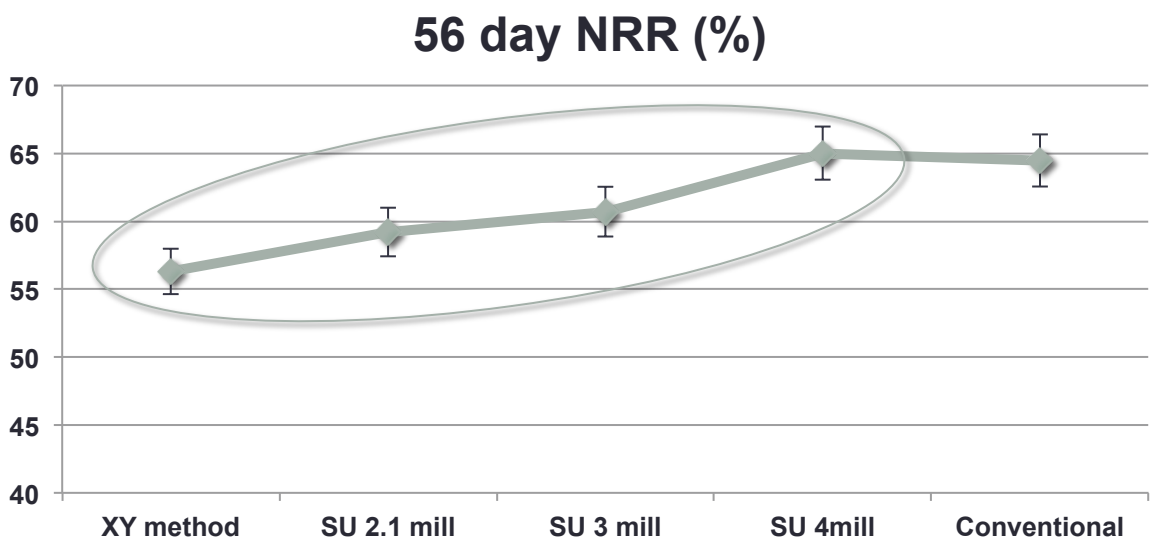
Field trials with a new and improved SexedULTRA™

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

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

Sexing Technologies

Dose rate trials with new SexedULTRA™



Treatment	Number of inseminations	56 day NRR (%)	Relative fertility (%)
XY method	1292	56.3^{A}	87%
SU 2.1 mill	1245	59.2^{A}	92%
SU 3 mill	1328	60.7^{AB}	94%
SU 4 mill	1182	65.0^{B}	***100%***
Conv (15 mill)	50,143	64.5^{B}	

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

Two important observations in this trial

- For the first time a dose response effect has been demonstrated with sex sorted sperm
- For the first time, parity in conception rates with conventional semen has been demonstrated.

GENETICS
MADE IN GERMANY

Sexing TECHNOLOGIES

Sex sorting technology – progress through the years

1990-1995	1995-2002	2002-2012
Sort speeds 200 to 400 cells per second, 83% purity 70% fertility of conventional	Sort speeds 1000 cells per second, 85% purity, 80% fertility of conventional	Sort speeds 5000 cells per second, 85% purity, 80% fertility of conventional
1000 conventional straws = 10 sex	1000 conventional straws = 50 sex	1000 conventional straws = 400 sex

Sexing™ TECHNOLOGIES

Last two years

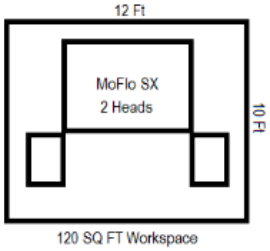
- Improvements in sorter technology as well as semen processing methods.

- 2012 - 2014
  10,000-20,000 cells per second
  >93% purity
  92% fertility of conventional semen

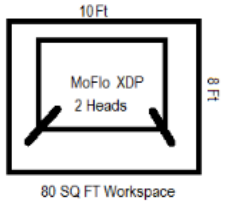
1000 conventional straws = 1100 sex

Sexing Technologies

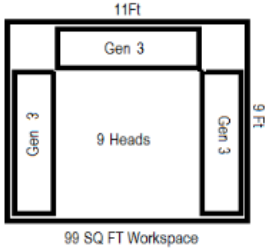
a



LEGACY MOFLO SX
Analog Sorting
20 Million Sexed per hour
Yield 10% of Ejaculate



MOFLO XDP SX
Digital Sorting
45 Million Sexed per hour
Yield 15% of Ejaculate



Genesis III
Digital Sorting/Application Specific
250 Million Sexed per hour
Yield 17-20%

b

MoFlo XDP – twin head

c

Cytonome/ST LLC – Genesis III

Sexing TECHNOLOGIES

In Conclusion

- **Sex sorting process + cryopreservation alters the heterogeneity of the sperm population.**
- **Imaginative field trials to dissect this response.**

Sexing™ TECHNOLOGIES

- **New SexedUltra™ process - Marked lift in fertility with sex sorted frozen semen**
- **No perceptible loss in fertility with fresh sex sorted semen.**
- **Fertility loss primarily due to the interaction between sex sorting and cryopreservation**

Sexing™ TECHNOLOGIES

For the first time, comparable conception rates for sex sorted sperm and conventional

Sexing™ TECHNOLOGIES

Acknowledgements

- The talented R&D team at Sexing Technologies
- The Artificial Breeding Companies who have participated in the trials

Questions ?

Sexing™ TECHNOLOGIES