Application of sexed sperm for dairy cattle production

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SEX RATIO AT BIRTH—PROSPECTS FOR CONTROL

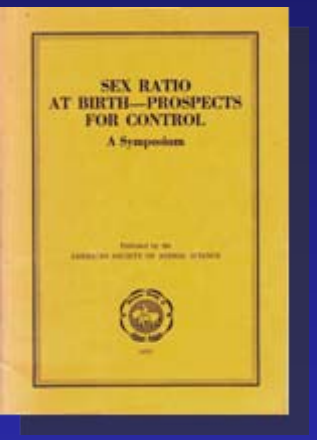
A Symposium

Published by the
AMERICAN SOCIETY OF ANIMAL SCIENCE

1971

PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK JULY 1970

Factors affecting application of sex preselection in livestock animals RH Foote and P Miller



- The degree to which sex ratio is altered
- The fertility or number of progeny/female per year
- The number of progeny per top sire possible from processed sperm
- The direct cost of the sex control technology
- The opportunity to combine sex control with other procedures
- Convenience
- Traditional ethical and moral considerations

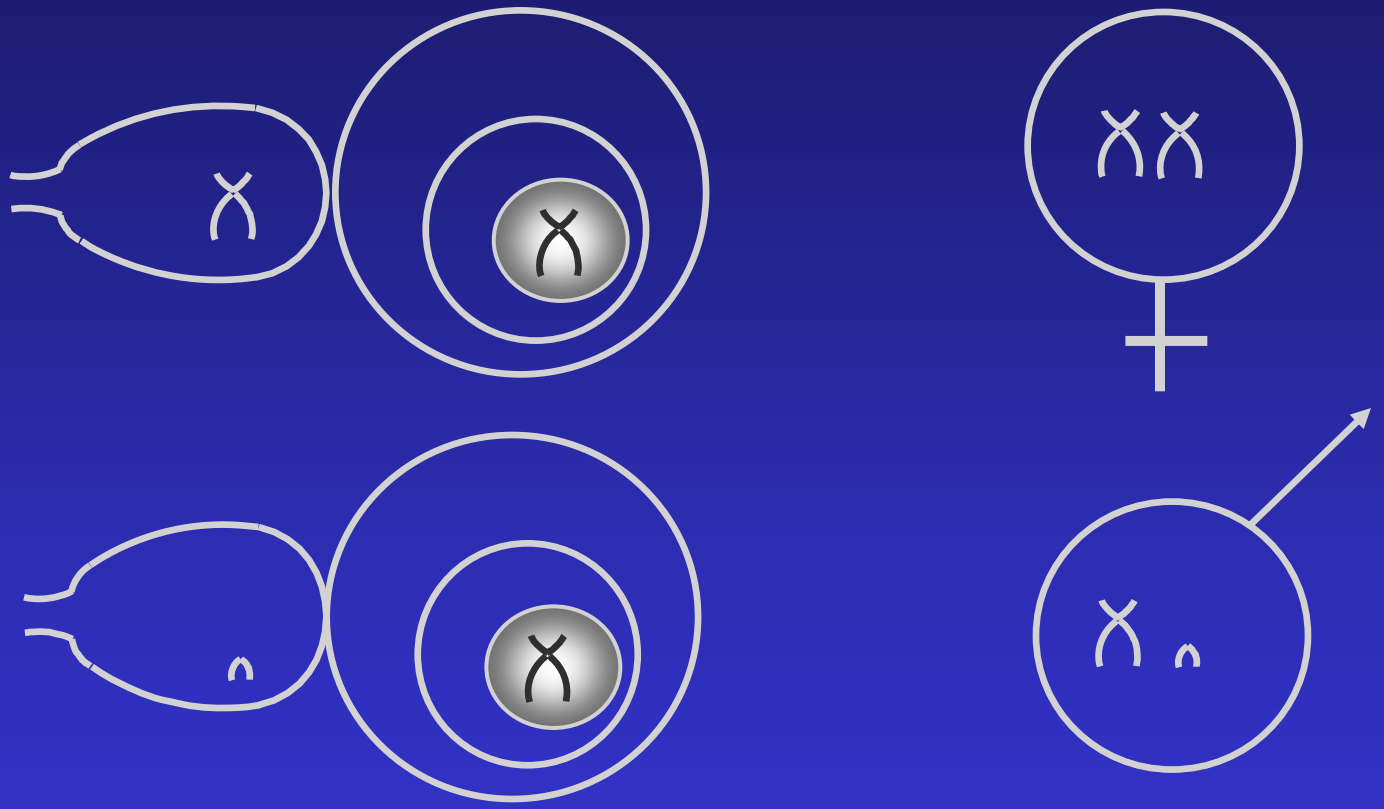
Pre-requisites for application of sex-sorted sperm

- Sorting protocols
- Sort purity
- Sperm quality
- Availability

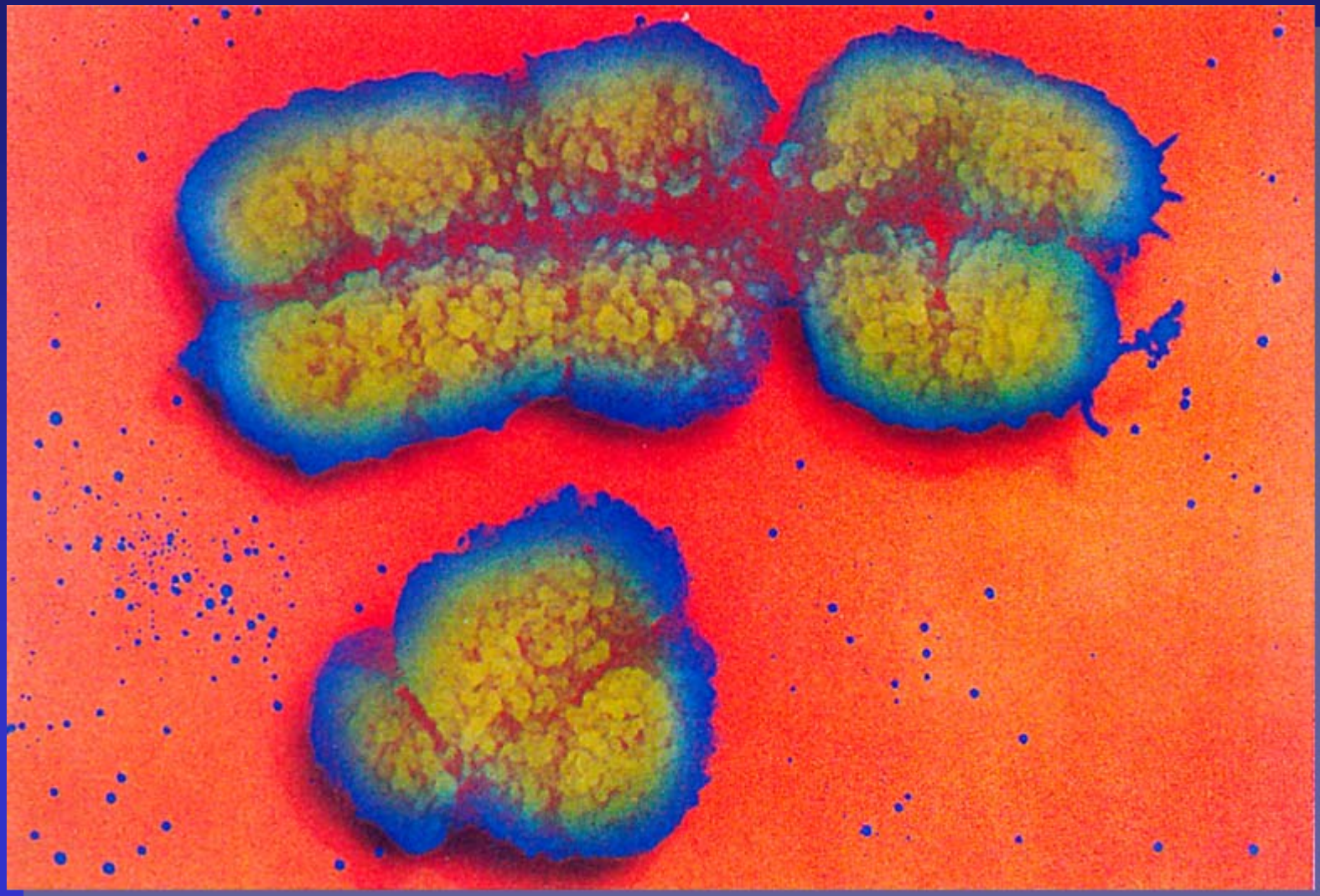
Sex Determination
Possible Methods

- Embryo biopsy and PCR (followed by ET)
- Selective abortion (fetus or embryo)
- Pre-selection of X or Y spermatozoa:
  - -Protein gradients (motility)
  - -Density gradients (density)
  - -Electrophoresis (surface charge)
  - -Immuno-separation (surface antigens)
  - -Flow cytometry (DNA). Beltsville sperm sexing technology (Johnson & Pinkel, 1986)

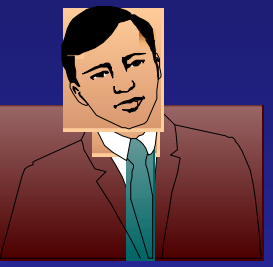
In mammals sex is determined by the heterogametic sperm population



X-and Y-chromosome differ in size



Differences of the relative DNA-content between X-and Y- chromosome bearing sperm



2.9%

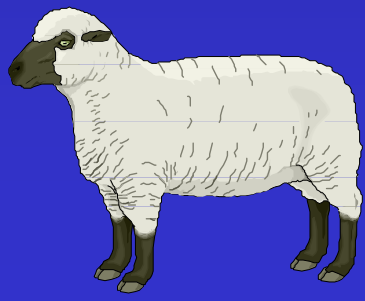
3.0%

3.2%

3.6%

3.8%

4.1%

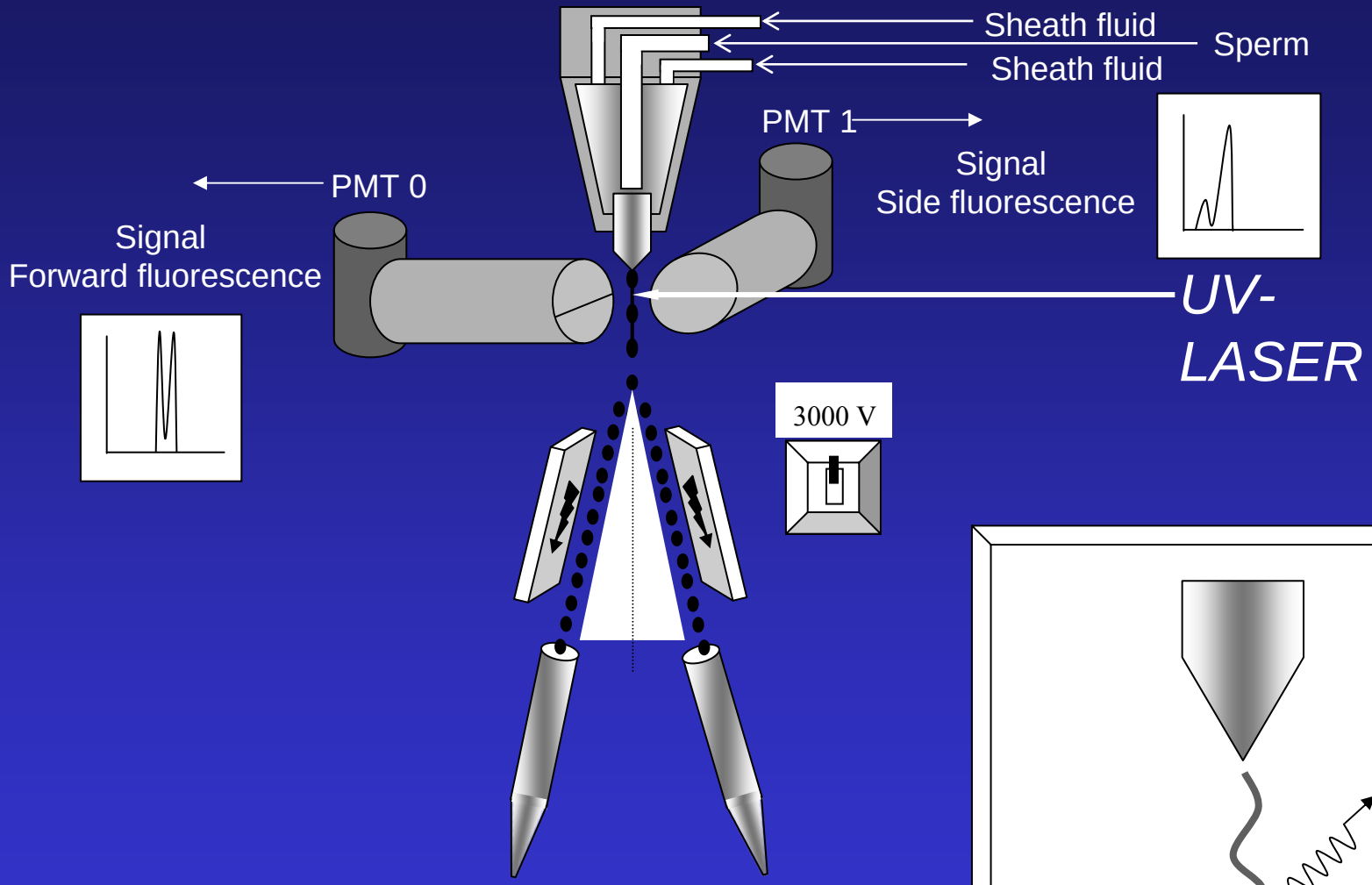


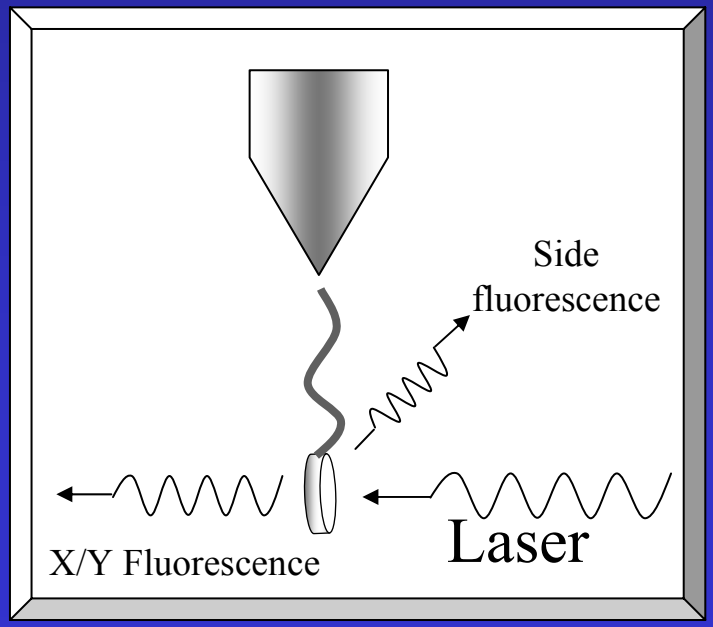
4.2%

Basic principles

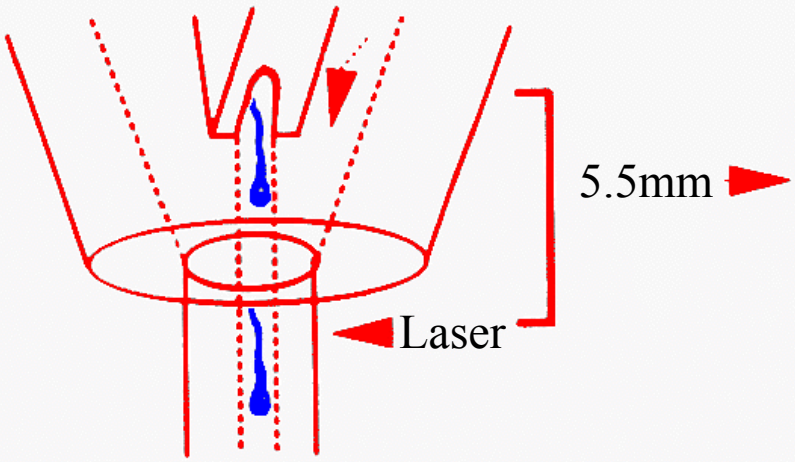
- DNA staining distinguishes X and Y sperm
- Modified flow cytometer sorts two sperm populations according to fluorescence
- Orientation of sperm to the laser

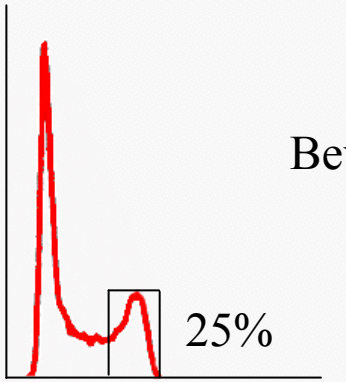
Flow cytometric principles



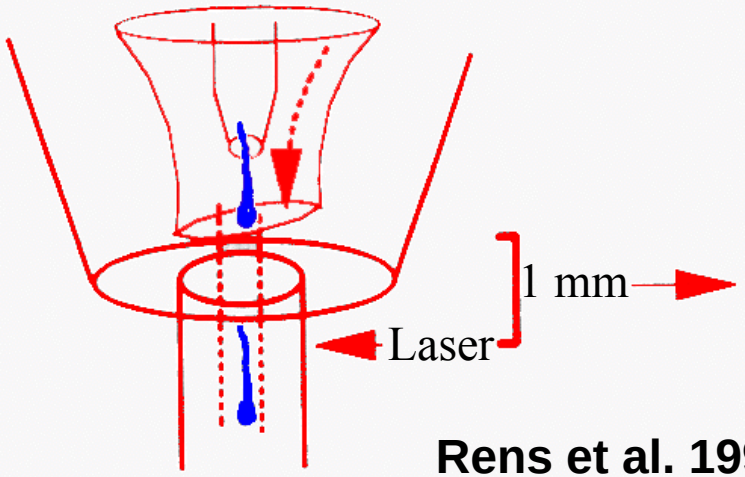


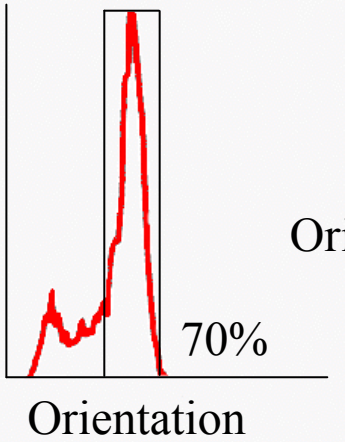
Improved sperm orientation





Beveled Nozzle

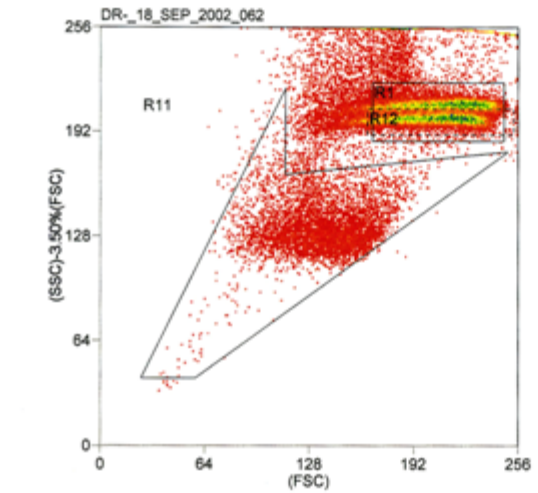




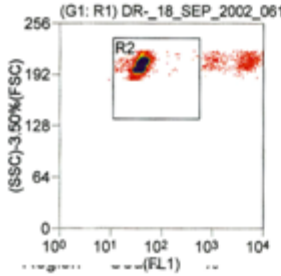
Orienting Nozzle

Rens et al. 1999

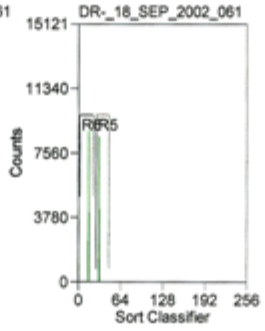
Sort histograms

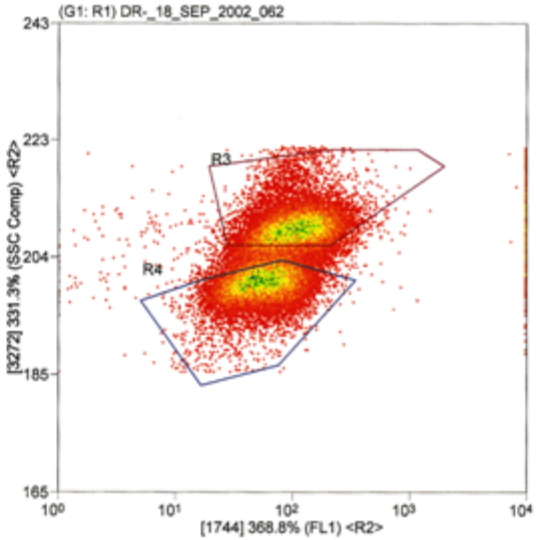


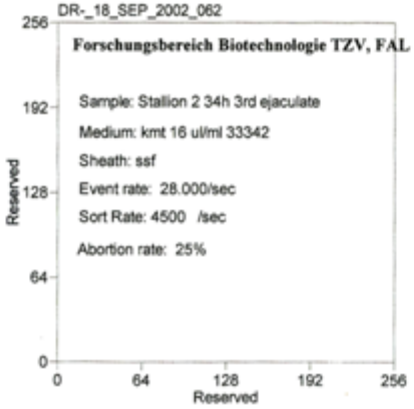
Total	40000	100.00	8.00190.65, 1...	39.40, 3...20.67...
R1	23290	58.23	4.66206.21, 2...	20.63, ...10.01...
R11	6563	16.41	1.31135.65, 1...	23.88, 1...17.58...
R12	13460	33.65	2.69204.11, 1...	21.03, ...10.30...

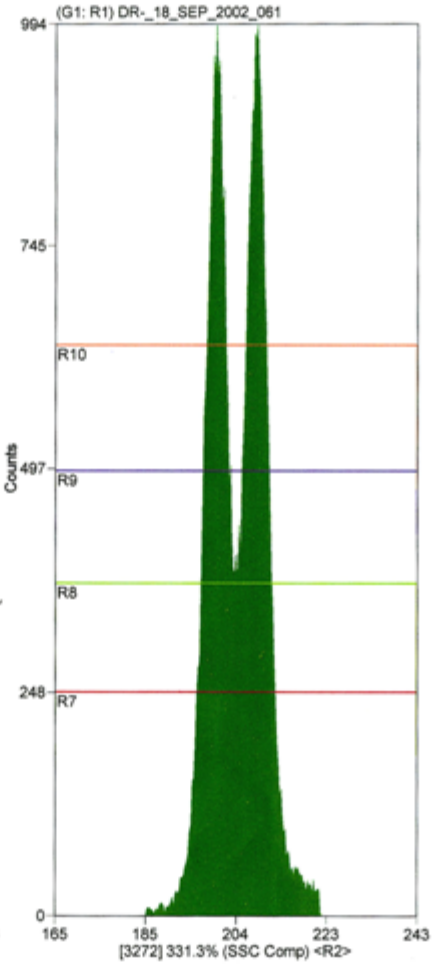


Total	40000	100.00









Comparison of normal and high speed flow cytometer

	EPICS V/753	MOFLO high speed Sorter
Pressure	20 psi	52 psi
Flow Rate sperm/sec/sex	2000	~ 30000
Sort Rate sperm/sec/sex	100	~ 6000
No. of sorted sperm/h/sex	0.35×10^6	15×10^6


MoFlo
MoFlo

Factors influencing sorted sperm viability

INSULTS

- High dilution
- Nuclear staining and incubation
- Mechanical forces
- Exposure to UV laser & electric charge
- Projection into collection medium
- Post-sorting centrifugation
- Freezing and thawing

PROTECTION

- Special media, including antioxidants
- Egg yolk and seminal plasma

Factors influencing sorted sperm viability

PRE- and POST-SORT HANDLING

- Time between collection and sorting
- Time between sorting and centrifugation
- Storage fresh/freezing-thawing protocol and insemination


Historical development and application of sperm sexing

Sex Determination – History

- 1982: analysis of sperm DNA difference
- 1987: separation of sperm nuclei
- 1989: pre-sexed offspring (rabbits)
- 1993: pre-sexed calf born (IVF & ET)
- 1996: pre-sexed lamb born (ICSI & ET)
- 1997: pre-sexed calves born (Fresh AI)
- 1997: pre-sexed lamb born (Fresh AI)
- 2002: pre-sexed lambs born (Frozen AI)
- 2003: pre-sexed lambs born (Frozen-sexed-refrozen)
- 2004: pre-sexed calves (AI & MOET)
- 2008: pre-sexed calf born (Frozen-sexed-refrozen AI)

Efficiency of sperm sorting
Single insemination dose
One desired sex

Species	Dose ($x10^6$)[1]	Pregnancy[2]	Time[3]	Time[4]
Cattle	2	10-20% ↓	8 min	3 min
Sheep	20	Similar	1 hr	25 min
Horses	25	Similar	1.6 hr	40 min
Pigs	50	40-50% ↓	3.3 hr	1.3 hr

[1] Live sperm
[2] Compared with unsorted sperm and conventional deep uterine AI, all sorted-frozen-thawed
[3] Sort speed of $15x10^6$ sperm/h
[4] Maximum theoretical sort speed current technology ($36x10^6$sperm/h)
(Garner & Seidel, 2003)

Application and commercialisation in dairy cattle

Patents and animal licenses

Because Your Business Begins at Concepti

Sexing technologies

GENETIC RESOURCES GRI INTERNATIONAL

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SEXED SEMEN

"The original and the best!"

Cogent Sexed Semen:

- Sexed Semen TV
- The Science
- Benefits
- Getting it right
- Nutrition & Health
- Handling Sexed Semen
- Service line
- Success Story's UK
- Success Story's Inter
- F.A.Q's

Since the advent of Artificial Insemination in dairy cattle, farmers and breeding companies alike have been dreaming of selecting the sex of the calves born. Every dairy farmer, wherever in the world, is looking to breed replacement heifers from the best animals. After extensive research and development, Cogent Breeding became the first breeding company in the world to offer Sexed Semen commercially in July 2000.

Seven years on Cogent now offer the most robust and successful Sexed product available on the market. Although the technology for sorting sperm has not changed, Cogent has been able to improve the product greatly from its original form with the implication of various new techniques including world leading quality controls, a world exclusive freezing technique and 7 years of on farm experience.

Cogent Sexed Semen is now the management tool all dairy farmers have been dreaming of, offering 90% female calves and conception rates achievable on a par with conventional semen.

So why now is Sexed Semen more important than ever before?

The global demand for milk and other dairy products is growing daily. No longer is over supply forcing prices down, the opposite is now true with supply not being able to keep up with ever increasing demand.

Replacement heifer prices are at all time highs across the world, with farms expanding in an attempt to meet the global demand for milk.

The demand for beef from the dairy herd is high with buyers seeking top quality crossbred calves rather than Holstein bull calves.

Animal health & welfare rises as a priority at consumer, supermarket and government level, making easily calved heifers a priority.

SOIL ASSOCIATION ORGANIC STANDARD — SOIL ASSOCIATION APPROVED

G

SHAWANO, W
specific line of
Technologies. G
Jersey sires.

Topping the GenC
are the 90th percei
1HO7290 OBRIAN.
APPARITION leads
additional dairy and b

The technologies used
percent purity, meaning
should produce a femal
be used for artificial insemination of bovine heifers that are not part of a timed A.I. synchronization program or being flushed for embryo transfer.

For specific sires and availability, contact your local Genex representative.

Cooperative Resources International

Inseminations:

Dansire

A minimum of 6 board meetings annuall
have 3-10 committee meetings annually.

Furthermore, a number of employee me

Three A.I. Technicians work in two d
work in three districts.
Each district has approx. 5,000 first ins
A.I. Technician. The A.I. Technici
to 8 a.m. and on his mobil
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Semen is
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Press release

Arnhem, 27 June 2007

HG introduces...SiryX

unce the start of sexed semen production from HG
nternationally under the name SiryX and its launc

SiryX

s get the unique opportunity to breed female offspri
h a very high reliability of at least 90%.
in a special laboratory in Deventer (The Netherlands) by us
achines are able to seperate sperm cells with the female
ose with the male chromosome (Y). They are the most advanced a
ailable at the moment and the operators responsible for the production
ilist training in the United States.

ryX
ntages for dairy farmers and has the capacity to maximise farm profit

- higher turn over: by inseminating part of the herd with SiryX, a larger proportion of the herd can be inseminated with beef bull semen. Farmers can profit from this because they can boost their income from the sale of cross-bred calves.
- assurance of heifer calves for replacement or expansion: the number of heifer calves born can be optimised by using SiryX. Farmers who want to expand their herd with their own stock can do this more efficiently with SiryX.
- faster genetic progress: using SiryX on the highest genetic merit cattle in the herd to bree the next generation milking cows will result in faster genetic progress. Theoretically this effect is the highest when using SiryX on heifers, because of the genetic superiority of heifers on most farms compared to the rest of the herd.
- reducing difficult births: heifer calves are smaller than bull calves and by using SiryX, particularly on heifers, the number of difficult births will decrease. As a result, the heife will have a better start to their lactation, with lower veterinary costs, and will get back i more easily.

Advice for use on heifers

To produce SiryX, the semen goes through several treatments. Because of these treatr the virility of the semen will be lower compared to conventional semen. In heifers this e expected to be limited, but the effect will be bigger in cows. Therefore HG advises farr

NEED 90% OR MORE HEIFERS?

JULY 2008

More Selection From the Industry Leader

Keys to Success

Select Sires continues to be the industry leader for sexed semen. Our gender SELECTed lineup is the most expansive of any A.I. stud with elite proven sires in the Holstein, Jersey and Brown Swiss breeds. And Select Sires is currently the only company to also offer elite young sires.

Our growing list of gender SELECTed bulls is backed by industry-leading research and experience. We understand the value of a heifer calf to your bottom line. After extensive field trials, the facts prove our technology can sex-sort sperm with 90 percent purity. Wouldn't you be tickled pink to get 9 heifers out of every 10 calves? Producers who pay strict attention to the details of semen handling and timing of insemination will be pleased with the results.

Turn to Select Sires, your experienced source for gender SELECTed semen!

gender SELECTed

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ABS SEXATION™

headquarters in DeForest, Wisc.

an additional management tool with sex sorted semen S SEXATION Marketing Specialist. "This will allow beef globally recognized product offering and give them the

st studied in U.S. government research laboratories in the this procedure was born in the 1990's, and since this time efficiencies of the semen sorting process. ABS now has a laboratory producing ABS SEXATION which will yield on he desired sex.

"Research has shown that flow cytometry technology will sort semen at an average of 90 percent purity for the desired sex," stated ABS Scientist Dr. Marj Faust. "ABS SEXATION is recommended for use in virgin heifers, with insemination occurring 12 hours after an observed or expressed heat. Proper semen handling and thawing is always an important step in a successful insemination, and is especially important with sex sorted semen. Even with proper care and handling, conception rates will average 75-90 percent of conventional semen."

"Through ABS SEXATION, producers will reduce disease risk, have the ability to control heifer replacement costs, increase heifers from within and more profitably manage their business," says Scott Bentley, Global Dairy Product Manager.

Meeting the varying needs of beef producers, ABS will be offering sorted female and male semen. Beef Product Manager Doug Frank states, "ABS will be bringing beef producers some of the top bulls in the industry, like the reigning number one bull in the Angus breed NEW DESIGN 878 and the new record seller IN FOCUS."

ABS SEXATION is based on a strong tradition of quality and excellence. The inspiration for the product line name, 29H2960 Ocean-View SEXATION, was an ABS customer-satisfaction sire from the early 1980's. Hailing from the Steps cow family at Ocean View Farms, SEXATION's black frames and outstanding udders can be seen in many of today's dominant pedigrees including ROY and JUROR. ABS SEXATION - a name with a great history - represents an exciting future of ABS products.

Headquartered in DeForest, Wisconsin, ABS Global, Inc. is the world-leading provider of bovine genetics, reproduction services, technologies and uddercare products. Marketing in more than 70 countries around the globe, ABS has been at the forefront of Animal genetics and technology since its founding in 1941. ABS Global, Inc. is a division of Genus plc.

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INTERNATIONAL OVERVIEW OF PRODUCERS OF SEXED BULL SEMEN

Center	working since
Cogent	2000
Select Sires	2006
CRI	2008
Alta	2008
ABS	2007
Dansire	2006
Holland Genetics	2007
Semex	2007
TWG/ST	2008

Rath and Johnson 2008, Reprod Dom Anim 34, 338-346.

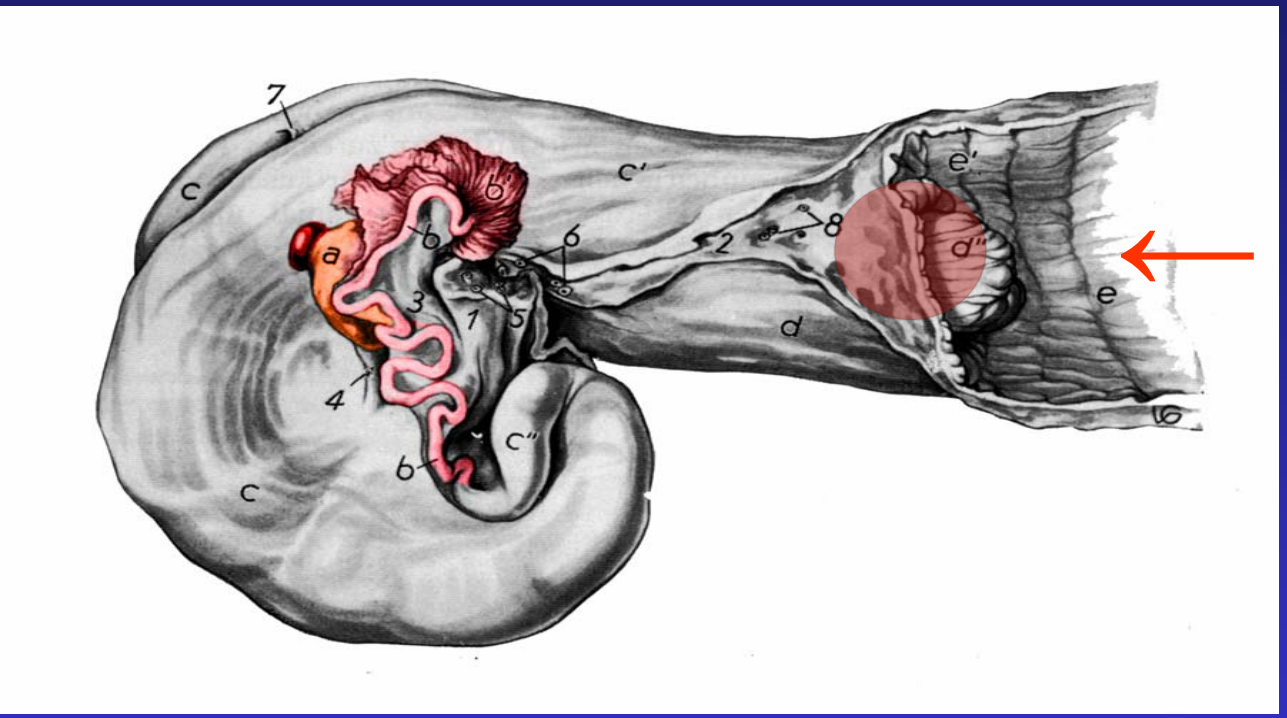

Cogent, UK

Why sex dairy bull sperm?

- Increase supply of replacement heifers
  - Reduce heifer sale and purchase prices
  - Reduce introduction of heifers into closed herds
- Increase availability of heifers for export
- Increase efficiency of progeny testing
- Increase efficiency of IVF programs
- Increase efficiency of MOET programs
- Include heifers in genetic selection and accelerate genetic progress
- Increase number of superior bulls
- Reduce cases of dystocia

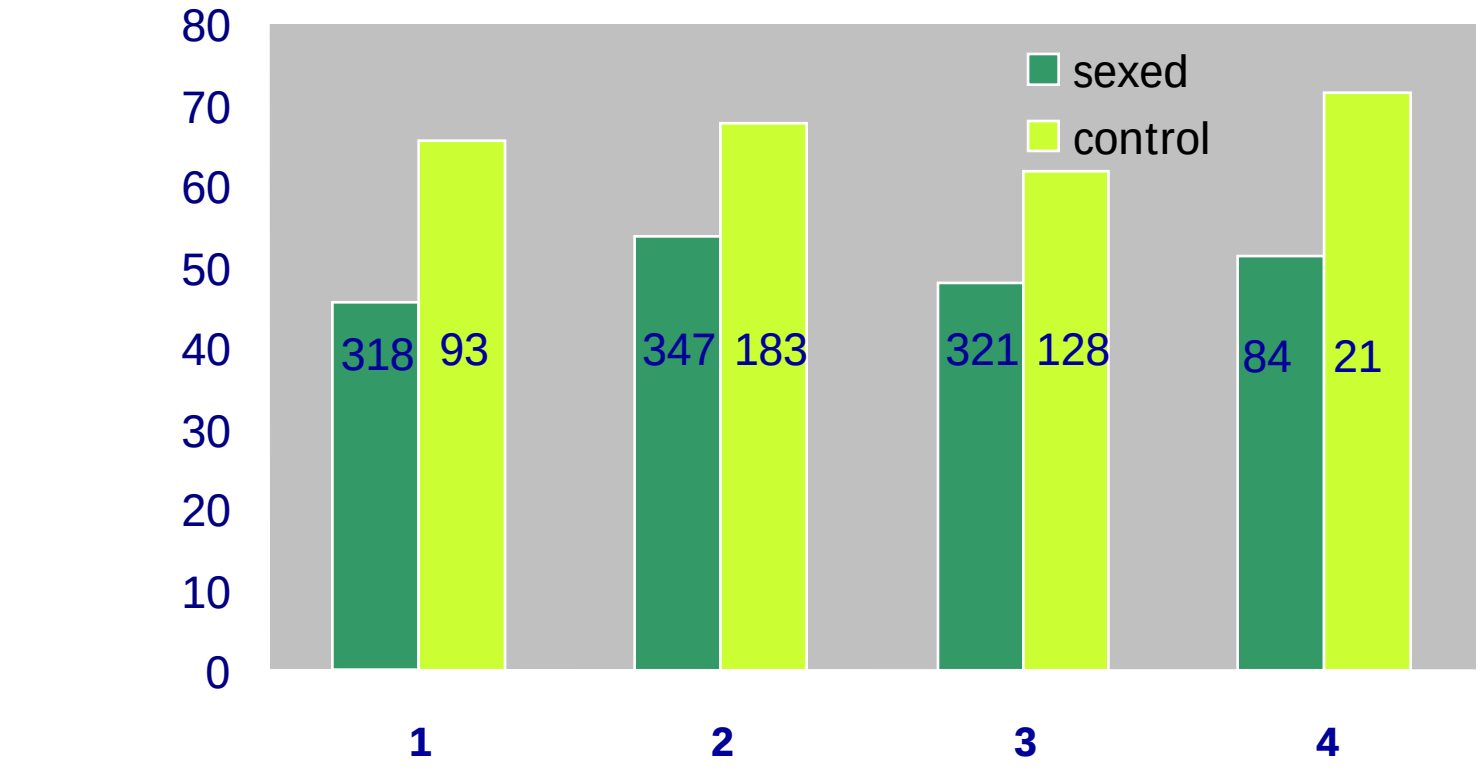
The quality (fertilising ability) of sex sorted sperm and its availability are decisive factors for its wide spread application in dairy cattle

Natural sperm selection



2 million instead of 20 million sperm

Summary of pregnancies after AI with sex sorted frozen bull sperm (Garner and Seidel 2003)



n = 16 trials

Sperm dose: **sexed: 1 – 3 x 10^6/uterine body or horn**
control: 20 x 10^6/uterine body

Table 1. Pregnancy and calving rates after AIs with sexed or unsexed sperm as well as distribution of gender and health of the calves born

	Sexed	Unsexed	p-value
Pregnancy rate			
No. of AIs	157	149	< 0.001
No. pregnant	33	69	
% pregnant	21	46	
Calving rate			
No. of AIs	157	146[a]	< 0.001
No. calved	32	65	
% calved	20	45	
Gender of calves			
No. calves[b]	33	67[c]	< 0.01
No. female calves	27	33	
% female calves	82	49	
Health of calves			
No. calves[d]	31	62	> 0.05
No. healthy calves	31	60	
% healthy calves	100	97	

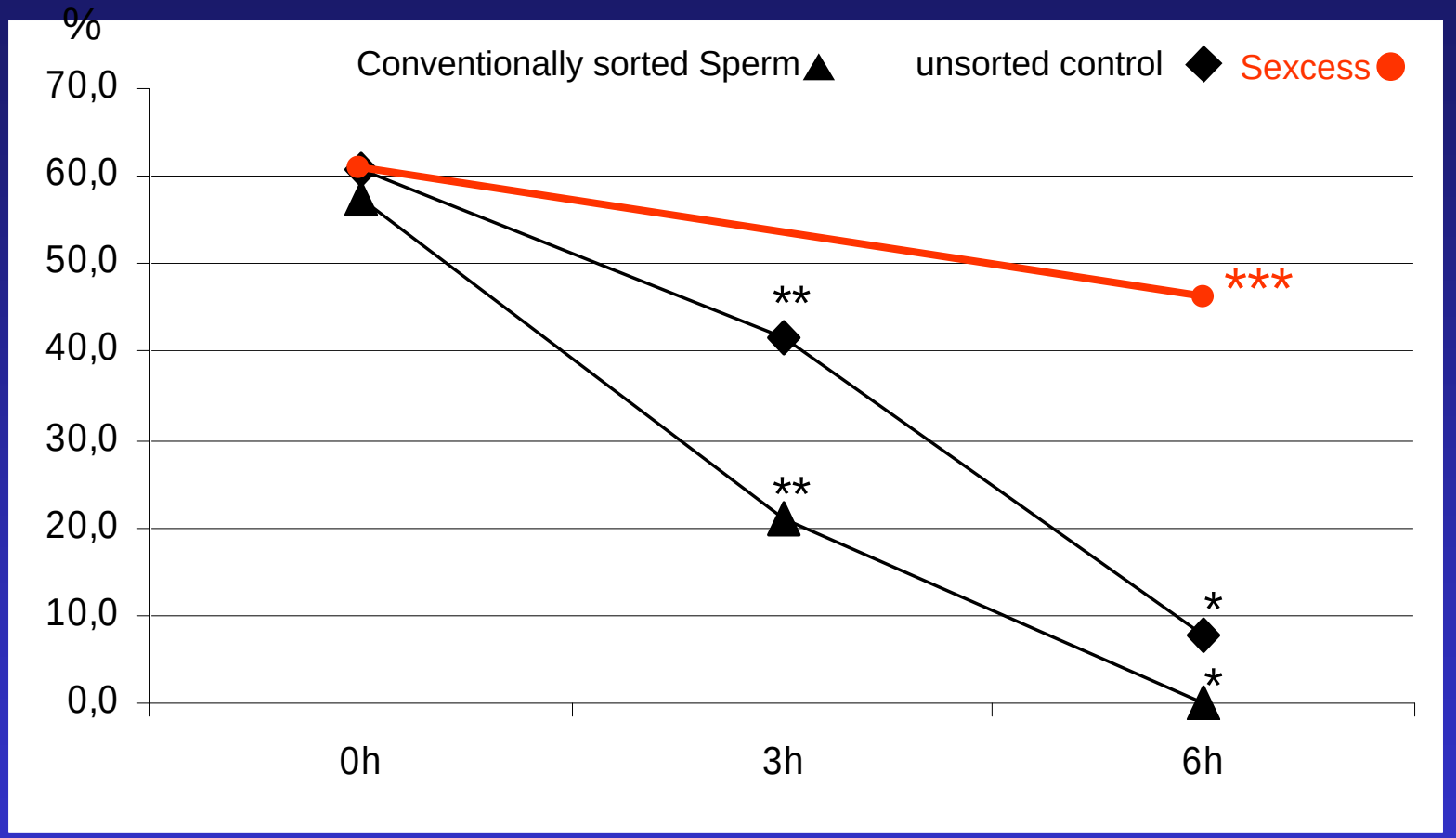
Andersson et al. 2006, Reprod Dom Anim 41, 95-97.

New sorting and processing protocols

Sexcess®

Klinc et al. 2007, Reprod Dom Anim 42, 58-62.

Motility of thawed sperm during incubation at 37ºC



Klinc et al. 2007, Reprod Dom Anim 42, 58-62.

PREGNANCY RATES WITH SEXED BULL SPERMATOZOA USING SEXCESS®

	Unsorted Control	Sexcess®
HF-Bull (SL)	75.5%	73.5%
Limousine (SL)	79.2%	73.3%
Limousine (D)	71.4%	73.9%
+/total	89/116	85/116
Total	76.7%	73.6%

Thawing: 37°C 20 sec
AI: 12-24h after onset of heat; AI into body or distal horn

Klinc et al. 2007, Reprod Dom Anim 42, 58-62.

COMPARATIVE DATA OF EJACULATES FROM A BULL, SORTED COMMERCIALLY AND UNDER OPTIMAL CONDITIONS

	0h	3h	6h
Mot (%) commercial	58	27	0
Mot (%) research	70	60	60

Abnormal sperm: post sort/post thawing commercial: 11%
Abnormal sperm: post sort/post thawing using experimental: 7%

Sort purity commercial: 88%
Sort purity experimental: 98%

Rath and Johnson 2008, Reprod Dom Anim 34, 338-346.

MINIMAL REQUIREMENTS OF SEMEN QUALITY AFTER SORTING

- Post thaw motility: (CASA; fluorescent image) ≥ 50%
- Thermo Tolerance Test: (6h; 37°C;CASA; fluorescent image) ≥ 30%
- Morphological abnormal sperm: ≤ 20%
- FITC-PNA/Syto 17/PI: ≥ 70% intact
- Sort purity: ≥ 90%
- Sperm number/straw: ≥ 2 million intact sperm

Rath and Johnson 2008, Reprod Dom Anim 34, 338-346.

STATUS OF SEXED SPERM APPLICATION

- Fertility	variable
- Sperm quality	needs improvement
- Sort purity	high
- Sort speed	improvable
- Usability of bulls	improvable
- Economic benefit	depends on fertilizing abilities and pricing conditions


Economics

Economics of sexed sperm in commercial dairies

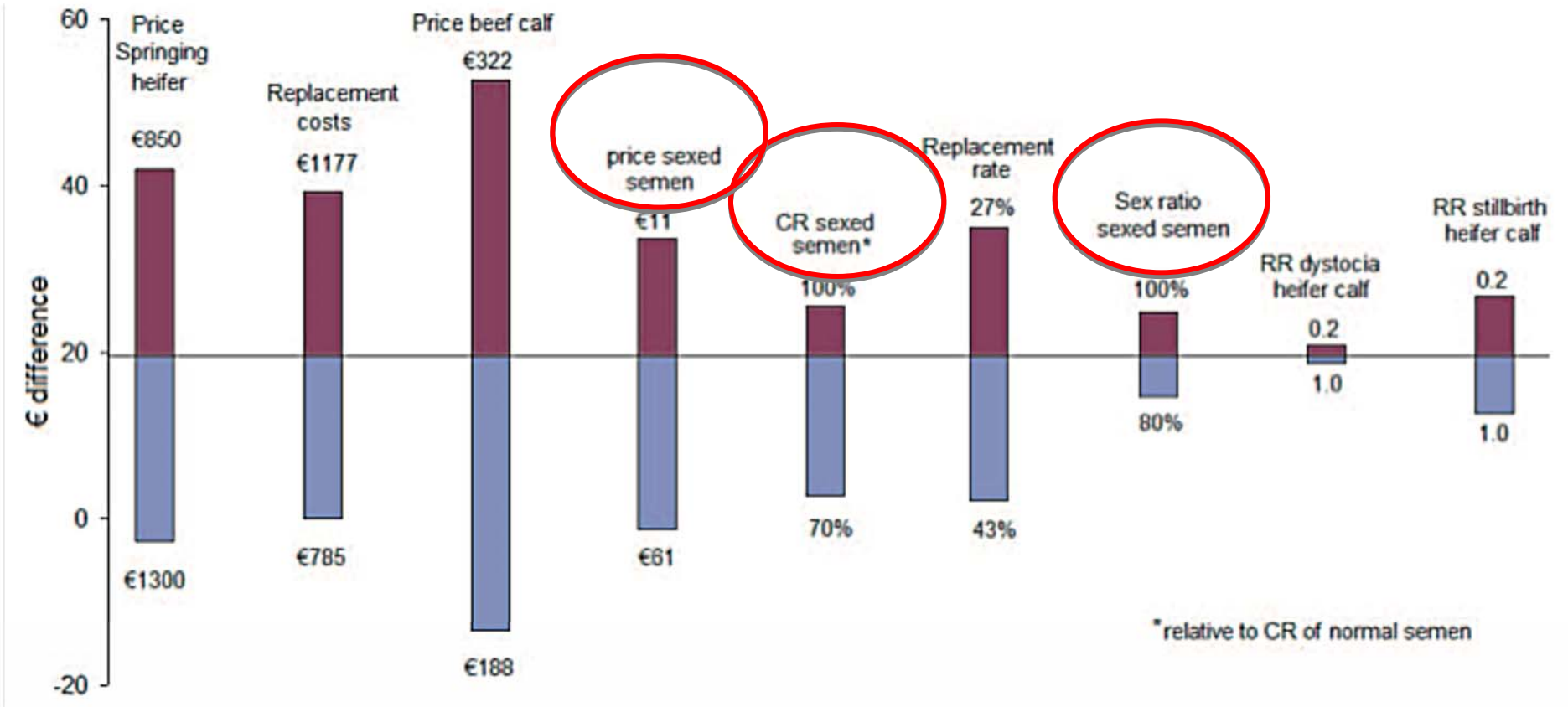


Figure 1: Difference between control and sexed semen when using lowest and highest estimate for each parameter. Base line represents the difference (€19.6) when using each parameter's best estimates (as presented in Tables 1 and 2).

Ettema et al. 2007, Western Dairy News 7, 67-68.

Example of a premium farm in Germany

	Pregnancy unsorted		Pregnancy sorted	
Insemination index	1.5		2.0	
Semen costs/Pregnancy (€)	40.00		129.35	
Costs /AI	9.75		13.00	
AI costs/calf	49.75		142.35	
	male	female	male	female
Sex ratio	53	47	10	90
€/calf/sex	120	300	120	300
€/calf in average	204.60		282.00	
Profit/sexed calf	77.40			
additional cost with sexed sperm/calf	92.65			

deficit per calf	**-15.25 €**

Rath and Johnson 2008, Reprod Dom Anim 34, 338-346.

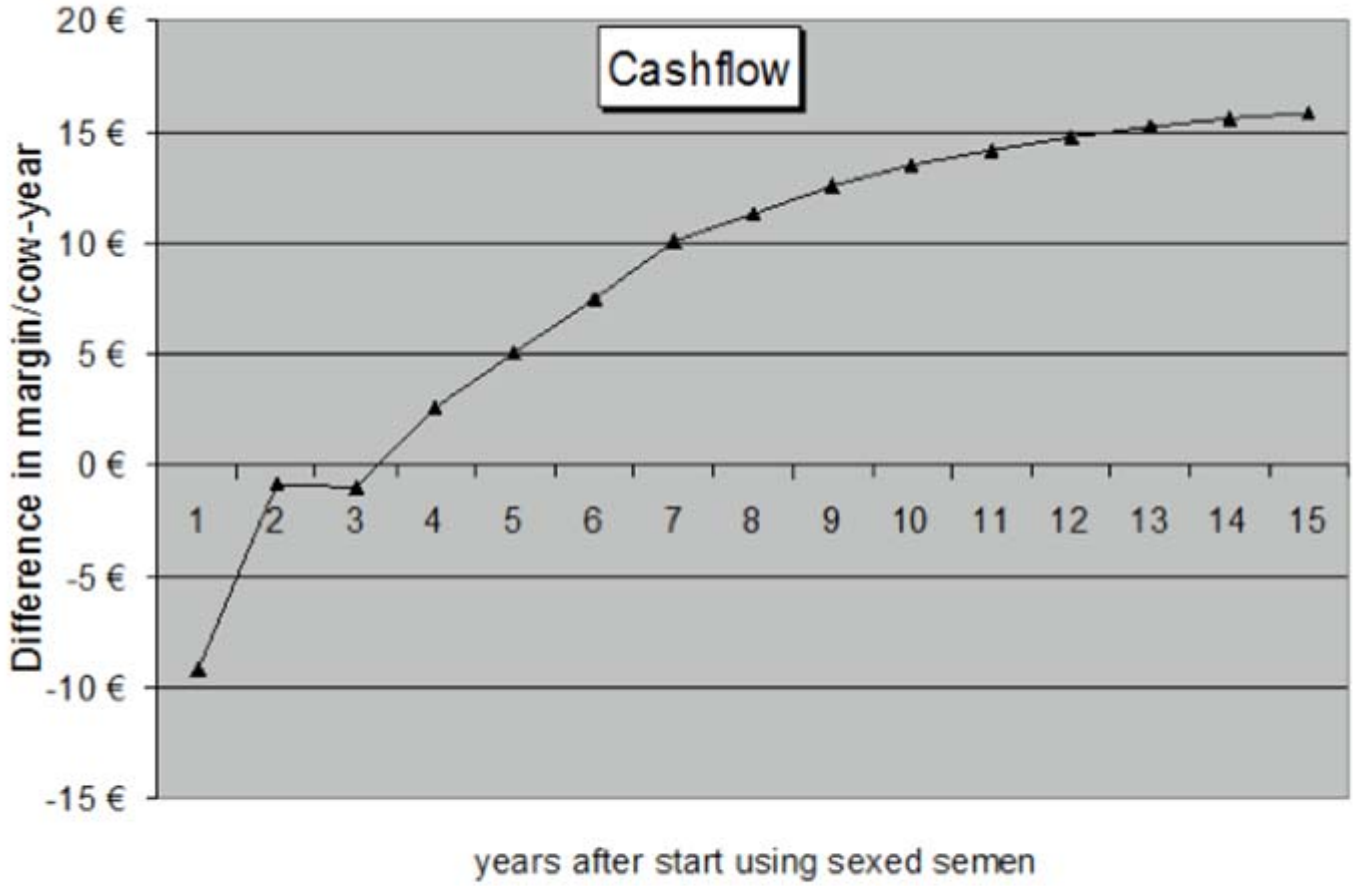


Figure 2: Difference in margin per cow-year between a scenario with and without the use of sexed semen.

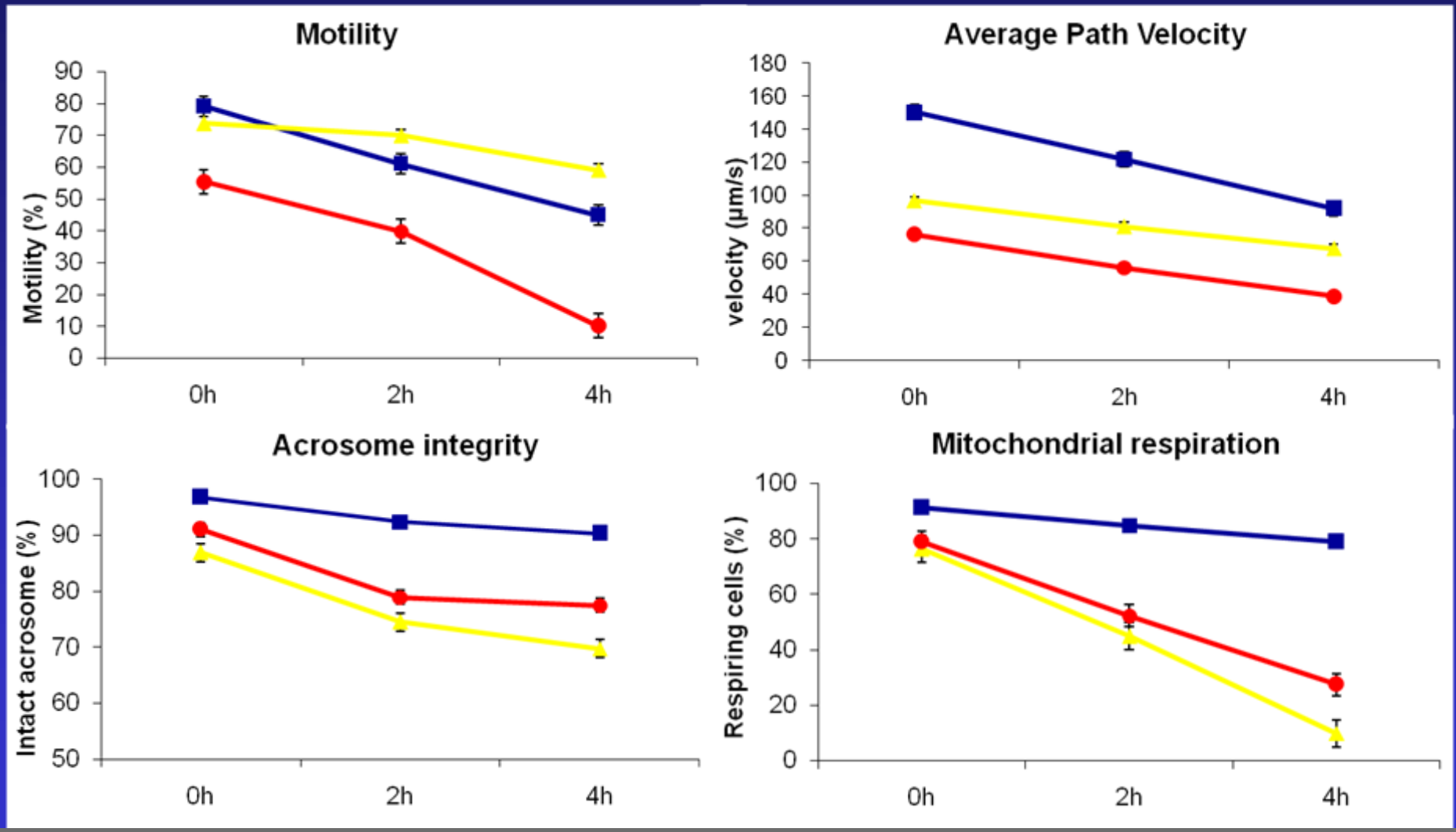
Ettema et al. 2007, Western Dairy News 7, 67-68.


Future development: Sexing of already frozen cattle semen

Advantages

- Sexed sperm is currently available only from bulls located at sex-sorting labs.
- Limited no. bulls available for sexing due to cost of each facility and the small number of labs worldwide.
- Sex-sorting already frozen semen would allow the use sexed sperm from any bull anywhere in the world.
- Over 1,000 lambs already born from this procedure.

In vitro results - cattle



Frozen-thawed non-sorted, Control or frozen-thawed, sex-sorted bull spermatozoa either refrozen (FSF) or incubated post-sort at 37C (FSFresh)

AI of frozen-thawed, sex-sorted, refrozen-thawed sperm

TYPE/DOSE SPERM	NO. HEIFERS INSEMINATED	NO. HEIFERS PREGNANT
FSF 4×10^6	12	0 (0%)[a]
FT (control) 4×10^6	8	6 (75%)[b]

Monitor growth of pre-ovulatory follicle and inseminate close to time of ovulation (Trial 1)

Increase sperm dose and AI 24h post-standing heat + introduce FSFresh treatment (Trial 2)

TYPE/DOSE SPERM	NO. HEIFERS INSEMINATED	NO. HEIFERS PREGNANT
FSF 10×10^6	7	1 (14.3%)
FSFresh 4×10^6	7	0 (0%)
FT (control) 10×10^6	7	4 (57.1%)

First calf born following artificial insemination of frozen-thawed, sex-sorted, refrozen-thawed sperm

Xena born 10 August 2008

Conclusions

- Sorting technology is available for application in dairy cattle.
- However, significant attention is required on sperm quality for commercial application.
- Sex sorting of already frozen semen may be available in the future.

Acknowledgements

Funding: XY Inc., Gardiner Foundation, Bioniche

Colleagues (former & current):

- Academic colleagues: Gareth Evans, Larry Johnsin, Detlef Rath, Juan-Maria Vazquez, Chris Grupen, Ros Bathgate, Simon de Graaf.
- Technical assistance: Monica Ruckholdt, Steven Burgun, Kim Heasman, Byron Biffin and Keith Tribe
- Postgraduate students: Shelley Underwood, Kiri Beilby, Tamara Leahy, Zamira Gibb, Fiona Hollinshead, Jenny Clulow.